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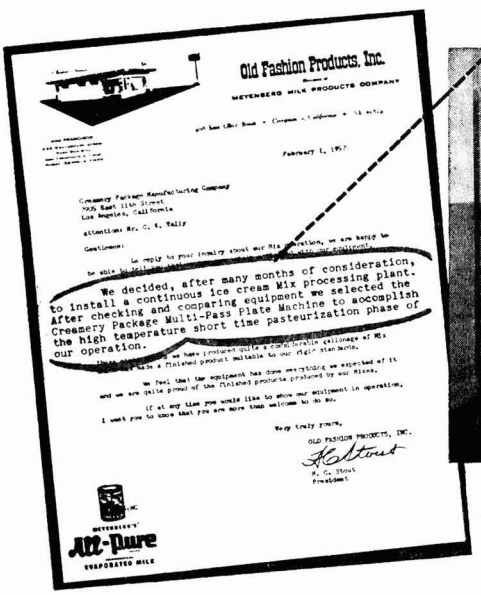
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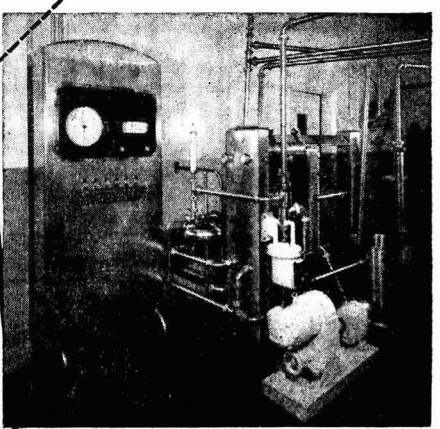

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THE USE OF INDICATOR METHODS IN MEASURING THE CONTRIBUTION OF TWO FORAGES TO THE TOTAL RATION OF THE DAIRY COW [1]

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The rapid expansion of dairying in the Southeast during the last several years has created a heavy demand on the available feed supply in the area. Because the prevailing climate is favorable, chief emphasis has been placed on the production of pasture forages on a 12-month grazing basis. To meet this demand, workers in the field of agronomy have carried out an extensive program of research to find, develop, or introduce plants suitable for the area, as well as to obtain information on the husbandry of the plants found to be adapted. Since there is frequently a vast difference between the desirable agronomic characteristics of a plant and its usefulness as a source of feed for dairy cows, investigators in the field of animal nutrition are faced with the necessity of determining which of the plants found to be adapted are also capable of permitting desired levels of livestock performance.

The work in this paper is a portion of long-time experiments designed to compare forages in use in the Piedmont area on the basis of their nutritive and other specific characteristics, milk producing ability, and methods for insuring optimum utilization. It seemed desirable to compare the forages under actual grazing conditions, which made direct measurement of digestibility and rate of consumption impossible. Reid (8) has outlined an indirect method for obtaining these two measures by grazing animals. The methods are commonly referred to as "index" or "indicator" techniques. Chromic oxide (Cr_2O_3) was used to obtain a measure of the feces dry matter voided per unit of time, and chromogen was employed to measure the degree of indigestibility of the consumed forage. With the above data the consumption of forage dry matter was calculated by the formula:

$$\text{Dry matter intake/unit} = \frac{\text{Amount of feces voided / unit of time}}{\text{\% indigestibility of forage dry matter}}$$

Crampton *et al.* (3) and Kane *et al.* (4) have discussed the use of chromic oxide and its use in ruminant digestion trials, and Reid *et al.* (9) have discussed the use of chromogen. The general methods employed in these experiments have been discussed in an earlier publication (5).

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[1] Journal Series Paper No. 231, Georgia Agricultural Experiment Station.

EXPERIMENTAL PROCEDURE

Two winter forages available for use were fescue, in a nearly pure stand, and a temporary forage mixture of oats, rye grass, and crimson clover. The experiment consisted of two 14-day periods beginning the last week in January and ending late in February. Six producing Guernsey cows were placed on the temporary forage and four on the fescue. The cows were selected and grouped according to stage of lactation, milk production, and body weight.

The cows had free access to the pasture with the exception of 2 hours each morning and evening, when they were in the barn for feeding and milking. Grain was fed according to the Morrison standard for good pasture forage (6). It was planned to feed hay at the rate of 1 lb. per 100 lb. body weight. When it was observed that the cows on the temporary forage refused much of their hay, both groups were limited to the amount the cows on the temporary forage would consume. Milk production was recorded at each milking, and butterfat tests were made at the beginning and end of each period.

A combination of indicator techniques as reported by Reid (8) was used to determine the contribution of two pasture forages to the total ration. Chromic oxide was fed at the rate of 15 g. per cow daily for a 7-day period midway in each 2-week period. Beginning 96 hours after the first chromic oxide feeding, fecal samples were collected from each cow on three successive days just prior to the evening milking. These samples were composited for each cow, and chromic oxide content was determined by the technique suggested by Schurch *et al.* (10). In addition, the chromogen technique of Reid *et al.* (9) was used to determine the apparent digestibility. The fecal samples were dried and ground, and a routine proximate feed analysis was made, using the A.O.A.C. technique (1). Feed and fecal gross energy values were determined in a bomb calorimeter and expressed as therms (T)) of energy.

RESULTS AND DISCUSSION

The average composition of the feeds used is shown in Table 1. The soybean hay was of very high quality, as indicated by its high crude protein content (17.69 per cent), its low fiber (25.5 per cent), the observation that most of the leaves were retained, and the fact that it had been cut before the leaves had matured.

TABLE 1

Proximate composition of feeds used (dry basis)

Feed	Protein	E. extract	Fiber	N.F.E.	Ash	Energy
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(T/lb.)*
Grain mixture	10.21	3.45	2.96	74.11	9.24	1.99
Soybean hay	17.69	2.30	25.50	45.87	8.62	1.86
Fescue forage	18.64	3.73	24.53	46.37	8.71	2.18
Temporary forage	30.10	5.60	13.99	43.41	6.88	2.04

The fescue forage had a desirable protein content but was high in fiber and ash. The temporary forage contained an excessive amount of crude protein (30

per cent) but probably represented a typical winter forage in this area. Large quantities of nitrogen fertilizer were used to promote growth, and the forages tended to remain in a state of vegetative growth throughout the winter months. These and other factors combined to produce a lush, highly nutritious, high-protein forage.

The cows on the fescue forage consumed an average of 13.17 lb. dry matter per cow per day from the forage; they also received a daily barn ration of 7.85 lb. grain and 4.93 lb. hay, making a total ration of 25.95 lb. dry matter per day. The cows grazing the temporary forage had a total daily dry matter intake of 30.45 lb. consisting of 6.77 lb. grain, 4.19 lb. hay, and 18.76 lb. forage.

TABLE 2
Composition and digestibility of total rations fed

Ration	Dry matter	Protein (Nx6.25)	Fat	Fiber	N.F.E.	Ash	Energy
	(lb.)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(therms)*
Temporary forage ration							
Av. composition	30.45	24.89	4.89	12.80	47.68	9.72	63.15
Av. digestibility	21.26	76.78	39.59	48.47	80.71	41.89	43.82
Fescue forage ration							
Av. composition	25.93	16.53	3.66	19.73	52.75	7.32	54.64
Av. digestibility	16.07	55.94	45.26	54.88	74.28	13.68[a]	33.57

[a] Feces samples indicated contamination with sand.

The average composition of the total ration supplied each cow is shown in Table 2 along with the apparent digestibility of each component. No special discussion is needed on this point other than to mention the apparent superiority in the digestibility of the temporary forage ration over that of the fescue ration.

The value usually termed "dairy merit" was calculated for each group from the formula suggested by Brody (2). The cows receiving the ration which included fescue forage returned an average of 27 per cent of their apparent TDN intake as milk, whereas the cows on the temporary forage returned 26 per cent. This close agreement between the two groups would seem to indicate no apparent difference in the producing ability of the two groups of cows.

At the beginning of the trial, both groups were selected to have an average daily production of 25 lb. of 4 per cent milk. The cows on the temporary forage ended the experiment with an average daily production of 29 lb., and the fescue group with 20.3 lb. Using the level of production at the end of the experiment and the average body weight (870 lb. on fescue and 919 lb. on temporary) daily TDN and digestible protein requirements were calculated from the National Research Council standards (7). The apparent utilization of the available food nutrients is shown in Table 3.

The fescue ration supplied the cows with 16.34 lb. TDN, 2.4 lb. digestible protein, and 35.57 lb. total digestible energy per day, and the temporary forage ration supplied 20.75 lb. TDN, 5.82 lb. digestible protein, and 43.82 lb. total digestible energy per day. The cows required 43 per cent of the TDN on the fescue and 35 per cent of the TDN on the temporary pasture for maintenance. This

difference obviously was due to the larger quantity of TDN supplied by the oats, rye grass, and crimson clover, since there was only 0.35 lb. difference between the requirements of the groups. As would be expected, the reverse would be true

TABLE 3

Apparent utilization of the nutrients supplied

Item	Fescue ration			Temporarary ration		
	TDN	Dig. protein	Dig. energy	TDN	Dig. protein	Dig. energy
Supplies by the ration	16.34 lb.	2.40 lb.	33.57 T	20.75 lb.	5.82 lb.	43.82 T
Required for maintenance	7.00 lb.	0.48 lb.	12.69 T[a]	7.35 lb.	0.55 lb.	13.33 T
% of ration	43	20	38	35	9	30
Required for milk production	6.51 lb.	0.92 lb.	11.80 T	9.30 lb.	1.31 lb.	16.87 T
% of ration	40	38	35	45	22	38
Excess	2.83 lb.	1.00 lb.	9.08 T	4.10 lb.	3.96 lb.	13.62 T
% of ration	17	42	27	20	69	32

[a] Energy values calculated for maintenance and production requirements allowing 1814 C per lb. TDN.

for milk production since the temporary pasture group produced at a consistently higher level of production and used 45 per cent of the supplied TDN for milk production as against 40 per cent by the fescue-fed group. The 17 per cent and 20 per cent apparently wasted TDN probably was not available for use but represented the normal loss as methane, specific dynamic action, and urine, which was included on the calculated TDN supplied. The 20 per cent and 32 per cent loss of energy undoubtedly fell in the same category as the excess TDN.

The usage of the available protein best illustrated the problems encountered in using winter forages. Fifty-eight per cent of the available digestible protein was used by the cows receiving the fescue ration and only 31 per cent by the cows on temporary forage. This large difference would indicate that the cows were producing at the general level of available energy in the ration irrespective of the protein intake, since neither ration supplied protein below that required.

The point of practical importance remains that the higher protein percentage of the temporary forage did not permit an increased milk production above the level of the usable TDN in the total ration consumed.

SUMMARY

An example of a combination of indicator techniques used to determine the contribution of two pasture forages to the total ration is reported. Two 14-day digestion trials involving six cows on a temporary winter forage mixture of oats, rye grass, and crimson clover, and four cows on fescue grass, each group being fed hay and grain in addition to having free access to the respective forages, are reported as supporting data. Within the range of the data, the following preliminary observations appear justified.

1. The cows had an average total dry matter intake of 25.9 lb. on the fescue and 30.4 lb. on the temporary pasture mixture and received 16.34 lb. and 20.75 lb. TDN, respectively, from the rations.
2. The milk production level on each ration apparently was limited by the amount of energy-producing nutrients consumed.
3. Increasing the protein content of the total ration from an average of 16 per cent on the fescue to 25 per cent on the temporary forage increased the wastage of protein from 42 to 69 per cent of the total digestible protein.
4. The apparent nutrient deficiency of winter forages under the conditions of this experiment is in energy-producing fractions.
5. The use of indicator techniques offers an apparently reliable measure of the contributions of forages to the total ration of a dairy cow under grazing conditions.

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A STUDY OF CERTAIN TECHNIQUES USED IN TESTING MILK BY THE BABCOCK TEST

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Since 1938, a number of committees of the American Dairy Science Association have been studying various aspects of the Babcock test. Recently, committee work has been concerned with the preparation of a complete set of recommendations which are intended to minimize the diversity of techniques existant among the various states. There are, however, few published works — particularly since 1900 — which contain data concerning certain phases of sampling and testing milk (7). This paper is presented, therefore, in order to supply data which may be found useful in the establishment of tolerances for certain practices followed in the testing of milk.

It is realized that much of the data contained in this paper probably is not new to most workers in this field. Many people undoubtedly have obtained related data which, if published, would be of value to committees concerned with standardizing the Babcock test.

It is a distinct tribute to the genius of Dr. Babcock, who developed his test 60 years ago, that the only subsequent modifications relate to narrowing the limits of tolerances permitted in the performance of the test. However, the attainment of consistently clear tests remains in the category of an art rather than a science. Furthermore, the general rule that an accurate test is one which is translucent, of golden-yellow or amber color, and free from curd or charred matter has not been verified as a criterion of precision.

Since the various references generally include data on more than one technique, each relevant reference will be quoted in the following sections. Herreid (7) has reviewed the literature on the Babcock test up to 1942. Consequently, the number of literature references prior to that time has been minimized in this work.

1. *Speed of Centrifuge*

REVIEW

Babcock (2) first specified 700 to 800 r.p.m. as the correct speed for his centrifuge, which was of the angle type, but the diameter was not given. He later gave 700 to 800 r.p.m. as correct for an 18-in. diameter wheel (3). According to Herrington (9), Farrington and Woll used this as a basis for calculating a table of speeds at which wheels of various diameters should be operated. Herrington pointed out that centrifugal force should be calculated from a point 1.5 in. up from the bottom of the extended cups and proposed a revised table to correspond to this method of calculation.

Baily (4) reported an average of six tests run at speeds from one-fourth to

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full speed and noted an increase in test with increasing speeds. The diameters of the centrifuges used were not given. Wilster (11) reported results obtained with a 20-in. wheel. The average of 12 tests made at normal (760 r.p.m.) was 3.808 per cent. When the speed was increased to 960 r.p.m. the average of these 12 samples was 3.817 per cent. When the speed was decreased to 560 r.p.m., the average test was 3.696 per cent.

APPARATUS

The study reported in this paper was made with three electrically heated machines. One was a 20 inch tester driven directly by a synchronous motor. The speed was varied by means of a rheostat. The other two centrifuges were 24 in. in diameter and were belt driven. The speed of each tester was determined with a precision tachometer and was reproducible to ± 3 r.p.m. The temperature of each tester was maintained between 130 and 150° F. Two centrifuging periods of 5 minutes and 3 minutes were used.

Except in trials concerned with the study of particular variables, the techniques which were followed were those described in Circular 618 of the University of Missouri (10). Tests were read to the nearest 0.1 per cent in front of a source of diffused light, using a 5-in. lens. Test bottles were retested using a specially designed burette with mercury as the calibrating liquid.

RESULTS

The results given in Table 1 show the influence of the speed of the centrifuge on the Babcock test.

If a figure of ± 0.01 per cent fat is considered acceptable as a reasonable standard of precision of the Babcock test (as measured by the averages of a large number of tests), calculations may be made, using the data in Table 1, to arrive at tolerances for centrifuge speeds. This was done, using the procedure of Herrington and the table of standard r.p.m. found in A.O.A.C. (1). These results are given in Table 2.

Based on the calculations in Table 2, two 24-inch centrifuges were altered by changing pulley sizes to yield an r.p.m. of about 720 on one machine and about 660 on the other. The actual r.p.m. were 732 and 665.

The results of tests made on each machine are given in Table 3. Using Stu-

TABLE 1

Effect of speed of 20-inch diameter centrifuge on the Babcock test

(Average of 18 tests at each speed)

r.p.m.	% of standard	Average test	Difference from standard
		(%)	
835	+ 10	3.694	+ 0.016
759	0	3.678	.....
683	— 10	3.633	— 0.045
607	— 20	3.572	— 0.106

TABLE 2

Calculated effect of variation in speed of centrifuges with various diameters on the Babcock test

Diameter of wheel	Calculated speed [a] of centrifuge required to:		[b]	[c]
	increase test by 0.01%	decrease test by 0.01%		
16	914	839	848	859
18	850	781	800	800
20	799	734	759	751
22	756	694	724	711
24	719	660	693	677

[a] Calculated r.p.m. based on A.O.A.C. values.
[b] A.O.A.C. values.
[c] Herrington's calculated values.

TABLE 3

Effect of speed of 24-inch centrifuge on the Babcock test

(Average of 72 tests on each machine)

r.p.m.	Average Test
	(%)
732	4.461
665	4.450

dent's T test, no significant difference can be demonstrated between the two centrifuging speeds. However, the trend would indicate a slightly lower value for the lower speed.

SUMMARY

These data indicate that Herrington's values could be followed as minimum centrifuging speeds with no significant effect upon the precision of the Babcock test. In the case of the 16-in. diameter centrifuge, the A.O.A.C. value of 848 r.p.m. is likely to result in tests averaging 0.01 per cent too low when a large number of tests are run and compared with the average obtained on an 18-in. diameter centrifuge operated at 800 r.p.m. A 16-in. machine was not available to verify this conclusion.

Since Herrington's values are correctly calculated, and since tolerances of 40 to 55 r.p.m. higher than his values may be considered safe, it is suggested that Herrington's values be used as minimum speeds not to be exceeded by 40 r.p.m. In effect, this suggestion accepts the A.O.A.C. values as nearly central values with the exception of the 16-in. and 18-in. machines.

2. *Time of Centrifuging.*

REVIEW

Babcock (2) originally specified 6 or 7 minutes for the first period, 1 or 2 minutes for the second, and a short time for the final period. Later (3) he centrifuged for 5 and 1 minutes, using only two centrifuging periods. Farrington specified periods of 5, 2, and 1 minutes, and since that time this procedure has

been followed in most published works (7). Gould (6) quotes data from the Dairy Inspection Service of Maryland and states that shortening the first period to 3 minutes, omitting the second period, and centrifuging 1 minute for the final period lowered the test by 0.024 per cent. The data indicate that there is no decrease in test, providing that the total centrifuging time is 6 minutes or more.

METHODS

The A.O.A.C. procedure was followed, except that 14 ml. of acid was used and that the centrifuging period was varied. Tests were read to the nearest 0.1 per cent. A quantity of milk was well mixed and divided into seven portions. Twenty tests were made on each portion for each centrifuging technique. A 24-in. centrifuge was used.

RESULTS

Table 4 shows that as total centrifuge time decreases, the fat test is decreased. However, a decrease of 2 minutes in the time of the first centrifuging period, or the elimination of the third centrifuging period, is apparent only in the third decimal place, and Student's "T" test does not reveal any significant difference. In Table 5, the results on 216 samples of milk show that the effect of eliminating the final 1 minute centrifuging period decreased the average butterfat test by 0.004 per cent. This difference is not statistically significant, nor is there any difference attributable to the freshness of the milk being tested. In another trial, the use of two centrifuge periods of 5 minutes and 3 minutes resulted in an increase of 0.011 per cent over the average test of the same samples centrifuged 5, 2, and 1 minutes. All tests were free from curd regardless of centrifuging procedure.

TABLE 4

Effect of various centrifuging techniques on the Babcock test
(Average of 20 tests for each technique)

First Period	Second Period	Third Period	Average Test
5	2	1	4.507
3	2	1	4.500
1	2	1	4.451
5	2	....	4.505
5	1	....	4.488
3	1	....	4.446
1	1	....	4.342

TABLE 5

Effect of three centrifuging techniques on the fat test of 108 samples of fresh milk and 108 samples of milk preserved 15 days at 50° F.

Samples	Centrifuged 5-2-1	Centrifuged 5-2	Centrifuged 5-3
108 (fresh milk)	4.285	4.281	.....
108 (composite samples)	4.853	4.849	.....
108 (fresh milk)	4.245	.....	4.256

SUMMARY

The data presented in this and the preceding sections show that the Babcock test is influenced by the amount of centrifugal force exerted over a period of time. Both of these factors are empirical. With a 24-in. heated centrifuge, operated at 693 ± 3 r.p.m., two centrifuging periods of 5 minutes and 3 minutes yielded results which were not significantly different from three centrifuging periods of 5 minutes, 2 minutes, and 1 minute. Two centrifuge periods are more simple than three and, when a large number of tests are made, a considerable saving in time results.

3. *Specific Gravity and Amount of Sulfuric Acid.*

REVIEW

Babcock (2) originally specified a specific gravity of 1.82 and 17.5 cc. of acid and later (3) recommended the same amount of acid with a specific gravity of 1.82 – 1.83. Baily (4) used 15 cc. of 1.83 for most of his work, but found that acid with specific gravity of 1.80-1.81 gave lower results. His data also indicate that 10 and 12.5 cc. of acid with a specific gravity of 1.83 gave lower results (.07 and .02 respectively) than 15, 17.5, and 20 cc. Herreid (8) found a decrease of 0.04 to 0.05 per cent in the fat test as a result of decreasing the amount of acid from 17.5 to 15 ml. From 1925 to 1945, A.O.A.C. specified 17.5 ml. of specific gravity 1.82 to 1.83 at 20° C. The specific gravity is not specified in the 1950 edition (1). Wilster (11) stressed the importance of controlling the specific gravity between 1.825 and 1.830.

METHODS

Reagent grade sulfuric acid was used in preliminary trials. This acid had a specific gravity of 1.836 at 68° F., as determined by a hydrometer. It was adjusted to the required specific gravity with distilled water. The temperature of both the acid and the milk was adjusted to 70° F. before mixing. In later trials, commercial sulfuric acid having a specific gravity of 1.833 was standardized to 1.830 and 1.820 at 68° F. In these trials, as in the previous ones, fresh milk samples were used. The required volume of acid was added, the test bottle immediately shaken, and shaking continued until a dark brown color was obtained. The time of shaking, recorded in Table 6, is correct within 5 seconds and corresponds to the interval between starting to mix and placing the test bottle in the centrifuge. Attempts to obtain fat colums free of curd or charred material were unsuccessful in trials using 17.5 ml. of 1.830 specific gravity acid. However, all other fat columns obtained in these trials were clear but showed varying degrees of color. The color of the fat columns ranged from lemon yellow to orange yellow. No amber colored tests were obtained.

SUMMARY

The results presented in Table 6 show that decreasing the specific gravity of acid from 1.830 to 1.820 results in a slightly lower test. If the acid is 1.820, low

TABLE 6

Effect of varying amount and specific gravity of sulfuric acid on the Babcock test

(Average of 12 tests in each trial)

Ml. Acid Used	Specific gravity of acid at 68° F.			
	1.820		1.830	
	Av. Test	Mixing time	Av. Test	Mixing time
	(%)	*(Sec.)*	*(%)*	*(Sec.)*
10 (Av. for 24 trials using 10 ml: 4.868%)	4.844	240	4.892	60
12 (Av. for 24 trials using 12 ml: 4.908%)	4.893	60	4.922	25
14 (Av. for 24 trials using 14 ml: 4.960%)	4.943	30	4.978	15
16 (Av. for 24 trials using 16 ml: 4.946%)	4.928	20	4.963	8
17.5 (Av. for 12 trials using 17.5 ml: 4.954%)	4.954	15	x	—
	(Av. for all trials using 1.820: 4.913, N = 60)		(Av. for all trials using 1.830: 4.939, N = 48)	

TABLE 7

Comparison of the effect of 17.5 ml. acid (sp. gr. 1.820 at 68° F.) with 14.0 ml. acid (sp. gr. 1.830 at 68° F.), 15 sec. mixing time.

(average of 36 samples in each trial)

Volume and sp. gr. acid	Average Test
	(%)
17.5 ml. — 1.820 at 68° F.	5.292
14.0 ml. — 1.830 at 68° F.	5.318

results will be obtained if less than 14 ml. is used, whereas equivalent results are obtained if 14, 16, or 17.5 ml. are used. If the acid is 1.830 sp. gr., 17.5 ml. results in "burnt" tests.

Although this article is concerned only with means of increasing the precision of the Babcock test, it is necessary to point out that inaccurate tests may be obtained if rule-of-thumb methods are followed. All tests reported in Table 6 would pass the inspection of an experienced tester, yet some of them are obviously inaccurate. It is also obvious that the volume of acid, specific gravity of acid, temperatures of milk and acid, and mixing time of milk and acid are interrelated factors. From a precision standpoint these can be controlled by arbitrarily establishing very narrow limits of tolerances. From the standpoint of accuracy, however, some other criterion, such as the gravimetric method, must be used. The criterion of judging accuracy based upon the appearance of the fat column at the time of measurement does not appear to be reliable. The criterion of mixing milk and acid to a dark chocolate color likewise does not appear to be a satisfactory criterion of accuracy. (The assumption is made that an acceptable standard of precision and of accuracy is $\pm$ 0.01 per cent fat as determined by averages of a large number of tests.)

The data presented in Table 7 show that in using the same mixing time, slightly higher results are obtained with 14.0 ml. of acid (sp. gr. 1.830) than with 17.5 ml. of acid (sp. gr. 1.820) on samples of fresh milk. The higher results

obtained with 14 ml. are statistically significant at the 90 per cent level of probability.

These results indicate that if mixing time is varied, 14 to 17.5 ml. of acid with a specific gravity of 1.820 may be used with substantially the same results. Likewise, 14 to 16 ml. of acid with a specific gravity of 1.830 may be used. Tests made using acids with different specific gravities may or may not yield results which are substantially the same.

4. *Mixing Milk Prior to Pipetting.*

REVIEW

According to Herreid's review of the literature on this subject (7), most investigators mixed the sample by pouring back and forth three or four times. This procedure is obviously necessary if the sample bottle is full or nearly full of milk. It appeared, however, that a quicker procedure might be found for mixing samples of milk in bottles not more than two-thirds full.

METHODS

In a preliminary survey, 36 8-oz. rubber-stoppered composite sample bottles were filled with 160 ml. of the same sample of fresh milk. One 0.5 g. tablet of mercuric chloride was added to each sample and the 36 bottles were stored 5 days at 45° F. They then were divided into three groups of 12 each and warmed to 90° F. for pipetting. There was no visible "oiling off" on any of the samples. The first group of 12 samples was subdivided into three groups of four each. All of these 12 samples were shaken back and forth (the bottle being held horizontally) six times in 2 to 3 seconds through a stroke of 5 to 8 in. Immediately after shaking each of the first four bottles, a pipette was inserted about ½ inch below the surface of the milk, and the sample for testing was withdrawn. The next four bottles were shaken in a similar manner, and the pipette was inserted to approximately one-half the depth of the milk. The remaining four bottles were shaken, and the tip of the pipette was inserted to the bottom of the milk.

The 12 samples in the second group were poured back and forth two times (four pourings), and those in the third group were poured back and forth one time (two pourings). The pipette was inserted to the same three levels as described for shaking procedure.

In subsequent trials, fresh milk warmed to 90° F. was used. Three persons were engaged in the process of pipetting. One poured or shook the sample while the other two pipetted simultaneously, one from the top ½ in. of the milk, the other from the bottom ½ in.

RESULTS

The results obtained by mixing the sample three different ways prior to pipetting are presented in Table 8.

TABLE 8

Effect of three methods of mixing preserved samples prior to pipetting using 8 oz. sample bottles ⅔ full

(average of 4 samples each trial)

Method of mixing								
Shaken horizontally 6 times			Poured back and forth 2 times			Poured back and forth 1 time		
Pipetted from			Pipetted from			Pipetted from		
Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
4.38%	4.35%	4.33%	4.35%	4.33%	4.33%	4.38%	4.35%	4.33%

1. Average test (12 trials) samples pipetted from top ½ in. milk: 4.366%
2. Average test (12 trials) samples pipetted from middle of ½ in. milk 4.342%
3. Average test (12 trials) samples pipetted from bottom ½ in. milk: 4.325%

(For means of 1 and 3, N = 22, t = 2.02, P = .05, difference between pipetting from top and pipetting from bottom is significant).

TABLE 9

Effect of two different methods of mixing fresh milk prior to pipetting, using 8 oz. sample bottles ⅔ full

(average of 36 tests in each trial)

Method of mixing			
Shaken back and forth 6 times, then pipetted from:		Poured back and forth 4 times, then pipetted from:	
Top	Bottom	Top	Bottom
4.272	4.244	4.274	4.235

1. For shaking, N = 70, t = 2.10, P = .04, difference between pipetting from top and pipetting from bottom is significant.
2. For pouring, N = 70, t = 3.33, P = .01, difference between pipetting from top and pipetting from bottom is significant.
3. Difference between methods of mixing: Variation of mean difference between top and bottom for pouring: 0.0389 ± .0117. Variation of mean difference between top and bottom for shaking: 0.0278 ± .0132. For N = 140, t = 0.630, P = 0.5 and difference between methods is not significant.

SUMMARY

The results presented in Tables 8 and 9 indicate that there is no difference between two methods of mixing milk in an 8-oz. composite sample bottle containing 160 ml. of milk. The method of shaking horizontally six times (12 strokes) in 2 to 3 seconds provides a rapid means of mixing samples. At temperatures of 90 to 95° F., foaming did not present a problem. No churning was observed on any of the samples tested.

The difference observed between inserting the pipette to a depth of ½ in. from the top of the milk and inserting the pipette to a depth of ½ in. from the bottom was unexpected. Calculations would indicate that if six clusters of 1,000 micron diameter escape the tip of the pipette near the bottom of the milk sample and if six extra clusters of the same size enter the tip of the pipette near the top of the milk sample, the result would be a difference in test of about 0.03 per cent.

Regardless of the explanation, the data indicate that in order to obtain the greatest precision, the tip of the pipette should be inserted into the well-mixed milk to a point equal to about one-half the depth of the sample.

CONCLUSIONS

Data are presented concerning centrifuge speeds, centrifuging procedures, specific gravity and amount of sulfuric acid, and procedures for mixing milk samples before pipetting for the Babcock test.

Herrington's calculated speeds for centrifuges from 16 in. to 24 in. in diameter may be used as minimum speeds not to be exceeded by 40 r.p.m.

Centrifuging for two centrifuging periods of 5 and 3 minutes yielded results equivalent to three periods of 5, 2, and 1 minutes.

The use of 17.5 ml. of acid with a specific gravity of 1.820 yielded results which were slightly lower than those obtained by using 14 ml. of acid with a specific gravity of 1.830.

If an 8-oz. sample bottle contains 160 ml. of milk or less, adequate mixing may be obtained by shaking the bottle horizontally six times in 2 to 3 seconds or by pouring back and forth four times.

For greatest precision, the tip of the pipette should be inserted to a point equal to about one-half the depth of the milk in the sample bottle.

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THE LIPASE OF *PSEUDOMONAS FRAGI* [1]
I. CHARACTERIZATION OF THE ENZYME

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Pseudomonas fragi has been described by several workers and has been known under different names; this early work has been reviewed by Hussong *et al.* (11). The organism was studied in detail by Hussong (10), who proposed the present name. Long (15) confirmed these studies and found that all the lypolytic cultures studied were consistent in the production of a diffusible fat-hydrolyzing enzyme.

The ability of the growing cells of *P. fragi* to produce lipase and cause defects in dairy products, especially butter and cream, has been the object of much study (4, 6, 10, 11, 16, 17). However, little information is available concerning the characteristics of the extracellular lipase independent of the parent cells.

EXPERIMENTAL METHODS

Phosphate-buffered 1 per cent Bacto-peptone broth or nutrient broth was employed as growth substratum. After incubation at 15° C. for 3 days, the culture was centrifuged to remove cells. Preliminary results showed over 90 per cent of the total lipase activity was in the broth, indicating the predominantly extracellular character of the enzyme. Although several million bacteria per milliliter of medium remained after centrifugation, these were shown to be responsible for no measurable lipase activity during the reaction period. The clear supernatant, which was used as the enzyme preparation, was preserved with 0.1 per cent of 36 per cent formaldehyde and held at 3 to 5° C. for further use. Attempts to obtain cell-free lipase preparations by running through Selas microporous filters were unsuccessful because of loss of enzyme activity. Lipase preparation for use as a blank was heated in boiling water for 15 or 20 minutes, the longer time being used in the latter part of this study. Most preparations were obtained by use of *P. fragi* strain 0-1 and occasionally strain E-1. These strains were chosen because of their high lipolytic activity.

Unless otherwise stated, the lipase test substratum was composed of 4 g. carbonate-washed coconut oil, 4 ml. 0.5 *M* KH_2PO_4, 14 ml. 0.1 *N* NaOH, 0.04 ml. formaldehyde (36 per cent), 0.16 g. sodium taurocholate, and enough distilled water to give 40 ml. final volume when the enzyme preparation plus some water to standardize amounts had been added. A hand homogenizer was used for emulsification. A concentration of 0.1 per cent formaldehyde (36 per cent) prevented bacterial growth during the reaction period, but higher concentrations were found somewhat inhibitory to lipase activity. The pH of the resulting

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routine emulsion was 7.2 ± 0.05. The lipase preparation was allowed to react on the test substratum for 24 hours at 36° C.

Emulsions of the different oils and fats were prepared by substituting the test material for the 10 g. of coconut oil in the usual test substrate. In testing the action of lipase on pure triglycerides, 1 per cent of tributyrin or equivalent molar concentrations of the other triglycerides was used in lieu of coconut oil. Trilaurin solidified on cooling, and this solidification was more extensive with trimyristin. With tripalmitin and tristearin no satisfactory emulsion could be obtained.

The extraction-titration procedure employed in this study was a modification of the method proposed by Johnson and Gould (12, 13). A mixture of two volumes of ethyl ether and three volumes of petroleum ether (Skellysolve B) was adopted, although any ratio of the two ethers within 1:2 to 2:1 resulted in practically the same net activity. However, the greater the quantity of ethyl ether in the mixture, the higher were the blank titrations. The routine extraction procedure used throughout this work was as follows: At the end of the reaction period, one or more 10-g. samples were weighed on a torsion balance into regular Mojonnier butterfat extraction flasks. The flask contents were acidified with 25 per cent H_2SO_4, until a definite pink color of thymol blue persisted after subsequent extractions (usually 0.25 to 0.3 ml. of acid would suffice). After 10 ml. of 95 per cent ethanol were added, the flasks were shaken vigorously for 15 seconds, and then let stand for 5 minutes. Two successive extractions with 10-ml. portions of ether mixture were made, the flasks being shaken vigorously for 30 seconds, and allowed to stand for 0.5 to 1 minute until the ether layer became clear. The ether layer was poured off into dry 125-ml. Erlenmeyer flasks and titrated with 0.05 *N* KOH in absolute methanol, using ten drops of 1 per cent phenolphthalein in absolute ethanol as an indicator. Duplicate titrations usually checked within 0.02 ml., and the variation very seldom exceeded 0.03 ml.

Recovery of fatty acids by this extraction-titration procedure was tested by adding 0.5 ml. of 0.2 *N* solution of the acids in absolute ethanol to 10 g. of test substrate. Recoveries of butyric and caproic acids were rather low (Table 1), but since coconut oil contains only traces of these two fatty acids (9), the low extraction of these two acids was not considered important. When the length of the fatty acid was eight carbon atoms or more, recovery of the added fatty acids was reasonably satisfactory.

TABLE 1

Recovery of added fatty acids by the extration-titration procedure

Trial No.	Per cent recovery of fatty acids				
	Butyric	Caproic	Caprylic	Lauric	Stearic
1	44.3	76.1	91.5	97.2	96.2
2	42.8	75.6	91.5	96.6	97.3
3	45.3	.....	91.5	98.2	.....
4	44.8	.....	91.0	97.2	.....
Av.	44.3	75.9	91.4	97.3	96.8

Heat inactivation studies were made on lipase produced by cultures growing in phosphate-buffered peptone broth. To 50 ml. of the lipase preparation, adjusted to pH 7.0 by addition of 0.1 *N* HCl, was added 10 ml. 0.5 *M* KH_2PO_4 and enough 0.1 *N* NaOH so that when the final volume was made up to 100 ml. with distilled water the pH of the diluted preparation was 7.0 ± 0.05. This preparation was pipetted in 10-ml. portions into 125 × 16 mm. Pyrex screw-cap test tubes which were completely immersed in constant-temperature water baths at 61.6 or 71.6 ± 0.1° C. Temperatures of 98 to 99° C. were obtained by immersing the test tubes in a bath of boiling water. Aliquots of the lipase preparation before heating, after the desired temperature was reached and after prompt cooling at the end of the heating period, were examined for lipase activity.

