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## Contents






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# JOURNAL OF DAIRY SCIENCE

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## THE VITAMIN A POTENCY OF FRESH AND OF STORED BUTTER MADE FROM SWEET AND FROM NEUTRALIZED SOUR CREAM[1]

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Butter has always been considered an important source of vitamin A. More butter is made from sour cream than is made from sweet cream. Before sour cream is pasteurized for butter-making, it is necessary to reduce the acidity to avoid churning losses and to improve the keeping quality and flavor of the finished product. Both soda and lime types of neutralizers are used for this purpose. A considerable amount of both sweet and neutralized sour cream butter is held in cold storage for periods ranging from 2 to 6 mo. before being consumed. Considering these factors, it should be of interest to the butter industry to know the effect of neutralization of cream on the vitamin A potency of fresh and of storage butter. Baumann and Steenbock (2) stated that the acidity of the cream at churning time was without effect on the vitamin A activity of the butter. Butter made from neutralized cream contained the same amount of carotene and vitamin A as that made from cream with an acidity of 0.42 per cent lactic acid.

Herzer and Gieger (4) made five trials using sweet cream as a control and allowing the remainder of the cream to sour to various acidities. The cream was then neutralized, pasteurized, and made into butter. Two trials were made while the cows were on green pasture and three while the cows were on dry feed. There were no significant differences in vitamin A or carotene content in the butters made from the sweet cream and that made from the sour neutralized cream.

Ashworth *et al.* (1) Hathaway and Davis (3) and Kemmerer and Fraps (5) found no correlation between the acidity of the cream, the type of neutralizer used and the vitamin A activity of the butter.

In order to determine the effect of neutralizing cream on the vitamin A potency of fresh and of stored butter made under Montana conditions, the following experiment was undertaken.

### EXPERIMENTAL PROCEDURE

Two different trials were made with high quality sweet cream and the same cream after it was allowed to sour, and one trial was made with low grade sour cream. The cream was treated as indicated in table 1 and churned in a small

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[1] Contribution from Montana State College, Agricultural Experiment Station. Paper no. 246 Journal Series.

TABLE 1

*The carotene and vitamin A content of fresh and stored butter made from different qualities of cream standardized for acidity with different brands and types of neutralizer*

| Quality of cream | Treatment of cream | Types of different brands of commercial neutralizers used | Fresh butter | | | Stored butter | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 60 d. at 40° F. | | | 180 d. at 0° F. | | |
| | | | Carotene | Vitamin A | | Carotene | Vitamin A | | Carotene | Vitamin A | |
| | | | γ/g. fat | γ/g. fat | I.U./lb. butter | γ/g. fat | γ/g. fat | I.U./lb. butter | γ/g. fat | γ/g. fat | I.U./lb. butter |
| *Trial 1.* | | | | | | | | | | | |
| **Fresh**, sweet cream. | Pasteurized to 170° F., held 30 min. | | | | | | | | | | |
| **Acidity**, 0.13%. | Churned sweet | None-acidity 0.13% | 4.8 | 7.0 | 14,000 | 4.6 | 7.0 | 13,900 | 4.9 | 7.9 | 15,600 |
| | Held at room temperature | Soda—Brand A | 4.8 | 7.2 | 14,300 | 4.3 | 6.6 | 13,100 | 4.5 | 7.2 | 14,100 |
| | until the acid increased to | Lime—Brand B | 4.9 | 8.2 | 16,000 | 4.7 | 6.3 | 13,000 | 4.6 | 8.0 | 15,600 |
| | 0.90%. Acid reduced to | Soda—Brand C | 4.8 | 6.6 | 13,500 | 4.6 | 6.8 | 13,600 | 4.7 | 6.9 | 13,800 |
| | 0.25%. Pasteurized to | Lime—Brand D | 4.5 | 7.0 | 13,900 | 4.6 | 6.5 | 13,100 | 4.6 | 6.7 | 13,500 |
| | 170° F., held 30 min. | Soda—Brand E | 4.5 | 7.3 | 14,400 | 4.6 | 6.3 | 12,800 | 4.7 | 6.8 | 13,700 |
| | | Soda—Brand F | 4.6 | 7.2 | 14,200 | 4.6 | 6.0 | 12,400 | 4.8 | 6.4 | 13,100 |
| | | Lime—Brand G | 4.6 | 7.7 | 15,100 | 4.5 | 6.0 | 12,300 | 4.6 | 7.2 | 14,200 |
| | | None-acidity 0.90% | 4.6 | 7.4 | 14,700 | 4.3 | 6.1 | 12,400 | 4.5 | 8.6 | 16,400 |
| | | | | | | 30 d. at 40° F. | | | 75 d. at 0° F. | | |
| *Trial 2.* | | | | | | | | | | | |
| Fresh, sweet cream. | Pasteurized to 158° F., held 30 min. | | | | | | | | | | |
| Acidity, 0.13%. | Churned sweet | None-acidity 0.13% | 4.9 | 5.0 | 11,000 | 5.1 | 5.0 | 11,000 | 4.8 | 4.9 | 10,800 |
| | Acidity developed to | Soda—Brand A | 5.1 | 5.3 | 11,600 | 5.1 | 5.6 | 12,000 | 5.0 | 5.7 | 12,200 |
| | 0.62% reduced to 0.22%. | Lime—Brand H | 5.0 | 5.5 | 11,800 | 5.2 | 5.2 | 11,400 | 4.6 | 5.3 | 11,300 |
| *Trial 3.* | | | | | | | | | | | |
| Old, sour, metallic cream. | Pasteurized to 160° F., held 30 min. | | | | | | | | | | |
| Acidity, 0.39%. | Acidity reduced to 0.25% | Lime—Brand H | 3.4 | 6.1 | 11,900 | 3.3 | 5.0 | 9,900 | 3.2 | 5.4 | 10,500 |
| | Acidity reduced to 0.32% | Soda—Brand A | 3.5 | 6.0 | 11,700 | 3.3 | 5.1 | 10,200 | 3.4 | 5.2 | 10,300 |
| Old yeasty, metallic cream. | Acidity reduced to 0.27% | Lime—Brand H | 1.4 | 6.3 | 10,800 | 1.4 | 6.3 | 10,800 | 1.4 | 6.0 | 10,300 |
| **Acidity**, 0.48%. | Acidity reduced to 0.28% | Lime—Brand B | 1.6 | 6.0 | 10,500 | 1.5 | 6.9 | 11,800 | 1.3 | 6.0 | 10,300 |
| Old, sour, metallic cream. | Acidity reduced to 0.31% | Soda—Brand A | 1.5 | 5.3 | 9,300 | 1.5 | 4.2 | 7,600 | 1.3 | 4.8 | 8,400 |
| **Acidity**, 0.54%. | Acidity reduced to 0.32% | Lime—Brand B | 1.5 | 5.4 | 9,400 | 1.5 | 4.5 | 8,100 | 1.2 | 4.8 | 8,300 |

experimental churn. The sweet cream was pasteurized and churned without reducing the acid in it. The acid in the sour cream was neutralized with either a soda- or a lime-type commercial neutralizer.

*Trial I:* The high quality fresh sweet cream was produced by the College dairy herd. The cream was divided into nine portions. The acid in seven of the sour samples was adjusted with seven different brands of commercial neutralizers and treated as shown in table 1.

*Trial II:* The high quality sweet cream used in this trial also was produced by the College dairy herd. The sweet cream acidity was 0.13 per cent. The cream was divided into three portions. The acid in the two sour portions was standardized with two brands of commercial neutralizers and processed as shown in table 1.

*Trial III:* Three samples of low grade cream, having objectionable flavor defects, were procured from a butter plant. Each sample was divided into two portions and the acid in each portion was standardized with a commercial brand of neutralizer. Three different brands of commercial neutralizers were used in these six portions and the creams handled as indicated in table 1.

The carotene and vitamin A contents of the butters were determined by the method outlined by the Technical Committee on Vitamin Research in Butter (7). The composition of the butter was determined by the Kohman method (6) and the composition results were used in calculating the vitamin A and the carotene content per gram of fat.

The samples of butter in trial I were analyzed for carotene and vitamin A when fresh, after holding 60 days at 40° F., and after storage for 180 days at 0° F. The butters in Trials II and III were analyzed for carotene and vitamin A when fresh, after holding 30 days at 40° F., and after a 75-day storage period at 0° F. Each International Unit represents 0.6 $\gamma$ of carotene or 0.25 $\gamma$ of vitamin A. These are the factors recommended by the Technical Committee (7).

## RESULTS

The carotene and vitamin A content found in the fresh and in the stored butter made from different qualities of cream, standardized for acidity with the two different types of neutralizers, are given in table 1.

The amount of carotene and vitamin A found in the fresh butters made from high quality fresh sweet cream, butters made from the same cream allowed to sour, and the acid standardized with a soda- or lime-type neutralizer, and butter made from the same cream churned sour, was fairly uniform. The same holds true for the butter samples from these same churnings held for 30 and 60 days at 40° F. and for 75 and 180 days at 0° F.

The carotene and vitamin A content found in the fresh butters made from low quality cream and in the same butters held for 30 days at 40° F., and stored for 75 days at 0° F., showed only slight variations.

## SUMMARY

Three trials, consisting of 18 churnings of butter, were made from sweet cream, the same cream soured and churned at a high acidity; from high grade

and from low grade sour cream neutralized with either soda- or lime-type commercial neutralizer.

No appreciable differences were found in the carotene and vitamin A contents of the fresh butters made from the same creams but standardized with different commercial neutralizers in the carotene and vitamin A content of the butters after holding for 30 and 60 days at 40° F., and after storage for 75 and 180 days at 0° F.

From the results obtained in this experiment, it is indicated that neither the acidity of the cream at churning nor the kind or type of commercial neutralizers commonly used to standardize the cream acidity, have any appreciable effect on the carotene and vitamin A content in the resulting fresh butter or butter held in cold storage.

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# SEPARATION AND RECOMBINATION AS A MEANS OF DEFERRING AN OXIDIZED OR CARDBOARD FLAVOR IN MILK DURING FROZEN STORAGE[1]

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Mechanical separation of milk was found by Roland and Trebler (5) to produce a marked decrease in its sensitivity to copper-induced oxidized flavor as evidenced by tests on milk made by recombining the skimmilk and cream. The milk was pasteurized but it was not homogenized nor was it frozen. They concluded that removal of lecithin or related substances by the separator or changes in their distribution between fat and aqueous phase may have been responsible for the decreased sensitivity.