The lipase was salted out by bringing 50 ml. of peptone broth preparation to nearly full saturation with $(NH_4)_2SO_4$ (about 27 g.) in 100-ml. centrifuge tubes. The tubes stood at 5 to 8° C. for 16 to 18 hours and then were centrifuged at 4000 r.p.m. for 30 minutes, after which the supernatant was decanted. The last portion of the supernatant was run through Whatman No. 30 filter paper. Precipitates from both filter paper and centrifuge tube were dissolved in cold distilled water. All these steps were done at 5 to 8° C. because the enzyme is highly unstable at higher temperatures.

The trials on the effect of temperature upon lipase activity were carried out by setting the lipase test substrate in constant-temperature water baths with deviations not to exceed 0.2° C.

For testing enzyme stability, preparations at pH 7.6 to 7.8 obtained after the growth of culture 0-1 in peptone broth were used. Lipase preparations were dispersed in 50-ml. portions, and 0.05 ml. of 36 per cent formaldehyde was added to prevent bacterial growth during holding.

RESULTS

Sodium taurocholate was the only compound among the several tried that gave a stable coconut oil emulsion, did not interfere with the extraction precedure, and did not inhibit lipase activity. Sodium glycocholate in concentrations of 0.3 and 0.5 per cent inhibited lipase action. Based on the data of Table 2, 0.4 per cent sodium taurocholate was used as an emulsifier throughout this study.

TABLE 2

Effect of concentration of sodium taurocholate [a] *upon lipase activity (2 ml. lipase preparation per 40 ml. test substrate)*

Per cent Sodium taurocholate	Active [b]	Blank [b]	Net activity
0.1	3.20	0.14	3.06
0.2	4.19	0.21	3.98
0.4	4.98	0.32	4.66
0.6	5.09	0.41	4.68
0.8	5.09	0.52	4.57

[a] Described by manufacturers as "pure."
[b] Av. of duplicate titrations.

The relationship between lipase activity at 36° C. and reaction time during the first 24 hours, although quite regular, deviates very slightly from a straight-line function (Figure 1). This also was the case during reaction periods extending to 48 and 72 hours. Even though a reaction period of 24 hours at 36° C. is beyond the straight-line portion of the time-activity curve, this period was chosen for routine tests for lipase activity in order to allow better comparison between different lipase preparations, especially those possessing low activities.

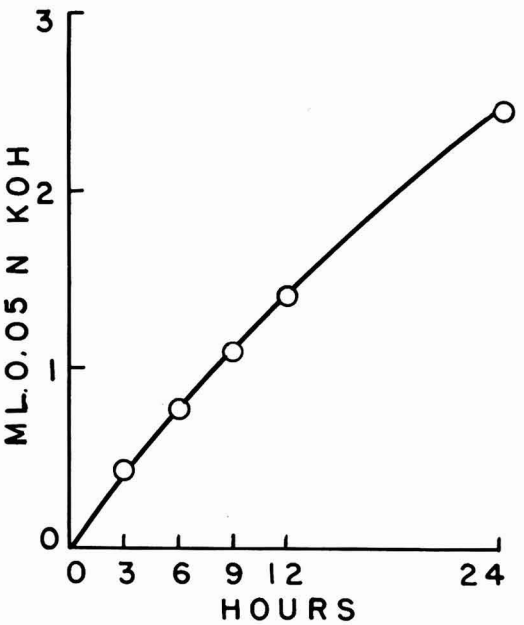

FIG. 1. Relation of lipase activity at 36° C. to time.

The influence of the temperature of the enzyme reaction upon lipase activity is shown in Figure 2, data from two series being included. The optimum temperature for lipase activity in 24 hours was 40° C. Although 36° C. was not optimum for enzyme action, it gave results not greatly less than those obtained at 40° C., and this temperature was employed routinely because of its convenience.

Representative data on variations in the action of the lipase upon some natural fats and oils are presented in Table 3. Coconut oil was hydrolyzed most rapidly and also gave a colorless ether extract, whereas several other fats and oils, particularly soybean oil, yielded colored ether extracts, thus masking the phenolphthalein endpoint in the final titration.

Somewhat surprisingly, tricaprylin underwent the greatest degree of hydrolysis, with some of the triglycerides both above and below tricaprylin being affected less the further removed they were in respect to molecule size (Table 4). Tricaprin was not available at the time these trials were carried out.

The relationship between the amount of lipase preparation and its demonstrable activity is shown in Figure 3. Either inactivation of the enzyme or in-

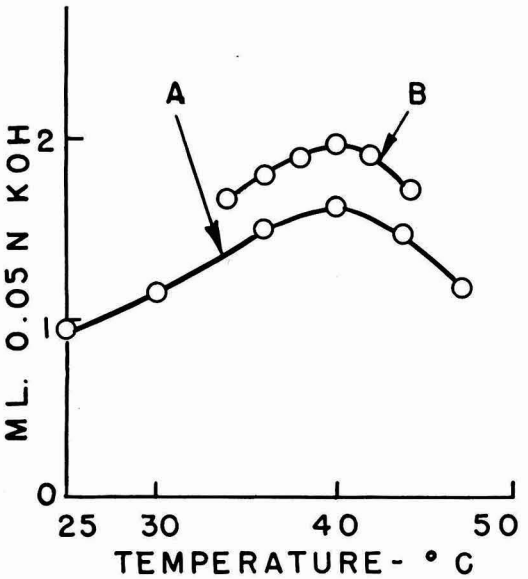

FIG. 2. Effect of temperature of test substrate upon lipase activity (24 hr. incubation). A and B represent determinations on two different preparations, using smaller temperature intervals with B.

TABLE 3

Action of lipase on some natural fats and oils
(2 ml. lipase preparation per 40 ml. test substrate)

Fat or oil	Active [a]	Blank [a]	Net activity	Color of ether extract
Butterfat	1.84	0.46	1.38	Amber
Coconut oil	2.21	0.41	1.80	Colorless
Corn oil	1.83	0.31	1.52	Faint yellow
Cottonseed oil	1.78	0.27	1.51	Colorless
Olive oil	1.89	0.59	1.30	Light green
Soybean oil	1.67	0.28	1.39	Greenish yellow

[a] Average of duplicate titrations.

TABLE 4

Action of lipase on some pure triglycerides

Triglyceride	Net activity			Average [a] corrected values	Per cent [b] hydrolysis
	Trial 1	Trial 2	Trial 3		
Tributyrin	0.77	0.49	0.46	1.28	5.28
Tricaproin	1.81	1.34	1.35	1.97	7.63
Tricaprylin	3.56	2.95	2.85	3.41	15.85
Trilaurin	2.00	1.71	1.67	1.85	7.98
Trimyristin	1.26	.....	.....	.....	.....
Triolein	1.48	0.92	0.80	1.10	4.98

[a] Corrected to 100 per cent recovery (See Table 1).

[b] Calculated on the basis of corrected values.

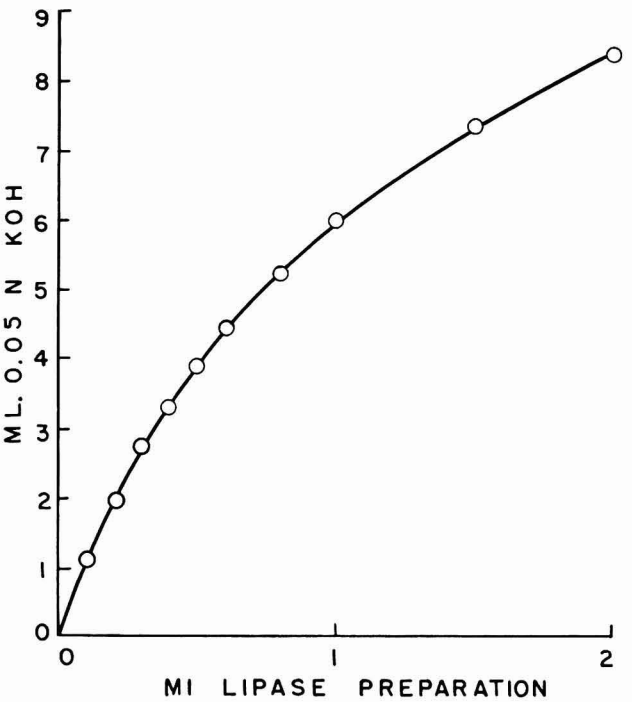

FIG. 3. Effect of lipase concentration upon its activity.

hibition of enzyme action occurs at the higher levels. The curve is reproducible, so that net activity values can be used to find the concentration of the lipase by insertion on this curve. However, this curve was not employed for correcting the lipase activities reported in this study, because it seemed desirable to use actual experimental data for direct comparisons.

In the studies on the effect of pH of the test substrate on lipase activity, the pH shifted during the reaction period to the extent of 0.1 to 0.3 unit, with a minimum shift at about pH 7.0 when phosphate buffers were used. Because of these shifts, only final pH values were plotted. Representative results are presented in Figure 4. The three curves are not directly comparable because they represent enzyme preparations with different levels of lipase activity. The optimum reaction for lipase action lies between pH 7.0 and 7.2, using either Clark and Lubs or McIllvain buffer. Lipase from culture E-1 decreased in activity more rapidly as the pH was raised above 7.2 than did the lipase of culture 0-1, which was used for obtaining the data presented in Figure 4. Absence of lipase activity at pH 5.75 or below possibly is due to instability of the coconut oil emulsions at these pH levels.

The effect of heat on inactivation of lipase is shown in Figure 5 by three representative curves taken from a family of ten very similar ones. In every case, considerable lipase activity was destroyed during the time required to

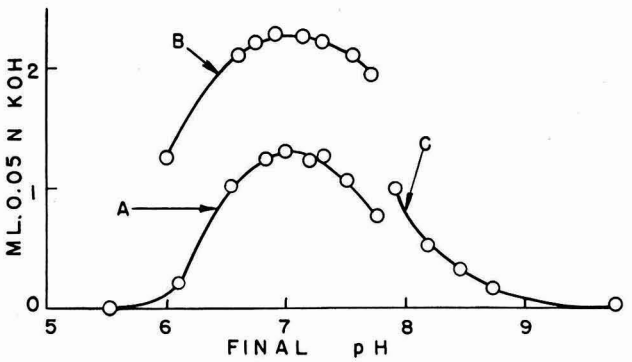

FIG. 4. Effect of pH of test substrate upon lipase activity.
A—Citric acid–Na_2HPO_4 buffer
B—KH_2PO_4–NaOH buffer
C—Boric acid–NaOH buffer

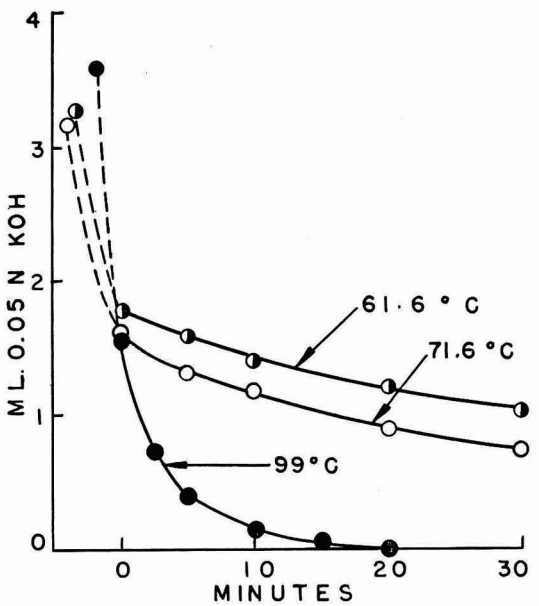

FIG. 5. Representative data on the effect of heat upon lipase activity.

bring the enzyme preparations up to the desired temperatures. The percentage destruction of lipase during the "coming up" time varies somewhat from trial to trial, depending upon the original lipase activity, but it was higher at 61.6° C. than at 71.6° C. The decline in lipase activity during the coming up time was rapid, but inactivation rate decreased after the holding temperatures were reached, especially at 61.6 and 71.6° C., so that appreciable lipase activ-

TABLE 5

Stability of lipase in peptone broth at various temperatures (0.3 ml. lipase preparation per 40 ml. test substrate)

Holding time (Days)	Net[a] activity at		
	3-5° C.	15° C.	36° C.
0	5.08	5.08	5.08
1	4.66	4.41	1.04
3	4.46	3.55	0.26
7	4.33	2.55	0.12

[a] After deduction of blanks (0.40-0.42).

ities were detectable after holding at these temperatures for 30 minutes. Complete inactivation of the lipase required heating at 99° C. for 20 minutes.

As Table 5 shows, the stability of lipase in broth varies considerably, depending upon the holding temperature. A relatively small loss of lipase activity occurred during a period of 7 days at 3 to 5° C., and an appreciable loss was detected at 15° C. in 24 hours, whereas a pronounced decline in lipase activity occurred at 36° C. in 24 hours.

The lipase could be salted out from a peptone broth preparation by nearly saturating it with $(NH_4)_2SO_4$. This procedure had to be carried out in the cold to prevent loss of lipase activity. In three trials, out of original lipase activities of 4.73, 3.66, and 3.38, values of 4.59, 3.24, and 3.38, respectively, were recovered when the salted out material was made up with distilled water to the original volume and then retested. A fluffy white material that invariably formed at the surface immediately after saturation with $(NH_4)_2SO_4$ accounted for about half of the enzyme activity, the other half being contributed by the brownish precipitate that sank to the bottom of the centrifuge tubes while the saturated solution stood at 5 to 8° C. for 16 to 18 hours. If this precipitate was dissolved in a small amount of water and reprecipitated with $(NH_4)_2SO_4$, most of the lipase activity was recovered in a floating white floc. Further purification was not achieved. The enzyme activity is destroyed easily by even mild protein precipitants, such as ethanol and ether, and is very sensitive to changes in pH. Chromatographic procedures of many types were tried unsuccessfully in attempting to obtain further purification.

Cupric, cobaltous, manganous, ferrous, calcium, or magnesium ions did not show any activation of the crude lipase preparations at concentrations ranging from 0.01 to 0.001 *M*, but were somewhat inhibitory at the higher concentrations. Cysteine hydrochloride and sodium thioglycollate at 0.01 to 0.001 *M* concentrations had no appreciable effect on lipase action.

DISCUSSION

The use of an extraction-titration procedure allows for acidification of the lipase test substrate to pH 2 or below, rendering all the released fatty acids in the medium in the free state prior to the extraction of the fat and fatty acids and subsequent titration. It also permits the titration with KOH in absolute methanol of a homogenous ether solution of fat and fatty acids, thus min-

imizing fading of the titration endpoint. In addition, since the buffers used are not extracted by this procedure, the blank titrations are lowered considerably.

Coconut oil was hydrolyzed by the lipase of *P. fragi* more rapidly than the other natural fats and oils tried. A similar observation was reported in the case of pancreatic lipase by Hartwell (8). The high lipase activity with coconut oil probably is due to its high content of esterified caprylic acid. The lipase of *P. fragi* apparently has some substrate preference, since tricaprylin was hydrolyzed to a greater extent than the other triglycerides tried. These findings appear contradictory to those of Collins and Hammer (4) and Long (15), who showed that tripropionin and tributyrin were hydrolyzed more easily than the higher triglycerides by *P. fragi* and other lipolytic bacteria. The discrepancy may lie in the fact that these workers were determining lipolysis by growing cells of the organisms rather than the action of the lipolytic enzyme independent of growth. However, the studies of Nelson (21) indicate greater activity against some lipid substrata than others in the case of the extracellular lipase of *Geotrichum candidum*.

Reports on the effect of bile salts on the activity of various animal and microbial lipases are not in agreement. Weinstein and Wynne (28) found bile salts without effect on pancreatic lipase, whereas Mallenby and Wolley (19) reported that the enzyme activity was stimulated. However, Fodor and Chari (5) found that the lipases of *Penicillium roqueforti* and *Aspergillus niger* were inhibited by sodium taurocholate in concentrations of 0.18 per cent. In the present studies the lipase of *P. fragi* apparently was activated by sodium taurocholate in concentrations of 0.1 to 0.4 per cent, whereas sodium glycocholate was markedly inhibitory in similar concentrations. The possibility exists that the increase in lipase activity resulted from a more efficient emulsification of the oil, yet sodium glycocholate gave coconut oil emulsions indistinguishable from those obtained with sodium taurocholate.

The relationship between demonstrable lipase activity and reaction time or the quantity of the lipase preparation was found to be slightly curvilinear. Similar relationships have been reported in the case of concentrations of pancreatic lipase (3, 29). This may be due to inhibition by the endproducts of the enzymatic reaction, temperature inactivation, or action of coexisting proteases. The enzyme preparations used in this study represent crude lipase with undetermined impurities, which may be one reason for not obtaining more linear relationships at least over part of the curves. Evidence of appreciable proteolytic activity in the present lipase preparations was found by van der Zant (27), using a modification of the method proposed by Anson (1), but no study was made to determine whether the inactivation rate of lipase paralleled proteolytic activity of the preparation.

The optimum for lipase activity at pH 7.2 is somewhat different from that reported for the lipases from many other microorganisms. Nearly all bacterial lipases previously studied have optima at pH 7.8 or above (2, 18, 24, 25). Although Fodor and Chari (5) observed that the optimum reaction for the extracellular lipases of *Aspergillus niger* and *Penicillium roqueforti* was about pH

8.0, lipases of most of the molds and yeasts have maximum activity at slightly acid reactions (5, 7, 14, 22, 26). Substratum, buffer, time and temperature of reaction, and other conditions prevailing in the test may influence considerably the optimum pH range for lipase activity. The various studies have been made, employing a wide variety of conditions; the observed differences between enzymes from various microorganism sources might not be substantiated if all were studied under uniform test conditions. The high activity at neutral reaction of the *P. fragi* lipase and the marked reduction of activity under acid conditions are important in the dairy industry, and further data on these points will be presented in another paper of this series (20).

Most lipases tested have been reported as sensitive to heat. Avery and Cullen (2) inactivated the lipase of *Pneumococcus* by heating it for 10 minutes at 70° C. Stevens and West (24) were able to destroy the lipase activity of a hemolytic streptococcus by heating at 55° C. for 10 minutes. However, Söhngen (23) observed the production by *Bacillus fluorescens liquefaciens (Pseudomonas fluorescens)* of a lipase that could withstand heating at 100° C. for 5 minutes, whereas the lipases of *Oidium lactis (Geotrichum candidum)* and *Penicillium glaucum* were inactivated at 80° C. Tammisto (25) found that the lipase activity of *B. fluorescens liquefaciens* was diminished by about 30 per cent after heating at 95° C. for 10 minutes. The present studies add one more lipase to that small group which has shown considerable resistance to high temperatures.

The rate of loss of lipase activity during the time required to heat the lipase preparations to 61.6, 71.6, and 99° C. was disproportionately greater than that during the subsequent holding period, even though the latter period was several times longer than the former. In most trials on different lipase preparations there was apparently more loss of lipase activity during the time required to reach 61.6° C. than during the corresponding period to reach 71.6° C. Possibly some inactivating agent is destroyed to a greater extent at 71.6 than at 61.6° C.

Probably a pure lipase preparation would have slightly different characteristics than those reported for the impure ones used. However, the lipase as it ordinarily is encountered functions in a complex medium with many impurities. Its reactions under these conditions undoubtedly are much the same as those which have been reported from these studies. Purification beyond precipitation with $(NH_4)_2SO_4$ was not achieved, although many procedures were tried.

SUMMARY AND CONCLUSIONS

A modified extraction-titration method, using coconut oil as substratum, was developed for measurement of lipase activity.

Sodium taurocholate showed some activation of the lipase, whereas sodium glycocholate was inhibitory in the concentrations tried, although both gave good substrate emulsions.

Maximum lipase activity on coconut oil was observed at a temperature of 40° C. and at pH 7.0 to 7.2, when a 24-hour test period was used.

Coconut oil was hydrolyzed at a more rapid rate than were some other natural

fats and oils. Tricaprylin was hydrolyzed to a greater extent than the other triglycerides tried, indicating probable substrate preference.

Appreciable lipase activity remained after heating the enzyme preparations at 61.6 or 71.6° C. for 30 minutes. Complete inactivation of the lipase required heating at 99° C. for 20 minutes.

Lipase preparations were more stable when allowed to stand at 3 to 5° C. than at 15 or 36° C.

Almost quantitative salting out of lipase could be accomplished by fully saturating the preparations with $(NH_4)_2SO_4$ below 7° C., but further purification was not achieved.

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THE LIPASE OF *PSEUDOMONAS FRAGI* [1]
II. FACTORS AFFECTING LIPASE PRODUCTION

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Quantitative studies on lipase production and the factors affecting the liberation of the enzyme in growth media by *Pseudomonas fragi* have not been reported. This study was undertaken to determine the effect of certain physical, physicochemical, and nutritional factors upon lipase production by this organism.

METHODS

The cultures used in this study were obtained from the stock culture collection of the Dairy Bacteriology Laboratories at Iowa State College. Stock cultures were carried on tryptone-glucose-beef extract agar slants which were stored at 2 to 3° C. and were transferred at monthly intervals. Before inoculating test media, the cultures were transferred twice in broth at 21° C. for 24 hours; one drop of the second transfer was inoculated into the growth medium, which was incubated at 15° C. for 3 days unless otherwise stated. The nutrient broth used contained 0.5 g. peptone, 0.3 g. beef extract, 10 ml. 0.5 *M* KH_2PO_4, enough 0.1 *N* NaOH to give the desired pH and distilled water to a total volume of 100 ml. Peptone broth was prepared as above except that 1 g. of peptone was used and the beef extract was omitted. All the culture media were sterilized by autoclaving at 15 lb. for 15 minutes.

Bacterial counts were made according to Standard Methods for the Examination of Dairy Products (1), except that no milk was included in the agar. Duplicate plates were used and plates were counted after incubation at 21° C. for 48 hours.

The lipase test substrate and the determination of lipase activity were the same as outlined previously (6).

RESULTS

Representative data obtained with culture 0-1 showing the effect on lipase production of incubation time at four temperatures are presented in Figure 1. At the lower temperatures the time required for the population to reach a level which gave good lipase yields increased appreciably. At the higher temperatures both maximum population and maximum enzyme activity were reached considerably earlier than at low temperatures and remained relatively constant thereafter. Although maximum organism populations were almost the same at all temperatures, the highest levels of enzyme activity per unit volume of medium or per cell were reached at the lower temperatures. Culture E-1 did

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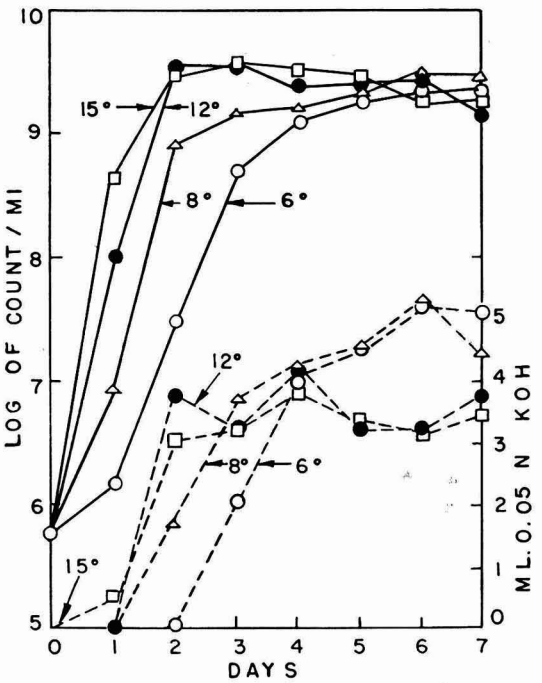

FIG. 1. Relation of time of incubation at various temperatures to count and lipase production by culture 0-1 at low temperatures.

———— Log of bacterial count / ml.
– – – – – Ml. of 0.05 *N* KOH

not produce lipase as rapidly as culture 0-1 at incubation temperatures below 12° C. In other trials with these two cultures, little or no lipase activity was found after 3 days at 30° C., although the bacterial counts were over a billion per milliliter. At 17 to 18° C. and at 21 to 22° C., lipase production after 3 days was much less than at 16° C., but the counts of bacteria were somewhat less than at the lower temperatures, indicating that enzyme production per cell had not been affected to any appreciable degree.

An appreciable shift in pH occurred during growth of *P. fragi* in most of the culture media tried, rendering the study of the effect of pH on lipase production rather difficult. Media containing sodium citrate, lactate, or protein digests or hydrolyzates shifted toward alkaline reaction, while glucose in the medium resulted in a shift to the acid side. Attempts to minimize this change in pH were not successful, since lipase production was lowered when the concentration of phosphate buffer was increased above 0.05 *M*. Periodic adjustment of pH was inadvisable, since periodic shaking of the media reduced demonstrable lipase activity. Detailed studies were made only on nutrient and peptone broths. Representative data on the effect of pH of nutrient broth on lipase production by culture E-1 are given in Table 1. In these studies the concentration of

TABLE 1

Effect of pH of nutrient broth upon lipase production by culture E-1 (1 ml. lipase preparation per 40 ml. test substrate)

Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
5.14	6.34	1,800	0.17
5.59	6.47	1,600	0.16
6.08	6.68	2,100	0.27
6.48	6.99	1,200	0.82
6.97	7.47	1,900	0.74
7.47	7.99	2,500	1.97
8.03	8.34	2,200	0.76
8.14	8.35	1,800	0.17
8.39	8.40	2,000	0.00

[a] Av. blank deducted = 0.26

KH_2PO_4 was kept constant at 0.05 *M*, and the desired pH was obtained by the addition of NaOH or HCl. There were two peaks of lipase activity, a slight one at an initial pH of 6.5 and the other at pH 7.5. However, with culture 0-1, lipase production was higher at the former than at the latter pH. In other studies *P. fragi* grew in peptone broth initially at pH 4.5 but only produced lipase in broth of which the initial reaction was pH 4.7, or above; in both cases the pH was raised considerably by the organism growth. Apparently no close relationship exists between count and lipase production per unit volume of medium at the different pH levels.

Shaking of the growth medium twice daily during incubation usually lowered both cell population and lipase production. This seems logical, since *P. fragi* is notably aerobic and usually forms a pellicle on the surface of the undisturbed liquid medium. Similarly, the surface-volume ratio of a liquid medium is important. When 100-ml. portions of peptone broth were dispensed into 1-1. Erlenmeyer flasks and into 34 × 300 mm. test tubes, surface areas of 114 cm.^{2} and 9 cm.^{2} were exposed, with depths of 1 cm. and 10.5 cm., respectively. The three cultures tried all showed considerably higher counts and greater lipase activities per unit volume where the greater surface area was exposed (Table 2). However, the differences in lipase activities were less than the differences in count. Oxygen relationships apparently were of some importance, since enzyme

TABLE 2

Effect of ratio of surface area to volume of peptone broth upon lipase production by three cultures (0.3 ml. lipase preparation per 40 ml. test substrate)

Culture	Container	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
0-1	Test tube [b]	7.18	7.32	1,500	3.65
0-1	Flask [c]	7.18	7.68	3,600	5.82
E-1	Test tube	7.18	7.34	1,200	2.64
E-1	Flask	7.18	7.72	4,600	4.83
10	Test tube	7.18	7.33	1,000	0.78
10	Flask	7.18	7.77	3,900	1.06

[a] Av. blank deducted = 0.40
[b] Depth of medium = 10.5 cm.; surface area = 9 cm.^{2}
[c] Depth of medium = 1 cm.; surface area = 114 cm.^{2}

TABLE 3
Effect of concentration of sodium chloride in peptone broth upon lipase production by culture 0-1 (0.3 ml. lipase preparation per 40 ml. test substrate)

Per cent NaCl	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
0	7.30	7.80	5,500	5.28
1	7.17	7.74	2,900	3.12
2	7.19	7.68	1,800	0.70
3	7.16	7.78	1,200	0.07
4	7.16	7.75	330	0.00

[a] Av. blank deducted = 0.40

activity per billion count was higher for the test tube preparation than for the flask culture in all three comparisons.

P. fragi is reportedly very sensitive to low concentrations of sodium chloride (2, 4). Culture 0-1 showed hardly any detectable lipase activity in peptone broth containing 3 per cent NaCl, as shown by representative data in Table 3. Even 1 per cent of this salt had a considerable effect in reducing both enzyme activity and population level. The effect of NaCl on lipase production is proportionally much greater than the effect on cell population, at salt concentrations of 2 per cent or above.

Phosphate-buffered nutrient broth was used in the early part of this study as a medium for lipase production. This medium was modified by using 0.5 g. of beef extract instead of the usual 0.3 g. per 100 ml. in order to determine which constituent was responsible for lipase production by *P. fragi*. As shown in Table 4, peptone was the ingredient which supported high lipase production. Further study with six cultures of *P. fragi* showed considerable differences in ability to produce lipase in the broth containing only 0.5 per cent peptone and the buffer, but measurable lipase was produced by each of these cultures in this growth medium. However, 1 per cent peptone gave maximum lipase production in 3 days at 15° C., and this higher concentration was used in subsequent studies.

The suitability of the various commercial protein digests and hydrolyzates as media for lipase production by culture 0-1 was tested using a phosphate-buffered medium containing 1 per cent of the particular digest or hydrolyzate.

TABLE 4
Effect of omission of individual components of nutrient broth upon lipase production by two cultures of P. fragi (1 ml. lipase preparation per 40 ml. test substrate)

Culture	Medium	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
0-1	Nutrient broth	7.56	7.93	3,700	3.89
0-1	Peptone omitted	7.63	8.05	640	0.38
0-1	Beef extr. omitted	7.63	7.82	2,400	6.74
E-1	Nutrient broth	7.56	8.11	3,500	2.69
E-1	Peptone omitted	7.63	8.13	2,200	0.09
E-1	Beef extr. omitted	7.63	7.83	2,600	4.55

[a] Av. blank deducted = 0.35

TABLE 5

Lipase production in some protein digests and hydrolyzates by culture 0-1 (0.3 ml. lipase preparation per 40 ml. test substrate)

Medium	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
Casamino acids [b]	7.39	7.93	3,600	2.00
Tryptone	7.46	7.82	2,800	1.96
Proteose-peptone	7.44	7.65	3,900	3.43
Tryptose	7.51	7.76	1,900	0.24
Peptone	7.49	7.84	4,500	3.60
Trypticase	7.48	7.93	3,200	0.71

[a] Av. blank deducted = 0.43
[b] Vitamin-free casamino acids (Difco)

The results presented in Table 5 show that peptone and proteose-peptone gave the highest net lipase activity.

Since peptone is a commercial peptic digest of protein, its composition probably is variable and is not precisely known. This product was compared with a more defined product known as Bacto vitamin-free casamino acids, which was substituted for peptone in the growth medium for five test cultures. For this comparison, NaCl was added to the peptone medium in quantities comparable to those present in the casamino acids medium. Casamino acids medium supported appreciable lipase production but, except with culture 10, lipase activity was considerably less than on peptone broth, as shown in Table 6.

Most attempts to increase production of lipase by culture 0-1 in vitamin-free casamino acids medium by supplementation with various compounds met with little success. Addition of peptone ash or calcium and magnesium ions decreased rather than increased lipase production. Supplementation with thiamin and niacin in quantities of 200 γ per 100 ml. of medium slightly increased lipase production, but pantothenic acid and pyridoxine in similar concentrations had no effect. Addition of uracil, thymine, guanine, adenine, cystine, or tryptophan or supplementation with the ten "indispensable" amino acids in groups or together, supported no appreciable increase in lipase production by culture 0-1 over that of the control in vitamin-free casamino acids medium.

TABLE 6

Lipase production in vitamin-free casamino acids medium and in peptone broth by four cultures (0.5 ml. lipase preparation per 40 ml. test substrate)

Culture	Medium	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
0-1	Peptone	7.38	7.71	5,700	4.65
0-1	Cas. acids [b]	7.41	7.97	4,700	2.31
E-1	Peptone	7.38	7.71	3,600	6.03
E-1	Cas. acids	7.41	8.11	5,100	3.66
C	Peptone	7.38	7.87	5,200	1.21
C	Cas. acids	7.41	8.09	.....	1.06
10	Peptone	7.38	7.87	4,100	1.14
10	Cas. acids	7.41	8.07	3,000	1.88

[a] Av. blank deducted = 0.32
[b] Vitamin-free casamino acids (Difco)

TABLE 7

Effect of addition of 0.2 per cent L-*leucine to a defined citrate-containing medium upon lipase production by three cultures (1 ml. lipase preparation per 40 ml. test substrate)*

Citrate medium with	Culture	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
.....	10	6.04	8.00	3,400	0.22
L-leucine	10	6.07	7.79	3,400	0.97
.....	C	6.04	7.81	3,900	0.09
L-leucine	C	6.07	8.02	6,300	1.74
.....	K-1	6.04	7.83	1,500	0.09
L-leucine	K-1	6.07	7.53	1,200	0.24

[a] Av. blank deducted = 0.39

Several chemically defined media were tested for lipase production by *P. fragi* during the course of this study. A citrate medium composed of 0.4 g. KH_2PO_4, 0.05 $MgSO_4 \cdot 7H_2O$, enough 0.1 *N* NaOH to give the desired pH, and distilled water to 100 ml. supported good growth but little lipase production. However, appreciable increase in lipase production was secured by supplementing this medium with a combination of L-leucine, DL-isoleucine and DL-valine, when each amino acid was used in quantities of 20 mg. per 100 ml. Data in Table 7 show that addition of 0.2 per cent L-leucine to the citrate medium caused a pronounced increase in lipase production by the three cultures tested, although the values still were not high. The combination of these three amino acids could serve as the sole source of nitrogen and reduced carbon and yet support good growth and lipase production, but L-leucine was the only single amino acid tested capable of supporting appreciable growth and lipase production. A modified glucose medium (2) composed of 0.5 g. glucose, 0.2 g. NH_4Cl, 0.05 g. $MgSO_4 \cdot 7H_2O$, 10 ml. 0.5 *M* KH_2PO_4, enough 0.1 *N* NaOH to get the desired pH and distilled water to make 100 ml. supported lipase production somewhat below that in a protein-hydrolyzate medium. Optimum glucose concentration was 0.5 per cent. Addition of the combination of L-leucine, DL-isoleucine and DL-valine together, or L-leucine alone, appreciably increased lipase production in the glucose medium (Table 8), but DL-isoleucine or DL-valine had no consistent effect.

A lactate medium consisting of 0.8 g. sodium lactate (50 per cent), 0.2 g. NH_4Cl, 0.05 g. $MgSO_4 \cdot 7H_2O$, 10 ml. 0.5 *M* KH_2PO_4, 0.1 *N* NaOH to give the desired pH and distilled water to make 100 ml. supported fairly good lipase production by five cultures of *P. fragi* but enzyme production on this medium did not approach the levels attained in peptone broth.

P. fragi elaborates lipase in many media devoid of triglyceride substrates. It remained to be seen whether the presence of triglycerides might induce greater lipase production. To the usual 1 per cent vitamin-free casamino acids medium, several pure triglycerides and butterfat were added at the rate of 0.1 per cent. Results of four trials are presented in Table 9. A great increase of lipase production was obtained in the presence of tricaprylin. Tricaprin caused slight stimulation. The effect of trilaurin was variable, apparently mostly

TABLE 8

Effect of addition of some amino acids to a defined glucose-containing medium upon lipase production by culture 0-1 (1 ml. lipase preparation per 40 ml. test substrate)

Glucose medium with	Initial pH	Final pH	Count/ml. (millions)	Net [a] activity
.....	7.32	6.74		0.91
L, Il, V [b]	7.18	6.55	2,300	1.80
L-leucine	7.22	6.58	1,600	1.52
DL-isoleucine	7.21	6.56	2,100	0.96
DL-valine	7.22	6.61	2,200	0.46

[a] Av. blank deducted = 0.38

[b] L = L-leucine; Il = DL-isoleucine; V = DL-valine

because of an inhibitory effect upon organism growth in some trials. The lower triglycerides had little effect on lipase production, while the triglycerides higher than trilaurin, and also butterfat, were inhibitory to lipase production, even though the bacterial counts either were increased or were unaffected. The extraordinary effect of tricaprylin was due primarily to caprylic acid, since the fatty acid caused much the same degree of stimulation as did the triglyceride, while glycerol was without effect. The stimulatory action of tricaprylin and caprylic acid on lipase production in both vitamin-free casamino acids and peptone media was verified with several cultures of *P. fragi*.

The optimum concentration of caprylic acid in vitamin-free casamino acids media was shown to be 0.0035 *M*. Several other fatty acids were tested using the same concentration. The results presented in the two trials in Table 10 are representative of several trials on cultures 0-1 and E-1. The pronounced stimulation of lipase production is caused only by caprylic and capric acids; other acids had little or no effect or were somewhat inhibitory.

DISCUSSION

Not only do different strains of *P. fragi* exhibit unequal abilities to produce lipase under the same conditions of growth, but also physical, physico-chemical,

TABLE 9

Effect of addition of some pure triglycerides and butterfat to vitamin-free casamino acids medium upon lipase production by culture 0-1 (0.3 ml. lipase preparation per 40 ml. test substrate)

Casamino acids medium with	Net activity			
	Trial 1	Trial 2	Trial 3	Trial 4
Control	3.36	3.84	1.74	3.36
Triacetin	3.95	.....	.....	.....
Tributyrin	3.44	2.31	2.95	2.87
Tricaproin	3.86	.....	.....	.....
Tricaprylin	8.56	.....	9.61	9.76
Tricaprin	4.20	.....	.....	.....
Trilaurin	0.03	9.33	0.74	6.62
Trimyristin	0.20	.....	.....	.....
Tripalmitin	.....	.....	0.09	0.73
Triolein	0.22	.....	.....	.....
Butterfat	0.04	.....	0.03	0.26

TABLE 10

Effect of addition of some fatty acids to vitamin-free casamino acids medium upon lipase production by culture 0-1 (0.2 ml. lipase preparation per 40 ml. test substrate [a])

Fatty acids added	Initial pH	Final pH	Count/ml. (millions)	Net [b] activity
		Trial 1		
Control	7.15	7.86	5,100	2.90
Butyric	7.16	7.81	5,200	2.45
Caproic	7.14	7.78	5,300	3.08
Caprylic	7.13	7.57	6,200	7.95
Capric	7.12	7.43	5,200	8.62
Lauric	7.11	7.11	260	0.85
		Trial 2		
Control	7.26	7.79	2,300	2.68
Myristic	7.24	7.75	3,900	3.91
Palmitic	7.24	7.63	5,300	1.32
Stearic	7.24	7.71	3,800	0.10
Oleic	7.23	7.70	2,800	1.09

[a] 0.2 per cent formaldehyde was used.
[b] After deduction of blanks (0.32-0.36)

and nutritional factors materially affect lipase production. Some of the effects are only upon the number of cells produced, lipase production per cell not being influenced appreciably; in other cases, growth is affected much less than is lipase production.

The fact that maximum lipase production per volume of culture occurs at temperatures of 15° C. and below during incubation periods of 3 days or longer possibly is related to the relatively higher rate of enzyme inactivation at the higher temperatures (6). The greater activity per unit volume at low temperatures is significant from the standpoint of the dairy industry where lipolytic activity by this organism is an important cause of product deterioration. This organism is a common contaminant in dairy products, and prolonged holding of contaminated products at low temperatures will inhibit but not prevent growth of this organism.

Lipase production by *P. fragi* in nutrient broth was favored in the neutral pH range, with no detectable enzyme being produced at initial pH levels of 4.5, or below. This would indicate that sweet cream and other less acid dairy products would support lipase production, but the concurrent growth of acid-producing organisms could appreciably lower lipase production by *P. fragi*. Hussong *et al.* (4) reported that when butter cultures were added to cream, the resultant butter developed rancid flavor somewhat less rapidly than when the butter was made without culture. Part of this effect may have been due to reduced lipase production, but an inhibition of enzyme activity by low pH probably also was a major factor.

P. fragi is a non-fastidious organism and can grow fairly well in simple defined media although lipase production in some such media commonly is at a low level. High levels of lipase production could be obtained only in media containing protein digests or hydrolyzates, although considerable differences in the yields of lipase on media containing the various commercial protein breakdown

products were observed. Maximum lipase production during these studies was obtained by supplementing Bacto-peptone with certain pure triglycerides or their component fatty acids, indicating that the character of the nitrogen source was not necessarily the only important factor.

The presence of specific substrate in the growth medium frequently stimulates the production of the enzyme which acts upon that substrate. Baker (1) found that the addition of some fatty acids to a synthetic basal medium caused appreciable increase in the ability of *P. fragi* cells to oxidize the salts of these fatty acids, using the Warburg respirometer. Cutchins *et al.* (3) believe that bacterial lipase is adaptive and that lipase production is stimulated by certain lipid substrates as carbon sources in the growth medium. The lipase of *P. fragi* does not appear to be an adaptive enzyme in the strict sense of the term, as the enzyme may be produced in high yield in media devoid of materials that ordinarily serve as substrates for lipase action; however, enzyme production has been stimulated by the presence of some triglycerides, particularly tricaprylin. Also the ineffectiveness of the triglycerides containing fatty acids of carbon-chain lengths shorter than that of tricaprylin and the marked inhibition of lipase production by the triglycerides of long-chain fatty acids and by butterfat does not support the explanation that the triglyceride structure of tricaprylin was responsible for the extraordinary increase in lipase production in media supplemented with that triglyceride. The effect of tricaprylin in increasing lipase production probably is due to the action of the component fatty acid which is liberated by the lipase elaborated during the incubation period. That this may be the case is demonstrable by the similarity of effect on lipase production by most triglycerides and by their component fatty acids. The data available give no clue as to why caprylic or capric acids have marked stimulatory action, while other fatty acids have no consistent stimulatory action or are inhibitory to lipase production, although nearly all seem somewhat stimulatory to organism growth. Attempts to purify the enzyme sufficiently to be able to determine if caprylic or capric acid is incorporated preferentially into the enzyme molecule have not been successful.

SUMMARY AND CONCLUSIONS

Maximum production of extracellular lipase by *P. fragi* in 3 days occurred at 15° C. or below, the exact optimum depending upon the organism strain. Little or no detectable lipase was produced at 30° C. or above. With longer incubation times the lower temperatures were increasingly more favorable for lipase production.

Lipase production was favored in nutrient broth with initial pH levels of 6.5 and 7.5.

Sodium chloride inhibited lipase production to a greater extent than growth was inhibited in peptone broth.

P. fragi produced considerably more lipase in shallow layers of peptone broth than in deep layers. The greater lipase production was associated with increased counts but not closely proportional thereto.

Appreciable lipase was produced by *P. fragi* in glucose and lactate defined media containing NH_4Cl as the only source of nitrogen. Good growth but little or no lipase production occurred in a similar medium with citrate as a source of carbon. Lipase production in glucose and citrate defined media was increased materially by supplementation with L-leucine or a combination of L-leucine, DL-isoleucine and DL-valine.

Lipase production in some protein digests and hydrolyzates, particularly peptone, was much higher than in the chemically-defined media tested.

Addition of small amounts of tricaprylin, caprylic acid or capric acid to vitamin-free casamino acids or peptone media caused a pronounced increase in lipase production.

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THE LIPASE OF *PSEUDOMONAS FRAGI* [1]
III. ENZYME ACTION IN CREAM AND BUTTER

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The presence of *Pseudomonas fragi* lipase in cream and butter causes development of objectionable flavors, thus lowering the quality of these products considerably. The growth of *P. fragi* and the subsequent breakdown of butterfat in cream and butter has been studied by several workers, chiefly Hussong (3), Hussong *et al.* (4), Long and Hammer (6, 7) and Fouts (1). However, the action of the lipase, independent of the growing organisms, upon the butterfat in cream and butter has not been studied.

The common belief is that lipases are inactivated by the pasteurization procedures to which the cream is subjected during the buttermaking process. Since the lipase produced by *P. fragi* is not completely inactivated by such pasteurization procedures (8), it was necessary to examine the behavior of enzyme in cream and butter under simulated commercial butter making and handling conditions.

EXPERIMENTAL PROCEDURES

The lipase test substrate for the usual measurement of lipase activity and the modified extraction-titration procedure employed with the above lipase test substrate were those reported previously (8).

In the case of determination of lipase activity in butter, the sample was melted quickly at 50 to 55° C. and mixed well, and 10-g. portions were weighed.

Due to the high fat content of butter, 20-ml. portions of ether mixture were used instead of the usual 10-ml. portions. This was followed by whirling in a Mojonnier hand centrifuge for 30 seconds after each extraction to provide good separation of layers.

For studying the effect of pH of cream on lipase activity, the cream was adjusted to the desired reaction by addition of 1 *N* lactic acid or 1 *N* NaOH. The pH was measured by the use of a Beckman glass-electrode potentiometer. At the end of the reaction period, 10-g. portions of cream were weighed into 100-ml. centrifuge tubes. After addition of 10 ml. of 95 per cent ethanol, shaking for 15 seconds and standing for 5 minutes, 10 ml. of ether mixture were added and the mixture was shaken for 30 seconds. The centrifuge tubes were whirled in an International centrifuge with an angle head 14 in. in diameter at 1500 r.p.m. in order to break the emulsion, and the ether layer was siphoned off into 125-ml. Erlenmeyer flasks. The extraction with ether was repeated and the combined ether extracts were titrated with 0.05 *N* KOH in absolute methanol, using 10 drops of 1 per cent phenolphthalein in absolute

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ethanol as indicator. Homogenized whipping cream obtained from the College creamery and standardized to about 30 per cent butterfat was used as the substratum.

The heat resistance of the lipase produced by *P. fragi* in cream was determined by inoculating each 100-ml. portion of sterile 30 per cent cream in a 300-ml. Erlenmeyer flask with one drop of a 24-hour culture in broth and incubating at 15° C. for 3 days. Portions of the cream then were pipetted into 125 × 16 mm. screw-cap test tubes and heated at 71.5° C. in a constant-temperature water bath for 30 minutes after reaching temperature and quickly cooled to below 20° C. in ice water. The lipase activity was tested on coconut oil emulsion before and after pasteurization.

For studies on the residual lipase in butter, 500-ml. portions of nonhomogenized approximately 30 per cent cream in 2-l. flasks were sterilized by autoclaving at 15 lb. for 15 minutes in trial 1 and by steaming on two consecutive days for 1 hour with holding at room temperature between steamings in trial 2. After cooling to 15° C., each flask was inoculated with five drops of a 24-hour broth culture of *P. fragi* and incubated at 15° C. for 3 days.

Pasteurization of the inoculated cream in trial 1 was carried out after incubating by aseptically combining the two 500-ml. portions of cream in one 2-l. flask, which then was set in a constant-temperature water bath at 71.6 ± 0.1° C. The cream reached 70° C. in 17 minutes, after which it was left for 30 minutes with frequent stirring. The cream temperature reached 71.5° C. in 20 minutes after timing was begun. After pasteurization, the incubated cream was cooled in ice water to below 10° C., split into 500-ml. portions and churned in sterile quart jars simultaneously with the uninoculated cream. It took 42 minutes for the incubated cream to churn, as compared to 25 minutes for the control cream. The butter, while still in the jars, was washed with sterile cold distilled water, and the test butter in the two jars was combined. The same procedure was followed with the control butter from the other two jars. Each combined sample was worked thoroughly and finally dispensed into sterile 2-oz. sample jars, which were stored at —10, 2-5, 21, and 36° C. for various intervals.

The same procedures were followed in trial 2, except that pasteurization consisted of heating the inoculated cream in a water bath at 80-85° C. to bring the temperature to 71.5° C. in 8 minutes, following which the flask containing the cream was transferred to the constant-temperature water bath at 71.6° C. and held for 30 minutes. Also, the inoculated cream, after pasteurization and subsequent cooling to below 10° C. in an ice-water bath, was held overnight at 4-6° C., together with the control cream, before being churned. The churning times in trial 2 were 38-44 minutes for the inoculated cream and 35-37 minutes for the control cream.

Butterfat in cream was determined by the Babcock test (2). Testing butter for moisture content, butterfat, and curd was carried out according to the modified Kohman procedure (2). The butter samples were checked organoleptically for flavor defects at the beginning and end of the holding periods.

In trials where *Streptococcus lactis* was used in combination with *P. fragi*, one drop of a culture of the coccus incubated 16 hours at 21° C. in litmus milk was used to inoculate 100 ml. of sterile cream to which one drop of a 24-hour culture of *P. fragi* also was added.

RESULTS

In preliminary studies on cream containing 10 per cent butterfat, the lipase of *P. fragi* was active at pH 5.5 and slightly lower, whereas it had not been active on coconut oil substratum at those pH levels (8). Further studies were made on homogenized cream testing approximately 30 per cent butterfat. Table 1 gives data representative of several trials. An appreciable change in pH occurred during the reaction period, especially when the initial reaction was above pH 6.5. There was pronounced lipase activity between pH 5.3 and pH 8.16, with an optimum pH between 5.7 and 6.56 or possibly slightly higher. In additional trials, little lipase activity was demonstrated below pH 4.9 or above 8.6.

P. fragi usually has to compete with acid-producing organisms in cream. Table 2 shows data representative of three trials in which *S. lactis* W2 was grown with *P. fragi* 0-1. Lipase production by *P. fragi* was suppressed markedly

TABLE 1

Effect of pH of cream upon lipase activity (0.2 ml. lipase preparation per 40 ml. cream)

pH of control cream		pH of test cream after reaction period	Net [a] activity
Before reaction period	After reaction period		
4.52	4.63	4.69	0.00
5.34	5.18	5.13	1.85
5.73	5.72	5.59	2.26
6.56	6.35	6.25	2.24
7.22	7.00	6.76	2.01
8.16	7.85	7.44	1.51
9.13	8.98	8.72	0.00

[a] Expressed as ml. of 0.05 *N* KOH to titrate the acids extracted from 10 g. of cream. Av. blank deducted = 0.50.

TABLE 2

Lipase production in cream by P. fragi 0-1 in association with S. lactis W2 (1 ml. cream per 40 ml. coconut oil substrate)

Cream inoculated with	Initial pH	Final pH	Count/ml. (millions)				Net [a] activity
			Initial		Final		
			T.G.E.M.	T.G.E. [b] with cr. violet	T.G.E.M.	T.G.E. [b] with cr. violet	
0-1	6.45	6.03	1.20	0.20	480	380	1.07
0-1 + W2	6.45	4.51	1.60	0.87	2,000	360	0.19
W2	6.45	4.39	0.88	0	1,300	0	0.01
Uninoculated	6.45	6.32	0	0	0	0	0.00

[a] Av. blank deducted = 0.33.

[b] Tryptone-glucose-extract agar with 1:120,000 crystal violet; incubated at 21° C. for 3 d.

in the presence of *S. lactis*. This reduction occurred even though no appreciable decrease in the count of *P. fragi* was demonstrable. The concentration of crystal violet used in plating completely inhibited the growth of *S. lactis*, but it was only slightly inhibitory to *P. fragi*.

Data representative of two trials on lipase production in cream by six cultures of *P. fragi* are given in Table 3. The cultures varied in their abilities to produce lipase under the conditions of the experiment. Bacterial counts did not correlate with the lipase activities produced in cream. Data on residual net activities after pasteurization reveal that over 50 per cent of the original lipase activities remained after heating at 71.5° C. for 30 minutes. The percentage loss of activity during the heating period varied somewhat between the six cultures tried, although there were no indications that particular significance should be attached to variations of the magnitude of those observed.

The role of the residual lipase in the deterioration of the quality of butter during storage was investigated. Two trials on sterile sweet cream were made. The growth of culture 0-1 in cream for a period of 3 days at 15° C. resulted in net lipase activities of 0.52 and 0.65 per milliliter in trials 1 and 2, respectively. The corresponding residual activities after pasteurization at 71.5° C. were 0.20 and 0.41. During the incubation period, appreciable fat degradation occurred due to the action of the lipase. The extraction and titration of fat and fatty acids from 10-g. portions of cream showed increases of 1.13 and 1.65 ml. 0.05 *N* KOH in the inoculated cream over those of the controls in trials 1 and 2, respectively. These fat degradation products were carried over to the butter churned and were responsible for the differences between the titrations of butter from inoculated and control cream samples at zero time (see Table 5).

There were greater variations between the composition of butter obtained from the inoculated and control cream in trial 1 than in trial 2, Table 4 shows. The butter made from inoculated cream in trial 1 had a relatively high moisture content, because the cream was not held cold for more than a few minutes before churning.

Considerable increases in the titration values of butter churned from inoculated cream occurred after storage at all the temperatures used, whereas

TABLE 3

Lipase production in cream by 6 cultures of P. fragi and the residual lipase in cream after pasteurization at 71.5° C. for 30 min. (1.0 ml. cream per 40 ml. coconut oil substrate)

Culture	Initial pH	Final pH	Count/ml. (millions)	Net activity [a]	
				Before pasteurization	After pasteurization
0-1	6.42	6.29	380	1.05	0.64
E-1	6.42	6.32	250	1.22	0.67
C	6.42	6.37	430	0.52	0.32
10	6.42	6.41	370	0.73	0.49
K-1	6.42	6.36	270	0.82	0.64
P	6.42	6.40	910	0.36	0.26

[a] Av. blank deducted = 0.39

TABLE 4

Composition of experimental butter

	Percentage composition of butter		
	Moisture	Fat	Curd
		Trial 1	
Inoculated	22.1	75.3	2.6
Uninoculated	17.4	81.0	1.6
		Trial 2	
Inoculated	18.4	80.0	1.6
Uninoculated	17.8	80.5	1.7

TABLE 5

Effect of residual lipase upon the flavor and titratable free fatty acids of butter after holding

Storage temp.	Period of holding	Butter from inoculated cream		Butter from uninoculated cream	
		Titration [a]	Flavor	Titration [a]	Flavor
(° C.)	*(d.)*				
			Trial 1		
–	0	4.27	Cooked	0.87	Sl. tallowy
36	2	9.17	Rancid	1.12	Sl. tallowy
21	7	13.66	V. rancid	1.37	Sl. tallowy
2-5	30	10.59	V. rancid	1.29	Sl. tallowy
–10	60	6.26	Sl. rancid	1.13	Oxidized
			Trial 2		
–	0	8.48	Rancid	1.51	Cooked, feed
36	2	22.50	V. rancid, peppery	1.20	Cooked, feed
21	7	38.16	Extremely rancid and peppery	1.30	Cooked, feed
2-5	30	29.12	V. rancid and peppery	1.36	Oxidized
–10	60	15.28	V. rancid and peppery	1.43	Oxidized, tallowy

[a] Av. of duplicate titrations of free fatty acids extracted from samples, expressed in ml. 0.05 *N* methanolic KOH.

there was no appreciable change in titration values of the control butter (Table 5). Butter containing residual lipase showed the greatest increase in titration when held at 21° C. for 7 days. Butter held at 36° C. melted during the holding period with partial separation of butterfat and butter serum, a condition which undoubtedly reduced lipase activity considerably. Organoleptically, all the butter samples containing residual lipase became rancid or the rancidity increased markedly after holding at the temperatures used. However, control butter samples were criticized only for being oxidized or having a cooked flavor; in no case were these butter samples detectably rancid.

An additional trial was made to determine the effect of normal salt content of butter upon the activity of the residual lipase in the test butter samples. The same procedures for handling cream as for trial 2 were followed. One of each pair of samples of the inoculated and control butter was salted and the other left as a control. The resultant butter was of average composition, the

salted butter made from inoculated cream containing 1.8 per cent salt and 16.9 per cent moisture. Immediately after churning the test butter, samples gave titrations of 6.09 and 6.14 for the salted and unsalted butter, respectively. Both butter samples were slightly rancid. The corresponding titrations for the control butter samples were 1.47 and 1.33. All the samples then were held at 21° C. for 7 days, during which time the titrations rose to 26.89 and 29.95, respectively, for the salted and unsalted butter samples containing residual lipase, while those for the corresponding control samples were 1.42 and 1.35. The test butter samples had a very pronounced rancid flavor, whereas the control samples were not detectably rancid.

DISCUSSION

The earlier studies on the pH limits for lipase activity on coconut oil emulsified by means of sodium taurocholate (8) had indicated that the enzyme had little lipolytic activity below pH 6.0 and that the optimum reaction was slightly above neutral. The differences between these values and those observed in cream in the present study undoubtedly are attributable mostly to the differences in the character of the aqueous phase, particularly the active emulsifying agents. The coconut oil emulsion containing taurocholate was somewhat unstable at acid reactions and oiled off just below pH 6.0; no such instability was noted in the case of the natural cream emulsion. Factors other than emulsion stability undoubtedly also are operative.

The fact that the optimum reaction for activity of the *P. fragi* lipase in cream was in the range from pH 5.6 to 6.8 and that the enzyme showed no measurable activity below pH 4.9 in cream are of great practical significance. Cream in which there has been any considerable growth of lactic-acid producing flora will be at a pH level unfavorable for activity of this bacterial lipase. Holding cream at temperatures of 10° C. and below will permit considerable development of *P. fragi* (4). The lower temperatures also are favorable to greater production of lipase by *P. fragi* than would be found at temperatures of 20° C. and above (9). Not only is the presence of acid unfavorable to the activity of the enzyme, but it also is unfavorable to production of the enzyme, even though the growth of the lipolytic organism is essentially unaffected (Table 2).

Kester (5) and others have noted that many samples of cream several days old but with relatively low acidities contain considerable quantities of water-insoluble acids. Not only will lack of extensive acid development favor production and activity of the *P. fragi* lipase, but it also will permit considerable activity by the natural milk lipase.

The several strains of *P. fragi* tested invariably grew more slowly in cream than in peptone broth. The plate counts after incubation for 3 days at 15° C. usually were less than 500 million per milliliter of cream, whereas they commonly reached several billions in peptone broth under similar conditions of incubation (9). The factors responsible for this difference in population were not investigated. As would be expected from the lower organism populations, lipase production in cream was considerably less than in peptone broth. The

amounts of lipase produced in cream were sufficient to cause considerable fat degradation, and the free fatty acids thus produced were found to a considerable degree in butter made from such cream.

The relatively high heat stability of the lipase under conditions commonly used for commercial pasteurization of cream for buttermaking and the considerable activity of the residual resistant lipase which is carried over into butter are important for the butter industry. Pasteurization commonly has been depended upon not only to destroy *P. fragi* and many other defect-producing bacteria but also to inactivate many of the potentially deleterious enzymes which may have been produced by the microorganisms. Pasteurization does destroy *P. fragi,* but much of the lipase produced by this organism remains active after this treatment and, as Table 5 shows, may cause pronounced rancidity in butter made from properly pasteurized cream. Salting of the butter has no appreciable effect upon the activity of the residual enzyme. These observations may explain why difficulty sometimes is encountered in isolating *P. fragi* or other related bacteria from butter which has developed rancidity during storage or handling; the causative bacteria would have been destroyed but the enzyme which they produced before being destroyed would be able to cause the lipolysis.

These results provide additional bases for the common axiom that pasteurization should be used only to protect a good product, rather than to attempt to make a good product from inferior raw material.

SUMMARY AND CONCLUSIONS

The lipase of *P. fragi* is active in cream between pH 4.9 to 8.2 with an optimum between pH 5.7 and 6.6, using incubation at 36° C. for 24 hours.

Growth and lipase production of *P. fragi* in cream at 15° C. for 3 days is rather slow. However, enough lipase is produced to cause extensive fat breakdown in the cream and the resultant butter.

Over 50 per cent of the lipase is not inactivated by pasteurizing the cream at 71.5° C. for 30 minutes.

Extensive growth of *S. lactis* in cream, resulting in lowering of pH, does not cause any appreciable inhibition of the growth of *P. fragi* but markedly reduces lipase production and activity.

Butter containing residual lipase undergoes considerable fat degradation during storage, even at —10° C., developing pronounced rancid flavor, especially at 5° C. and higher temperatures.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. VIII. DIGESTIBILITY OF COLOSTRUM AND MILK BY CALVES DURING THE EARLY POSTNATAL DAYS OF LIFE [1]

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It is well known that colostrum is a rich source of nutrients and that the new-born calf usually does best when it receives this food. Although the digestibility of nutrients in milk by calves has been studied (1, 4, 6), reports of digestion trials in which colostrum and transition secretions were collected and fed in the normal sequence to new-born dairy calves have not been found in the literature.

The investigation reported herein was designed to ascertain the apparent digestibility of the nutrients of colostrum and also whether changes occur in digestibility when the mammary products of the early phases of lactation are collected and fed in the normal sequence to the growing dairy calf at four different intervals during the first seventeen days of life.

EXPERIMENTAL

Feeding and management of calves. The one Jersey and eight Holstein male calves used in this investigation were removed from their dams before they had a chance to nurse and placed in a metal metabolism cage (Figure 1) located in an artificially heated room. In a few cases when two calves were on experiment at the same time, the older calf was moved to a different cage for the completion of the trial. The calves were muzzled to keep them from ingesting foreign material. Each calf was removed from the cage once a day for exercise.

During the first 8 days, each calf received the mammary secretions produced by its own dam, and after that whole mixed herd milk. The dam was milked completely each time, and if the milk was not fed immediately, it was refrigerated. Milk was warmed to 98° F. and well mixed before feeding. It was fed from nipple bottles to minimize the chance of it entering the rumen instead of the abomasum (11). The calves were fed three times daily for the first few days and then twice daily, at the rate of 8 per cent of body weight for the Jersey and 10 per cent of body weight for the Holsteins, to a maximum of 9 lb. of milk daily. In cases of digestive disturbance, milk was reduced to one-half the normal amount at the subsequent feeding and then gradually increased to normal. No other feed supplement or medication was used, except that a capsule containing 400 units vitamin D was given daily. As noted in Table 1, a few cases of severe scours were observed during the trials.

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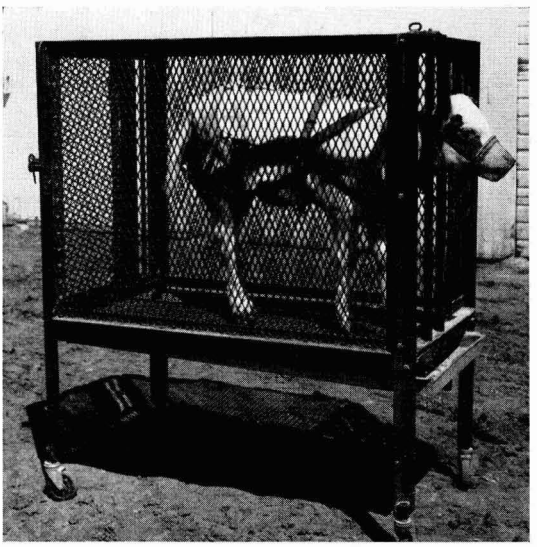

FIG. 1. A young calf, wearing the feces collection bag and muzzle, is shown in the metabolism cage used in the digestion studies.

Design of trials and collection of feces. The trials were conducted at four separate periods in order to determine whether there is any difference in the ability of the calf to digest the mammary secretions of a markedly changing composition when they are fed in the normal sequence to the growing calf during the first 17 days of life.

Carmine was used as a food-feces marker to permit the division of an uninterrupted feeding of the diet of milk of variable composition into four separate digestion trial periods, which were intended to be when the calf was 1-2, 3-4, 5-8, and 14-17 days of age. In order to simplify identification and separation of feces of the respective periods, the last normal feeding of each period was not given. The first milk fed in each period and the first after the last period contained one-fourth teaspoonful of carmine marker. Carmine was not needed to differentiate the meconium present in the gut at birth (7) from residues associated with the first feeding, as previous experience enabled the authors to make this separation satisfactorily.

Feces were collected in plastic bags inserted as a liner in small canvas bags constructed to fit around the tail and over the rump of the calf. The bags were held in place by adjustable harness (Figure 1). The plastic bags were changed twice daily and the feces were refrigerated until the end of each collection period, when a composite sample was prepared for analysis. When carmine-marked and unmarked feces appeared in the same bag, they were separated as completely as possible with a spatula.

TABLE 1

Apparent coefficients of digestion of nutrients of colostrum and milk by dairy calves

Period	Days of age	Calf number 1	2	3	4	5	6	7	8	9	Average
					Dry matter						
I	1-2	....	94	94	95	98[f]	97	91[a j]	94	95	95
II	3-4	86[a b]	89[a]	91	....	93[g]	98	....	85	95	91
III	5-8	99[c]	96[d]	97[e]	95	94[b]	....	88[a]	90	95	94
IV	14-17	96	98	97	98	95[i]	97[k]	98	....	97	97
					Protein						
I	1-2	79[a]	92	90	93	96	96	92	93	94	92
II	3-4	70	78	87	....	85	95	....	71	89	83
III	5-8	99	90	92	88	83	....	74	76	87	86
IV	14-17	94	96	93	95	89	90	95	....	93	93
					Ether extract						
I	1-2	92	96	97	97	99	98	96	95	96	96
II	3-4	96	90	98	....	98	99	....	96	97	96
III	5-8	100	98	98	96	98	....	85	96	98	96
IV	14-17	90	99	99	99	99	99	99	....	98	98
					Carbohydrate						
I	1-2	....	94	99	100	100	98	81	98	99	96
II	3-4	90	100	99	....	98	99	....	97	98	95
III	5-8	100	99	100	100	96	....	100	97	99	98
IV	14-17	99	99	100	100	98	99	100	....	100	99
					Ash						
I	1-2	86	91	91	96	99	98	86	97	96	93
II	3-4	86	88	93	....	94	98	....	91	95	91
III	5-8	100	95	95	93	92	....	80	91	91	92
IV	14-17	76[l]	97	94	97	90	95	97	....	93	95

[a] Severe scours.
[b] 3rd day only.
[c] 4-7 days.
[d] 5-9 days.
[e] 5-10 days.
[f] 1-3 days.
[g] 4-6 days.
[h] 7-9 days.
[i] 13-15 days.
[j] 1-4 days.
[k] 15-18 days.
[l] Dirt through muzzle (value not included in average).
Note: Superscripts on dry matter values apply to corresponding data on other nutrients.

Apparent digestion of each nutrient was calculated as follows: Percent digested $= \frac{\text{(wt. nutrient in feed–wt. nutrient in feces)}}{\text{wt. nutrient in feed}} \times 100.$

Analytical methods. Crude protein was determined by the Kjeldahl method; solids, by heating overnight at 100° C.; and ash, by heating at 500° C. The ether extract of colostrum and milk was determined by grinding 1.50 g. of a well mixed sample with anhydrous sodium sulfate in a mortar, transferring to a corundum extraction thimble, and completing the analysis in the usual manner. A similar procedure was used for feces, except that the aliquot extracted represented 2.00 g. of feces. Carbohydrate was determined by difference [CHO = solids — (crude protein + E.E. + ash)].

Methods that were used for determining vitamin A and carotenoids of milk

and colostrum have been described (8). Feces were analyzed for vitamin A and carotenoids in essentially the same manner as for milk, except that 10.0-g. samples were saponified by refluxing for 30 minutes with 20 ml. alcoholic KOH previous to the extraction.

RESULTS AND DISCUSSION

The nutrients of the mammary secretions fed during each of the four respective periods (days of age 1-2, 3-4, 5-8, 14-17) were digested well by the newborn-calf, most of the averages being above 90 per cent (Table 1). No marked differences in average digestibility during the different periods were apparent, except that possibly the digestion of protein during periods II and III (days 3-4 and 5-8) was somewhat reduced. The digestion of the nutrients appeared to have been lowered in some cases of severe scouring. It is possible that the small decrease in apparent digestion of protein during periods II and III was the result of incomplete adaptation of the digestive system to the markedly changing composition of the protein fraction of the mammary products (9) consumed by the calf during the trial. Other possible reasons might be an increased output of metabolic nitrogen after the first few feedings following intrauterine life, and/or the digestive upsets previously mentioned.

Carmine marker has been reported to alter the rate of passage of food through various parts of the alimentary tract of children (5). To what extent carmine affected rate of passage and digestion of nutrients by calves in this study is not known, but the possibility should be recognized.

It will be noted that in some cases the days of actual collection did not fit exactly the periods as set forth in the design, and that a few samples were missed. These variations in the planned procedure were due to changes necessitated by the inability to separate the feces representing various consecutive collection periods, or to accidents that could not be controlled. Some of the variations found in digestion of nutrients by different calves and at different periods might have been due to incomplete seperation of feces of the various periods. However, digestion usually was so complete and fecal residues relatively so small that any such errors would have had little effect on the calculations of the digestion coefficients.

Hydrolyzed sugars were determined on a few samples of feces by a modification of the method of Potter (10) for hydrolyzed lactose in milk. Results indicated that mere traces of monosaccharides were present. Only traces of reducing sugars were found in tests of extracts of feces with Fehling's solution.

Colostrum is a rich source of nutrients, as is well known; furthermore, as judged from these data, it is digested to a remarkably high degree by the newborn calf. This was not entirely unexpected, since work reviewed previously (7) indicated that many of the digestive mechanisms of the new-born are capable of functioning at birth.

In the literature it was found that Frenzel (3) had reported on a colt, 3-7 days of age, that digested about 90 per cent of the nutrients of mare's milk, except for ash, which was only 79 per cent digested. Digestibility decreased

gradually with increasing age of the new-born animal. Wöhlbier (12) stated that during the first week of life, suckling pigs digested about 89 per cent of the protein of the dam's milk and in the second week, 85 per cent. The apparent digestion of the nutrients of colostrum and milk by the young calf is similar to that reported elsewhere for older calves fed milk (1, 4, 6), except for protein digestion during periods II and III.

Apparent absorption of vitamin A averaged 81 to 95 per cent at the different periods of the trial (Table 2). Vitamin A absorption was lowest during periods

TABLE 2

Apparent absorption of vitamin A and carotenoids of colostrum and milk by dairy calves

Period	Days of age	Calf number								Average
		2	3	4	5	6	7	8	9	
					Vitamin A					
I	1-2	....	98	88	94[b]	97	85[c]	96	89	92
II	3-4	45[a]	91	....	91	93	....	68	87	81
III	5-8	97[d]	96[e]	93	90[f]	....	84[a]	81	87	89
IV	14-17	94	95	98	94[g]	97[h]	98	....	92	95
					Carotenoids					
I	1-2	....	70	52	96	80	51	55	50	65
II	3-4	....	45	....	....	65	....	25	21	38
III	5-8	34	82	31	4	....	19	47	68	42
IV	14-17	59	49	74	48	53	59	....	58	57

[a] Severe scours
[b] 1-3 days
[c] 1-4 days
[d] 5-9 days
[e] 5-10 days
[f] 7-9 days
[g] 13-15 days
[h] 15-18 days
Note: Superscripts on vitamin A values apply to corresponding data on carotenoids.

II and III, as was noted for protein. Apparent carotenoid absorption was not as great as, and varied more than, that of vitamin A, but little can be offered by way of explanation. Carotenoid absorption also was lowest during periods II and III. Carotene averaged 78 per cent (range 51-89) of the total carotenoids in feces samples that were studied chromatographically (mostly samples from calves 8 and 9). It is recognized that in studies of this type apparent digestion includes nutrients absorbed as well as those lost in passing through the alimentary tract. Therefore, any vitamin A lost by oxidation after ingestion would be recorded as digested. It was not possible to determine what part, if any, of the vitamin A in the feces represented that which was formed from carotene by conversion in the intestinal contents (2) and what part was that secreted back into the contents after systemic conversion. Further studies are needed to determine whether carotene and other yellow carotenoid pigments are differentially absorbed or excreted by the young calf.