Since the work of Roland and Trebler (5) seemed to indicate a practical way to defer the development of an oxidized (cardboard) flavor in milk during frozen storage (2), it was decided to compare the flavor stability of pasteurized and homogenized whole milk with that of milk made by recombining its cream and skimmilk. Either the recombined milk was pasteurized and homogenized or the cream was pasteurized and homogenized before being added to the pasteurized skimmilk.

In connection with these experiments various factors such as the cream homogenization temperature and pressure and fat content were studied.

Doan (4) has described studies on recombined or "viscolized" milk in which milk or cream containing 8 per cent or more fat was homogenized and mixed with pasteurized skimmilk. A deep cream layer formed on standing as the result of fat clumping. Elsewhere, Doan (3) reports that the clumping tendency increases as the homogenization pressure and concentration of the fat are increased. Babcock (1) has shown that the degree of clumping in 20 per cent cream increases above and below an homogenizing temperature of about 167° F. Rehomogenization of the cream caused many of the clumps to be broken up.

Homogenized milk generally is considered less likely to develop an oxidized flavor than is unhomogenized milk (6). According to Spur (7) homogenized milk can be heated at a higher temperature for a longer time without developing a heated flavor than can unhomogenized milk. Creaming in the usual sense is not a factor. Some dairies pasteurize milk to be homogenized by holding it at 155° F. or higher for 30 min. instead of 143° F. for 30 min., the minimum recommended by the United States Public Health Service (8).

Although homogenized milk is more resistant than unhomogenized milk, to the development of an oxidized flavor, it may develop this undesirable property in frozen storage and so be unsatisfactory not only as a beverage but also for other purposes (2).

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[1] This work was done with funds from the Research and Marketing Act of 1946.

## METHODS

Most of the milk was less tran 6 hr. old and none was more than 18 hr. old when processing was begun. All surfaces with which the milk came in contact during processing were stainless steel.

All samples were frozen in sealed cans of 160-ml. capacity in a 1° F. ice cream hardening room. At the end of the storage period, the cans were left overnight in a room that was maintained at 36° F., then warmed to 70° F. and examined. Body stability is recorded as the number of milliliters of deposit from 50 ml. of milk after whirling at 1,000 r.p.m. for 5 min. in a centrifuge that had a 15.5-in. head, measured from the inside bottoms of opposite cups when they were in a horizontal position.

The creams of different fat content were prepared by mixing high-fat cream and skimmilk obtained during the same separation. Recombining of cream and skimmilk to the original fat content was done manually with the aid of a suitable stirrer.

## EXPERIMENTAL

*Cream homogenization temperature.* Sufficient cream was prepared so that a portion of it could be homogenized at 170° F., the remainder cooled to 160° F., a portion of that homogenized and the stepwise cooling process repeated until homogenization at the several temperatures had been completed. Cooling was accomplished by placing the cream container in ice water and stirring the contents until a desired temperature was attained.

The pasteurized skimmilk and the pasteurized and homogenized cream were at room temperature when they were mixed. The following samples were prepared: (1) Original milk containing 4.0 per cent fat was heated to 170° F., homogenized at 2,000 lb. per in.$^2$ (p.s.i.) and cooled; (2) Milk prepared by mixing 30 per cent raw cream and raw skimmilk and containing 4.0 per cent fat was treated like 1; (3) 30 per cent cream was pasteurized and homogenized at 170° F., cooled and mixed with pasteurized skimmilk; (4–7) Same as 3, except that the cream was homogenized at 160, 130, 100 and 70° F., respectively; (8) Same as 7, except that the cream was held 3 hr. at 70° F. before it was homogenized at that temperature.

A set of samples that was examined at the end of 61-days storage at 1° F. showed no apparent deterioration in body. However, sample 2 was strongly oxidized and 8 was judged slightly stale. At the end of 140 days in storage there was no further significant change in flavor. However, the thawed milk was flaky.

After 244 days in storage samples 1, 3, 4 and 5 still had an acceptable flavor; sample 2 was strongly oxidized, 6 tasted old, 7 was very slightly oxidized and 8 was definitely (slightly) of this flavor. All the thawed samples wheyed off on standing and on being centrifuged formed a deposit of 13 to 16 ml. in the 50-ml. tubes.

Judged by experience in recent years, the original milk had excellent flavor stability during frozen storage. The data resemble that of other experiments of a similar design and purpose, except that after prolonged storage, sample 1

or the control milk of the other experiments usually was inferior to sample 3, the one made from cream that was homogenized at the pasteurizing temperature and pasteurized skimmilk. The flavor of the control samples of these experiments was not as stable as that of the milk described above.