SUMMARY

Studies were made of the apparent digestibility of nutrients in the natural food of new-born calves, colostrum, and early milk, when these products were fed in the normal sequence from birth to 17 days of age. The feeding and the feces collection were divided into four trial periods, days 1-2, 3-4, 5-8, and 14-17, after birth of the calf. Average apparent digestion of dry matter, carbohydrate, ether extract, and ash at each of the periods was 90 to 99 per cent. The apparent digestion of protein, however, was less than 90 per cent in periods II and III. Average apparent absorption of vitamin A was 81 to 95 per cent during the four periods, whereas apparent absorption of carotenoids was only 38 to 65 per cent.

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ANTIBIOTICS IN RUMEN DIGESTION AND SYNTHESIS. II. THE EFFECT OF AUREOMYCIN ON THE CONCENTRATION OF SOME AMINO ACIDS AND B VITAMINS IN THE RUMEN [1]

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The microbial synthesis of amino acids and B vitamins by ruminants on natural or supplemented rations has been indicated, and the literature has been comprehensively reviewed by McNaught and Smith (23) and Kon and Porter (14). Loosli *et al.* (19) were the first to show the synthesis by rumen microorganisms of the ten amino acids essential for the growth of the rat by feeding a purified ration to sheep. Urea supplied the only essential source of dietary nitrogen. Duncan *et al.* (9) fed essentially the same ration to fistulated steers and confirmed the synthesis of the ten amino acids and also found, with the exception of histidine, that the amino acid pattern of the mixed proteins in the ingesta of the steers on a purified ration was fundamentally similar to that found for a steer on a natural ration. Block and Stekol (3) and Block *et al.* (4) demonstrated that methionine and cystine are synthesized in the rumen from radioactive $Na_2S^{35}O_4$ at approximately the same rate and are used by the tissues to synthesize new protein.

As early as 1928, Bechdel *et al.* (2) observed that cows fed rations deficient in the vitamin B-complex produced milk which contained a vitamin B potency equal to that of herd milk from cows on a good winter ration. Ruminal synthesis of riboflavin (20, 21) and pantothenic acid in sheep and cattle (21, 22, 17, 29) have been reported. Hunt *et al.* (11) fed a ration of corn, alfalfa hay, and a protein supplement to steers and found that the ingesta which had been in the rumen for 12 to 16 hours showed a greater riboflavin content than the feed. An increase in riboflavin content did not occur when an all-alfalfa ration was fed.

Wegner *et al.* (28) found a three- to fourfold increase in the nicotinic acid content of the dried rumen ingesta over that in the feed. Lindahl *et al.* (18) reported that the excretion of nicotinic acid was higher when sheep received a ration containing casein than when receiving a low protein ration. Slaughter tests by Kesler and Knodt (12, 13) indicated that the concentrations of nicotinic acid and riboflavin were higher in the small intestine than in the feeds the calves received. Agrawala *et al.* (1) presented quantitative evidence to show that the

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bovine rumen microorganisms can utilize urea-nitrogen to synthesize appreciable amounts of riboflavin, niacin, and pantothenic acid. More recently, Gall *et al.* (10) have been successful in isolating some organisms responsible for the synthesis of various B vitamins.

In view of the above reports in the literature supporting microbial synthesis of protein and certain B vitamins in the rumen, feeding experiments were conducted to study the influence of aureomycin on the microorganisms as determined by the concentration of amino acids and riboflavin, nicotinic acid and pantothenic acid in the rumen at various intervals after feeding.

EXPERIMENTAL

The rumen contents from two rumen fistulated steers and the feeds used in this investigation were obtained from the same samples that were collected and used in previous work (7). The steers received a ration of 4 lb. of ground corn and 15 lb. of alfalfa-brome hay once daily. The corn was consumed in about 10 minutes and the hay in about 3 hours. Water was available in a drinking cup at all times. Crystalline aureomycin-HCl was mixed with the corn just before feeding and fed at the rate of 0.5 g. per day for 15 days and then increased to 1.0 g. per day for the next 15 days. At the completion of each 15-day period, the rumen was completely emptied of all solid and liquid material three times in 12 hours. The contents were weighed and thoroughly mixed, and a 500-g. aliquot was taken for analysis. The remaining contents were replaced immediately in the rumen. The contents that were removed before feeding are designated as 0-hour samples, and those collected after feeding are called 6- and 12-hour samples. Actually, the 0-hour sample was obtained 24 hours after feeding, but since most of the digestion occurs soon after the ingestion of the feed, the time immediately prior to feeding (0-hour) was selected as the most logical time to start to follow the progress of digestion. Each steer was its own control when maintained on the aureomycin-free hay and corn ration.

Hydrolyzates for the determination of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine were prepared according to the procedure of Stokes *et al.* (27) for feed, and tryptophan was prepared by the alkaline hydrolysis method outlined by Kuiken *et al.* (16).

The amino acids and B vitamins were determined by microbiological assay. The organisms, media, concentration ranges, and assay procedures for the amino acids were the same as those compiled by Duncan *et al.* (8). The hydrolytic and assay procedures outlined by Snell and Strong (26) were used for the determination of riboflavin, and the method of Krehl *et al.* (15) was used for nicotinic acid. The procedure designed by Skeggs and Wright (25) as modified by Buskirk *et al.* (5, 6) was used for the preparation of the material and assay for pantothenic acid. The organism *L. casei* (7469) was used in the assay for riboflavin, and *L. plantarum* 17-5 (8014) was used in the assays for nicotinic acid and pantothenic acid. The samples were ether extracted before they were analyzed for their amino acid and B vitamin contents.

The amino acid and B vitamin content of the corn and hay used in this work is given in Table 1.

TABLE 1

Amino acid and B-vitamin content of the ration

	Corn	Alfalfa-brome hay
	Amino acid	
	(%)	*(%)*
Arginine	0.48	0.73
Histidine	0.36	0.34
Isoleucine	0.42	0.77
Leucine	0.96	0.99
Lysine	0.19	0.61
Methionine	0.14	0.08
Phenylalanine	0.36	0.59
Threonine	0.33	0.65
Tryptophan	0.07	0.14
Valine	0.38	0.70
	B Vitamins	
	(γ/g.)	*(γ/g.)*
Riboflavin	1.65	6.48
Nicotinic acid	23.24	25.89
Pantothenic acid	2.02	22.44

TABLE 2

The essential amino acid composition of the dried rumen contents from steers fed aureomycin (All values expressed on the dry matter basis)

Time	Aureomycin	Arg [a]	His	Isol	Leu	Lys	Met	Phe	Thr	Try	Val
(hr.)	*(g.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
					Steer 707						
0	0	0.59	0.27	0.85	0.93	0.56	0.08	0.51	0.63	0.13	0.69
0	0.5	0.40	0.22	0.62	0.68	0.39	0.03	0.37	0.48	0.08	0.49
0	1.0	0.39	0.23	0.62	0.72	0.43	0.05	0.44	0.41	0.09	0.50
6	0	0.68	0.38	0.99	1.18	0.75	0.12	0.66	0.77	0.16	0.83
6	0.5	0.46	0.31	0.87	0.97	0.59	0.08	0.42	0.65	0.09	0.68
6	1.0	0.58	0.33	0.83	0.86	0.64	0.10	0.60	0.62	0.12	0.70
12	0	0.56	0.32	0.93	1.12	0.60	0.09	0.60	0.70	0.14	0.79
12	0.5	0.45	0.26	0.89	0.98	0.59	0.08	0.44	0.66	0.10	0.70
12	1.0	0.54	0.30	0.81	0.90	0.61	0.07	0.54	0.62	0.11	0.65
					Steer 714						
0	0	0.59	0.30	0.74	0.93	0.54	0.08	0.52	0.67	0.13	0.66
0	0.5	0.50	0.26	0.77	0.85	0.59	0.06	0.49	0.61	0.11	0.62
0	1.0	0.43	0.22	0.69	0.80	0.47	0.05	0.45	0.50	0.10	0.53
6	0	0.69	0.40	0.98	1.26	0.73	0.10	0.71	0.79	0.15	0.98
6	0.5	0.56	0.33	0.91	1.07	0.65	0.09	0.62	0.71	0.12	0.74
6	1.0	0.58	0.35	0.89	1.00	0.62	0.09	0.58	0.66	0.12	0.69
12	0	0.70	0.41	1.06	1.32	0.80	0.12	0.69	0.83	0.18	0.93
12	0.5	0.54	0.31	0.95	1.05	0.73	0.09	0.61	0.67	0.13	0.75
12	1.0	0.51	0.32	0.83	0.98	0.61	0.09	0.58	0.58	0.12	0.68

[a] The first three letters of the amino acid are used as the symbol.

RESULTS AND DISCUSSION

The percentage composition of the various amino acids in the dried rumen contents is shown in Table 2. The data are arranged by the hour of collection for each level of aureomycin intake. There was a decrease in the percentage of amino acids in the rumen ingesta of both steers when 0.5 g. of aureomycin was included in the ration and a tendency for a further decrease when 1.0 g. of aureomycin was fed to steer 714. The higher level did not cause a further decrease in the amino acid percentages insofar as steer 707 was concerned. These observations appear to be correlated with the consistency of the rumen contents of the two steers; those from steer 707 were finer in texture and more completely macerated than those from steer 714. A possible explanation for the decrease at each collection period when 0.5 g. of aureomycin was fed may be associated with a stimulatory effect of the antibiotic on the cellulolytic bacteria and, consequently, a more rapid passage of rumen nutrients into the remainder of the digestive tract. The feeding of 1.0 g. of aureomycin, however, appeared to affect the two steers differently. The microflora in the rumen of steer 707 apparently was unaffected by the higher concentration of the antibiotic because the percentages of the vari-

TABLE 3

Comparison of the total amounts of dried rumen contents and amino acids in the rumen dry matter before, 6 and 12 hr. after feeding the natural and aureomycin-supplemented ration (All values expressed as grams)

Aureomycin	Time	Wt. of rumen D.M. [a]	Arg	His	Isol	Leu	Lys	Met	Phe	Thr	Try	Val
(g.)	*(hr.)*											
						Steer 707						
0	0	4862	87.1 [b]	43.0	101.5	130.2	72.2	12.2	71.8	81.2	17.3	88.5
0	6	8514	58.1	32.2	84.2	100.7	63.9	9.9	56.6	65.3	13.6	70.4
0	12	7203	40.4	22.9	67.1	80.6	43.3	6.4	43.2	50.7	10.4	57.4
0.5	0	4636	76.6	39.8	88.9	116.2	62.7	9.8	63.8	72.4	14.5	77.1
0.5	6	7555	35.0	23.3	65.4	73.1	44.4	6.3	31.6	48.9	7.0	51.1
0.5	12	5761	25.9	14.8	51.5	56.8	34.0	4.9	25.3	38.3	5.8	40.3
1.0	0	6201	82.6	43.8	98.6	129.5	71.2	11.2	73.8	75.4	16.6	85.3
1.0	6	9378	54.1	30.3	76.6	80.2	59.2	9.0	55.6	57.2	10.7	64.8
1.0	12	7697	41.3	23.3	61.9	68.9	46.8	5.1	41.4	47.5	8.7	50.0
						Steer 714						
0	0	5511	90.2	45.9	100.9	135.6	74.2	12.6	75.3	87.0	17.9	90.8
0	6	10196	69.8	40.7	100.0	128.5	74.5	10.4	71.8	79.9	15.1	88.8
0	12	8591	60.2	35.5	91.4	113.8	69.2	10.4	59.5	71.2	15.2	80.0
0.5	0	6286	89.6	46.3	108.7	138.2	81.8	11.9	77.6	88.3	18.0	93.3
0.5	6	10384	58.3	34.7	94.7	110.7	67.6	9.6	64.4	73.7	12.3	77.3
0.5	12	8742	47.3	26.7	83.4	92.1	63.8	7.9	53.0	58.9	11.3	66.0
1.0	0	7387	89.7	46.3	111.2	143.5	79.5	11.8	79.9	87.3	18.4	94.0
1.0	6	12359	71.6	42.7	110.2	123.6	76.7	10.9	71.8	81.0	15.1	85.0
1.0	12	9684	48.9	31.4	80.3	95.1	58.8	8.5	55.7	56.2	11.4	65.4

[a] 7700 g. of moisture-free ration was fed to each steer immediately after each 0-hr. evacuation.

[b] All of the 0-hr. values include the amount present in the rumen before feeding plus that ingested in the feed.

ous amino acids remained relatively unchanged when compared to those obtained when 0.5 g. was fed. In steer 714, the percentages tended to decrease. This decrease may have been due to an inhibitory effect of the antibiotic on the cellulolytic bacteria and the accumulation of crude fiber and dry matter in the rumen. The decrease in the degradation of dry matter (cellulose) would tend to dilute the protein in the rumen, and samples taken for analysis would contain a higher percentage of dry matter and a lower percentage of protein. This observation is in accord with experimental data reported previously (7).

The number of grams of the various amino acids present in the rumen at the various collection periods are compiled in Table 3. In all cases, the amount indicated for the 0-hour period represents the amount in the rumen at the 0-hour collection plus that ingested in the feed. In general, the feeding of 0.5 g. of aureomycin decreased the amount of the various amino acids in the rumen at both the 6- and 12-hour collections over that observed when aureomycin was omitted from the ration. This decrease is assumed to be due to a more rapid removal. When 1.0 g. was fed, the amounts present at 6 and 12 hours were approximately the same as those obtained when no aureomycin was fed. More amino acids disappeared from the rumen of steer 707 on both the supplemented and unsupplemented rations than from the rumen of steer 714, but the trend was in the same direction for both steers. The difference in response between the two steers may be due to breed differences; steer 707 was a Guernsey and steer 714 was a Holstein. The type of ration also may have had some influence on the amount of amino acids in the rumen just as the type of ration influences the kind and numbers of rumen microorganisms. Reed *et al.* (24) found that the

TABLE 4

The B vitamin composition of dried rumen contents from steers fed aureomycin

Animal	Time	Aureomycin	Riboflavin	Pantothenic acid	Nicotinic acid
(no.)	*(hr.)*	*(g.)*	*(γ/g.)*	*(γ/g.)*	*(γ/g.)*
707	0	0	7.3	12.2	11.9
	0	0.5	6.6	39.0	29.7
	0	1.0	4.0	11.3	17.5
707	6	0	8.0	24.2	51.9
	6	0.5	7.2	36.3	52.6
	6	1.0	5.2	26.7	40.6
707	12	0	5.8	21.0	41.5
	12	0.5	5.4	28.1	69.2
	12	1.0	3.9	18.4	42.6
714	0	0	7.4	10.3	10.7
	0	0.5	7.8	27.0	40.8
	0	1.0	4.1	14.0	26.7
714	6	0	8.4	26.5	46.3
	6	0.5	5.9	25.1	41.3
	6	1.0	4.2	22.8	44.2
714	12	0	8.0	29.2	65.7
	12	0.5	5.4	26.5	60.3
	12	1.0	3.8	22.7	50.9

bacterial protein obtained from sheep receiving either dry or green feed contained approximately the same amount of cystine, but more methionine was present when green feed was fed.

The results of this experiment failed to indicate that amino acids were synthesized, but this does not mean that synthesis did not occur. There is no suitable quantitative method available to measure synthesis in a continually moving system under the condition of this experiment.

Table 4 presents the concentrations of riboflavin, nicotinic acid and pantothenic acid in the rumen ingesta at each collection period and for each level of aureomycin intake. The amount of riboflavin decreased progressively with each increase in aureomycin intake, whereas the concentration of nicotinic acid and pantothenic acid was approximately threefold greater at the 6-hour collection than that obtained at 0-hour. When 1.0 g. of aureomycin was ingested, the concentration of these two vitamins at each collection period approximated that obtained on the aureomycin-free ration. There was relatively little difference between any of the values obtained at the 6- and 12-hour collections.

The total number of milligrams of each of the three B vitamins in the rumen at each collection period is shown in Table 5. The values for riboflavin and pantothenic acid support the data in Table 4, in that both concentrations of aureomycin caused a more rapid removal of these two vitamins than was observed on the aureomycin-free ration. No evidence could be detected for the synthesis of riboflavin in either steer.

TABLE 5

Comparison of the amounts of riboflavin, pantothenic acid and nicotinic acid in the rumen dry matter before, 6 and 12 hr. after feeding the natural and aureomycin-supplemented ration

Steer	Aureomycin	Time	Riboflavin	Pantothenic acid	Nicotinic acid
(no.)	*(g.)*	*(hr.)*	*(mg.)*	*(mg.)*	*(mg.)*
707	0	0	82.6[a]	216	277
	0	6	68.3	206	443
	0	12	42.0	152	300
	0.5	0	77.6	337	356
	0.5	6	54.4	274	397
	0.5	12	31.4	163	400
	1.0	0	72.0	226	328
	1.0	6	47.8	248	377
	1.0	12	29.8	141	327
714	0	0	87.6	213	277
	0	6	85.6	270	470
	0	12	68.8	252	566
	0.5	0	96.2	327	476
	0.5	6	60.8	261	430
	0.5	12	46.9	232	528
	1.0	0	77.3	260	416
	1.0	6	51.7	282	545
	1.0	12	36.7	220	493

[a] All of the 0-hr. values include the amount present in the rumen before feeding plus that ingested in the feed.

In the case of nicotinic acid, there was an accumulation because of either rapid synthesis or delayed removal. The highest synthesis occurred when no aureomycin was included in the ration. The ingestion of both levels of aureomycin reduced the synthesis or removal of nicotinic acid to less than that obtained on the aureomycin-free ration. The concentration of nicotinic acid at the 0-hour collection was higher when aureomycin was included in the ration than when no aureomycin was fed. This increase may have been due to a delayed synthesis of the vitamin by the rumen microorganisms or to a decreased absorption from the rumen.

Some synthesis of pantothenic acid was evident, although the rate was low. A small accumulation occurred during the first 6-hour period when 1.0 g. of aureomycin was fed, but the quantity of this vitamin was higher at the 0-hour collection when 0.5 g. was fed because of delay in synthesis or decreased absorption from the rumen. The pantothenic acid data also suggest that there may be an individual difference in the rate of removal of this vitamin from the rumen when a natural ration is fed. The data for steer 707 showed a disappearance of pantothenic acid, whereas the data for steer 714 indicated an accumulation and/or synthesis during the first 12 hours after feeding. The individual differences were less noticeable when the antibiotic was fed.

The final interpretation of these data must wait until more specific information is available concerning the nutritive requirements of the rumen microorganisms and the synergistic or antienergistic properties of antibiotics. Under the conditions of this investigation, the ingestion of 0.5 g. of aureomycin promoted the removal of amino acids and riboflavin from the rumen.

SUMMARY

Two fistulated steers were used to determine the amount of amino acids and B vitamins present in the rumen at two intervals after feeding when aureomycin was fed at the rate of 0.5 and 1.0 g. per day.

The concentration of the ten essential amino acids in the rumen 6 hours after feeding was less when 0.5 g. of aureomycin was included in the ration. The data suggest that the rate of removal from the rumen was accelerated. When 1.0 g. was fed, the rate of passage was slightly higher than that obtained on the aureomycin-free ration, but there was an individual difference when either level was fed. Direct evidence of the synthesis of amino acids was lacking. The increased amounts in the rumen at both collection periods could be accounted for from the amounts ingested.

The amount of riboflavin in the rumen was lower when 0.5 g. of aureomycin was fed than when the aureomycin-free ration was fed.

Synthesis of nicotinic acid was indicated during the first 12 hours after feeding the aureomycin-free ration, whereas both levels of aureomycin tended to reduce the amount of synthesis.

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EFFECTS OF VACUUM LEVEL AND MILKING DURATION ON UDDER HEALTH IN MASTITIS-FREE FIRST CALF HEIFERS[1]

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The use of the mechanical milker has been associated many times with an increased incidence of mastitis. Burkey and Sanders (3) and Little and Plastridge (14) indicate that the main factors in machine milking that may contribute to mastitis are excessive vacuum, leaving the machine attached after milk flow has ceased, and attaching the machine before adequate let-down has occurred. Dodd *et al.* (7) found a higher incidence of clinical mastitis in 19 first calf heifers subjected to an 8-minute milking duration for an entire lactation than in a similar group of heifers subjected to a 4-minute duration.

The occurrence of teat lesions and injuries has been found to coincide with an increased incidence of infection (1, 3, 13, 21). Espe and Cannon (8) and Kennedy (13) have associated irritation and teat erosion with machine milking, and other workers (3, 12) have attributed erosions specifically to abnormally high vacuum.

In view of the lack of adequately controlled experiments on the significance of the milking machine as related to bovine mastitis, this project was undertaken in an attempt to establish an optimum vacuum level for the bucket-type milker used and to determine the possible detrimental effects of leaving the milker attached after cessation of milk flow.

EXPERIMENTAL

Animals and treatments. From September 1950, through March 1951, 22 first calf heifers, four Jerseys from the University herd and 18 Holsteins from the University and four other herds, were placed on experiment. For 4 weeks prior to the calculated calving date all heifers were maintained under uniform conditions. The first 4 weeks after parturition made up the standardizing period during which all heifers were milked normally at 13 in. of vacuum, and quarter milk samples from each heifer were tested twice weekly for mastitis. Heifers were considered mastitis-free when they showed no clinical mastitis or teat or

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udder injury during the standardizing period and had leucocyte counts of one million or less on all quarter samples obtained during the third and fourth weeks postpartum (only 11 of 192 samples contained more than 300,000 leucocytes per milliliter).

To obtain 12 heifers which met all requirements, 22 heifers were placed on experiment. Of the ten discarded, one was removed for a Caesarian section and resulting complications, one for being unmanageable, one for a teat injury, two for high leucocyte counts, two for acute coliform mastitis, and one for acute staphylococcic and two for sub-clinical staphylococcic mastitis.

Within a breed the first three heifers to calve which met the above restrictions made up a replicate and were assigned at random to one of three vacuum levels. Assignment of mastitis-free heifers continued until three Holstein replicates and one Jersey replicate were filled. Halves of udders were randomly assigned to a milking duration for the first and third replicates, and within a vacuum the opposite halves for the second and fourth replicates were assigned to the same milking duration. This was necessary to insure even numbers of right and left halves being milked at each duration.

The vacuum levels used were 10, 13, and 17 in. of mercury. The actual values as measured twice weekly throughout the experiment were 10.3 ± 0.0, 13.2 ± 0.0, and 17.2 ± 0.0 at the line end and 10.1 ± 0.0, 13.0 ± 0.0, and 16.8 ± 0.0 at the teat cup. The pulsations per minute for these vacuums were 48 ± 0, 48 ± 0, and 49 ± 0, respectively.

The milking durations were designated as "normal" and "twice-normal." Normal milking duration was defined as the length of time from application of the machine until the rate of flow for a given half fell ≤ 0.1 lb. in a 15-second interval (2). The "twice-normal" duration had the machine left on for a total of twice its own normal milking duration. During the comparison period, the average of the total daily milking time (not including machine strippings) was 6.8 ± 0.5 minutes for the normal halves and 12.9 ± 1.1 minutes for the twice normal halves.

Milking. Beginning with the first regular milking after calving and continuing to the end of the fourth week, all heifers were milked normally at 13 in. vacuum. Milking was performed by two operators with goat-claw, bucket-type milkers, using two machines on each cow.

At the start of the fifth week each heifer was milked at its assigned vacuum and each half for the duration specified. On the first 2 days of each week in the comparison period normal milking duration was determined by suspending each milker unit from a dairy scale and recording the milk flow for 15-second intervals with the aid of a stopwatch (10). At the end of normal milking duration, the half designated as "normal" was immediately machine stripped and the machine removed. The other half had the machine left on for twice its own normal milking duration before machine stripping was begun. The average of the A.M. and P.M. durations required for the first 2 days were adhered to for the respective milkings on the 5 days following. The interval between A.M. and P.M. milkings was 10 hours and between the P.M. and A.M. 14 hours. Cows were milked in the order

in which they were assigned to treatment with the exception that during a period of clinical mastitis that particular cow was milked last.

To insure adequate let-down and provide proper sanitation, each udder was washed for a full 30 seconds with two Kowtowels taken from hot water containing 0.5 per cent Phenolor.[3] Two or three streams of milk were taken from each teat into a strip cup. One minute after the end of stimulation, the machines were attached. During the standardizing period, the machine was removed at the milker's discretion, but in the comparison period each half was milked for its specified duration with the aid of interval timers before machine stripping was begun. Between cows the teat cups were dipped in cold water and then in hot water containing 1 per cent Phenolor. The cows were milked in the manner described to the end of the 44th week of lactation.

Feeding. A 13.6 per cent crude protein grain ration was fed at 1 lb. per 100 lb. live weight from 30 days prior to the calculated calving date to the end of the 6th week postpartum, based on the live weight 31 days prior and the first Tuesday after calving. For the seventh and remaining weeks it was fed at 1 lb. for each 4 lb. fat corrected milk (11), based on an average of the previous 3 weeks' production. Corn silage was fed at 2 lb. per 100 lb. live weight before calving and at 3 lb. per 100 lb. after calving. U. S. No. 1 alfalfa hay was fed at 1 lb. per 100 lb. live weight prepartum and *ad libitum* after calving. Silage and hay were adjusted weekly for live weight. In summer, bluegrass-ladino pasture replaced the silage in the ration.

Observations and analyses. Twice each week, 4 to 5 hours after the morning milking, each cow's udder was washed with a 1 to 2 per cent Phenolor solution in hot water, and each teat then was swabbed with a pledget of cotton saturated with 70 per cent alcohol. Immediately after this treatment, quarter milk samples of approximately 40 ml. were obtained aseptically. A 5- to 10-ml. portion was transferred aseptically to a small tube to be examined for leucocyte content and presence of mastitis organisms. This portion was incubated at 37° C. for 18 hours. Films were prepared and stained, and the leucocyte count was determined to the nearest 100,000 per milliliter by the Prescott-Breed method (14). Counts of less than 100,000 were recorded as 50,000 and all values were converted to 2-place logarithms of the count $\times 10^{-4}$ for the purpose of statistical analysis. A small amount of each incubated milk sample was streaked on blood agar to determine presence or absence of haemolytic staphylococci and coliform organisms, since no streptococci were observed in the films.

On part of the unincubated portion, pH was measured on a Beckman pH meter (Lab. model G) and two aliquots were titrated for chloride content, using essentially the method of Rosell (19) modified by the use of the adsorption indicator, dichlorofluorescein.

During the 4th and 44th week after calving, each udder was given a physical examination and classified according to Udall (22).

[3] Squibb's Phenolor contains 13.00 per cent orthophenyl phenol, 13.00 per cent soap, 3.80 per cent (by wt., 5 per cent by vol.) isopropyl alcohol, and 70.20 per cent inert ingredients.

Leucocyte counts, chloride and pH values, freedom from mastitis organisms, udder fibrosis, and teat erosion were the criteria used to evaluate effects of treatment.

An analysis of variance was run on each criterion, and individual half udder differences were partially accounted for by using the values obtained during the 3rd and 4th week of the standardizing period to adjust all values obtained during the comparison period by covariance (5).

The analysis was as follows:

Source of Variation	*Degrees of Freedom*
Between Cows	
Replicates	3
Vacuum levels	2
Replicates × vacuum levels (error A)	6[a]
Within Cows	
Normal vs. twice normal	1
Right vs. left	1
Normal vs. twice normal × vacuum	2
Right vs. left × vacuum	2
Residual (error B)	6[a]
Total	23

[a] Degrees of freedom were reduced to 5 for both error A and error B when the adjustment was made.

Time trends for the comparison period were explored by the separate analyses of first, second, and third order orthogonal polynomial coefficients (6, 9) which were adjusted to the values for the 3rd and 4th weeks of the standardizing period.

The values presented in Tables 1, 2 and 3 are arithmetic means ± their standard errors.

RESULTS AND DISCUSSION

Leucocytes. Statistical analysis of the average logarithms of the leucocyte count (Table 1) showed no difference among the three vacuum levels. The halves milked for normal duration had a greater average log count than the twice normal halves ($P < 0.05$). Investigation of time trends (Figure 1) showed no differences in the linear rates of increase for vacuum levels, whereas the normal halves increased at a greater rate than the twice normal halves ($P < 0.01$). The normal halves also showed a greater tendency ($P < 0.10$) to rise to a peak (at about 28 weeks) and then decline in the later stages of lactation. Right and left halves by vacuum levels also differed in their tendency toward this type of curvature ($P < 0.05$).

TABLE 1

Effects of vacuum level and milking duration on leucocyte values

	Mean log of the Leucocyte Count $\times 10^{-4}$			Mean Linear Orthogonal Polynomial Coefficient of the Log of the Leucocyte Count $\times 10^{-4}$	
	Standardizing Period	Comparison Period	Comparison Period Adjusted for Mean of Standardizing Period	Comparison Period	Comparison Period Adjusted for Mean of Standardizing Period
Vacuum Level					
10 in.	0.96 ± 0.05	0.98 ± 0.11	0.97	0.0006 ± 0.0016	0.0001
13 in.	0.95 ± 0.05	1.14 ± 0.13	1.14	0.0087 ± 0.0033	0.0081
17 in.	0.84 ± 0.03	0.91 ± 0.07	0.92	0.0039 ± 0.0020	0.0052
Milking Duration					
Normal	0.91 ± 0.03	1.08 ± 0.11*	1.08*	0.0069 ± 0.0024**	0.0066**
Twice Normal	0.93 ± 0.04	0.94 ± 0.06	0.94	0.0019 ± 0.0017	0.0022

* $P < 0.05$
** $P < 0.01$

TABLE 2

Effects of vacuum level and milking duration on chloride values

	Mean Chloride Mg. Per Cent			Mean Linear Orthogonal Polynomial Coefficient of the Chloride Value	
	Standardizing Period	Comparison [a] Period	Comparison Period Adjusted for Mean of Standardizing Period [b]	Comparison Period	Comparison Period Adjusted for Mean of Standardizing Period
Vacuum Level					
10 in.	101 ± 3	119 ± 4	118	0.7998 ± 0.1285	0.7843
13 in.	93 ± 5	110 ± 4	112	0.8226 ± 0.0660	0.9304
17 in.	106 ± 8	122 ± 5	120	0.7042 ± 0.0941	0.6119
Milking Duration					
Normal	100 ± 5	119 ± 5*	120**	0.8472 ± 0.0857	0.8573*
Twice Normal	101 ± 5	114 ± 3	114	0.7038 ± 0.0693	0.6937

* $P < 0.05$
** $P < 0.01$
[a] Right *vs.* left was significant in this term ($P < 0.01$).
[b] Right *vs.* left was significant in this term ($P < 0.05$).

Chloride. As with the leucocyte count, the average chloride content (Table 2) and the time trends for this criterion (Figure 2, Table 2) failed to show any significant differences among vacuum levels. Also in agreement with the leucocyte results, the average chloride for the normal halves was significantly greater ($P < 0.01$) than for the twice normal halves and the linear rate of increase (Figure 2, Table 2) was also greater ($P < 0.05$). Right halves were significantly higher for average chloride than left halves ($P < 0.05$). The normal halves tended to increase with time in an almost linear manner, whereas the twice normal halves increased more rapidly at first, tended to level off at about the 19th week of the comparison period, and then rose again more rapidly in later lactation (Figure 2). The two differed ($P < 0.01$) in their tendency toward this type of curvature.

pH. The average pH for the three vacuum levels, as well as that for milking durations (Table 3), did not differ significantly. In agreement with the two previous criteria, the normal halves increased at a greater linear rate ($P < 0.05$)

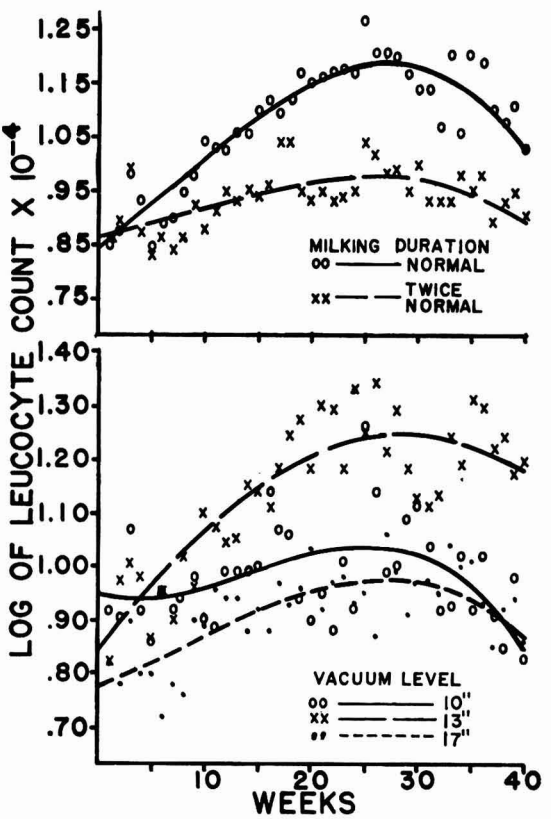

FIG. 1. Effects of vacuum level and milking duration on time trends of the leucocyte values.

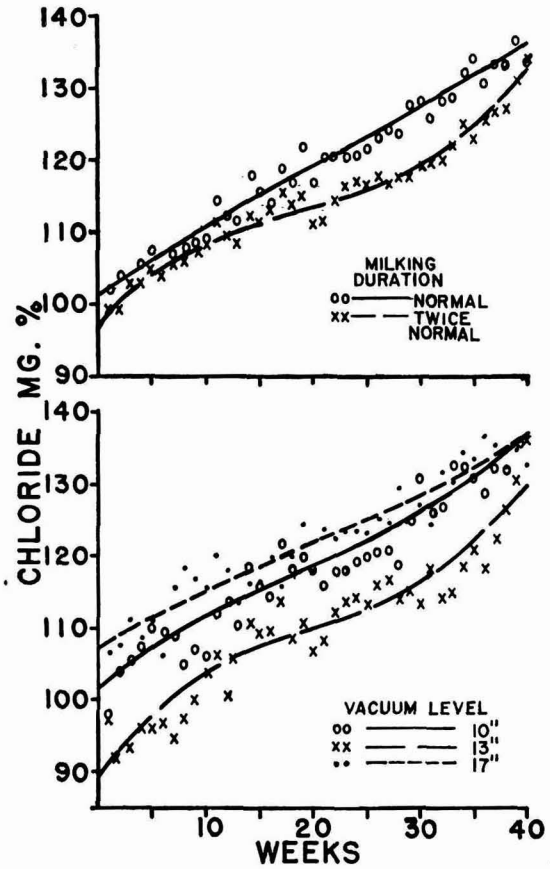

FIG. 2. Effects of vacuum level and milking duration on time trends of the chloride values.

over the lactation than the twice normal halves. The vacuum levels differed ($P < 0.05$) only in their tendency to curvature with time (Figure 3, Table 3). The disagreement of mean pH with the leucocyte and chloride values for the normal and twice normal halves is not unexpected in view of the published evidence of the rather poor agreement between pH and other indirect tests for abnormalities of milk (3, 4, 15, 17).

Mastitis organisms. The only mastitis organisms encountered during the comparison period were haemolytic staphylococci, with the exception of three samples which contained coliform organisms. When the per cent of total samples free from mastitis organisms for the comparison period and the 3rd and 4th weeks of the standardizing period were converted to the arc sin $\sqrt{\text{Percentage}}$ according to Snedecor (20) and these values were subjected to statistical analysis, no differences were revealed for either vacuum levels or milking durations.