*Cream homogenization pressure.* All milk, skimmilk and cream were pasteurized by heating to 160° F. and holding for 15 sec. That portion of the raw milk which was to be separated was warmed to 100° F. and its cream was standardized to 30 per cent fat content with raw skimmilk. In homogenizing this cream at 160° F., the initial pressure was 500 p.s.i. Then, as the homogenizer continued to operate, the pressure was increased by steps to 1,000, 1,500, 2,000 and finally 2,500 p.s.i. Since the homogenizer had a capacity of 125 gal. per hour and only 1 or 2 l. of each cream were required, there was little difference in the time the samples were at the homogenizing temperature. When each of these creams was mixed with pasteurized skimmilk to obtain a product of the same fat content as the original milk, the temperature of the cream was 120° F.; that of the skimmilk was 80° F.

At the end of 91 days in storage at 1° F., the body of all the samples was good, but the recombined milk made by mixing cream that had been homogenized at 500 p.s.i. with pasteurized skimmilk was slightly oxidized. After 249 days at 1° F. this recombined milk was strongly oxidized and milk that was prepared by mixing cream that had been homogenized at 1,000 p.s.i. and skimmilk was slightly oxidized. The other samples were free of this defect. These results resemble those of Ross (6) who, working with unfrozen milk, concluded that "pressures of 500 and 1,000 lbs. per square inch were partially effective in preventing development of oxidized flavors, but could not be classed as dependable."

*Fat content of cream.* Milk of 4.4 per cent fat content was separated to yield cream that contained more than 35 per cent fat. Then this cream was standardized with the skimmilk to obtain creams of the desired composition. Again, cream and skimmilk were mixed when their temperature was 120 and 80° F., respectively.

The following samples were prepared for frozen storage: (1) Whole milk heated to 160° F., homogenized at 2,500 p.s.i. and cooled in ice water; (2) Recombined raw milk from 35 per cent cream and skimmilk processed as in no. 1; (3) Recombined milk from pasteurized (160° F.) and homogenized (2,500 p.s.i.) 10 per cent cream and pasteurized (160° F.) skimmilk; (4–8) Same as 3, except that 15, 20, 25, 30 and 35 per cent cream, respectively, was used.

At the end of only 80 days at 1° F., the first 3 samples were strongly oxidized, 8 was slightly so and the others were more or less stale with samples 6 and 7 considered the best. Other similarly planned and executed experiments gave variable results. However, the accumulated evidence is that best results may be expected when pasteurized and homogenized 25 or 30 per cent cream is used in preparing the recombined milk. To use cream of 40 or more per cent fat content would be impractical because of the high viscosity of the homogenized product.

The effect of the homogenizing pressure and the method of preparing recombined milk on the tendency of the milk to become oxidized in flavor are shown in more detail in the following experiment. Milk of 3.7 per cent fat content and its skimmilk and cream fractions were pasteurized by holding them at 160° F. for not less than 15 sec. Only 30 per cent cream was used. The temperature of the cream and skimmilk when they were mixed was above 100° F.

At the end of 114 days in frozen storage the recombined samples made by mixing pasteurized and homogenized cream and pasteurized skimmilk had the best flavor (table 1). Earlier examinations revealed that those samples which were prepared from raw recombined milk were less stable in flavor relative to the whole milk samples than is shown in the table and markedly less stable than those that were a mixture of pasteurized and homogenized cream and pasteurized skimmilk.

Since the cream that was used in preparing all of the recombined milks was

TABLE 1

*Effect of the homogenizing pressure and the method of preparing recombined milk on the tendency of the frozen milk to develop an oxidized flavor. (Storage temperature, 1° F.; age of samples, 114 d.)*

| Homogenizing pressure | Whole milk | Raw recombined milk, pasteurized and homogenized | Cream pasteurized and homogenized, then mixed with pasteurized skim milk |
|---|---|---|---|
| (*p. s. i.*) | (*flavor*) | (*flavor*) | (*flavor*) |
| 500 | St.[a] ox. | St. ox. | Oxidized |
| 1,000 | St. ox. | St. ox. | Tr. ox. |
| 1,500 | Ox. | St. ox. | Normal |
| 2,000 | Stale, old | Ox. | Normal |
| 2,500 | Old | Sl. ox. | Best of all samples |

[a] St. = strongly; tr. = trace; sl. = slightly

from the same well-mixed supply, there was no difference in the amount of lecithin and related substances in the recombined samples.

Because experiments showed that recombined milk prepared by mixing pasteurized and homogenized 30 per cent cream and pasteurized skimmilk was less susceptible to the development of an oxidized flavor during frozen storage than was the milk of origin, an attempt was made to measure this property. This was done in terms of Cu tolerance as follows: (1) Prepared by holding 4.4 per cent fat content milk at 160° F. for 15 sec., homogenizing at 2,500 p.s.i. and cooling; (2) Similar to 1 except that Cu, in the form of a $CuSO_4$ solution, was added to the pasteurized milk at the rate of 0.5 ppm.; (3) A 4.4 per cent fat content mixture of raw 30 per cent cream and raw skimmilk processed as in 1; (4) Similar to 3 except that 0.5 ppm. of Cu was added to the processed milk; (5) A 4.4 per cent fat content mixture of pasteurized and homogenized 30 per cent cream and pasteurized skimmilk; (6) Similar to 5 except that 0.5 ppm. of Cu was added to the processed milk.