TABLE 3

Effects of vacuum level and milking duration on pH values

	Mean pH			Mean Linear Orthogonal Polynomial Coefficient of the pH Value	
	Standardizing Period	Comparison Period	Comparison Period Adjusted for Mean of Standardizing Period	Comparison Period	Comparison Period Adjusted for Mean of Standardizing Period [a]
Vacuum Level					
10 in.	6.33 ± 0.09	6.58 ± 0.01	6.58	0.0037 ± 0.0008	0.0028
13 in.	6.55 ± 0.04	6.58 ± 0.03	6.58	0.0052 ± 0.0022	0.0057
17 in.	6.52 ± 0.04	6.57 ± 0.02	6.57	0.0057 ± 0.0013	0.0061
Milking Duration					
Normal	6.47 ± 0.06	6.58 ± 0.02	6.58	0.0053 ± 0.0013	0.0052*
Twice Normal	6.46 ± 0.05	6.57 ± 0.01	6.57	0.0045 ± 0.0012	0.0046

* $P < 0.05$

[a] Right *vs.* left was significant in this term only ($P < 0.05$).

Physical examination of udders. At the end of the comparison period teat ends were observed for redness, openness, vegetation, and proliferation and were arbitrarily scored for these characteristics to obtain a numerical value for each half. Analysis of variance indicated no significant differences among vacuum levels nor between milking durations for this estimate of teat erosions. Left halves scored significantly higher than right halves ($P < 0.01$), and some interaction of normal vs. twice normal with vacuum was indicated ($P < 0.05$). These results agree with the report by Dodd *et al.* (7) of equal occurrence of erosions in heifers milked for an 8-minute duration and those milked for a 4-minute duration for an entire lactation. The observations of other workers (3, 12) that high vacuum caused teat erosions were not supported in this study.

The results of quarters scored for fibrosis were expressed numerically, and analysis showed no significant differences for vacuum levels or milking durations.

Clinical mastitis. A total of seven clinical cases occurred in the comparison period, five in normal halves and two in twice normal halves. The 10 in. vacuum group had five of these cases, three in the normal half and two in the twice normal half of the same cow. Two cases occurred in the 13 in. vacuum group,

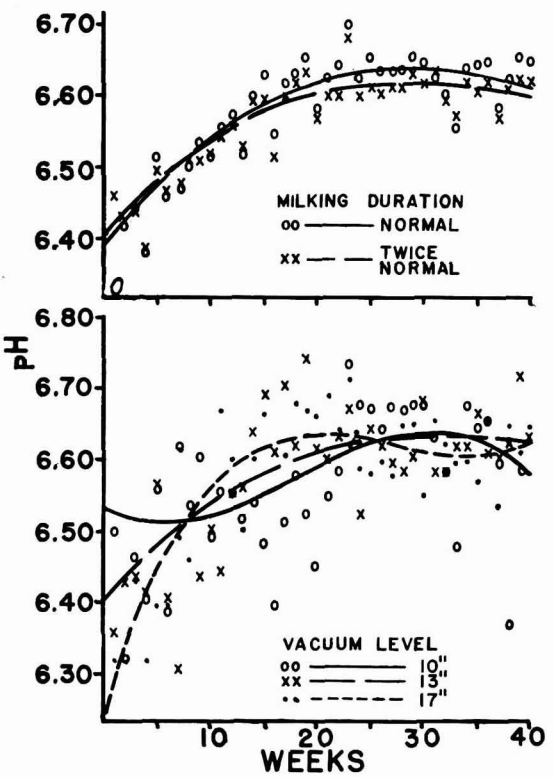

FIG. 3. Effects of vacuum level and milking duration on time trends of the pH values.

both in the normal half of the same cow. Although no cases occurred in the 17 in. vacuum group, one cow had a high leucocyte count (800,000 per milliliter or above) each week from the 10th to the 40th in milk from one quarter of the normal half, but no abnormal milk resulted.

Although five clinical mastitis cases occurred in normal halves while only two were present in the twice normal halves, this does not explain the significantly higher leucocyte and chloride values of the normal halves. Ten of the 12 normal halves had a higher mean log leucocyte count than the corresponding twice normal halves during the comparison period, but only four of these 12 were higher during the standardizing period. In the case of mean chlorides, values for seven of the 12 normal halves were higher than those for the corresponding twice normal halves during the comparison period, whereas only four of these 12 were higher in the standardizing period. Therefore, it seems probable that some factor other than clinical mastitis was responsible for the higher leucocyte and chloride values and the greater linear rates of increase obtained for the normal halves.

On the basis of reports reviewed earlier, the differences in leucocyte and chloride values for the normal and twice normal halves would have been expected to be opposite to those obtained. Latent infection or a previous mastitis history has been considered to affect the occurrence of clinical cases resulting from poor milking practices (16, 17, 18). The results of this experiment could be reconciled with most of the field reports if it is assumed that the clinical cases, apparently due to excessive vacuum or longer-than-necessary machine time, may have resulted from aggravation of an existing abnormality.

Since vacuum levels of 10, 13, and 17 in. did not result in significant differences in leucocyte count, chloride values, pH, freedom from mastitis organisms, teat erosion, or udder fibrosis, it appears that high vacuum *per se* had no measurable detrimental effect on the udder health of the 12 mastitis-free, first-calf heifers over one lactation period.

These results suggest that other factors, such as: individual cow susceptibility, laxity of the teat sphincter, residual infection of the environment, and sanitizing of equipment may be more important to udder health than the vacuum level used to milk or the length of time the machine is left on after milk flow has ceased.

The effect of leaving the machine on after milk flow ceases should be explored, using whole udders to eliminate any possible interaction of two different treatments occurring simultaneously in the same individual. The effects of vacuum and milking durations on animals in later lactations or on those with latent infections or previous history of infection also should be investigated.

SUMMARY

Three Jersey and nine Holstein first-calf heifers, free from mastitis for the first 4 weeks postpartum, were milked at 10, 13, or 17 in. of vacuum from the 5th through the 44th week of lactation. Each half of each cow's udder was milked at its designated duration of normal or twice normal for the entire comparison period.

Log of the leucocyte count, chloride and pH values, and freedom from mastitis organisms in twice-weekly quarter samples showed no significant differences in average response among vacuum levels. Neither were differences observed for teat erosions or udder fibrosis. Time trends for leucocyte and chloride values did not differ significantly for vacuum levels, which, in fact, differed only in their tendency to curvature for the pH criterion.

Halves milked for the normal duration had significantly greater mean leucocyte and chloride values and a greater linear rate of increase over the lactation for these two criteria and for pH than did halves milked for the twice normal duration. No significant differences were observed between milking durations for freedom from organisms, teat erosions, or udder fibrosis.

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PREPARATION OF MILK FAT.[1] III. PROPERTIES OF BUTTEROILS PREPARED BY THE USE OF SURFACE ACTIVE AGENTS[2]

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The efficiency of certain simple organic compounds and of more complex surface-active agents in the preparation of butteroil by de-emulsification of cream has been reported previously (4, 6, 7). However, the utility of such butteroil for either laboratory experimentation or commercial usage would depend upon the extent to which it resembled butteroil obtained by more conventional methods, such as the melting and refining of butter. To clarify this point, some physical and chemical constants of butteroils prepared by de-emulsification of cream were determined and compared with those of butteroil from butter.

EXPERIMENTAL

The same lot of fresh raw 40-per cent cream was used as the fat source for all samples of butteroil. The oil employed as a control was secured by churning a portion of the cream in a glass-walled Dazey churn at a temperature of 13-15° C. Additional samples were prepared by de-emulsification of cream with surface-active agents according to methods previously described (4, 7). The agents chosen for this purpose were Tergitol 7, Tergitol P 28, Aerosol OT, and the butylamine — butyl alcohol reagent proposed by Patton (4). Tergitols 7 and P 28 were used in their liquid commercial form at levels of 3 and 6 per cent, respectively, based on the weight of the cream. Aerosol OT was made to a concentration of 30 per cent by weight in butyl carbitol and this solution was then employed at a level of 3 per cent. The butylamine — butyl alcohol reagent was prepared and used as previously suggested (4). Prior to analysis, all the butteroils were washed five times with equal volumes of hot (95° C.) water and were dried while hot for 2 hours at a pressure of 15 to 20 mm. Hg. The sample prepared with the butylamine — butyl alcohol reagent was washed preliminarily with 1 per cent HCl, which procedure was observed to facilitate greatly the removal of any amine dissolved in the fat.

The butteroils were subjected to the following analyses and, unless otherwise stated, these determinations were carried out according to official methods of the A.O.A.C. (1): saponification number; unsaponifiable matter, by the procedure described by Winton (8); Reichert — Meissl number; Polenske number; free-fatty-acid number, calculated as the milligrams of KOH required to neutralize the free fatty acids in 1 g. of butteroil; iodine number, by the procedure of

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Rosenmund and Kuhnhenn (5); refractive index and melting point range. The latter value was obtained on samples that had been placed in thin-walled capillary tubes, sealed at one end and held at 5° C. for 48 hours. The results of these analyses, Table 1, show with perhaps one exception that the five butteroils are

TABLE 1

Fat constants of butteroils prepared from butter by refining and from cream by de-emulsification with surface-active agents

Constant	Type of butteroil [a]				
	CB	T 7	AOT	P 28	BA
Sap. No.	228.7	229.0	229.1	227.6	228.9
Unsap. (%)	0.31	0.37	0.30	0.57	0.39
Reichert-Meissl No.	28.2	27.9	27.7	27.9	29.3
Polenske No.	1.8	1.8	1.9	1.8	2.0
F. F. A. No.	0.86	0.79	0.88	0.85	1.05
Iodine No.	32.17	32.11	32.10	31.69	31.80
R. I. n^D 40	1.4548	1.4547	1.4547	1.4552	1.4549
Melting range (°C.)	34-36	34-35	34-36	34-36	34-36

[a] Butteroils prepared by churning cream (40% fat) and refining the resulting butter (CB) and by de-emulsifying cream with Tergitol 7 (T 7), Aerosol OT (AOT), Tergitol P 28 (P 28), and butylamine — butyl alcohol reagent (BA).

similar. The large amount of unsaponifiable matter in the sample prepared with Tergitol P 28 suggests that solution of the agent in the oil occurred. The slightly higher free fatty acid value of the oil prepared with butylamine — butyl alcohol reagent possibly may have resulted from residual HCl remaining in the oil after washing.

The purity of surface active agent-prepared butteroil was studied further in a qualitative manner through determination of drop numbers. Since this value is influenced notably by the presence of surface active agents, it was felt that drop numbers might reveal the extent to which butteroils were contaminated with the agents. In these experiments, patterned on that described by Lisse (3), butteroil samples were drained from a Donnan pipette at a rate of 10 drops per minute under distilled water at 40° C. Drop numbers were obtained on oils made from butter, the same oils to which varied amounts of surface-active agents had been added and oils prepared from 40-per cent cream with a surface-active agent (Tergitol 7). All glassware involved was washed, rinsed, held overnight in chromic acid cleaner, and rinsed thoroughly before use. The Donnan pipette was rinsed with ether, cleaned, and dried after each determination. During use, this pipette was held in a clamp so that it would always be immersed the same depth in the distilled water. From representative results, Table 2, it is clearly evident that contamination of butteroils with surface-active agents, such as Tergitol 7 or Aerosol OT will affect the drop number. Addition of as little as 0.01 per cent of these agents gave increased values. Since Tergitol 7 and Aerosol OT, as employed here, were not 100 per cent active principle, the minimum amounts of the agents affecting the drop number were actually less than 0.01 per cent. The fact that butteroil prepared from cream with the aid of Tergitol 7 gave drop numbers in the same range of values as the control oil indicates that the agent was effectively removed from the oil during processing.

TABLE 2

The effect of adding certain surface-active agents to butteroil [a] on its drop number [b]

Agent	Percentage of agent added					
	2%	1%	0.5%	0.1%	0.01%	No Agent
Tergitol 7	245	74	53	41	35	25
Aerosol OT	92	58	50	44	40	25
T 7 butteroil [c]	.....	....	....	....	....	28

[a] Made by churning cream and melting, washing, and drying the resulting butter.
[b] Averaged from 5 to 10 determinations with a Donnan pipette at a rate of 10 drops per min. at 40° C.
[c] Made by de-emulsifying cream with 3% by weights of Tergitol 7, as previously described.

To study further the properties of surface-active agent-prepared butteroil as compared with that obtained from butter, determinations were made for phospholipids as lecithin. All samples were made from the same lot of cream, the control oil prepared by churning and refining of the resulting butter. This latter sample was subjected to the same temperature changes as the other oils and was washed in precisely the same manner. The agent-prepared oils utilized Tergitols 7 and P 28 and Aerosol OT, respectively. From the bulk supply of each butteroil, samples were removed for analysis with from 0 to 6 washings. For the analyses the procedure of Deniges as employed for dairy products by Horrall (2) was followed. The blue phosphomolybdate complex was measured in a Klett-Summerson colorimeter and the amount of phosphorus present calculated by comparison with a standard curve. Multiplication of the phosphorus content by the factor 25.94 yielded the lecithin content of the oils. These values are presented in Table 3. They show that butteroils prepared with the aid of some surface-active agents may show a considerably lower lecithin content than

TABLE 3

Lecithin contents of butteroils prepared by refining butter and by de-emulsification of cream with surface-active agents

Type of butteroil	Number of washings	Lecithin
		(%)
From butter	0	0.084
	1	0.022
	3	0.020
	6	0.019
Tergitol 7	0	0.010
	1	0.008
	2	*
	3	*
Aerosol OT	0	0.008
	1	0.007
	2	*
	3	*
Tergitol P 28	0	1.250
	1	1.000
	2	0.916
	3	0.721
	4	0.428

[a] Quantities too small to measure.

would be encountered in butteroil prepared from the same cream by churning. Since Tergitol P 28 contains sodium di (2 ethyl hexyl) phosphate, the phosphorus data for oil made with this agent suggest either that some of the agent was rather permanently incorporated or that a substantial amount of phospholipids was recovered in the oil. It was noted that the Tergitol P 28 butteroil was completely bleached and had developed an oxidized odor on standing for several weeks, whereas the other samples appeared normal in color and odor.

SUMMARY AND CONCLUSIONS

By use of an appropriate surface active agent (Tergitol 7, Aerosol OT, or a butylamine — butyl alcohol reagent) to de-emulsify cream, a butteroil can be prepared which is practically identical to butteroil obtained from butter. Determination of conventional fat constants revealed that the only significant difference between such oils concerns their phospholipid content. Even exhaustive washing of butteroil from churned cream failed to give a lecithin value as low as the unwashed samples made with Aerosol OT or Tergitol 7. The presence of surface-active agents in butteroil was detected in concentrations as low as 0.01 per cent by drop number determinations. This determination might be employed appropriately to reveal contamination of an oil with such agents.

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THREE SUCCESSFUL TRANSPLANTATIONS OF FERTILIZED BOVINE EGGS

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Since the report of the first calf developed from a transplanted bovine embryo and carried to term (14), two other calves have been produced by similar procedures, making a total of three calves from five transplantations. The authors believe that these calves are the first to have been produced in this way. This paper reports details of the techniques followed.

EXPERIMENTAL

With the exception of the first donor, all the heifers used in the transplantations were grade yearling Holsteins with markings characteristic of the breed. The first donor, which was the dam of the first calf, was one-fourth Shorthorn and three-fourths Holstein and solid black except for a few white spots on the body and head. All of the sires were purebred Holsteins.

With the first calf, the estrual periods of the donor and recipient were synchronized by administering to both animals 50 mg. progesterone-equivalent daily (11, 13) in the form of progestationally active concentrate (PAC) in corn oil. For each of the two later successful transplantations, pairs of heifers with heats occurring at approximately the same time were available so that administration of progesterone was not necessary.

The donors were superovulated by injecting subcutaneously an FSH extract of sheep pituitaries daily for 5 days followed on the 6th day by an intravenous injection of unfractionated sheep pituitary extract (15). These treatments were so timed that the intravenous injection was administered on the day each heifer was expected to be in estrus. Each donor was inseminated on the day of the intravenous injection and again the day following.

Blood serum from the respective donors was the medium for the eggs. The serum was prepared by incubating the blood at 37° C. for 30 to 60 minutes, centrifuging twice, and then storing the serum in a refrigerator for 1 or 2 days prior to the transplantations. This storage period should remove possible ovicidal factors (2).

The transplantations were made on the 4th day following the second insemination. The donor was killed, and the reproductive organs were removed. The eggs were flushed out with blood serum and held at room temperature in this medium throughout the operation. Each unbred recipient was anesthetized by administering intravenously the following: 50 g. chloral hydrate, 25 g. magnesium sulfate, 100 ml. Nembutal containing 60 mg. per milliliter, and distilled water to make a total volume of 1 l. Anesthesia was given slowly until the heifer was no longer able to stand. Anesthetization was completed with ether. The uterus

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was exteriorized following mid-ventral laparotomy. The egg was inserted into the uterus near the tubo-uterine junction by puncturing the wall with a glass micro-pipette containing the egg. Single eggs with 8, 10, and 12 cells were transplanted in the three respective successful transplantations.

The heifer which carried the third calf was in estrus 1 day later than the donor, and the egg was placed in her uterus 4 days after she had been in heat. For this reason 50 mg. progesterone-equivalent of PAC was given on the 2nd and 3rd days following heat.

RESULTS AND DISCUSSION

Pregnancy in the three recipients was established by palpation 33 to 40 days after the intravenous injection and the first insemination of the donor. The number of days from the intravenous injection and the first insemination of the donors to the birth of the calves were 278, 280, and 278, respectively. The three calves were normal at birth and in later development.

Three successful transplantations from five attempts (60 per cent) are as good or better than has been reported in the literature with other species (1, 3, 4, 5, 6, 12), and this rate of success is close to pregnancy rates with cows in general. A few statements concerning the two unsuccessful transplantations may suggest causes for failure. The procedures differed from the successful ones in two ways: (a) The recipients were given no ether but were completely anesthetized with Nembutal, chloral hydrate, and magnesium sulfate. (b) Three eggs were transplanted in each recipient, one in one horn and two in the other. In addition, 16 and 18 corpora were in the ovaries of the respective donors as contrasted with 9, 6, and 3 in the ovaries of the three dams of the calves. Furthermore, the three eggs in one unsuccessful transplantation each had four blastomeres and the three in the other unsuccessful transplantation had four, four, and eight. As mentioned above, the three successfully transplanted eggs had eight, ten, and twelve blastomeres. In the two failures, PAC was administered to both the donors and recipients to synchronize their heats. More work is needed to determine the optimum time to discontinue administration of PAC or progesterone when such treatment is to be followed by gonadotrophins to produce superovulation. Later experience has suggested also that PAC or progesterone needs to be given only to the recipient to synchronize her estrus with that of the donor.

The first calf had black feet and switch, thus indicating that the donor was the dam. With all three calves born it was possible by use of the cattle blood-typing test (9) to exclude the recipients as the dams of the calves and thus provide evidence that the calves developed from the transplanted eggs. This test for exclusion is based upon the well-established principle that an individual possesses a particular blood group only if one or both of its parents has it.

The cells of the first calf possessed the blood groups *A*, *W*, and *S*, which were not carried in the cells of either the sire or the recipient. These three blood groups represent gene products at three different loci (8). Therefore, the only reasonable explanation is that these factors were contributed by the donor to the calf.

The cells of the second calf reacted with the specific blood-typing fluids (reagents) for the antigenic factors, P, Q, and I′. This complex of antigenic factors is inherited as a unit and represents the product of one of the genes ($B^{PQI'}$) at the B-locus (10). This complex was absent in the cells of both the recipient and the sire. In neither of the above cases were the cells of the donor typed.

The antigenic complex at the B-locus in the cells of the third calf provided evidence for an exclusion of maternity of the recipient of the fertilized egg. In this case, however, the cells of the donor were typed. The cells of both the calf and the donor reacted with the reagents for the factors O_3, Y_2, J′, and K′ representing the B-allele $B^{O_3Y_2J'K'}$. This gene product was not present in the cells of either the recipient or the sire. The logical conclusion is that the calf received this allele from the donor of the fertilized egg.

Since the donors were slaughtered to obtain the eggs and since surgery was necessary to transplant the eggs, the techniques reported in this paper leave much to be desired with respect to practical application of superovulation and egg transplantation for obtaining an increase in the number of offspring from outstanding cows. Such a procedure may become more feasible with additional research and refinement of techniques. The present technique with some possible improvements could, however, be useful in physiologic or genetic studies. It would enable one to determine whether the egg or the uterus is at fault in hard-to-settle cows. Transplantation of eggs also would enable one to study the influence that uterine environment might have upon the developing embryo and fetus as evidenced by physical and physiological characteristics, including subsequent milk production. That uterine environment might be of importance is suggested by the work of Russell (7), who has demonstrated by interstrain transplantation of ovaries that the number of vertebrae of mice is influenced by uterine environment.

SUMMARY

Techniques employed in the first three successful transplantations of bovine eggs are described in detail. A total of five transplantations were made. Blood typing showed definitely that the heifers carrying the calves through pregnancy were not their true dams.

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FERTILITY OF BOVINE SPERMATOZOA IN BUFFERED WHOLE EGG EXTENDERS CONTAINING PENICILLIN, STREPTOMYCIN, SULFONAMIDES AND ADDED GLUCOSE

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Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

Fertility results from two field experiments previously reported by Dunn *et al.* (5) indicated a slight difference in favor of the standard citrate-sulfanilamide-yolk extender over the citrate-succinylsulfathiazole-whole egg extender. At about the same time that these first whole egg experiments were being conducted, favorable results on the fertility of semen obtained by the addition of penicillin and streptomycin to the standard citrate-yolk extender was reported by Almquist (1, 2), Easterbrooks *et al.* (7) and Foote *et al.* (8). These results indicated the need for fertility studies comparing yolk and whole egg extender when each contained similar amounts of penicillin and streptomycin. The results of three field experiments with whole egg extenders containing these two antibiotics, and referred to herein as the third, fourth and fifth fertility experiments, are reported in this paper.

EXPERIMENTAL PROCEDURES

In general, the design of each of the three field experiments was similar. Semen from bulls of the five major dairy breeds was used. Ordinarily, only one ejaculate was collected from each bull at approximately weekly intervals but occasionally a second ejaculate was collected and mixed with the first when one ejaculate was insufficient to meet the semen requirements for the day. Each ejaculate of semen was divided into two portions. One portion was extended in the yolk (control) extender and the other portion in the whole egg (experimental) extender, and both at rates to give 15×10^6 motile spermatozoa per milliliter of extended semen.

The control and experimental portions of each ejaculate were shipped to different groups of field technicians affiliated with the New York Artificial Breeders' Cooperative, Inc., during a 2-week experimental period. The technicians receiving the control portion the first week received the experimental portion the second week, and vice versa. In this way all technicians had equal opportunities to use the semen of each bull in each treatment. Processing of the semen was accomplished as reported previously (5).

The third experiment, which included ejaculates from 27 bulls, was conducted in April and May, 1950, and consisted of a comparison of the control treatment, 2.9 CSAY, and the experimental treatment, 2.9 CSSWE, with both extenders containing 500 units each of penicillin and streptomycin per milliliter of extender.

The fourth experiment was conducted in July, 1950, with semen from 20 bulls, and included the following modifications of the whole egg extender.

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Succinylsulfanilamide replaced the succinylsulfathiazole (sulfasuxidine) in experiment 3 and those previously reported (5) in order to have a more comparable sulfa in both the yolk and whole egg extenders. One per cent of glucose was added to the buffer on the basis of the good results from laboratory storage experiments wtih a modification of Bogart's diluent for stallion spermatozoa (4). To maintain the isotonicity of the extender containing the added glucose the citrate in the buffer was reduced to 2.32 per cent. Thus, in this experiment, the experimental extender was 2.32 CSSAWEG and the control extender 2.9 CSAY. Both extenders contained the same amounts of penicillin and streptomycin as were used in the third experiment.

TABLE 1

Composition of buffers and extenders

Extenders [a]	Concentration of citrate, sulfonamides and glucose in buffers					Proportion of buffer to egg material		Concentration of citrate, sulfonamides and glucose in extenders				
	C	SA	SS	SSA	G	Y	WE	C	SA	SS	SSA	G
	(%)	(%)	(%)	(%)	(%)			(%)	(%)	(%)	(%)	(%)
2.9 CSAY	2.90	0.60	.....	.....	.....	1:1	.....	1.45	0.30	.....	.....	.....
2.9 CSSWE	2.90	.....	0.40	.....	.....	.....	3:1	2.175	.....	0.30	.....	.....
2.32 CSSAWEG	2.32	.....	.....	0.40	1.00	.....	3:1	1.74	.....	.....	0.30	0.75

[a] 2.9 and 2.32 represent the percent of sodium citrate dihydrate in the buffer. C = citrate; SA = sulfanilamide; SS = succinylsulfathiazole (sulfasuxidine); SSA = succinylsulfanilamide; G = glucose; Y = egg yolk; WE = whole egg. (500 units of sodium penicillin-G and 500 units of streptomycin-calcium chloride complex were added to each milliliter of extender).

The fifth experiment was conducted in September, 1950, with semen from 18 bulls and the same control and experimental extenders as in the fourth experiment.

The fertility of spermatozoa preserved in these extenders was measured by the 60- to 90-day and 150- to 180-day non-returns to first services. Because of the nonorthogonality of the data within experiments, the estimate of the treatment effects on fertility was made by the method of least squares (10).

RESULTS AND DISCUSSION

The fertility results of the three experiments are summarized in Table 2.

In the third experiment, the least squares estimates of the 60- to 90-day and 150- to 180-day per cent non-returns to first service cows were, respectively, 70.6 and 68.1 for the 2.9 CSAY extender and 68.4 and 65.5 for the 2.9 CSSWE extender. Only the difference of 2.6 percentage units for the 150- to 180-day non-returns is significant ($P < .05$).

In the fourth experiment, the least squares estimates of the 60- to 90-day and 150- to 180-day per cent non-returns to first service cows were, respectively, 71.4 and 69.5 for the 2.9 CSAY extender and 71.1 and 69.0 for the 2.32 CSSAWEG extender. None of these differences approach statistical significance at the 5 per cent level.

TABLE 2

Summary of fertility data and statistical analysis

Experiment No.	Extenders [a]	No. 1st services / treatment	60- to 90-day non-returns			150- to 180-day non-returns		
			Treatment [b] mean in %	Error mean square	F values for [c] treatment mean squares	Treatment [b] mean in %	Error mean square	F values for [c] treatment mean squares
3	2.9 CSAY	2388	70.6	0.19	2.84	68.1	0.20	3.94 [d]
	2.9 CSSWE	2315	68.4			65.5		
	Differences		2.2			2.6 [d]		
4	2.9 CSAY	1759	71.4	0.20	0.02	69.5	0.21	0.10
	2.32 CSSAWEG	1748	71.1			69.0		
	Differences		0.3			0.5		
5	2.9 CSAY	918	74.2	0.19	7.51 [e]	70.2	0.21	8.48 [e]
	2.32 CSSAWEG	965	68.6			64.0		
	Differences		5.6 [e]			6.2 [e]		

[a] 2.9 and 2.32 represent the per cent of sodium citrate dihydrate in the buffer. C = citrate; SA = sulfanilamide; SS = succinylsulfathiazole (sulfasuxidine); SSA = succinylsulfanilamide; G = glucose; Y = egg yolk; WE = whole egg. (500 units of sodium penicillin-G and 50 units of streptomycin-calcium chloride complex were added to each ml. of extender).

[b] The per cent non-returns are reported as the least squares estimate of the mean plus the treatment effect.

[c] In binomially distributed data with large numbers of observations and with probabilities not close to 0 or 1.0 the F test, which assumes the normal distribution, is a close approximation to an exact test.

[d] $P < .05$

[e] $P < .01$

In the fifth experiment the least squares estimates of the 60- to 90-day and 150- to 180-day per cent non-returns to first service cows were, respectively, 74.2 and 70.2 for the yolk extender and 68.6 and 64.0 for the whole egg extender. The differences between extenders are highly significant ($P < .01$) for both the 60- to 90-day and 150- to 180-day per cent non-returns.

In the three experiments reported herein, the average 60- to 90-day per cent non-returns for approximately 5,000 first service cows per treatment was 71.5 for the semen extended in the yolk formula and 69.4 for that extended in the whole egg formulae, with an average difference of 2.1 percentage units in favor of the yolk formula.

Although fertility information on the yolk and whole egg extenders without the antibiotics penicillin and streptomycin would have been desirable in these experiments, the authors chose not to jeopardize unnecessarily the over-all breeding efficiency of the New York Artificial Breeders' Cooperative operations by omitting these antibiotics from the extended semen used in the field. However, it is concluded from the results reported herein and those reported previously (5) that the addition of penicillin and streptomycin to the whole egg extender has about the same effects on fertility as has their addition to the yolk extender.

In experiments 4 and 5 the effects of glucose and succinylsulfanilamide are confounded. Consequently, their independent effects are not ascertainable. Yet it appears that adding glucose, reducing the citrate concentration, and substituting succinylsulfanilamide for succinylsulfathiazole in the whole egg formulae containing penicillin and streptomycin did not improve it in comparison with the standard yolk formula containing penicillin and streptomycin.

No definite explanation can be given at the present time for the difference in non-return rates of spermatozoa stored in yolk and whole egg extenders. However, one postulation may be made, namely, that the amount of yolk (approximately 8.3 per cent) in the whole egg extenders (6) has been too low for maximum fertility. In experiments comparing 50 per cent and 25 per cent yolk extenders Almquist (3), Olds *et al.* (9) and Stewart *et al.* (11) reported differences in non-returns of 2.8, 1.6, and 1.1 percentage units, respectively, in favor of the higher percentage of yolk in the extender. Almquist (3) also reported a difference in non-returns of 0.7 percentage units in favor of 50 per cent yolk over 12.5 per cent yolk. These differences are similar in magnitude to the average difference between the 50 per cent yolk extenders and the whole egg extenders studied in these and previously reported experiments (5).

In view of the fertility results from all of the experiments from this laboratory in which yolk and whole egg extenders have been compared, there appears to be a difference in non-return rates of about 2 percentage units in favor of egg yolk. In large scale operations, any semen treatment accompanied by an average increase in conception rate of 2.0 percentage units probably would be used unless there were other overriding considerations. On the other hand, if operations were on a small scale and the cost and availability of eggs were deciding factors, the whole egg extender still might be used with expectations of satisfactory conception rates.

SUMMARY

Three experiments were conducted, using the split sample technique, for comparing the fertility of bovine semen extended in 2.9 per cent citrate-sulfanilamide-yolk, 2.9 per cent citrate-succinylsulfathiazole-whole egg, and 2.3 per cent citrate-succinylsulfanilamide-whole egg-glucose. Each extender contained 500 units each of penicillin and streptomycin. The average 60- to 90-day per cent non-returns to a total of approximately 5,000 first services per treatment was 71.5 for the yolk extenders containing antibiotics and 69.4 for the whole egg extenders containing antibiotics.

From these results and those previously reported, it appears that the standard 2.9 per cent citrate-sulfanilamide-yolk extender is about 2.0 percentage units superior to the whole egg extenders when measured by the 60- to 90-day per cent non-returns to first services. Furthermore, it is concluded that together, penicillin and streptomycin have similar effects on fertility when they are added to either the yolk or the whole egg formula.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of the management of and the technicians associated with the New York Artificial Breeders' Cooperative, Inc. in furnishing the semen samples and in performing the inseminations. Also, the IBM summarization of data from all experiments by Leslie Phillips and coworkers and the assistance of R. H. Foote in the preparation of the manuscript are gratefully acknowledged.

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PROGRAM

FORTY-EIGHTH ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF WISCONSIN

MADISON, WISCONSIN

JUNE 21-24, 1953

Program Committee

H. A. BENDIXEN, Washington, Chairman

O. F. GARRETT, Ohio

GEORGE HYATT, JR., North Carolina

I. E. PARKIN, Pennsylvania

K. G. WECKEL, Wisconsin

GENERAL PROGRAM

Sunday, June 21, 1953

8:00 A.M. on **Meeting of the Executive Board,** *Room 201, Babcock Hall*

2:00 P.M. on **Registration,** *Babcock Hall*

8:00 P.M. **Informal Get-Together,** *Babcock Hall*

Monday, June 22, 1953

8:00 A.M. on **Registration,** *Babcock Hall*

9:45 A.M. **Opening Session,** *Wisconsin Union Theatre*

Dr. H. C. Jackson, *Chairman, Department of Dairy and Food Industries, University of Wisconsin*, Presiding.

NATIONAL ANTHEM

Leader—Emmett R. Sarig, *Associate Professor of Music, Extension Division, University of Wisconsin.*

INVOCATION

Dr. Charles A. Puls, *Pastor, Luther Memorial Church, Madison.*

WELCOME ADDRESS

Dr. E. B. Fred, *President, University of Wisconsin.*

PRESIDENTIAL ADDRESS

Profesor H. B. Henderson, *University of Georgia.*

ADDRESS: OVER THE FENCE

Dr. W. E. Krauss, *Ohio Agricultural Experiment Station, A.D.S.A. Representative on the National Research Council.*

Section Meetings

1:30–4:30 P.M. MANUFACTURING SECTION
Symposium: Nonfat Milk Solids—Analytical Methods—The Economic Situation
Agriculture Hall Auditorium

4:30 P.M. Manufacturing Section Business Meeting

1:30–5:00 P.M. PRODUCTION SECTION A
Artificial Breeding
Room 205, Babcock Hall

PRODUCTION SECTION B
Feeding and Techniques
Room 101, Biochemistry

1:20–4:30 P.M. EXTENSION SECTION
Papers E1 to E7
Room 206, Soils Building

7:30 P.M. **Officers' Reception** (Informal)
Great Hall, Wisconsin Union Building, followed by entertainment, *Wisconsin Union Theatre.*

An informal round-table discussion on methods of detecting the presence of substitute fats in dairy products will be held in *Room 119, Babcock Hall* immediately following the the Officers' Reception. Those interested are invited to participate in the discussion.

Tuesday, June 23, 1953

Section Meetings

8:30–11:00 A.M. MANUFACTURING SECTION A
Chemistry, Milk Fat
Room 119, Babcock Hall

MANUFACTURING SECTION B
Microbiology
Room 205, Babcock Hall

MANUFACTURING SECTION C
Equipment, Dried Products
Building T16 Auditorium

8:30–11:00 A.M. PRODUCTION SECTION A
Genetics and Reproduction
Room 101, Biochemistry

PRODUCTION SECTION B
Rumen Physiology and Milk Secretion
Room 302, Hiram Smith Hall

PRODUCTION SECTION C
Calf Nutrition and Antibiotics
Agriculture Hall Auditorium

9:00–10:45 A.M. EXTENSION SECTION
Papers E8, E9, and Teaching Aids Exhibits
Room 206, Soils Building

11:00 A.M.–NOON MANUFACTURING SECTION BUSINESS MEETING
Building T16 Auditorium

11:00 A.M.–NOON PRODUCTION SECTION BUSINESS MEETING
Agriculture Hall Auditorium

10:45 A.M.–NOON EXTENSION SECTION BUSINESS MEETING
Room 206, Soils Building

1:30–4:30 P.M. MANUFACTURING SECTION A
Chemistry, Milk Fat, Proteins
Room 101, Biochemistry

MANUFACTURING SECTION B
Microbiology, Milk
Building T16 Auditorium

MANUFACTURING SECTION C
Cheese
Room 205, Babcock Hall

4:30 P.M. **Cheddar Cheese Judging Clinic**
Room 222, Babcock Hall

1:30–4:30 P.M. **Joint Session of Production and Extension Sections**
Symposium on Dairy Cattle Housing
Joint Committee Reports
Report of the Purebred Dairy Cattle Association
Agriculture Hall Auditorium

7:30 P.M. **Recognition Program and Entertainment**
Presentation of Honor Awards and Installation of Officers
Wisconsin Union Theatre

Wednesday, June 24, 1953

8:45–9:15 A.M. **Manufacturing Section**
Report of Subcommittee III of Curriculum Committee on Dairy Manufacturing Curricula
Building T16 Auditorium

8:45–9:15 A.M. **Joint Session of Production and Extension Sections**
Report of Subcommittee II of Curriculum Committee on Dairy Production Curricula
Agriculture Hall Auditorium

9:30 A.M.–NOON **General Association Program and Business Meeting**
Building T16 Auditorium

9:30 Reports of Subcommittees I, IV, and V of the Curriculum Committee and of the General Curriculum Committee.