At the end of 31 days at 1° F., sample 1 had a clean oxidized flavor, 2 a strong unclean one, 3 resembled 1, 4 was strongly oxidized and unclean, 5 was flat and

6 was slightly oxidized. At the end of 2 mo. of storage, the first four samples were strongly oxidized; the last two were oxidized, 6 slightly more than 5. Furthermore, 5 and 6 showed the least deposit after centrifuging 50 ml. of each for 5 min. at 1,000 r.p.m. This superiority in flavor and body stability was evident after 4 mo. storage.

This experiment was repeated except that pasteurized and homogenized creams of 35 and 25 as well as 30 per cent fat content were used and no samples were prepared from raw cream and raw skimmilk. Thirty-five per cent cream was used in preparing samples 3 and 4, 30 per cent in preparing 5 and 6 and 25 per cent in preparing samples 7 and 8. At the end of 77 days at 1° F. sample 1 was oxidized, the three recombined samples to which no Cu had been added were flat and clean in flavor and the four samples which contained 0.5 ppm. of added Cu were strongly oxidized. At this stage the control sample one and its duplicate, no. 2, to which 0.5 ppm. of Cu had been added, yielded 8 ml. of

TABLE 2

*Effect of the homogenizing pressure upon the tendency of recombined milks to develop an oxidized flavor during frozen storage and to form a deposit and a cream plug after thawing and centrifuging. Milk, cream and skimmilk were pasteurized by holding them at 160° F. for 15 sec. Milk and cream were homogenized at 2,500 p. s. i. at pasteurizing temperature. (Temperature of storage, 1° F.; length of storage, 93 d.)*

| Sample | Homogenizing pressure | Deposit | Cream plug | Flavor |
|---|---|---|---|---|
| | (*p. s. i.*) | (*ml.*) | (*ml.*) | |
| Control | 2,500 | 4.0 | 1.0 | St.[a] ox. |
| Recombined milk | ..... | 2.0 | 5.0 | Old |
| Recombined milk | 250 | 2.0 | 5.0 | St. ox. |
| Recombined milk | 500 | 2.2 | 4.0 | Ox. |
| Recombined milk | 1,000 | 2.8 | 3.0 | Sl. ox. |
| Recombined milk | 2,000 | 2.8 | 2.0 | Tr. ox. |
| Recombined milk | 2,500 | 3.0 | 1.0 | Old |

[a] See table 1.

deposit from 50 ml. of milk after centrifuging under the uniform conditions; the recombined milks yielded only 4 ml. One month later the figures were 14.0 for 1 and 2, 11.0 for 3 and 4, 10.0 for 5 and 6 and 9.0 ml. for 7 and 8.

The increased stability of this recombined milk with respect to the onset of an oxidized flavor was not of sufficient magnitude to overcome the catalytic effect of 0.5 ppm. added Cu. On the other hand, the recombined milks apparently had a measurably more stable body during storage at 1° F.

While there are flavor and body stability advantages in storing recombined milk that is made by mixing pasteurized and homogenized cream and pasteurized skimmilk, there is at least one disadvantage, namely, the formation of a cream-like layer in the thawed product. This layer is of sufficient depth and fat content to prevent the milk from coming within the definition of homogenized milk, since, after 48-hr. storage, the fat percentage in the top 100 ml. of milk in a quart bottle differs by more than 10 per cent of itself from the fat percentage

of the remaining milk as determined by thorough mixing (8, as amended). In an effort to overcome this property, recombined milk, consisting of pasteurized and homogenized 30 per cent cream and pasteurized skimmilk, was homogenized at pressures ranging from 250 to 2,500 p.s.i. At the lowest pressure there was no effect on the volume of the cream layer that formed when a 50-ml. portion of the thawed milk was centrifuged (table 2). As the pressure was increased, the depth of this cream layer decreased until, at an homogenizing pressure of 2,500 p.s.i., it was the same as that of the control sample.

The work of Doan (4) and of Babcock (1) suggested that the fat in the thawed recombined sample caused a deep cream layer or cream plug to form during centrifuging because its globules were clumped. Being clumped, these globules had a sweeping effect and, as they rose, carried some of the destabilized solids towards the surface of the milk. Thus, the depth of the cream layer was increased, while that of the deposit was decreased. As the increased pressure of homogenization broke up the fat clumps in the recombined milk, less fat and other solids collected near the surface and more insoluble solids were deposited in the bottom of the tube.

While the volume of the cream layer in the thawed recombined milk that was homogenized at 250 p.s.i. was as great after centrifuging as that in the recombined milk that was not homogenized, the sensitivity of this milk to the development of an oxidized flavor was greater than that of the control. A strongly oxidized flavor developed in less than 60 days, whereas the control did not reach this off-flavor intensity until it was 93 days old. It was not until the homogenizing pressure was increased to 2,500 p.s.i. that the recombined milk again had a flavor stability during frozen storage equal to that of the recombined milk that had not been homogenized.

The recombined milk that contained clumped fat globules was less susceptible to the development of an oxidized flavor than the control milk in which the globules were not clumped. And yet, as these clumps were broken up by homogenization, the milk at first became very susceptible and then, as the homogenizing pressure was increased, more and more resistant to the onset of this off flavor.