10:00 American Dairy Association—Research. Lester J. Will, *General Manager, ADA.*

10:15 Nutrition Research and the Dairy Industry. Dr. Zoe E. Anderson, *Director of Research and Nutrition Service, National Dairy Council.*

10:30 Association Business Meeting

1:30–4:30 P.M. **Section Meetings**

Joint Session of Manufacturing and Extension Sections
Building T16 Auditorium
Symposium: Bulk Handling of Milk on the Farm and in Transit to the Plant. Installation and Maintenance of In-Place Sanitary Pipelines—Farms and Plants.

PRODUCTION SECTION A
Mastitis, Ketosis, Management, and Reproduction
Agriculture Hall Auditorium

PRODUCTION SECTION B
Calf Nutrition and Silage Preservatives
Room 101, Biochemistry

ENTERTAINMENT PROGRAM

Entertainment for Men and Women

Sun., June 21 8:00 P.M.—INFORMAL GET-TOGETHER
Babcock Hall

Mon., June 22 7:30 P.M.—OFFICERS' RECEPTION AND ENTERTAINMENT
(Informal)
Great Hall, Wisconsin Union Building

Tues., June 23 7:30 P.M.—RECOGNITION PROGRAM AND ENTERTAINMENT
Wisconsin Union Theatre

Entertainment for Women

Mon., June 22 2:30 P.M.—STYLE SHOW
Wisconsin Union Theatre, followed by TEA, *Great Hall, Wisconsin Union*

Tues., June 23 1:00 P.M.—LUNCHEON
Great Hall, Wisconsin Union

Wed., June 24 2:30 P.M.—CONVERSATION AND BRIDGE
Elizabeth Waters Hall Lounge

Children's Program

NURSERY SCHOOL (Age group 3-5)

Monday 1:30 to 4:30 P.M., *Wisconsin High School Gymnasium*

Tuesday 9:00 A.M. to 4:00 P.M., *Wisconsin High School Gymnasium*

ORGANIZED ATHLETICS AND SWIMMING (Age Group 6-9)

Monday 1:30 to 4:30 P.M., *Wisconsin High School Gymnasium*

Tuesday 9:00 A.M. to 4:00 P.M., *Wisconsin High School Gymnasium*

PLANNED TOURS (Age Group 10 and up)

Monday 1:30 to 4:30 P.M., Meet at *Wisconsin High School Gymnasium*

Tuesday 9:00 A.M. to 4:00 P.M., Meet at *Wisconsin High School Gymnasium*

MANUFACTURING SECTION

Monday, June 22, 1953

1:30–4:30 P.M.

Symposium: Nonfat Milk Solids—Analytical Methods—The Economic Situation.

O. F. GARRETT, *Presiding.*
Agriculture Hall Auditorium

Introduction of the Subject. R. E. Remaley, *American Dry Milk Institute.*

Determination of Fat and Solids-Not-Fat in Mixed Milk by the Lactometer Method. P. F. Sharp, *University of California.*

New Work on the Use of a Lactometer for Determining the Total Solids in Milk. Paul D. Watson, *Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.*

Measurement of Solids in Milk by Oxidimetry. A. G. Leggatt, *Ontario Agricultural College.*

The Economic Situation. Hugh L. Cook, *University of Wisconsin.*

4:30 P.M.

Section Business Meeting

Tuesday, June 23, 1953

8:30–11:00 A.M.

Section A. **Chemistry, Milk Fat.**

G. H. HARTMAN, *Presiding.*

Room 119, Babcock Hall

M1 Chromatographic Studies of Reducing Sugars, other than Lactose, in Raw and Autoclaved Milk. C. J. Honer and S. L. Tuckey, *University of Illinois.*

M2 The Effect of Cationic and Anionic Resins on the Salt Content of Raw Skimmilk. G. K. Murthy and R. McL. Whitney, *University of Illinois.*

M3 An Ion-Exchange Resin—Contact Time Method for the Study of Ionic Equilibria in Complex Systems. (A) Preliminary Studies on Raw Skimmilk. J. M. Baker, C. W. Gehrke, and H. E. Affsprung, *University of Missouri.*

M4 The Use of Ion-Exchange Resin Membranes in the Study of the Ionic Equilibrium in Milk. H. E. Affsprung, C. W. Gehrke, and J. M. Baker, *University of Missouri.*

M5 A Study of the Ionic Equilibria in Raw Skimmilk and Heat Treated Milks. C. W. Gehrke, H. E. Affsprung, and J. M. Baker, *University of Missouri.*

M6 Some Factors Affecting the Water Insoluble Acid Content of Cream and Butter as Determined by Hillig Method. L. K. Crowe, *University of Nebraska.*

M7 The Effect of Some Feeds upon the Characteristics of the Butterfat Produced. C. E. Parmelee and H. A. Hollender, *Purdue University.*

M8 A Study of Milk Fat from Cows on Special Roughage Diets. W. H. Chilson and H. H. Sommer, *University of Wisconsin.*

M9 The Influence of Certain Surface Active Compounds upon the Accelerated Oxidation of Butteroil. H. A. Hollender, *Purdue University.*

M10 An Improved Method of Determining Peroxide Values of Butterfat in Dry and Fluid Milk. C. M. Stine, H. A. Harland, S. T. Coulter, and R. Jenness, *University of Minnesota.*

8:30–11:00 A.M.

Section B. **Microbiology.**
A. J. MORRIS, *Presiding.*
Room 205, Babcock Hall

M11 Non-sporulating Anaerobic Bacteria from Dairy Products. C. A. Claybaugh and F. E. Nelson, *Iowa State College.*

M12 Activity of Bacteria and Enzymes in Raw Milk Held at 40° F. F. J. Babel, *Purdue University.*

M13 The Rate of Heat Inactivation of Several Strains of Brucella Abortus in Milk. F. R. Kronenwett, S. A. Lear, and H. J. Metzger, *Rutgers University.*

M14 The Proteolytic Activity of *Bacterium Linens.* M. E. Friedman, W. A. Wood, and W. O. Nelson, *University of Illinois.*

M15 Proteolysis by *Streptococcus Lactis* Grown in Milk with and without Controlled pH. W. C. van der Zant and F. E. Nelson, *Iowa State College.*

M16 A Study of the Bactericidal Effectiveness of Ultra-violet Light in Terms of the Energy Absorbed by the Milk. W. H. Burgess and B. L. Herrington, *Cornell University.*

M17 Influence of Host on Adaptations of Bacteriophage Active against Lactic Streptococci. E. B. Collins, *University of California.*

M18 Type and Frequency of Mutation to Bacteriophage Resistance in Pure Cultures of Lactic Streptococci. D. M. Graham and F. E. Nelson, *Iowa State College.*

M19 Some Factors Influencing the Growth and Toxin Production of *Clostridium botulinum* Experimentally Inoculated into Surface Ripened Cheese. R. O. Wagenaar and G. M. Dack, *University of Chicago.*

M20 A Plate Culture Technique for the Quantitative Determination of *Leuconostoc citrovorum* in Cultured Buttermilk with Observations on the Progressive Changes in Numbers During the Fermentation Process. C. C. Prouty and Wilburn Glenn, *State College of Washington.*

8:30–11:00 A.M.

Section C. **Equipment, Dried Products.**
O. F. GARRETT, *Presiding.*
Building T16 Auditorium

M21 Operating Characteristics of Some Commercially Available Homogenizer Valves. D. A. Seiberling, *Ohio State University.*

M22 A Comparison of Tinned Steel and Stainless Steel Milk Cans. L. J. Hansen and W. C. Winder, *University of Wisconsin.*

M23 Laboratory Studies of Mixing Liquids by Agitation with Air. R. L. Perry, W. L. Dunkley, and Catherine Campbell, *University of California.*

M24 Mixing by Air Agitation in Horizontal Cylindrical Milk Tanks. W. L. Dunkley and R. L. Perry, *University of California.*

M25 A Study of the Effect of the Temperature of Water on Ease of Redispersion of Spray-dried Whole Milk Powder Using Low Energy Agitation. F. S. Hirt, W. K. Stone, K. R. Wood, and J. M. McIntire, *Quartermaster Food and Container Institute, Chicago, Ill.*

M26 Certain Factors Influencing the Self-Dispersion of Whole Milk Powder with and without Added Surfactants. D. W. Mather and H. A. Hollender, *Purdue University.*

M27 Some Factors Involved in the Wettability and Dispersibility of Dried Whole Milk. J. J. Janzen, W. A. McGugan, and A. M. Swanson, *University of Wisconsin.*

M28 Emulsion Stability of Cream Dried by Sublimation. W. D. Rutz and W. C. Winder, *University of Wisconsin.*

M29 The Effect of the Fat Content of the Milk on the Keeping Quality of the Dried Product. G. R. Greenbank and C. F. Hufnagel, *Bureau of Dairy Industry, U.S.D.A.*

11:00 A.M.

Section Business Meeting
Building T16 Auditorium

1:30 P.M.–4:30 P.M.

Section A. **Chemistry, Milk Fat, Proteins.**
O. F. GARRETT, *Presiding.*
Room 101, Biochemistry

M30 Detection of Foreign Fats in Dairy Products. W. H. Chilson and H. H. Sommer, *University of Wisconsin.*

M31 A Rapid Chromatographic Method for the Detection of Foreign Fats in Dairy Products. W. J. Harper and T. V. Armstrong, *Ohio State University.*

M32 Fractionation by Selective Solidification as an Aid in Detecting Butterfat Adulteration. W. A. Krienke, *University of Florida.*

M33 A New Method for the Detection of Substitute Fats in Dairy Products. V. Bhalerao and F. A. Kummerow, *University of Illinois.*

M34 Improved Techniques Make Cryoscopic Values Reliable. W. A. Krienke, *University of Florida.*

M35 The Electrophoretic Properties of Casein and Whey Proteins from Skimmilk, Buttermilk and Butterserum from the Same Whole Milk. V. H. Nielsen, J. L. Kucera, and E. W. Bird, *Iowa State College.*

M36 The Distribution of Casein and Noncasein Proteins, Calcium and Phosphorus among Skimmilk, Buttermilk and Butterserum from the Same Whole Milk. V. H. Nielsen and E. W. Bird, *Iowa State College.*

M37 The Denaturation of Milk Serum Proteins at Temperatures Ranging from 180° to 290° F. H. A. Harland, S. T. Coulter, V. H. Townley, and R. Jenness, *University of Minnesota.*

M38 Study of Denaturation of B-Lactoglobulin. Virginia Mularz and A. M. Swanson, *University of Wisconsin.*

M39 Changes in Casein Brought about by the Prolonged Action of Rennet. E. C. Hagberg and R. A. Sullivan, *National Dairy Research Laboratories, Inc., Oakdale, N. Y.*

M40 Characterization of the Heat-labile Loaf Volume Depressant of Milk Serum Proteins. A. L. Gordon, E. J. Guy, R. Jenness, and W. F. Geddes, *University of Minnesota.*

M41 The Effect of Storage upon the Nitrogen Distribution in Ice Cream. J. B. Mickle and J. A. Meiser, Jr., *Michigan State College.*

1:30–4:30 P.M.

Section B. **Microbiology, Milk.**
F. E. NELSON, *Presiding.*
Building T16 Auditorium

M42 Observations on Bacterial Population and Characteristics of Bottled Milk under Refrigerated Holding. H. V. Atherton, F. J. Doan, and C. W. Watrous, Jr., *Pennsylvania State College.*

M43 Influence of Time and Temperature of Plate Incubation upon Bacterial Counts of Market Milk and Related Products. F. E. Nelson and M. P. Baker, *Iowa State College.*

M44 The Destruction of Psychrophilic Bacteria in Milk by HTST Pasteurization Based on Thermal Death Time Studies. R. H. Andrews and O. W. Kaufmann, *University of Illinois.*

M45 The Role of Psychrophilic Bacteria in the Keeping Quality of Commercially Pasteurized and Homogenized Milk. J. C. Boyd, C. K. Smith, and C. M. Trout, *Michigan State College.*

M46 Effect of Incubation or Storage Temperatures on the Growth of Psychrophilic Bacteria. E. M. Mikolajcik and L. H. Burgwald, *Ohio State University.*

M47 Effects of Chelating Compounds upon Oxidized Flavor of Milk. L. R. Arrington and W. A. Krienke, *University of Florida.*

M48 Ascorbic Acid and Oxidized Flavors. E. S. Guthrie, *Cornell University.*

M49 An Origin of Sunlight Flavor in Milk. S. Patton and D. V. Josephson, *Pennsylvania State College.*

M50 A Study of the Transmission and the Reflectance of Light by Milk. W. H. Burgess and B. L. Herrington, *Cornell University.*

M51 The Stability of Added Vitamin A in Fluid and Dry Milks. D. H. Cox, S. T. Coulter, and W. O. Lundberg, *University of Minnesota* and *Hormel Institute, Austin, Minnesota.*

M52 Evaluation of the 2,4 Dinitrophenylhydrazine Test for Vitamin C in Milk. J. Tobias, D. W. Whitman, and E. O. Herreid, *University of Illinois.*

1:30–4:30 P.M.

Section C. **Cheese.**
A. J. MORRIS, *Presiding.*
Room 205, Babcock Hall

M53 A Short Activity Test for Starters. N. S. Golding, C. C. Prouty, P. R. Elliker, and B. P. Kirthisinghe, *State College of Washington.*

M54 A Study of Cottage Cheese Quality. D. D. Deane, F. E. Nelson, and R. W. Baughman, *Iowa State College.*

M55 A Miniature Cheese Technique and Its Application to Research Problems. H. Fram and F. W. Barber, *National Dairy Research Laboratories, Inc., Oakdale, N. Y.*

M56 Observations on Cheese Flavor Production by Pure Chemical Compounds. G. J. Silverman and F. V. Kosikowsky, *Cornell University.*

M57 The Effect of Heat Treatment upon the Acceptance of Process Cheese Products. R. I. Meyer and J. M. McIntire, *Quartermaster Food and Container Institute, Chicago, Ill.*

M58 The Influence of Processing on the Electrophoretic Pattern of Cheese Protein. E. C. Hagberg, R. A. Sullivan, and Margaret Fitzpatrick, *National Dairy Research Laboratories, Inc., Oakdale, N. Y.*

M59 An Early Gas Defect in Swiss Cheese Caused by *Bacillus polymyxa.* R. Tjepkema and W. V. Price, *University of Wisconsin.*

M60 Effects of pH on the Growth of *Propionibacterium shermanii* and its Relation to the Quality of Swiss Cheese. R. P. Tittsler and G. P. Sanders, *Bureau of Dairy Industry, U.S.D.A.*

M61 A New Method for Making Cheddar Cheese. H. E. Walter, A. M. Sadler, J. P. Malkames, Jr., and C. D. Mitchell, *Bureau of Dairy Industry, U.S.D.A.*

M62 An Interrelationship between Butyric Acid and Glutamic Acid in the Flavor Development of Provolone Cheese. J. E. Long and W. J. Harper, *Ohio State University.*

M63 A Soft-Ripened Cheese. D. M. Irvine and W. V. Price, *University of Wisconsin.*

4:30 P.M.

Cheddar Cheese Judging Clinic.

D. R. STROBEL, H. A. WILSON, W. V. PRICE, and G. M. TROUT.
Room 222, Babcock Hall

Wednesday, June 24, 1953

8:45–9:15 A.M.

Report of Sub-Committee III of Curriculum Committee on Dairy Manufacturing Curricula.

Discussion of Report.

O. F. GARRETT, *Presiding.*
Building T16 Auditorium

9:30–NOON

General Association Program and Business Meeting.

Building T16 Auditorium

1:30–4:30 P.M.

Joint Session of Manufacturing and Extension Sections.

Chairmen: O. F. GARRETT and I. E. PARKIN
Building T16 Auditorium

Symposium: Bulk Handling of Milk on the Farm and in Transit to the Plant. Installation and Maintenance of In-Place Sanitary Pipe Lines —Farms and Plants.

Moderator: H. F. JUDKINS, *National Dairy Products Company, Inc., New York City.*

Panel Members:

W. C. Frazier, *University of Wisconsin*
Paul Girton, *The Girton Manufacturing Company*
G. L. Hopson, *DeLaval Company*
L. H. Minor, *Wyandotte Chemical Company*
J. R. Perry, *National Dairy Products Company, Inc.*
Clarence Weber, *New York State Department of Milk Sanitation*
S. A. Witzel, *University of Wisconsin*

PRODUCTION SECTION

Monday, June 22, 1953

1:30–5:00 P.M.

Section A. **Artificial Breeding.**

GEORGE HYATT, JR., *Chairman*
Room 205, Babcock Hall

P1 Tenure and Turnover of Desirable Dairy Bulls in Artificial Studs. R. B. Becker and P. T. Dix Arnold, *University of Florida.*

P2 Effect of Transportation on Fertility of Bulls. E. L. Willett and G. L. Larson, *American Foundation for the Study of Genetics, Madison, Wis.*

P3 Observations on the Sexual Behavior and Semen Production of Dairy Bulls. E. B. Hale, J. O. Almquist, and D. L. Thacker, *Pennsylvania State College.*

P4 A Study of the Optimum Time for Insemination. Peter W. Aschbacher, Vearl R. Smith, and W. H. Stone, *University of Wisconsin.*

P5 The Effect of Dosage, Concentration, and Site of Depositing Semen on Fertility in Artificial Insemination. Durward Olds, D. M. Seath, M. C. Carpenter, and H. L. Lucas, *University of Kentucky.*

P6 Site of Semen Deposition as Related to Fertility in Dairy Heifers. Victor Hurst, *Clemson Agricultural College.*

P7 Extenders and Techniques for Freezing Bovine Spermatozoa. H. O. Dunn, H. D. Hafs, *American Foundation for the Study of Genetics, Madison, Wis.*

P8 Factors Affecting Survival of Bull Spermatozoa at Sub-Zero Temperatures. W. J. Miller and N. L. VanDemark, *University of Illinois.*

P9 The Storage of Bovine Semen at Low Temperatures (—15° C.). O. T. Stallcup, H. K. McCartney, and Lantis Ratcliff, *University of Arkansas.*

P10 Preliminary Breeding Results with Frozen Semen. H. O. Dunn, G. L. Larson, and E. L. Willett, *American Foundation for the Study of Genetics, Madison, Wis.*

P11 Evaporated Milk as a Semen Extender. W. J. Collins, *Mississippi State College.*

P12 Some Metabolic Measurements of Diluted Bovine Semen Adjusted to Various pH's. W. C. Kinney, Jr. and G. W. Salisbury, *University of Illinois.*

P13 Influence of Incubation Interval on Fructose and Lactic Acid of Bull Semen. M. H. Ehlers and F. H. Flerchinger, *State College of Washington.*

P14 Aerobic Uptake of Glucose-C^{14} by Bovine Spermatozoa. R. J. Flipse, *Pennsylvania State College.*

P15 The Occurrence of Penicillin and Streptomycin-resistant Microorganisms in Diluted Bull Semen. John A. Alford, *Mississippi State College.*

P16 Characterization of the Bovine Seminal Plasma Proteins. B. L. Larson and G. W. Salisbury, *University of Illinois.*

P17 The Conversion of Seminal Constituents to a Seminal Plasma Basis when Analyzed as Total Semen. F. H. Flerchinger and R. E. Erb, *State College of Washington.*

1:30–5:00 P.M.

Section B. **Feeding and Techniques.**

P. L. KELLY, *Chairman.*
Room 101, Biochemistry

P18 Blackstrap Molasses for Feeding Dairy Cattle. W. A. King and J. P. La Master, *Clemson Agricultural College.*

P19 Babassu Meal in Rations with and without Molasses for Milk Production. R. E. Mather, *New Jersey Agricultural Experiment Station, Sussex.*

P20 The Effects of Adding Ground Hay to Dairy Cattle Rations. Garland M. Bastin and D. M. Seath, *University of Kentucky.*

P21 Grain Rations for Cows on Pasture. A. D. Pratt and R. R. Davis, *Ohio Agricultural Experiment Station, Wooster.*

P22 The Value of Corn Cobs as a Partial Replacement for Hay in the Ration of Lactating Cows. G. C. Graf and R. W. Engel, *Virginia Polytechnic Institute.*

P23 Corn Cobs and Purdue Cattle Supplement A as a Ration for Dairy Cattle. D. L. Hill, B. Hatcher, N. S. Lundquist, and B. W. Crowl, *Purdue University.*

P24 Growth Stimulators and Growth Inhibitors in Forage and Forage Juice Concentrate. R. G. Hansen, B. L. Larson, P. Krichevsky, H. M. Scott, and T. S. Nelson, *University of Illinois.*

P25 Are There "Unidentified Lactation Factors" for Cows? R. F. Davis, J. K. Loosli, and R. G. Warner, *Cornell University.*

P26 The Utilization and Value of Ammoniated Industrial By-Products as Sources of Nitrogen for Dairy Cattle. N. D. Nagruder and C. B. Knodt, *Pennsylvania State College.*

P27 The Economy of Winter Feeding Thyro-active Supplement Under a Base-Surplus Marketing Plan. Eric W. Swanson and S. A. Hinton, *University of Tennessee.*

P28 The Estimation of the Dry Matter Consumption of Grazing Animals by Ratio Techniques. E. A. Kane, W. C. Jacobson, R. E. Ely, and L. A. Moore, *Bureau of Dairy Industry, U.S.D.A.*

P29 The Possible Use of Plant Pigments as a Marker in Digestion Trial Studies. H. M. Irvin, *University of Maryland,* and H. G. Wiseman, *Bureau of Dairy Industry, U.S.D.A.*

P30 A Procedure for Measuring Pasture Herbage Consumption. W. A. Hardison, J. T. Reid, and C. M. Martin, *Cornell University.*

P31 A Method for Estimating the Value of Corn Silage. K. E. Harshbarger, W. B. Nevens, and R. W. Touchberry, *University of Illinois.*

P32 Plasma Carotene and Vitamin A Levels of Dairy Cows Before and After Parturition. K. A. Kendall and K. E. Harshbarger, *University of Illinois.*

P33 Nitrate Poisoning in Cattle and the Use of Ammonium Nitrate as a Pasture Fertilizer. I. L. Hathaway and Leon Chesnin, *University of Nebraska.*

P34 Studies of Feeding Aldrin to Dairy Cows. Ray E. Ely and L. A. Moore, *Bureau of Dairy Industry,* and P. E. Hubanks, R. H. Carter, and F. W. Poos, *Bureau of Entomology and Plant Quarantine, U.S.D.A.*

Tuesday, June 23, 1953

8:30–11:00 A.M.

Section A. **Genetics and Reproduction.**

GEORGE HYATT, JR., *Chairman*
Room 101, Biochemistry

P35 Body Form in Relation to Production in Holstein and Jersey Cows. W. W. Swett and C. A. Matthews, *Bureau of Dairy Industry, U.S.D.A.*

P36 Some Factors Affecting Age at Puberty in Holstein-Friesian Dairy Heifers. H. W. Hawk, W. J. Tyler, O. T. Fosgate, D. G. Sprain, and L. E. Casida, *University of Wisconsin* and *Bureau of Dairy Industry, U.S.D.A.*

P37 A Study of Lactational Differences in Butterfat Percentage in Dairy Cattle. E. H. Voeller, T. M. Ludwick, C. M. Clifton, H. R. Donoho, and F. Ely, *Ohio Agricultural Experiment Station, Wooster.*

P38 Sire by Herd Interaction in Production Traits in Dairy Cattle. J. E. Legates and F. J. Verlinden, *North Carolina State College,* and J. F. Kendrick, *Bureau of Dairy Industry, U.S.D.A.*

P39 Comparative Heat Tolerance of Holstein and Crossbred Red Sindhi-Holstein Heifers when Exposed to Elevated Temperatures and Humidities. J. E. Johnston and J. B. Frye, Jr., *Louisiana State University.*

P40 Physiological and Hereditary Responses of Lactating Holstein-Friesian and Jersey Cows to Natural Environmental Temperature and Humidity. Cecil Branton, J. E. Johnston, and G. D. Miller, *Louisiana State University.*

P41 Urinary Estrogen Excretion During the Gestation Period of the Bovine. E. P. Smith and Wm. M. Dickson, *State College of Washington.*

P42 Some Causes of Infertility in Dairy Heifers. T. Y. Tanabe and J. O. Almquist, *Pennsylvania State College.*

P43 Bactericidal Activity of the Uterus in Different Endocrine States. W. G. Black, J. Simon, S. H. McNutt, and L. E. Casida, *University of Wisconsin,* and *Bureau of Dairy Industry, U.S.D.A.*

P44 Androgenic Substances in the Urine of Bulls. R. Wiseman and A. B. Schultze, *University of Nebraska.*

P45 Effect of Oxytocin and Epinephrine on the Conception Rate of Cows. R. L. Hays, N. L. VanDemark, and E. E. Ormiston, *University of Illinois.*

P46 Reproductive Rate in Holstein-Friesian Cattle. Mogens Plum and H. P. Davis, *University of Nebraska.*

P47 The Distribution of Alkaline Phosphatase, Glycogen, and Periodic Acid Schiff-positive Substances in Follicles of the Bovine Ovary. S. Moss, T. R. Wrenn, and J. F. Sykes, *Bureau of Dairy Industry, U.S.D.A.*

8:30–11:00 A.M.

Section B. **Rumen Physiology and Milk Secretion.**

P. L. KELLY, *Chairman*
Room 302, Hiram Smith Hall

P48 Comparison of Rumen Flora and Environment in Roughage vs. Grain-fed Animals. L. S. Gall, C. N. Huhtanen, R. Saunders, and W. Schmidt, *National Dairy Research Labs., Inc., Oakdale, N. Y.*

P49 The Dissimilation of Amino Acids by Bovine Rumen Bacteria. R. N. Doetsch, R. Q. Robinson, and J. C. Shaw, *University of Maryland.*

P50 The Catabolism of Carbon Compounds by Bovine Rumen Bacteria. R. N. Doetsch, R. Q. Robinson, and J. C. Shaw, *University of Maryland.*

P51 Nutritional Requirements of Bovine Rumen Bacteria. J. J. McNeill, R. N. Doetsch, and R. Q. Robinson, *University of Maryland.*

P52 The Bacterial Flora in the Rumen of Heifers Fed a Ration of Alfalfa Silage. M. P. Bryant and L. A. Burkey, *Bureau of Dairy Industry, U.S.D.A.*

P53 The Effect of Somatotropin upon Milk Production and Various Blood Substances of Lactating Cows. A. C. Chung, J. C. Shaw, and W. M. Gill, *University of Maryland.*

P54 The Rate of Cell Division in the Mammary Glands of Rats. Ralph P. Reece and Virgene Warbritton, *Rutgers University.*

P55 Perfusion Technique in Tracer Studies of Milk Secretion. S. Lakshmanan, S. Kumar, and D. R. Jacobson, *University of Maryland.*

P56 Carbohydrate Metabolism of Mammary Gland Homogenates. E. M. Craine, *University of Illinois.*

P57 The Conversion of Glucose to Galactose. R. G. Hansen, E. M. Craine, and Paul Krichevsky, *University of Illinois.*

P58 The Effect of Hourly Milking with the Use of Oxytocin on the Butterfat Percentage and Saponification Number. J. H. Koshi and J. D. Donker, *University of Minnesota.*

P59 The Effect on Milk Production in the Bovine when Milking Intervals were Varied and Intravenously Administered Oxytocin was Used for Milk Ejection. J. D. Donker and J. H. Koshi, *University of Minnesota.*

P60 Investigations on the Secretion of Calcium into Milk Using Radioactive Tracers. R. A. Monroe, W. J. Visek, E. W. Swanson, and C. L. Comar, *University of Tennessee.*

8:30–11:00 A.M.

Section C. **Calf Nutrition and Antibiotics.**

W. R. MURLEY, *Chairman.*
Agriculture Hall Auditorium

P61 The Production of a Magnesium Deficiency in the Young Calf Using a Semi-synthetic Milk Diet. J. W. Thomas and M. Okamoto, *Bureau of Dairy Industry, U.S.D.A.*

P62 Estimation of Vitamin A Depletion Time in Young Dairy Calves. R. Teichman, J. E. Rousseau, Jr., H. D. Eaton, and G. Beall, *University of Connecticut.*

P63 Lipid Requirements of the Young Dairy Calf. M. R. Lambert, N. L. Jacobson, R. S. Allen, and J. H. Zaletel, *Iowa State College.*

P64 Effect of Diet on the Diurnal Variation of Blood Plasma Lipides in Young Dairy Calves. H. A. Ramsey, S. B. Tove, and G. H. Wise, *North Carolina State College.*

P65 Chrystalline Vitamin B_{12} Requirement of the Young Dairy Calf. Charles A. Lassiter, G. M. Ward, C. F. Huffman, C. W. Duncan, and H. D. Webster, *Michigan State College.*

P66 The Value of Certain Surfactants as Growth Stimulants When Fed to Calves. Charles A. Lassiter, T. W. Denton, and G. M. Bastin, *University of Kentucky.*

P67 Effects of Various Antibiotics and a Detergent and of Frequency of Milk Replacement Feeding on Young Dairy Calves. H. Voelker and N. L. Jacobson, *Iowa State College.*

P68 B-Vitamin Levels in the Blood of Young Dairy Calves Fed a Milk Replacement Diet with and without Aureomycin. Q. T. Smith and R. S. Allen, *Iowa State College.*

P69 The Effect of Aureomycin upon the Growth of Dairy Calves when Administered Orally, Subcutaneously and Intramuscularly. C. W. Richardson, Magnar Ronning, E. R. Berousek, and C. L. Norton, *Oklahoma A. and M. College.*

P70 Oral Supplementation Versus Intramuscular Injection of Aureomycin to Young Calves. L. L. Rusoff, J. M. Fussell, C. E. Hyde, and R. M. Crown, *Louisiana State University.*

P71 The Effect of Feeding Aureomycin Supplement on the Performance of Calves Raised on the High Roughage System. J. W. Hibbs and H. R. Conrad, *Ohio Agricultural Experiment Station, Wooster.*

P72 Alfalfa vs. Prairie Hay for Calves with and without Aureomycin Supplement. G. R. Clawson, S. C. Musgrave, C. L. Norton, and W. D. Gallup, *Oklahoma A. and M. College.*

P73 The Long-time Effects of Aureomycin Feeding to Dairy Heifers. Robert C. Fincham and Howard H. Voelker, *Iowa State College.*

11:00 A.M.–NOON

Production Section Business Meeting
Agriculture Hall Auditorium

1:30–4:30 P.M.

Joint Session of Extension and Production Sections.
IVAN PARKIN and GEORGE HYATT, JR., *Co-Chairmen.*
Agriculture Hall Auditorium

Symposium: Dairy Cattle Housing

Our Dairy Cattle Housing Problems.
E. E. Heizer, *University of Wisconsin.*

Saving Money and Labor by Good Barn Planning.
Stanley Witzel, *University of Wisconsin*

Milking Equipment for all Types of Dairying.
R. P. Morrissey, *Manager, Dairy Division, International Harvester Company, Chicago, Illinois.*

Milk Sanitation Aspects of Dairy Cattle Housing.
Howard K. Johnston, *Milk Sanitation Bureau, Department of Health, Harrisburg, Pennsylvania.*

Joint Committee Reports—
Breeds Relations, R. E. Erb, *Chairman*
Dairy Cattle Breeding, C. D. McGrew, *Chairman*
Dairy Cattle Health, J. K. Loosli, *Chairman*
Type, Lynn Copeland, *Chairman*
Antibiotics, W. A. Krienke, *Chairman*

Report of Purebred Dairy Cattle Association, J. F. Cavanaugh, *Secretary*

Wednesday, June 24, 1953

8:45–9:15 A.M.

Joint Session of Extension and Production Sections

Report of Subcommittee II of Curriculum Committee on Dairy Production Curricula
Agriculture Hall Auditorium

9:30 A.M.–NOON

General Association Program and Business Meeting
Building T16 Auditorium

1:30–4:30 P.M.

Section A. **Mastitis, Ketosis, Management, and Reproduction.**
GEORGE HYATT, JR., *Chairman*
Agriculture Hall Auditorium

P74 A Comparison of the Resistance of Milk Samples from Cows on Pasture and Dry Feed to the Action of *Str. agalactiae*. W. D. Pounden and A. D. Pratt, *Ohio Agricultural Experiment Station, Wooster.*

P75 Use of the Whiteside Test in Designating Herds from which Mastitis Milk is Being Delivered to the Creamery. W. E. Petersen and I. A. Schipper, *University of Minnesota.*

P76 Antibiotic Levels of Milk Following Intramammary Administration of Various Antibiotics. I. A. Schipper and W. E. Petersen, *University of Minnesota.*

P77 Effects of Vacuum Level and Milking Duration on Udder Health and Milk Production. R. D. Mochrie, H. H. Hale, R. E. Johnson, and W. N. Plastridge, *University of Connecticut.*

P78 Effects of Vacuum Level and Prolonged Milking on Milk Production, Milking Time, and Rate of Milk Flow in First Calf Heifers. A. T. Gregoire, R. D. Mochrie, H. D. Eaton, F. I. Elliott, and G. Beall, *University of Connecticut.*

P79 Studies on the Use of Permanent Milk Pipelines in Dairy Barns. III. Labor (Time) Requirements for Operation of Pipeline and Conventional Installations in a Stanchion Barn. E. E. Ormiston, W. O. Nelson, and M. H. Alexander, *University of Illinois.*

P80 The Effect of Irrigation on Pastures for Dairy Cattle. A. G. Van Horn, M. W. Whitaker, R. H. Lush, and B. T. Throop, *University of Tennessee.*

P81 A Comparison of the Influence of Hard Water and Soft Water on the Milk Production of Dairy Cows. T. H. Blosser and B. K. Soni, *State College of Washington.*

P82 Further Studies on the Use of Sodium Propionate in the Control of Ketosis in Dairy Cattle. L. H. Schultz, *Cornell University.*

P83 Studies of the White Blood Cells at Parturition and after ACTH Administration. Vearl R. Smith and R. P. Niedermeier, *University of Wisconsin.*

P84 Rumen Studies on Normal and Ketotic Cows. R. E. Brown, *University of Maryland.*

P85 A Technique of Freezing and Factors Effecting the Revival of Bovine Spermatozoa. E. F. Graham and G. B. Marion, *University of Minnesota.*

P86 The Frequency of *In Utero* Vascular Anastomosis in Bovine Twins as Determined by Blood Typing. E. J. Lazear, L. C. Ferguson, and Fordyce Ely, *Ohio Agricultural Experiment Station, Wooster.*

P87 Variation in Fertility of Dairy Cattle in Alaska. W. J. Sweetman, *Alaska Agricultural Experiment Station, Palmer.*

P88 Chromatographic Separation of Bovine Urinary Adrenal Corticoids as Hydrolyzed by Beta-Glucuronidase or by Sulfuric Acid. John P. Mixner and William G. Robertson, *New Jersey Agricultural Experiment Station, Sussex.*

P89 Application of the Electroejaculation Technique to the Bull. P. J. Dziuk and E. F. Graham, *University of Minnesota.*

P90 Occurrence of Hereditary Edema in Ayrshires. Franklin E. Eldridge, and F. W. Atkeson, *Kansas State College.*

P91 Effect of the Ration on Volatile Fatty Acid Production in the Rumen. C. S. Gard and L. H. Schultz, *Cornell University.*

1:30–4:30 P.M.

Section B. **Calf Nutrition and Silage Preservatives.**

P. L. KELLY, *Chairman*
Room 101, Biochemistry

P92 The Influence of Diet on the Development of the Ruminant Stomach. R. G. Warner, H. F. Bernholdt, C. H. Grippin, and J. K. Loosli, *Cornell University.*

P93 The Use of Various Fats in "Filled Milk" Diets for the Production of Veal Calves. B. Connor Johnson, John H. Hopper, and K. E. Gardner, *University of Illinois.*

P94 Vegetable Oils versus Butterfat in the Diet of Dairy Calves. Thor. W. Gullickson, R. S. Adams, John Gander, and J. H. Sautter, *University of Minnesota.*

P95 Response of Dairy Calves to Whole Milk Replacements Containing Dried Whey Product, Dried Skimmilk, and Fat. F. B. Young, *Iowa State College.*

P96 Use of Special Processed Soybean Oil Meal in Feeds for Young Dairy Calves. J. F. Stein, C. B. Knodt, and E. B. Ross, *Pennsylvania State College.*

P97 The Effect of Rumen Inoculations and the Ratio of Hay to Grain Eaten on Digestion and Nitrogen Retention in High Roughage Fed Calves. H. R. Conrad and J. W. Hibbs, *Ohio Agricultural Experiment Station, Wooster.*

P98 Roughage: Concentrate Ratios for Young Dairy Calves. K. E. Gardner and G. S. Stuff, *University of Illinois.*

P99 Changes in Blood Reducing Sugar Levels Following Administration of Carbohydrates Directly into the Omasal-abomasal Cavity of Dairy Calves. H. J. Larsen and G. E. Stoddard, *Iowa State College.*

P100 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, *University of Wisconsin.*

P101 Relation of Breed and Free Gossypol Levels to Cottonseed Meal Toxicity in Dairy Calves. R. E. Leighton, W. B. Anthony, J. S. Huff, and I. W. Rupel, *Texas A. and M. College.*

P102 Outdoor Individual Portable Pens Compared with Calf Barn for Raising Dairy Calves. L. R. Davis, K. M. Autrey, H. Herlich, and G. E. Hawkins, Jr., *Bureau of Animal Industry, U.S.D.A.*, and *Alabama Polytechnic Institute.*

P103 Bacterial Activity on Forage Plants Before, During and After Ensiling as Indicated by Numbers. John T. Kroulik, *Bureau of Dairy Industry, U.S.D.A.*

P104 Sodium Metabisulfite as a Silage Conditioner. C. H. Gordon, J. B. Shepherd, H. G. Wiseman, and C. G. Melin, *Bureau of Dairy Industry, U.S.D.A.*

P105 Sodium Metabisulfite as a Preservative for Grass Silage. J. W. Bratzler, R. L. Cowan, and R. W. Swift, *Pennsylvania State College.*

P106 Bacterial Activity in Forage Crop Silage as Indicated by the Predominant Groups or Species of Bacteria at Different Stages of Curing. L. A. Burkey and John T. Kroulik, *Bureau of Dairy Industry, U.S.D.A.*

P107 Response of Calves to a Chromatographed Milk. E. G. Moody, N. S. Lundquist, and S. M. Hauge, *Purdue University.*

P108 Effect on Growth of Feeding Aureomycin to Dairy Calves from Birth to Thirteen Months of Age. E. E. Bartley, F. C. Fountaine, and F. W. Atkeson, *Kansas State College.*

P109 Histology of the Pituitary Gland as Related to Reproduction in Dairy Cattle. P. T. Cupps, S. W. Mead, and R. C. Laben, *University of California.*

EXTENSION SECTION

Monday, June 22, 1953

1:20–4:00 P.M.

Presentation of Papers.

I. E. PARKIN, *Presiding.*

Room 206, Soils Building

E1 Brucellosis in the United States. C. G. Bradt, *Cornell University.*

E2 The Place of Television in the Extension Program. Floyd J. Arnold, *Iowa State College.*

E3 Grassland Farming. J. M. Fry, *Pennsylvania State College.*

E4 Pasture Management in the Rotation Plan. Lyman H. Rich, *Utah State Agricultural College.*

E5a Irrigation—Eastern Presentation. D. C. Sprague, *G. L. F. Farm Products, New York.*

E5b Irrigation—Western Presentation. H. P. Ewalt, *Oregon State College.*

E6 Evaluating the Type and Production of Offspring Resulting from Sires Used in Artificial Breeding. Clyde N. Hall, *Pensylvania State College.*

E7 Influence of Environment and Test Intervals of Estimation of Yields of Dairy Cows. R. E. Erb and A. O. Shaw, *State College of Washington.*

Tuesday, June 23, 1953

9:00 A.M.–NOON

Presentation of Papers and Business Meeting.

STANLEY N. GAUNT, *Presiding.*

Room 206, Soils Building

9:00 A.M.

E8 Demonstration and Discussion of a Detergent Test for Butterfat in Milk and Other Dairy Products. O. S. Sager, *Bureau of Dairy Industry, U.S.D.A.*

E9 Abnormalties of Reproduction. T. Y. Tanabe, *Pennsylvania State College.*

Teaching Aids Exhibits. Lawrence Johnson, *Michigan State College.*

10:45 A.M.

Extension Section Business Meeting

Reading of the minutes.

Roll call.

Committee reports:

Dairy Records—D. E. Voelker, *Chairman*
Teaching Methods—Lawrence Johnson, *Chairman*
4-H Club—C. W. Nibler, *Chairman*
Resolutions—Jerry Heebink, *Chairman*

Old business.

New business.

An Evaluation of the 1953 Extension Section Program.

1:30–4:30 P.M.

Joint Session of Extension and Production Sections

GEORGE HYATT, JR., and I. E. PARKIN, *Presiding.*
Agriculture Hall Auditorium

Symposium: Dairy Cattle Housing

Our Dairy Cattle Housing Problems. E. E. Heizer, *University of Wisconsin.*

Saving Money and Labor by Good Barn Planning. Stanley Witzel, *University of Wisconsin.*

Milking Equipment for All Types of Dairying. R. P. Morrissey, *International Harvester Co., Chicago, Illinois.*

E10 Milk Sanitation Aspects of Dairy Cattle Housing. H. K. Johnston, *Pensylvania State Department of Health.*

Joint Committee Reports:

Dairy Cattle Health—J. K. Loosli, *Chairman*
Dairy Cattle Breeding—C. D. McGrew, *Chairman*
Type—Lynn Copeland, *Chairman*
Breeds Relations—R. E. Erb, *Chairman*
Antibiotics—W. A. Krienke, *Chairman*

Report of the Purebred Dairy Cattle Association—J. F. Cavanaugh, *Secretary*

Wednesday, June 24, 1953

8:45–9:15 A.M.

Joint Session of Extension and Production Sections

Agriculture Hall Auditorium

Report of Sub-Committee II of Curriculum Committee on Dairy Production Curricula.

Chairmen: George Hyatt, Jr., and I. E. Parkin.

9:30 A.M.–NOON

General Association Program and Business Meeting
Building T16 Auditorium

1:30–4:30 P.M.

Joint Session of Extension and Manufacturing Sections
Chairmen: I. E. Parkin and O. F. Garrett
Building T16 Auditorium

Symposium: Bulk Handling of Milk on the Farm and in Transit to the Plant. Installation and Maintenance of In-Place Sanitary Pipe Lines. —Farms and Plants.

Moderator: H. F. JUDKINS, *National Dairy Products Company Inc., New York City.*

Panel Members:

W. C. Frazier, *University of Wisconsin*
Paul Girton, *Girton Manufacturing Company, Pennsylvania*
George L. Hopson, *DeLaval Company, New York*
Lee H. Minor, *Wyandotte Chemical Company, Michigan*
John R. Perry, *National Dairy Products Co., New York*
Clarence Weber, *New York State Department of Milk Sanitation*
S. A. Witzel, *University of Wisconsin*

PEOPLE and EVENTS
in the Dairy Science World

President Urges Full Attendance at Annual Meeting

I am sure you will be interested in a brief report on plans for the annual meeting. Officials of the University of Wisconsin have indicated that everything is in readiness for the largest delegation of dairy scientists ever to be assembled in this country. We may rest assured that the good people of Wisconsin will be ready for all who can arrange to be there.

The program committee reports the largest number of papers of any previous meeting, with a total of 185. In addition, there will be three papers by invitation and two symposia. This is some 80 papers more than in 1952 and 30 more than in 1951.

I would like to urge you to make a last minute effort to send to the Secretary the names of prospective members. SECRETARY ELLSWORTH reports that membership is already above the peak of 1952. In many states the membership campaign is moving ahead with remarkable success. If you have not done your part by getting at least one new member, please get busy and tell one of your associates about the merits of membership in A.D.S.A. If you have not received a copy of the new promotional literature about the Association, write the Secretary and ask for one.

Make your plans now to attend the 48th Annual Meeting of the American Dairy Science Association at the University of Wisconsin on June 22-24.

H. B. HENDERSON
President, A.D.S.A.

Hilton to become President of Iowa State

JAMES H. HILTON will become president of Iowa State College July 1, 1953, to succeed DR. CHARLES E. FRILEY who retires on that date. The president-elect was born on a farm near Hickory, North Carolina, in 1899. He entered North Carolina State in 1918, but finished his degree at Iowa State in 1923 in Animal Husbandry. He served as county agent in Iowa for three years before becoming a member of the Extension Service at Purdue University. He later became a member of the Dairy Husbandry teaching and research staff at Purdue during which time he received his M.S. and Ph.D. degrees from this institution.

In 1945 Dr. Hilton returned to North Carolina as head of the Animal Husbandry Department at State College and in 1948 he was made Dean of the School of Agriculture.

He is a member of a number of honorary and scientific societies, is a past member of the board of directors of the American Dairy Science Association, and in 1948 was chosen "Man of the Year" by the magazine, "The Progressive Farmer."

ADA Honors Dr. Ohlson

For her research in developing safe weight-reduction diets, DR. MARGARET A. OHLSON of the Department of Foods and Nutrition, Michigan State College, was given a distinguished service award by the American Dairy Association at a recent meeting in Chicago. The diet proposed by Dr. Ohlson includes dairy products in every meal. The age group studied by Dr. Ohlson and her associates included those in the early twenties and those over 70 years of age. The total number of calories consumed per person per day was approximately 1400.

The study showed that weight can be lost while the person is eating satisfying meals which include foods, such as dairy products, that people enjoy eating. Dr. Ohlson believes that milk is an important factor of safety in low calorie diets.

Cow Owned by Washington State College Makes Record

A Holstein cow, Chinook Imperial Catherine, 2609753 (VG), bred and owned by the State College of Washington, has ended a year with a production of 28,218 lb. of milk, 4.1%, and 1,160.8 lb. of fat.

This record places her 13th in the national fat list in Advanced Registry classification 3X, full aged group, yearly division. She has the highest herd test record in the state of Washington and is first on the list of college-owned cows in the national A.R. fat list. As a senior 3-yr.-old, she produced 741 lb. of fat, and last year in 305 days she produced 24,750.8 lb. of milk and 980.5 lb. of fat, which placed her as high A.R. cow in the 10-mo. division for the state of Washington. In five lactations she has produced 109,423 lb. of milk and 4,166 lb. of fat. She freshened first at 2 yr. 6 mo. of age and has had a calf every year.

Golding Will Attend Dairy Congress

DR. N. S. GOLDING, Department of Dairy Science, State College of Washington, will attend the 13th International Dairy Congress at

The Hague, June 24-26. He and Mrs. Golding will sail from New York on June 3 on the Queen Elizabeth.

Following the sessions of the Dairy Congress, Dr. Golding will tour Holland and study dairy technological practices in that country. After a brief visit in his native homeland, England, Dr. and Mrs. Golding will return to Pullman early in August.

ADA Awards Cowbells

At a recent meeting of the American Dairy Association several "Oscars" in the form of copper cowbells were awarded to certain commercial concerns, magazines, and newspapers for contributing to the greater utilization of milk and its products. Pillsbury Mills and General Foods were recognized for their promotion of "parfait pie," which involves the use of ice cream and which was considered to be the outstanding food recipe of 1952. General Mills won an award for their Bisquick promotion of shortcake with cream and butter. National Biscuit Company won an award for consistent national advertising of dairy foods with Nabisco products. Recognition was given to three magazines, Better Homes and Gardens, Good Housekeeping, and McCall's for featuring recipes using dairy products. Supermarket merchandising was given an award for promoting dairy products and related foods in special sales promotion in retail stores.

For promoting the use of dairy products in their special sections devoted to foods, "Oscars" were given the Chicago Daily News, the Chicago Tribune, the Milwaukee Journal, and the Salt Lake City Desert News.

Dr. Sommer Dies Suddenly

Dr. Hugo H. Sommer, 56, Professor of Dairy Industry at the University of Wisconsin, died at the University Hospital May 8, a few minutes after being stricken in his office. Dr. Sommer, an internationally known dairy chemist, was the author of a textbook on market milk and one on ice cream and had published extensively in the field of dairy technology. He was graduated from the University of Wisconsin in 1918 and obtained his advanced degrees at that institution. He is survived by his wife and two sons.

Atwood Leaves Rhode Island

John W. Atwood, Assistant Extension Professor of Animal and Dairy Husbandry, University of Rhode Island, has resigned his position effective July 1, 1953. He has accepted a position as Assistant Advertising Manager of the American Guernsey Cattle Club Breeders' Journal. Mr. Atwood expects to receive his M.S. degree prior to leaving the University.

Colvard is New Dean of Agriculture at N.C.

Dr. D. W. Colvard, head of the Animal Industry Department, North Carolina State College, will assume the duties of Dean of Agriculture on July 1, 1953, replacing Dr. J. H. Hilton, who has accepted the Presidency of Iowa State College.

Kurtz Named Extension Dairyman at S.D.

Ervin U. Kurtz has been appointed Extension Dairyman to replace Kenneth Gross, who resigned March 31. Mr. Gross is now farming near Ortonville, Minnesota. Mr. Kurtz, a native of South Dakota, was graduated from South Dakota State College in 1939. Before coming to South Dakota, he managed a Kraft Foods Company plant at Clare, Michigan.

Nebraska Industry Conferences

A Fluid Milk, Cream, and Butter Conference was held at Lincoln, March 4-5, under the sponsorship of the Dairy Husbandry Department, Nebraska Butter Institute, ADA of Nebraska, Nebraska Technology Society, and the State Department of Agriculture. The major subjects discussed included good housekeeping and sanitation, bulk handling of milk, employee-management relations, and federal food and drug regulations related to the butter industry.

An Ice Cream, Milk, and Milk Products Clinic was held at Scottsbluff April 24 for the benefit of western Nebraska processors. Included was a discussion of basic principles and problems as related to ice cream, market milk, cultured milk, chocolate milk and cottage cheese. Staff members conducting the clinic were L. K. Crowe and Fred Schultz.

Iowa to Hold Conference on Reproduction

The Department of Animal Husbandry, Iowa State College, is sponsoring a "Research Conference on Female Reproduction in Farm Animals" on July 7, 8, and 9, 1953. The program for this Conference was drawn up by Dr. Dennis T. Mayer, University of Missouri, Dr. Joseph Meites, Michigan State College, and Dr. R. M. Melampy, Iowa State College. The Conference is open to interested persons; further information may be obtained from the College.

During the 3-day conference the following topics are to be discussed by various authorities from agricultural colleges and universities: Growth of the follicle; Nervous factors in control of ovulation; Environmental influences on ovulation; Influence of nutrition on ovulation; Gonadotrophic activity of pituitaries and induction of ovulation; Estrogens and progesterone; Thyroid and thyroprotein; Egg transfer and superovulation in farm animals; Motility patterns in the female reproductive tract; Fetal mortality in farm animals; Factors involved in sterility of farm animals; and Problems in the field of physiology of reproduction of farm animals.

United States Delegation to the XIIIth International Dairy Congress

DR. RALPH E. HODGSON, Delegation Chairman
Assistant Chief, Bureau of Dairy Industry
U. S. Department of Agriculture
Washington 25, D. C.

COL. BENJAMIN F. CASTLE
Executive Director, Milk Industry Foundation
1625 Eye Street, N. W.
Washington 6, D. C.

DR. CHARLES W. ENGLAND
Director of Research, C. Y. Stephens Dairy Industries
Washington 3, D. C.

MR. BURDETTE S. GRAHAM
Executive Secretary, Central Oklahoma Milk Producers Assn.
Oklahoma City, Okla.

MR. T. KLINE HAMILTON
Past president, Milk Industry Foundation
2653 Fair Ave.
Columbus, Ohio

MR. MILTON HULT
President, National Dairy Council
111 North Canal St.
Chicago 6, Ill.

DR. OTTO F. HUNZIKER
103 Seventh Ave.
La Grange, Ill.

DR. EUGENE L. JACK
Head, Division of Dairy Industry, Univ. of California
Davis, Calif.

MR. LESTER S. OLSEN
President, Olsen Publishing Co.
1445 North Fifth St.
Milwaukee 12, Wis.

PROF. ARTHUR C. RAGSDALE
Chairman, Department of Dairy Husbandry
Univ. of Missouri
Columbia, Mo.

DR. FRANK E. RICE
Exec. Secretary, Evaporated Milk Association
228 N. LaSalle St.
Chicago 1, Ill.

DR. HARRY C. TRELOGAN
Assistant Administrator for Marketing
Agricultural Research Administration
U. S. Department of Agriculture
Washington 25, D. C.

DR. G. MALCOLM TROUT
Professor of Dairy Manufactures
Michigan State College
East Lansing, Mich.

DR. HERMAN D. WEIHE
Bureau of Dairy Industry
U. S. Department of Agriculture
Washington 25, D. C.

DISA Increases Cash Value of Dairy Fellowships for Winners of Judging Contest

An increase of nearly 30% in Dairy Industrial Fellowship funds to be disbursed annually by Dairy Industries Supply Association was approved by DISA's board of directors at a meeting in March. Three fellowships—each of which gives a year's graduate study in dairy industrial problems—are offered as top awards to winning undergraduate teams in the annual Collegiate Students' International Contest in Judging Dairy Products, which has been sponsored since 1930 by A.D.S.A. and DISA.

Top award for winning contestants is now a $1,380 fellowship for graduate study at a recognized dairy school; the award previously was $1,000. The second award is a $1,280 fellowship; formerly it was $950. The third award is increased to $1,180 from $900.

In increasing the cash value of the fellowships, DISA recognizes the continued need for more trained dairy personnel, as well as the higher cost of living. "Attracting young men and women of the highest calibre to the dairy industries is equally as important as fostering research into dairy problems," a spokesman of DISA said, "and we believe the increased fellowship grants will further both aims."

The next contest, which will be held in Boston during the week of the October conventions of the Milk Industry Foundation and International Association of Ice Cream Manufacturers, is open to dairy products judging teams from all land grant colleges in the United States and Canada and from similar institutions of higher learning abroad. Any college desirous of entering a team in the contest may obtain full details on entrance procedure by writing DISA, 1108 - 16th Street, N. W., Washington 6, D. C.

Hanson Addresses Ice Cream Group

The Northwest Association of Retail Ice Cream Manufacturers held its annual meeting at Gearhart, Oregon, March 17-19. Out-of-state speakers included WILLIAM CLEGG of London,

Ontario, national president of the group, and DR. HENRY HANSON, University of Idaho.

Animal Pathology Department Formed at Maine

Effective July 1, J. F. WITTER will head the newly created Department of Animal Pathology. Dr. Witter, a native of Maryland, has been a member of the University of Maine staff since 1932 and has served as teacher, extension worker, and research member.

The 46th Annual Farm and Home Week was held this year from March 30 to April 2. This event brings to the residents of Maine outstanding leaders in the many phases of agriculture and homemaking. Speaking on the Dairy Management Program were S. E. SMITH, Professor of Animal Husbandry, Cornell University; W. A. DODGE, Extension Dairyman, University of Vermont; and L. V. TIRRELL, Head, Department of Animal Husbandry, University of New Hampshire.

Cheese Industry Moves Toward Mechanization

GOTTFRIED HANNI of Mayville, Wisconsin, has installed in his factory a Steinecker cheesemaking machine, with which he is making Baby Goudas and Port du Salut cheese. This machine, which is believed to be the first of its kind used in this country, has a capacity of approximately 5,000 liters per batch. The machine is shaped somewhat like a vertical pasteurizer. It has a hinged suspension cover, cutting apparatus, agitators, and a unique vacuum-seal and outlet valve, through which curd and whey are drained into the hoops. The machine and its appliances can be cleaned easily. It can be operated by one man and can be used repeatedly during the day. This machine indicates the trend toward greater mechanization in the cheese industry.

Ohio News Notes

The Ohio Dairy Boosters Association has given the University a grant of $500 to increase its scholarship fund for deserving dairy technology students.

The high school scholarship program designed to attract more students to dairy technology is being carried out again this year. The Cleveland Dairy Technology Society has contributed two scholarships of $150 each; the Stark County Milk Distributors Association, one $150 scholarship; the Milk Dealers Association of the Akron area, $150; and the Columbus Milk Distributors Association $300.00 for this program. The recipients of these awards are selected on the basis of their high school record, a written examination, and a personal interview by representatives of the industry and the Dairy Technology Department.

The Ohio Dairy Products Association branches of Ice Cream, Butter, Milk, and Manufactured Products are contributing approximately $4,000 to the support of the dairy technology extension program for 1953.

A series of five sectional ice cream clinics have been scheduled throughout the state. They will be late afternoon and evening meetings, to which the plants will bring ice cream samples for examination and evaluation. A discussion will be held on current problems of the ice cream industry. This is a program designed to take the University to plant employees.

A permanent education committee with representatives from all areas of the State has been appointed by the Ohio Dairy Products Association to work with the Department of Dairy Technology to (a) review the curriculum periodically and advise in matters pertaining thereto and (b) aid in making more effective a high school recruitment program.

P. R. ELLSWORTH has been placed temporarily in charge of the Agriculture Extension television and radio, which has programs appearing regularly on TV Station WLWC and Radio Station WOSU. One highly effective TV series was handled by the staff of the Dairy Technology Department involving four half-hour shows dealing with the "Magic of Milk."

Three senior dairy technology students were honored by being awarded scholarships for their achievements while in college. They were B. W. TAYLOR, G. R. BAKER and E. J. HAYNES, JR. The selection was made on the basis of scholarship, leadership, and extracurricular activities.

H. J. BASSET has completed his phase of the work on the Quartermaster Corps project concerning the effect of fat on the dispersibility of whole milk powders and has accepted a position on the research staff of E. F. Drew and Company, Inc., New Jersey.

Two staff appointment changes have taken place in the Department as follows: J. T. SMITH from Assistant Professor in Dairy Technology Extension to Assistant Professor in Dairy Technology and R. B. DOUGLAS from Instructor in Dairy Technology to Instructor in Dairy Technology Extension.

Report From Arizona

There is a small surplus of locally produced fluid milk at this time. There is some evidence that Arizona may be following the national trend with a very slight increase in number of cows. The price paid to the producer was lowered 13 cents per pound fat on April 1, 1953. Some decreases in the retail prices of milk have followed. The Arizona legislature, which finished its regular session the last week in March, failed to take action on a bill introduced to create a state price control commission.

The Lucerne Milk Company's new plant in Phoenix finished over a year ago is not in operation, pending settlement of labor union arbitration on classification of delivery personnel.

The Arizona Dairy Technology Society announced on April 6 that 3 yearly scholarships of $150 each are to be awarded to outstanding juniors or seniors in the field of dairy technology.

U. S. Milk Ordinance and Code

The 1953 recommendations of the U. S. Public Health Service for a Milk Ordinance and code (Public Health Service Publication No. 229) has been released by the Federal Security Agency. According to JOHN D. FAULKNER, Chief, Milk and Food Branch, Division of Sanitation, as of September 1, 1952, the Milk Ordinance recommended by the Public Health Service has been adopted by 1,542 municipalities and 397 counties. It also serves as the basis for the state law or regulations of 34 states and two territories. In 11 of the states and in both of the territories, it is in effect statewide. Mr. Faulkner further states that milk-sanitation regulations to be effective must be applied and observed on a day-to-day basis by the dairy industry itself and that the success of sanitation programs depends upon the achievement of a high degree of cooperation between regulatory officials and the dairy industry.

Personnel Changes at Cherry-Burrell

JOHN G. CHERRY, President of the Cherry-Burrell Corp., has announced the retirement of CARL A. WOOD, Vice-President, Operations. He is to be succeeded by D. H. BURRELL, III. Burrell is the grandson of David H. Burrell, who organized one of the predecessor companies of Cherry-Burrell in 1869 at Little Falls, N. Y. Mr. Burrell served 4 years in the Navy during World War II.

Mr. Wood is serving his second term as President of DISA and is a member of the board of the National Dairy Council. He is also chairman of the 1953 June Dairy Month Committee. He plans to make his home in New Hampshire, his native state.

Foods for America

A guest editorial

Food technologists are asking this question: "Are Americans losing their taste for good foods?" Good 92-score butter is being replaced by a bland, almost tasteless oleo; rich, mellow ice cream is losing the battle to a bland vegetable-fat frozen dessert; fine high quality chocolate products are not able to compete with mild cocoa-flavored products; pure vanilla is losing the fight to synthetic vanillin and coumarin. If American people are actually losing their taste for good foods, then the dairy industry is wide open to substitution, and the future of the dairy cow may be in jeopardy.

There is good reason to believe that these trends are purely temporary and are based on simple, economic facts. It is believed that people are just as taste conscious as they ever were, and that there is no cause for worry on this point. Those of us in the food industry always have waged a constant battle for our share of the housewife's food dollar. During periods of economic inflation our values become upset and out of balance. The consumer has only a certain amount to spend for food. Income taxes, television, automobiles, new homes, etc., are exerting their influence on how our dollars are spent. When quality dairy products, pure chocolate, and pure vanilla get out of line on costs, consumer merely changes to a substitute which is "almost as good," well advertised, and cheaper. It is as simple as that.

R. J. Ramsey

Let us look for a moment at pure vanilla. At the present rate of food consumption, there is not enough pure vanilla in the world to begin to fill our needs. Synthetics, such as vanillin, are an absolute necessity. Even in our own dairy industry we would now be short of butterfat, were it not for oleo. There is not enough milk being produced to feed our increased population at the increased per capita consumption brought about by better economic conditions. When prices on dairy products reach a high level, we can expect food manufacturers to bring out substitutes—and the public will buy them if they are reasonably good, well advertised, and cleverly merchandised.

Unfortunately, dairymen are prone to rest on their laurels and to underestimate the competitive power of advertising and merchandising. People should not be expected to buy milk just because it is "man's most nearly perfect food." To remain competitive, dairymen must spend more money for advertising and merchandising and quit burying their heads in the sand at the first signs of danger. It is time they learned to stand their ground and to sell, advertise, and merchandise in a way that will secure their rightful portion of the consumer's food dollar. The modern trend is to sell dairy products in self-service food stores, where "impulse buying" is very important. Dairymen must become good merchandisers to remain in business. Substitute food manufacturers at present are winning the battle because of more advanced selling weapons and current economic advantages, rather than because of any current decline in the taste for good foods.

R. J. RAMSEY, *President*
Ramsey Laboratories, Inc.
Cleveland, Ohio

VOLUME XXXVI MAY, 1953 NUMBER 5

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

323. Dairy Engineering. 2nd ed. A. W. FARRALL. John Wiley and Sons, Inc., New York. 477 pp. $6.00. 1953.

The second edition of this text is a considerable improvement over the first edition from both the practical and the teaching aspects.

Mr. Farrall has included sections on 3-A standards for pumps, pipes, motors, homogenizers, and other processing equipment, which are of practical importance. The section on refrigeration has been expanded and now includes operational procedures for most types of refrigeration equipment. Also, the information on equipment in this section has been brought up to date to include booster systems and flash coolers. The section on water supply and treatment could be broadened to make it more inclusive for different types of water treatment for various uses.

From the teaching aspect the text has been considerably improved, especially in the sections dealing with plant layout and design, refrigeration, drying, pasteurization, and steam generation. One of the best changes made was to include in the text the various tables formerly in the appendix. The chapter on fluid flow is excellent from the plant aspect, but for teaching purposes it could be expanded to cover different types of flow, means of measuring velocities, pressure relationships to loss in heat, etc., since much of the equipment used today employs high velocities and temperatures.

It would be valuable if some of the principles of heat transfer were more closely correlated with such unit operations as refrigeration and pasteurization. C. G. Fortney

324. Commercial and Industrial Refrigeration. C. WESLEY NELSON. McGraw-Hill Book Company, Inc., New York. 1952.

A practical approach to refrigeration applications of commercial and industrial sized equipment. The subject matter of the book is based upon material the author has employed over a period of years in teaching refrigeration at Wentworth Institute, Boston, Mass. The body of the book comprises 18 chapters, bibliography, and appendix. It is well illustrated with photographs of equipment and diagrams. Each chapter has a number of review questions. This book could be of considerable value for an in-plant training program for ice cream manufacturers. L. M. Dorsey

BUTTER

H. A. HOLLENDER, SECTION EDITOR

325. Continuous buttermaking machine o.k.'d in Midwest trial. Food Eng. Staff. Food Eng., 25, 2: 47, 208, 209. Feb., 1953.

A small production model of the Westfalia (Fritz) continuous buttermaking machine is in operation at the Rising Sun (Indiana) Creamery. Advantages claimed for the machine are: excellent texture in the butter, small floor space requirements, and the possible use in manufacturing low-fat products. Plant separated sweet cream of about 50% butterfat is utilized. Churning is accomplished by a beating action in a brine cooled cylinder. Working is done in other cylinders with screws and perforated plates. Adjustments control the composition of the butter. The addition of salt posed a problem, which has been solved by adding a new device. Butter from the machine may be packaged immediately without conditioning. A package that has met much success is a 12-oz., aluminum foil-wrapped patty, which sells at a premium. T. J. Claydon

326. Butteroil process. I. J. LUNDAL and R. P. ROBICHAUX (assignors to Cherry-Burrell Corp.). U. S. Patent 2,626,217. 13 claims. Jan. 20, 1953. Official Gaz. U. S. Pat. Office, 666, 3: 821. 1953.

Dry butteroil is produced from butter by the following process: (1) Melt butter and mix with water at 110° F., (2) while agitating, neutralize the acidity to the neutral point, (3) heat to a temperature of 120-150° F., (4) filter, (5) immediately separate to obtain butteroil having 0-6% moisture, (6) heat to 170-180°, (7) pasteurize in the range of 195-205°, and (8) chill the oil to 60-70° F. R. Whitaker

327. Butter and milk fat concentrate system. I. J. LUNDAL and R. P. ROBICHAUX (assignors to Cherry-Burrell Corp.). U. S. Patent 2,630,-

059. 9 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 112. 1953.

A process for continuously converting cream to butter. The following steps are given: Sour cream is neutralized, agitated, and heated to a predetermined controlled temperature, passed through a centrifuge to concentrate the fat, pasteurized in a vacuum pasteurizer, standardized to a desired fat content, converted into butter under positive pressure at a prescribed temperature with a metered amount of gas, the butterfat crystallized under quiescent conditions, and the butter worked and then packaged directly into prints. R. Whitaker

328. Dairy system. H. C. HORNEMAN, R. V. HUSSONG, S. N. QUAM, and B. W. HAMMER (assignors to Cherry-Burrell Corp.). U. S. Patent 2,630,060. 5 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 113. 1953.

A continuous process for converting cream into butter, essentially the same as Abs. 327. R. Whitaker

CHEESE

S. L. TUCKEY, SECTION EDITOR

329. Ripening phenomena in cheeses of the Edam type with different water contents and different salt concentrations. C. W. RAADSVELD. Netherlands Milk & Dairy J., **6**, 4: 342-355. Oct.-Dec., 1952.

Proteolysis, lipolysis, and flavor development were studied during ripening of cheeses with different moisture content and different salt: water ratios. In cheeses with equal salt:water ratio greater protein breakdown was found with higher moisture content and higher pH values. The increases were more pronounced in the values for amino acid and ammonium N than for soluble N. Salt had a retarding effect on protein breakdown. No indication was found that a high moisture content or salt concentration increased fat hydrolysis during ripening. W. C. van der Zant

330. Het toepassen van antibiotica afscheidende melkzuurstreptococcen als zuursels by het tegengaan van boterzuurgisting in kaas (The inhibition of butyric acid fermentation in cheese by using antibiotic producing streptococci as starter). J. S. KOOY and J. W. PETTE. Netherlands Milk & Dairy J., **6**, 4: 317-322. Oct.-Dec., 1952.

Butyric acid fermentation was inhibited in cheeses prepared from milk inoculated with *Cl. tyrobutyricum* and an antibiotic producing strain of *S. lactis,* whereas gas defects were observed after 2 wk. when an inactive strain of *S. lactis* was used. In one experiment the butyric acid bacteria grew even though an antibiotic producing strain of *S. lactis* was used. *L. plantarum* was found to be responsible for the rapid destruction of the antibiotic in the cheese. W. C. van der Zant

331. Cheese stirrer. C. F. KRUCKER. U. S. Patent 2,630,303. 3 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 182. 1953.

A stirrer for round bottomed circular cheese kettles consisting of a rotating paddle, shaped to the contour of the kettle, and suspended from, as well as driven by, an overhead motor-powered gear box. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

332. Some aspects of the bacteriology of pasteurized milk. III. Influence of storage of raw milk at different temperatures on the thermoduric count. TH. E. GALESLOOT. Netherlands Milk and Dairy J., **6**, 4: 283-301. Oct.-Dec., 1952.

The effect of time of delivery of milk at the dairy and temperature and time of holding before pasteurization on the thermoduric count was studied. Thermoduric counts were determined of milk samples received at the dairy (a) after immediate pasteurization; (b) after delaying pasteurization for 3 hr., during which period the samples were held at 20, 27° C., and at atmospheric shade temperature; and (c) after delaying pasteurization for 1 to 2 hr., during which period the samples were held at 37 and 45° C. Laboratory pasteurization was carried out by holding the milk at 63° C. for 35 min. Plate counts were determined on T.G.E.M. agar with incubation at 27° C. for 4 d. Thermoduric counts after delayed pasteurization were higher than after immediate pasteurization. The tendency to give higher thermoduric counts increased with storage at higher temperatures. The organisms responsible for the increased thermoduric counts belonged in most cases to the *S. thermophilus* and *S. bovis* species. The increased thermoduric count after delayed pasteurization may be explained, according to the author, by the observation that during holding the responsible organisms enter another growth phase with a higher thermal resistance. Increased thermoduric counts were found only with pasteurization at low temperatures (phosphatase just negative). W. C. van der Zant

333. Remming van de groei van lactaatvergistende boterzuurbacterien door antibiotica van melkzuurstreptococcen (Inhibition of the growth of lactate-fermenting butyric acid bacteria by antibiotics from lactic acid streptococci). J. S. KOOY and J. W. PETTE. Netherlands Milk and Dairy J., **6**, 4: 302-316. Oct.-Dec., 1952.

Three hundred fifty strains of lactic acid streptococci were isolated from raw milk, cheese, and feces; 11 produced antibiotic substances against other lactic acid streptococci mainly *S. cremoris.* Antibiotic production was tested by the effect of culture filtrate on the growth of a test strain inoculated in litmus milk containing 20% culture filtrate. Culture filtrates were pre-

pared by filtration of heated coagulated cultures through paper. All antibiotic producing strains were classified as *S. lactis*. Lactate-fermenting butyric acid bacteria and starter organisms were inhibited by antibiotic containing filtrate; no inhibition of lactobacilli was found.
W. C. van der Zant

334. Diacetyl formation in starters. II. N. EVENHUIS. Netherlands Milk and Dairy J., **6**, 4: 331-341. Oct.-Dec., 1952.

According to the author, the formation of diacetyl from pyruvic acid appeared to be an oxidation-reduction (o-r) process. A high o-r potential favored the formation of higher oxidation products as diacetyl. Following addition to a starter of compounds with a high o-r potential as methylene blue or quinone much diacetyl was found anaerobically after 0.5 hr. With the exception perhaps of KIO_3, oxidizing agents as KNO_3 and $KClO_3$ did not increase the o-r potential sufficiently to allow much diacetyl to be formed. W. C. van der Zant

335. Stammen van Lactobacillus plantarum die antibiotica van Streptococcus lactis onwerkzaam maken (Strains of Lactobacillus plantarum which inhibit the activity of the antibiotics produced by Streptococcus lactis). J. S. KOOY. Netherlands Milk and Dairy J., **6**, 4: 323-330. Oct.-Dec., 1952.