With decreasing body stability, as a result of longer storage, the relationship between body stability and cream plug formation was less apparent. Finally, the amount of deposit in each centrifuge tube reached a maximum of about 14 ml. At the same time the volume of the cream plug in the thawed recombined milk continued to decrease with increasing homogenization pressure approximately as shown in table 2.

## SUMMARY

The development of an oxidized (cardboard) flavor in milk during frozen storage was deferred by separating the milk, pasteurizing and homogenizing the cream and mixing this cream with the pasteurized skimmilk. Best results were obtained when the temperature of the cream during homogenization was well above the melting point of the fat, the homogenizing pressure exceeded 1,500 lb. per square inch and the fat content of the cream was 25 to 30 per cent.

The increased flavor stability of this recombined milk was not sufficient to counteract the catalytic effect of 0.5 ppm. of added Cu.

Recombined milk prepared by mixing raw cream and raw skimmilk and then pasteurizing and homogenizing the mixture was more susceptible to the development of an oxidized flavor during frozen storage than the unseparated milk that was similarly pasteurized and homogenized.

The difference in susceptibility to the development of an oxidized flavor of the recombined milks prepared by these two methods was not due to a difference in content of lecithin and related substances because the milk was subjected to the same centrifugal forces during separation.

Low pressure homogenization of recombined milk made by mixing pasteurized and homogenized 25 to 30 per cent cream and pasteurized skimmilk, increased the susceptibility of the milk to the development of an oxidized flavor. As the pressure was increased the flavor stability of the milk increased until at 2,000 to 2,500 lb. it was no more likely to develop an oxidized flavor than was the same recombined milk before it was homogenized. At the same time, the body stability decreased and there was less tendency to form a cream plug.

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# A COMPARISON OF THE VITAMIN A POTENCY OF MILK FAT FROM COWS FED ON DRY FEED AND ON GREEN PASTURE[1]

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Milk fat always has been considered an important source of vitamin A. It has been shown by a number of investigators (1, 2, 4, 5, 6, 7, 8, 9, 11, 13, 16, 17) that the vitamin A potency of milk fat is directly related to the carotene content of the feed consumed by the cow. It also has been shown that some breeds of dairy cattle utilize the carotene of the feed more efficiently than others (1, 13, 14, 15, 17). A number of investigators have reported rapid increases in the vitamin A potency of milk fat when cows are given access to feeds high in carotene, such as green pasture grass. They also have reported on the length of time required for the milk fat to reach high vitamin A values after the cows have access to green grass. Treichler *et al.* (16) reported the results on one cow which had been on a vitamin A-free ration for 60 days. She then grazed on green grass 5 hr. a day for 3 days. The vitamin A content *per se* of her milk fat increased to approximately the normal level at the end of the 3-day grazing period. The carotene content steadily increased until she had been on green pasture for 70 hr. Baumann *et al.* (1) reported results on a Holstein cow that had been on a dry feed ration without silage for 6 mo. This cow showed maximum vitamin A values in 2 wk. after her ration was changed to 60 lb. of green alfalfa cut immediately before being fed.

Loy *et al.* (9) experimented with two cows and found that when the animals were changed from a low vitamin A ration to a high one, the color value and the carotene and vitamin A content of the milk increased rapidly, reaching equilibrium in about 10 days. Likewise, Peterson *et al.* (12), in A.I.V. silage feeding trials, observed that the maximum effect of the A.I.V. silage on the vitamin A content of the milk was reached within the first 30 days of feeding. Then there was a slight drop to a level which was maintained during the remainder of the experiment. The work of Fieger and Lewis (5) showed that varying periods of time are required for cows previously grazed on native grass pasture, to graze on oat pasture before the vitamin A potency is increased.

From the results of these experiments it is indicated that the maximum vitamin A potency of milk fat is reached shortly after cows have access to high carotene feeds such as green pasture. In view of the experimental results obtained by other investigators, this work on the comparison of vitamin A potency of milk fat from cows fed on dry feed and on green pasture was undertaken. The general objective was to see how the results obtained under Montana conditions would compare with the findings of investigators in other states.

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## EXPERIMENTAL PROCEDURE

After a preliminary study of 1 yr., 1-day composite milk samples were taken at irregular intervals for 5 yr. on the Montana State College dairy herd, composed of purebred Jerseys and purebred Holsteins. During the 5-yr. period when the herd was on dry feed, the milking cows were fed primarily second-cutting alfalfa hay, generally of good quality. Some good quality first-cutting alfalfa also was fed during this period. The best quality hay was fed in the late winter and early spring dry feeding periods. The hay ration was supplemented by a concentrate mixture of rolled barley, wheat bran, dried beet pulp, soy bean or cottonseed or mustard seed oil meal, or ground mustard seed to which small percentages of bone meal and salt were added.

During the same 5-yr. period when the milking herd was grazed on green pasture, they were fed a small supplement of the above concentrate but relied upon green pasture grass for the roughage. Occasionally in the late summer, a small amount of good quality second-cutting alfalfa hay was fed to supplement the diminishing quantity of pasture grass. The green pasture was composed primarily of blue grass with some orchard grass, white clover, Ladino clover and alfalfa. Blue grass was the most abundant, with the other grasses diminishing in quantity in the order named.

The 1-day composite herd milk samples were not taken on any definite day during the month. Emphasis was given to securing vitamin A data on the milk while the cows were still on dry feed just before they were turned out on green pasture and again as soon as they were on green feed and at short intervals thereafter until the cows became adjusted to pasture grass.

The composite herd sample either was analyzed at once for vitamin A and carotene or frozen and held at $-10°$ F. until the analysis was made. The milk samples analyzed the first 2 yr. first were churned into butter and the butter analyzed according to the method outlined by the technical committee on vitamin A research on butter (10). The vitamin A and carotene data on the samples of milk collected the last 3 yr. were obtained by direct analysis of the milk by the "Rapid Extraction Procedure" reported by Boyer *et al.* (3). In this method the carotene value obtained represents the total carotenoids of the milk, consequently the carotene reported for the milk analyzed the last 3 yr. includes the total carotenoids in the milk. The vitamin A values reported in table 1 for the first 2 yr. of the study were corrected for the vitamin A lost in analysis. The vitamin A values reported for the last 3 yr. were not corrected for the approximate 5 per cent loss in analysis as reported by Boyer *et al* (3). Each International Unit reported in the table represents 0.6 $\gamma$ of carotene or 0.25 $\gamma$ of vitamin A. These are the same factors used by the technical committee (10).

## DISCUSSION

The vitamin A and carotene content of the milk expressed in micrograms per gram of fat and in International Units per gram of fat found during the 5 yr., 1945 to 1949, inclusive, are given in the table. These values are given for the

TABLE 1

*The vitamin A and the carotene content expressed in micrograms per gram of fat and the vitamin A potency expressed in international units per gram of fat in milk produced by cows fed on dry feed and on green pasture*

| Yr. | (a) Butter analyzed for vitamin A and carotene | | | | | | | | (b) Milk analyzed for vitamin A and carotene | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry feed | | | | | | | | Dry feed | | | | | | | | | | | |
| | 1945 | | | | 1946 | | | | 1947 | | | | 1948 | | | | 1949 | | | |
| | Day | Vitamin A | Carotene | I.U. | Day | Vitamin A | Carotene | I.U. | Day | Vitamin A | Carotene | I.U. | Day | Vitamin A | Carotene | I.U. | Day | Vitamin A | Carotene | I.U. |
| October | 29 | 6.79 | 5.06 | 37.84 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| November | 28 | 6.58 | 3.87 | 34.90 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| December | 18 | 5.69 | 4.09 | 31.47 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 27 | 8.86 | 15.40 | 61.10 | ..... | ..... | ..... | ..... |
| January | 22 | 6.21 | 4.31 | 34.08 | 28 | 6.33 | 4.48 | 34.86 | 3 | 6.08 | 8.22 | 38.02 | 19 | 7.20 | 10.43 | 46.18 | ..... | ..... | ..... | ..... |
| February | 19 | 7.41 | 3.47 | 37.82 | 27 | 4.91 | 3.56 | 27.17 | ..... | ..... | ..... | ..... | 23 | 4.80 | 6.83 | 30.58 | 13 | 10.28 | 17.65 | 70.53 |
| March | 29 | 5.50 | 2.68 | 28.24 | 20 | 6.57 | 2.88 | 33.18 | ..... | ..... | ..... | ..... | 28 | 3.69 | 4.17 | 21.71 | 15 | 7.32 | 7.44 | 41.68 |
| April | 26 | 7.13 | 3.52 | 36.70 | 18 | 8.65 | 4.78 | 44.97 | 14 | 5.37 | 6.50 | 32.29 | 22 | 5.09 | 5.09 | 28.84 | 15 | 6.14 | 6.46 | 35.32 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 15 | 6.89 | 13.69 | 50.37 | ..... | ..... | ..... | ..... | 15 | 6.07 | 8.01 | 37.63 |
| May | 16 | 6.77 | 3.21 | 34.62 | 17 | 6.80 | 4.16 | 36.35 | 22 | 8.00 | 12.69 | 53.15 | 23 | 7.65 | 4.91 | 38.78 | 16 | 5.20 | 7.70 | 34.15 |
| AVERAGE | ..... | 6.51 | 3.78 | 34.46 | ..... | 6.65 | 3.97 | 35.31 | ..... | 6.58 | 10.28 | 43.46 | ..... | 6.22 | 7.81 | 37.87 | ..... | 7.00 | 9.45 | 43.86 |
| | Green pasture | | | | | | | | | | | | Green pasture | | | | | | | |
| May | 24 | 9.48 | 4.21 | 48.00 | 18 | 10.35 | 4.67 | 52.50 | 23 | 6.69 | 8.38 | 40.73 | 24 | 7.65 | 4.80 | 38.60 | 17 | 5.35 | 9.81 | 37.75 |
| May | 29 | 8.93 | 5.10 | 47.11 | 22 | 10.26 | 5.46 | 53.48 | 24 | 7.15 | 6.37 | 39.21 | 25 | 3.90 | 4.46 | 23.03 | 18 | 6.69 | 7.61 | 39.37 |
| May | ..... | ..... | ..... | ..... | 27 | 9.22 | 6.38 | 50.57 | 25 | 7.61 | 11.70 | 49.94 | 26 | 5.26 | 5.09 | 29.52 | 19 | 7.56 | 7.94 | 43.47 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 26 | 7.87 | 12.01 | 51.49 | 27 | 5.05 | 5.93 | 30.08 | 20 | 8.53 | 8.81 | 48.73 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 27 | 11.38 | 20.25 | 79.27 | 28 | 5.81 | 6.76 | 34.52 | 21 | 8.96 | 8.52 | 50.04 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 29 | 10.16 | 20.81 | 75.32 | 31 | 5.86 | 7.28 | 35.57 | 22 | 8.96 | 8.79 | 50.49 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 23 | 6.17 | 8.51 | 37.88 |
| May | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 27 | 4.54 | 5.01 | 26.51 |
| June | 4 | 10.44 | 4.67 | 52.90 | ..... | ..... | ..... | ..... | 3 | 5.60 | 7.35 | 34.65 | 8 | 6.00 | 8.33 | 37.88 | 2 | 7.21 | 8.96 | 43.77 |
| June | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 5 | 6.45 | 7.66 | 38.57 | 23 | 5.93 | 10.42 | 41.09 | 15 | 7.44 | 10.71 | 47.61 |
| June | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 23 | 8.11 | 10.14 | 49.34 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| July | 8 | 10.83 | 7.09 | 58.69 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 2 | 6.46 | 10.44 | 43.24 | ..... | ..... | ..... | ..... |
| August | 27 | 10.49 | 7.12 | 57.30 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 8 | 11.18 | 18.21 | 75.07 | ..... | ..... | ..... | ..... |
| September | 17 | 10.57 | 7.81 | 58.80 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 17 | 9.86 | 18.95 | 71.02 | ..... | ..... | ..... | ..... |
| AVERAGE | ..... | 10.12 | 6.00 | 53.80 | ..... | 9.94 | 5.50 | 52.18 | ..... | 7.89 | 11.63 | 50.95 | ..... | 6.63 | 9.15 | 41.78 | ..... | 7.14 | 8.47 | 42.56 |