Certain strains of *L. plantarum* isolated from raw milk and cheese destroyed the antibiotics produced by strains of *S. lactis*, whereas others with similar biochemical properties did not show any effect. No suppression of the destructive action of strains of *L. plantarum* could be obtained in milk by inoculation with one hundred times as much of strains which did not destroy the antibiotics. Inoculation with strains of *L. casei* prevented the destruction of the antibiotics by *L. plantarum* in mixed cultures. W. C. van der Zant

336. The influence of DDT wettable powder on the methylene blue reduction test in milk. S. J. MILLIAN and H. H. WEISER, Ohio State University, Columbus. J. Milk and Food Technol., **16**: 4-5, 8. Jan.-Feb., 1953.

The presence of appreciable quantities of DDT wettable powder in raw milk will interfere with the accuracy of the methylene blue reduction test. The inert constituents in DDT, not the active agent of the insecticide, are largely responsible for the precipitation of the dye. The authors suggest an inspection of the samples for the presence of precipitates when decolorization of milk has taken place rapidly. H. H. Weiser

337. Membrane filter method for determination of coliforms in pasteurized and certified milk. RICHARD EHRLICH. Am. Butter Institute, Chicago. J. Milk and Food Technol., 16: 6-8. Jan.-Feb., 1953.

The membrane-filter method permits more rapid counting of coliform organisms in certified and pasteurized milks than the standard plating technique. Large quantities of milk can be examined if the samples are heated to 40° C. and centrifuged in special centrifuge bottles for 10 min. at 2000-2500 r.p.m. The coliform colonies can be preserved on the membrane filter for future reference.
H. H. Weiser

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

338. Isolation of crystalline α-lactalbumin from milk. W. G. GORDON and W. F. SEMMETT. J. Am. Chem. Soc., **75**: 328-330. 1953.

Experimental procedure is given for the preparation of a protein fraction of whey which previously has been called "crystalline insoluble substance." The substance was characterized on its electrophoretic and ultra-centrifugal properties, solubility, optical rotation, elementary analysis, and tryptophan content. It was suggested, as the result of this study, that "crystalline insoluble substance" henceforth be called α-lactalbumin.

The protein comprises 12% of the total proteins of whey, has an approximate molecular weight of 16,000 and an electrophoretic mobility of —4.2 at pH 8.5. It contains approximately 7% tryptophan, 1.91% sulfur, only a trace of phosphorus, and no carbohydrates.
J. Tobias

DAIRY ENGINEERING

C. W. HALL, SECTION EDITOR

339. Commercial and industrial defrosting: General principles, C. F. HOLSKE; **Defrosting commercial equipment,** R. H. LUSCOMBE; **Water defrost of blower coils,** D. D. WILE; **Automatic hot gas defrosting,** S. C. SEGAL; **Warm air defrosting,** G. A. M. ANDERSON; **Defrosting cold storage equipment,** M. W. GARLAND; **Chemical prevention of frost formation,** E. A. WINDHAM. Refrig. Eng., **61**, 3: 261-274. 1953.

A symposium held at the 48th annual meetinug of the Am. Soc. of Refrig. Engineers to explain the principles and applications of the many and varied ways of defrosting commercial and industrial refrigeration equipment. Each of the principal methods of defrosting was presented by an expert in the particular field of application, and the techniques are presented in sufficient detail to be understood easily. L. M. Dorsey

340. Pressure operated milk metering device. W. H. HARSTICK and H. W. HEIN (assignor to International Harvester Co.). U. S. Patent 2,630,712. 12 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 379. 1953.

A device for measuring milk, consisting of a chamber in which a divided receptacle or cell

slides back and forth. In one position, one side is under a filling spout from an inlet pipe. As the milk fills that side of the receptacle, a float rises and activates a mechanism which slides the receptacle to the other position. In the second position, the first side of the cell, which is full of milk, is positioned over a drain from which the milk flows to an outlet pipe, and the second side of the cell is positioned under the filling spout from the overhead inlet pipe. It also has a float which in turn activates the sliding mechanism, thus completing the cycle by returning the cell to the first position.
R. Whitaker

341. Flow diversion valve. A. W. GRISWOLD (assignor to Taylor Instrument Companies). U. S. Patent 2,631,001. 3 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 464. 1953.

Details are given for the construction of a flow diversion valve. A piston operates in the tube formed by two hollow tees connected end to end, one above the other. The milk enters through the side of the upper tee and flows either upward or downward depending on the piston, which is caused to slide in the upper tee by a rod which passes through a stuffing box arrangement in the bottom outlet of the bottom tee. R. Whitaker

342. Ice cream carton lifting, scraping and cutting tool. S. SMITH. U. S. Patent 2,630,591. 1 claim. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 347. 1953.

This tool serves three purposes. It has a flat blade, slightly concave, for scraping ice cream from the side of bulk cans, a hook-like depression in one side of the blade for lifting cans in and out of the cabinet sleeve, and a sharpened portion of the blade for cutting off the empty portion of the paper can.
R. Whitaker

343. Diaphragm type milk releaser. F. G. HODSDON (assignor to International Harvester Co.). U. S. Patent 2,630,782. 5 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 400. 1953.

A device for discharging milk from a pulsating vacuum type of milking machine to atmospheric pressure. The release is accomplished by a combination of 2 diaphragms which operate 2 valves depending on the pulsations of the pressure on the milk line. R. Whitaker

344. Releaser assembly for continuous milking systems. R. E. REEVE. U. S. Patent 2,630,783. 2 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 401. 1953.

Details are given for a mechanism for releasing milk from a pulsating vacuum type of milking machine, consisting of a chamber into which the milk can collect at atmospheric pressure when released through a vertical flap valve operated by the pulsations of the pressure applied to the milk line. R. Whitaker

345. Stable gutter cleaner. D. H. MILLER (assignor to Cooperative Grange League Federation Exchange, Inc.). U. S. Patent 2,630,907. 3 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 437. 1953.

A portable stable gutter cleaner, which pulls a gutter belt up a chute and winds it on a reel. The device is nearly balanced on one wheel which acts as a fulcrum pivot. As the belt is wound on the reel, its added weight tends to tip the chute and dump the manure which has been dragged up on the belt. R. Whitaker

346. Receptacle filler. A. J. LIPPOLD (assignor to Cherry-Burrell Corp.). U. S. Patent 2,630,960. 2 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 452. 1953.

Details are given for the construction of a milk or other liquid bottle-filling valve. A vent is provided for allowing the air to escape. The valve is actuated by the bottle rising from below and pushing the valve upward to open and allow the liquid to flow into the bottle.
R. Whitaker

347. Centrifugal separator with adjustable supply can bracket. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,630,966. 4 Claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 454. 1953.

The supply tank for a small milk separator is mounted on a pedestal of tubular design, immediately above the bowl of the separator.
R. Whitaker

348. Method and apparatus for evaporating milk. R. O. HENSZEY. U. S. Patent 2,631,105. 13 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 493. 1953.

A method of evaporating, in which milk is rapidly preheated in a heat exchanger by the vapors from the first effect of the evaporator, which is operated at a high temperature. The temperature rise during the preheating step is completed before coagulation occurs.
R. Whitaker

349. Cream can emptying method. I. F. KING (assignor to Swift & Co.). U. S. Patent 2,631,112. 3 claims. March 10, 1953. Official Gaz. U. S. Pat. Office, **668**, 2: 494. 1953.

To facilitate dumping of thick sour cream, the cans are placed in a round tank in which there is a coil of pipe. The inside of the coil is provided with numerous holes for spraying very hot water on the can to soften the cream. Melting the outside layer makes the cream dump easily and cleanly. R. Whitaker

350. Butter. H. C. HORNEMAN, R. V. HUSSONG, S. N. QUAM, and B. W. HAMMER (assignors to Cherry-Burrell Corp.). U. S. Patent

2,630,388. 12 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 207. 1953.

The product produced by the processes described in Abs. 327 and 328. R. Whitaker

351. Brick slicing mechanism for ice cream sandwich machines. J. H. KEYSER and V. C. CURRY. U. S. Patent 2,629,342. 4 claims. Feb. 24, 1953. Official Gaz. U. S. Pat. Office, **667**, 4: 911. 1953.

Bricks of ice cream are conveyed on an endless belt under an oscillating blade, which slices the ice cream into portions suitable for sandwiches. The bricks are fed to the machine through a chute and held in position by lugs on the belt. R. Whitaker

352. Portable frozen confection dispenser. K. B. MAXWELL. U. S. Patent 2,629,344. 4 claims. Feb. 24, 1953. Official Gaz. U. S. Pat. Office, **667**, 4: 911. 1953.

A motor driven scoop to be held in the hand for forming individual spherical or semispherical shaped portions of ice cream.

R. Whitaker

353. Apparatus and method of ice cream bar manufacture. F. M. JOHANSEN. U. S. Patent 2,629,346. 10 claims. Feb. 24, 1953. Official Gaz. U. S. Pat. Office, **667**, 4: 912. 1953.

Stiff ice cream from the freezer is extruded upward through multiple openings in a head. A rack holding sticks is placed above, so that the ice cream is extruded around the sticks. A blade traveling along the extruder surface cuts off the individual portions. R. Whitaker

354. Bag dispensing apparatus for bagging machines. C. K. NELSON (assignor to Eskimo Pie Corp.). U. S. Patent 2,629,369. 2 claims. Feb. 24, 1953. Official Gaz. U. S. Pat. Office, **667**, 4: 918. 1953.

Bags, of the type used for frozen novelties, are placed in racks or chutes in an upright position. Compressed air, directed through a tube, opens the end bag in each rack. The bags are retained in place until filled, when they drop down by gravity, and another bag is extended. R. Whitaker

355. Apparatus for dispensing ice cream. W. S. FREDENHAGEN and M. S. SCHMIDT. U. S. Patent 2,630,083. 3 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 120. 1953.

A device for cutting rectangular shaped individual servings from bulk ice cream. The rectangular scoop is pressed down into the ice cream and the portion is ejected by pressing a shaft attached to a piston which slides in the scoop. A handle makes this a one-hand operation. R. Whitaker

356. Food handling implement. E. S. PRINCE. U. S. Patent 2,630,082. 11 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 119. 1953.

A device for cutting individual servings from bulk ice cream. Similar in mode of operation to device described in Abs. 355. The piston is operated by a pivoted bar attached to a lever which is squeezed by the fingers of the hand gripping the handle of the scoop.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

357. Weigh can selection for the dairy plant. CARL W. HALL, Mich. State College, East Lansing. Quart. Bull. 35, 3: 310-316. Feb., 1953.

Surveys in southern Michigan indicate that receiving room weigh cans are often improperly sized. The volume of producers' deliveries determines the most efficient size. Two interesting tables are shown. One of these is used for determining the years required in terms of labor saved to offset the additional cost of a 750-lb. weigh can in comparison with a 500-lb. weigh can in plants in which a certain percentage of their milk arrives in lots of over 500 lb. The second table lists standard time intervals for receiving room operations. Data are shown also to indicate that print-weigh devices pay for themselves in approximately 4.8 yr. when labor is $1.75 per hr. and at least 40,000 lb. of milk are received daily. L. C. Thomsen

358. They get top efficiency with one-floor straight-line operation. A. V. GEMMILL. Food Eng., **25**, 2: 51-55, 186, 187. Feb., 1953.

The new Riech-McJunkin Dairy Co. ice cream plant at Pittsburgh is designed for straight-line flow in a 1-floor operation. Processing systems are designed to handle frozen cream or butter, skimmilk powder, or pumpable ingredients, as desired. General details of processing and the equipment involved are given along with a floor plan of the layout indicating the flow of products. Several new types of equipment include the Bryant Centrifugal Liquefier for liquefying frozen products and an electric eye mold filler for popsicles. Utilization of push button mixing, automatic weighing, a new cold air distribution system, and improved construction has resulted in numerous benefits. T. J. Claydon

359. Mechanical milk men for apartment houses. Anonymous. Milk Dealer, **42**: 48, 62-63. Feb., 1953.

The city milk company of Maspeth, N. Y., is dispensing qt. containers of milk in 70 apartment buildings by the use of vending machines and is steadily expanding this distribution method. The advantages of this system are: (1) There is always milk immediately at hand. The machines are restocked every day, some twice a day. Space is saved in small apartment refrigerators. Labor of carrying milk from the store, and handling bottles is eliminated.

(2) The milk in standard qt. containers is delivered in insulated trucks to the refrigerated machines and kept at a thermostatically controlled temperature of about 38° F. (3) Because of the economy of the operation, the milk is sold at super market price, 3 or 4 cents less than home delivered milk, currently 22 cents against 26 cents per qt. The tenants' reaction has been enthusiastic. The daily milk sales through the mechanical vendors average 1 qt. per family. The machines are so constructed that they can sell butter, cream, cheese, and almost any packaged food that can be kept under refrigeration. C. J. Babcock

360. Physical layout of soft ice cream operation. C. B. WELLS, General Equip. Sales, Inc., Indianapolis. Southern Dairy Products J., **53**, 2: 36, 38, 39. Feb., 1953.

Important considerations in locating a soft ice cream operation are accessibility, parking facilities, attractiveness and visibility, sanitary surroundings, adequate supply of good water, sewage disposal, and amount of traffic by the site. The building should be planned in advance to meet sanitary regulations, should make abundant use of glass, have 2 rest rooms with outside entrances, furnish adequate space for storage and freezing and clean-up operations, and be designed for the equipment to be used. The freezing room is usually 18-24 ft. wide by 10-12 ft. deep; the whole building the same width by 20-36 ft. deep. Side lines are not favored, but if included should be located separately. Efficient equipment should be selected, laid out on the plans, and installed as the building is being constructed.

F. W. Bennett

361. Getting employee response. RAY BAER, Bowman Dairy Co., Chicago. Ice Cream Rev., **36**, 8: 58, 60-62. March, 1953.

Obtaining the right kind of employee response which manifests itself in good work, loyalty, enthusiasm, increased productivity, and reduced costs is too frequently neglected in ice cream plants. Such response constitutes the chief competitive advantage which one plant can hope to enjoy over another. Production facilities, raw product cost, distribution expense, and wage rates are very much the same for each market area.

If employees are expected to take an interest in their work, in the company and its accomplishments, the company in turn must reciprocate and take an interest in the employees, not only as a group but as individuals. A simple way of doing this is to talk to the individual employee with sincerity. In this manner the usual cold employer-employee relationship will be changed into a friendship which will express itself in higher production per man hour and lower production costs. A period of each day should be devoted by management to getting to know each employee. W. J. Caulfield

362. What's ahead for butterfat? RUDOLPH K. FROKER, Dean, College of Agriculture, Univer. of Wis. Milk Dealer, **42**, (5); 47, 84-87. Feb., 1953.

A general weakening in the demand for milk fat, especially in the form of butter, is one of the most important changes affecting the dairy industry during the last half century. In the 1930's about 45% of the milk fat used for human consumption in the U. S. was made into butter. In 1952, only about 25% was used for butter. The average American is eating only a little more than half as much butter as before World War II. During the period, 1921 to 1929 inclusive, retail butter prices and average hourly wages of all manufacturing industries were nearly identical for the nation as a whole. Wages and butter prices went up and down together. If that relationship held today, butter would be fully twice its present price. On this basis, butter prices are not high; they have not kept pace with the prices of many other foods, including fluid milk, cheese, and nonfat milk solids. The weakening in consumer demand for butter has been accelerated by the market situation during the war, the margarine legislation since the war, and the decline in the total demand for fat in the American diet. Because of these changing economic conditions, we need more emphasis on milk and less on milk fat in our breeding, production, and marketing programs. It is suggested that (1) our state and federal standards for composition of milk products be revised in light of the milk fat situation, (2) more effort be placed on promoting fluid milk, cheese, and ice cream, for which products substitution is less readily made and which stand high in the list of nutritional recommendations. C. J. Babcock

363. Mechanical refrigeration of milk trucks. D. W. GREENE, Biltmore Dairy Farms, Biltmore, N. C. Southern Dairy Products J., **53**, 2: 86, 87, 92-95. Feb., 1953.

Two types of refrigerated milk truck bodies are in use at Biltmore. For retail delivery, a 6-cyl. model with dual wheels and oversize tires, 4 in. of insulation on all sides of the body, 2 holdover plates, a ¾ h.p. compressor, sliding doors behind the driver, and double doors at the rear is used. It has a capacity of 80 cases. The compressor is on the floor behind the driver and is accessible from the ouside. The cost is about 185% of the conventional 4-cyl. truck. The wholesale truck has a 12-ft. van type body, 3 plates mounted against the walls, 1½ h.p. compressor swung below the body on the left side, a 2 or 2½ ton chassis, a double door in the rear, and a single door on the side. The cost is $1300-$2200 more than a conventional van on a 1½ ton chassis. Electrical outlets cost $15 each and power cost is about $5/mo. each. Truck operation cost is about $40/mo. above that of the conventional truck.

The advantages of these trucks include better

utilization of labor by loading in the afternoon and not handling ice, elimination of spoiled milk, elimination of cost of ice, provision of additional storage space, elimination of moisture in trucks, minimization of losses due to accidents, advertising value, and satisfaction of route men.

Some of the problems are increased weight, unbalanced load on truck, and tying the life of the retail body to the life of the truck. These may be solved best by the cooperation of refrigeration engineers and truck builders.

F. W. Bennett

364. Route efficiency as it affects sales costs. AL GILBERT, Philadelphia Dairy Products Co., Inc. Proc. 48th Ann. Convention I.A.I.C.M., 3: 5-7. 1952.

Important considerations regarding route efficiency as it affects sales costs of the ice cream are discussed. Planned delivery, balanced hours for the driver salesmen, and controlled delivery costs are the main topics considered.

J. Sheuring

365. Increasing route productivity through route supervisors. R. J. NUGENT, General Ice Cream Corp., Schenectady, N. Y. Proc. 48th Ann. Convention I.A.I.C.M., 3: 8-9. 1952.

The importance of route reports and the proper duties of the driver and supervisors are discussed. J. Sheuring

366. Increasing route productivity through labor relations. COURTNEY JOHNSON, Beatrice Foods Co., Chicago. Proc. 48th Ann. Convention I.A.I.C.M., 3: 9-12. 1952.

The importance of careful and intelligent selection and the proper education of the employee regarding management and industry problems and needs are discussed in relation to their effect on productivity. J. Sheuring

367. Increasing route productivity through the sales department. CLYDE JOHNSON, Beatrice Foods Co., Des Moines, Ia. Proc. 48th Ann. Convention I.A.I.C.M., 3: 12-15. 1952.

The importance of intelligent selection of personnel for the sales department and the driver-sales department and the establishment of definite duties and responsibilities for all members in each of the two departments are discussed in relation to their effect on productivity.

J. Sheuring

368. What are we selling? WELLINGTON PAUL, Foremost Dairies, Jacksonville, Fla. 48th Ann. Convention I.A.I.C.M., 3: 17-21. 1952.

The importance of adequate cost analysis and the proper rating as to profit margin of each item are discussed. Important information is given regarding the sale of items showing the highest profit margin. J. Sheuring

369. Why sell unprofitable items on routes? A. G. ANDERSON, General Ice Cream Corp., Schenectady, N. Y. Proc. 48th Ann. Convention I.A.I.C.M., 3: 21-24. 1952.

The advisability of selling or not selling unprofitable items is discussed. The author shows that in several instances the sale of an unprofitable item is justifiable. J. Sheuring

370. Cubic foot accounting for the distribution of delivery and selling expense. DONALD A. HALL, Brock Hall Dairy, Hamden, Ct. Proc. 48th Ann. Convention I.A.I.C.M., 3: 25-28. 1952.

This is an interesting discussion on three methods; namely, the space gallon, dollar cost, and the sales dollar, with regard to their application in the proper distribution of delivery and selling expense. Delivery expenses may well be assigned on the above basis. There is some question with respect to the allocation of selling expenses on this basis. J. Sheuring

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

371. Preparation of carotene concentrates from dehydrated alfalfa meal. H. L. MITCHELL, W. G. SHRENK, and R. E. SILKER, Kansas Agr. Expt. Sta., Manhattan. Ind. Eng. Chem., **45**: 415-417. Feb., 1953.

A method is described for preparing carotene concentrates from chlorophyll-containing plant tissues. Such concentrates are needed to supply vitamin A potency to feeds. A search was made for an adsorbent that would not adsorb carotene but would adsorb chlorophyll and xanthrophylls. Thicalcium phosphate met these requirements. Alfalfa was extracted with Skellysolve B and the extract treated with tricalcium phosphate to remove chlorophyll and xanthophyll. Carotene was not adsorbed but remained in the solution in high potency. The adsorbent was inexpensive, and large quantities of the carotene extract could be prepared by this simple procedure. B. H. Webb

372. Effect of a copper supplement to the rations of milking cows. G. DUNLOP. Nature, **171**, 19: 356. 1953.

A study was made of 19 herds in Scotland during the time the cows were stall-fed. In all but 3 of the herds, there was an increase in the amount of butterfat produced per cow when 10 g. of $CuSO_4 \cdot 5H_2O$ was included in the 3½ lb. of meal fed the cows, over the butterfat produced by a control group of cows in the same herds. The increase was shown to be significant statistically. R. Whitaker

373. Encapsulation in rumen bacterial fractions. P. N. HOBSON and M. J. MACPHERSON. Nature, **171**: 129. 1953.

Rumen liquor can be separated while cold

into bacterial-free liquid, a bacteria-rich layer, and a fraction containing plant residues by high speed centrifugation. The bacterial fraction shows encapsulated organisms. The largest capsules, up to 9 μ in diameter, were found when the animal was fed hay, and smaller ones when animals were on a starch ration. The capsular material appears to contain a polysaccharide which does not stain with iodine. The authors speculate on the availability of this capsular material as food for the host.

R. Whitaker

374. Effect of sulfur dioxide-silage on vitamin excretion by ruminants.. A. E. TERRI, D. JOSSELYN, N. F. COLOVOS, and H. A. KEENER, N. H. Agr. Expt. Sta., Durham. J. Animal Sci., **12**, 1: 15-18. 1953.

Rumen synthesis of nicotinic acid was apparently uninfluenced by the ration, the excretions tending to follow the intake of this vitamin. Silage apparently favored the rumen synthesis of riboflavin, this synthesis-favoring action being uninfluenced by SO_2 or molasses. Although SO_2 destroys much of the thiamine in silage, it apparently favors the synthesis of this vitamin, as indicated by thiamine excretion when the ration consisted of SO_2 silage. Molasses silage favors the synthesis of thiamine though to a lesser extent. The presence of limestone tended to nullify this effect. The vitamin content of various silages after storage for 6 mo. is reported.

O. T. Stallcup

375. Terramycin supplement for dairy calves. A. M. MACKAY, W. H. RIDDELL, and R. FITZSIMMONS, Vt. Agr. Expt. Sta., Burlington. J. Animal Sci., **12**, 1: 19-23. 1953.

A terramycin supplement was fed so as to supply 30 mg. terramycin hydrochloride per 100 lb. body weight daily to young dairy calves receiving liberal milk, calf starter, and good quality hay. The experimental group had a significant increase in growth, stimulated appetite, and improved appearance as compared to a control group. Feeding terramycin to calves 6 to 9 wk. of age had less effect. No conclusions were drawn as to the control of scours.

O. T. Stallcup.

376. Ruminant feed composition containing an amidine. K. E. WALKER (assignor to E. I. du Pont de Nemours & Co.). U. S. Patent 2,630,386. 10 claims. March 3, 1953. Official Gaz. U. S. Pat. Office, **668**, 1: 206. 1953.

A feed supplement for stimulating the growth of rumen microflora, consisting of creatine, creatinine and salts of guanidine. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

377. Effect of moisture-loss upon the body, flavor, and texture of packaged ice cream. J. A. MEISER and D. A. SEIFERT, Mich. Agr. Expt. Sta., East Lansing. Ice Cream Rev., **36**, 8: 46, 47, 116, 118, 120, 121. March, 1953.

Moisture loss, as well as changes in flavor and body, of ice cream stored at cabinet temperatures in different types of cartons was determined over a period of 8 wk. It was observed that ice cream packaged in paper containers loses weight during extended storage periods as a result of loss of moisture. This loss was due in part to movement of water vapor through apertures provided by the pores, seams, and unsealed closures of containers and to the absorption of moisture by the packaging material which was subsequently released to the atmosphere surrounding the container.

Flavor deterioration was observed in ice cream stored in untreated cartons for periods in excess of 3 wk. The deterioration was not as pronounced or rapid in ice cream stored in treated containers. The evidence indicates that flavor deterioration of ice cream is affected by moisture loss as well as by the duration of the storage period. Ice cream stored in untreated containers exhibited a slight gumminess after 3 wk. of storage, and after 8 wk. the ice cream exhibited a leathery film on the surface. Severely dehydrated samples were shrunken. Comparable samples in treated cartons could be stored for 6-8 wk. before gumminess was observed.

The data emphasize the importance of using properly treated cartons with tight fitting lids for prolonged storage of ice cream.

W. J. Caulfield

378. H.T.S.T. pasteurization of ice cream mix. J. A. WILDERMUTH, Cherry-Burrell Corp., Little Falls, N. Y. Ice Cream Rev., **36**, 8: 140, 142, 144, 145. March, 1953.

Advantages of pasteurization of ice cream mix at 175° F. for 25 sec. are discussed from the viewpoint of the public health officer and the processor. The milk sanitarian is finding H.T.S.T. pasteurization desirable because: (1) It provides for positive control of the time-temperature relationship. (2) The equipment can be more easily cleaned and sterilized by the circulation method of cleaning. (3) The pasteurization efficiency is equal to that of the batch method of pasteurization. The plant operator finds the method desirable because it: (1) establishes a definite production rate for the plant, (2) speeds up the operation of mix processing, (3) saves labor, (4) saves steam and water through regeneration, and (5) simplifies materials handling when combined with push button control.

Flow diagrams for several different types of installation using H.T.S.T. pasteurization are pictured in the article. W. J. Caulfield

379. Factory packaged carry-home sundaes. Anonymous. Ice Cream Rev., **36**, 8: 42, 43, 70. March, 1953.

A carry-home ice cream sundae package con-

sisting of eight 3½-oz. slices of vanilla ice cream, plus 8 transparent plastic envelopes of ½ oz. of chocolate sauce each, has been introduced by H. P. Hood and Sons of Boston. The single package container provides the consumer with complete ingredients for 8 chocolate sundaes at a cost of less than 10 cents each. The retail price of the sundae package is 75 cents at regular stores and 69 cents at chain stores.

Advertising and promotion stresses the time- and labor-saving features, as well as the economy, of the package. W. J. Caulfield

MILK SECRETION

V. R. SMITH, SECTION EDITOR

380. Sympathetico-adrenal inhibition of the neurohypophyseal milk ejection mechanism. B. A. CROSS, Physiological Laboratory and Dept. Veterinary Clinical Studies, Univ. of Cambridge. J. Endocrin., **9**: 7-18. 1953.

The injection of up to 50 micrograms of epinephrine into lactating female rabbits before nursing interfered with milk ejection. The injection of 150 milliunits of oxytocin either before or immediately after injecting epinephrine did not restore normal milk ejection. In anesthetized rabbits with cannulated teats, where 50 milliunits of oxytocin will normally stimulate milk ejection, the prior injection of 5 micrograms of epinephrine will prevent this response. A simultaneous injection of epinephrine and oxytocin reduces milk ejection in cannulated animals. An electrical stimulation of the supraoptico-hypophyseal tract will induce milk ejection. This can be inhibited by injecting oxytocin either before or after electrical stimulation. Electrical stimulation of the posterior hypothalamus produced inhibition of the milk ejection response to injected pitocin, together with pupillary dilatation and exopthalmos. Victor Hurst

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

381. Parabiosis in physiological studies. J. C. FINERTY. Physiol. Rev., **32**, 3: 277-302. 1952.

Parabiosis, the union of two living individuals, has been a profitable research tool to study hormone transmission and interrelationships. Materials are transmitted from one surgically joined partner to another after 3 to 4 days corresponding to the time required for revascularization of a wound. The ready effect of protein-like hypophyseal hormones as contrasted to the ineffective crossing over of steroid gonadel hormones was explained on the basis of different thresholds for effectiveness and rates of metabolism. Substances readily inactivated never reach a minimal effective level in the recipient, whereas materials more resistant are readily equilibrated. In cattle the freemartin (sterile female born twin to a bull) was attributed by Lillie to be due to common placental circulation due to fusion of the chorions within the uterus. Surgical parabiosis of male and female rats resulted in little change in gonads and secondary sex characteristics. Normal pregnancy occurred in the female. However, profound changes occurred in the ovaries and uterus of a female paired with a castrate (male or female) due to increased gonadotrophic hormone from the anterior pituitary of the castrate. Ovarian hormones stimulate mammary growth by acting directly on the mammary gland and indirectly by causing the release of an anterior pituitary factor (more in female than male) which acts synergistically with the ovarian hormone to promote mammary growth. The cases of parabiosis intoxication in which one of the pair will die due to unfavorable metabolic differences are comparable to the relation between mother and fetus; in erythroblastic fetalis the fetus is the intoxicated partner and in toxemias of pregnancy the mother is the victim. E. G. Moody

382. Correlation between serum protein-bound iodine levels and metabolic rates in male bovine. K. H. BURNS, R. W. COLBY, P. GOUGLER, and H. O. KUNKEL, Texas A. and M. Coll., College Sta. Am. J. Physiol., **172**: 107-108. 1953.

Three Hereford and 4 Santa Gertrudis bulls, 10-12 mo. of age, were used in these studies. The levels of serum protein-bound iodine were determined in conjunction with measurements of metabolic rates. A Benedict-Roth type 120 1.0_2 spirometer with a closed circuit spirographic-mask was used for metabolic determinations. A definite relationship was found between the average rate of metabolism and the serum protein-bound iodine levels. This indicates that the measurement of serum protein-bound iodine in the bovine could be used as a measure of thyroid activity. Victor Hurst

383. Repeatability, heritability, and the effect of level of milk production on the occurrence of first estrus after calving in dairy cattle. D. OLDS and D. M. SEATH, Ky. Agr. Expt. Sta., Lexington. J. Animal Sci., **12**, 1: 10-14. 1953.

The average length of time from parturition to first estrus was 32.1 ± 18.6 d. for 472 calvings of 210 cows. There was more variation among cows than within records of the same cow, the repeatability being 0.29 for single records. The correlation between milk production (M.E. FCM) for 120 d. postpartum and the number of days from calving to first heat was 0.095 ($P = < 0.05$). The heritability for time interval from calving to first estrus was estimated to be 27% (not significant) when based on the intra-sire regression of daughter on dam, using only the first records of each animal. When all records were used, the heritability estimate was 32.2% ($P = < 0.05$). The

correlation between half-sibs indicated a heritability too small to be measured when based on single records but was 31.1% when all records were used. O. T. Stallcup

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

384. Cleaning-in-place. C. A. ABELE, Diversey Corp., Chicago. Southern Dairy Products J., **53**, 2: 73, 74, 78-80, 82. Feb., 1953.

Cleaning and bactericidal treatment of milk pipelines in position has proven economical under some circumstances, and all opposition to the practice eventually will be dissipated. Plants with 50 ft. or less of piping may not find it economical. Pipe wash sinks for short lines and special fittings still will be necessary. More cleaning compound is needed than when the lines are completely disassembled.

Different metals in the same line must be avoided to prevent electrolysis, and dented pipe and scarred fittings should not be used. Glass pipe may be used to advantage. Lines must be rigidly held in place with pitch or drains for complete removal of bactericidal solutions. The 3-A suggested method for installation and cleaning should be followed. The 3-A pipe and fittings are recommended.

"Rabbits" are not generally used for scrubbing action. Five ft./sec., or faster, circulation is suggested. Cleaning solution tanks should be stainless steel. A head of 18 in. of solution above the pump intake should be maintained. A steam hose with a thermostatic valve should be provided for the solution tank. Cleaning compound should be completely predissolved. Recording thermometers in the circuit probably will be required by sanitarians. The piping should be subjected to the conventional bactericidal treatment after all branch lines and pumps have been connected. F. W. Bennett

385. The cleaning of glass piping in dairy plants. F. F. FLEISCHMAN and R. F. HOLLAND, Cornell Univ., Ithaca, N. Y. J. Milk and Food Technol., **16**, 1: 9-14. Jan.-Feb., 1953.

Water above 185° F. was comparable to 200 p.p.m. of hypochlorite solution circulated for 5 min. in sanitizing Pyrex piping cleaned in place. Nine commercial alkaline cleaners were studied and all were satisfactory in cleaning and sanitizing the piping. Each of 4 acid cleaners studied in conjunction with an alkaline cleaner satisfactorily cleaned the glass holding tube on the H.T.S.T. pasteurizer.

The quaternary-ammonium compounds gave high bacterial counts when used as cleaners or sanitizers in comparison to hot water or hypochlorite solution.

This study shows that glass piping cleaned and sanitized in place gave lower bacterial counts than did stainless steel sanitary pipe cleaned by daily dismantling and reassembling.
H. H. Weiser

386. Cleaning-in-place pipe lines. H. P. HODES, Tri-Clover Machine Co., Kenosha, Wis. Milk Dealer, **42**: 44-45, 74-78. Feb., 1953.

The successful operation of a cleaned-in-place pipe line results in a substantial saving in labor, maintenance, and material replacement costs. The successful operation depends upon a few basic rules, such as: (1) All pipe lines in the C-I-P circuit must be sloped at a minimum of 0.05 in. per ft. (2) Provisions should be made to bypass pipe lines, vats, tanks, vertical coolers, and other equipment which is not a part of the C-I-P cleaning circuit. (3) The average flow velocity of the cleaning solution should be 5 ft./sec. It may be as low as 1.5 ft./sec. and as high as 22 ft./sec. The velocity depends upon the proper formulation and concentration of the cleaner chemicals and the internal pressure at which they are recirculated. (4) Both the pump and the solution tank should be made of non-corrodible material. A movable unit is recommended. (5) Plans for the C-I-P circuit should include a temperature recording device; in multiple operations, temperature controlling devices are a must. The recording thermometer should have a scale range from at least 40° F. to 190° F. and should be protected against bulb damage at a temperature of 220° F. The thermometer bulb should be located in the return line as near the exit end as possible. The construction and installation of the pipe lines are discussed and directions for planning their installation are given. C. J. Babcock

387. Quarternaries and hypochlorites in mastitis sanitation. P. R. ELLIKER, Ore. Agr. Expt. Sta., Corvallis. J. Milk and Food Technol., **16**, 1: 22-25. Jan.-Feb., 1953.

Quaternaries have an advantage over the hypochlorites in that the former do not have a marked destructive action on the rubber inflations. Quaternaries tend to minimize the chapping of teat surfaces coming in contact with the germicide on the disinfected teat cups. The sanitation principles involved in the destruction of mastitis streptococci is applicable to other pathogenic bacteria, although *M. pyogenes aureus* may be more resistant to action of many germicides than is *S. agalactiae*.
H. H. Weiser

388. Power washing centrifugal separator. J. R. ORELIND (assignor to International Harvester Co.). U. S. Patent 2,629,547. 5 claims. Feb. 24, 1953. Official Gaz. U. S. Pat. Office, **667**, 4: 970. 1953.

A milk separator bowl so designed that after use a washing solution may be introduced through the top or inlet and deflected so as to flush the discs and interior surfaces of the bowl.
R. Whitaker

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