[a] Analysis made by the method outlined by the Technical Committee on Vitamin Research (10). Corrections made for loss of Vitamin A in analysis.
[b] Analysis made by the Method of Boyer *et al.* (3).

period when the cows were fed entirely on dry feed and when they were fed on green pasture supplemented with a small amount of a grain concentrate.

In 1945 and in 1946, the first and second years, the average vitamin A and carotene contents were lower when the cows were on dry feed than when they were on green pasture. The difference in the average vitamin A and carotene contents of the milk fat in the dry feeding and in the green pasture feeding periods in the third and fourth years, 1947 and 1948, was not so pronounced. In the fifth year there was not much difference in the averages of these two milk fat constituents. There was a tendency toward a slightly higher vitamin A potency in the dry feeding period.

The vitamin A potency of the milk fat in the samples taken in May 1945 and in 1946, in the dry feeding period just before the cows were turned out on green pasture, generally were lower than the potencies of the May milk samples for the same 2 yr. taken just after the cows were turned out on green pasture. In these 2 yr. the cows were fed good quality alfalfa hay in the dry feeding period previous to the time when they were turned out on green pasture. The vitamin A potencies in the successive samples taken in May and in the remaining months of the green pasture feeding period were higher than that found in the first sample taken in May when the cows were on pasture. In 1945, the cows were on grass 4 days and in 1946, the cows were on grass only 5 hr. before the first sample was taken.

In 1947 the herd was fed very high quality alfalfa hay and was producing heavily in the dry feeding period just before the cows were turned out on green pasture. The vitamin A potency of the milk fat in the two samples taken in May in the dry feeding period of that year before the cows were turned out on pasture, were much higher than that of the first two samples taken after the cows were on green grass for 1 and 2 days for 1.5 and 3.5 hr. respectively. The vitamin A potency increased on the 4 succeeding days to a level approximately 50 per cent higher than found in the May samples taken during the dry feeding period. The samples taken during the month of June of that year showed a definite decrease from the high level.

In the third year 1948, the vitamin A potency of the milk sample taken in May in the dry feeding period immediately before the cows were turned out on pasture, was practically the same as that found in the first sample taken after the cows were on pasture grass for 1.5 hr. the first day. The alfalfa hay fed to the cows in the dry-feed period just before the cows were turned out on green grass pasture was of good quality. The vitamin A potency of the milk fat went down to a lower level in the succeeding milk samples taken in May and raised to a higher level again in samples taken in June, July, August and September. The highest vitamin A potencies found during this year were in August, September and December. In December, the cows were on all dry feed which included high quality alfalfa hay. In September, the cows were on pasture but were fed a small amount of good alfalfa hay between the night and morning milkings to supplement the pasture grass, which was not sufficient to satisfy the roughage requirement in the ration.

In 1949, the vitamin A potency behavior was different than in any of the other 4 yr. Good alfalfa hay was fed during the dry feeding period.

The highest vitamin A potency was found in the sample taken in February. The vitamin A potency declined each succeeding month of the dry feeding period. It then raised slowly to a higher level when the cows were turned out to pasture, reaching the highest for the green pasture feeding season on the fifth and sixth days. The average vitamin A potency for the green pasture period never did reach the average for the dry-feed period.

## SUMMARY AND CONCLUSIONS

One-day composite samples of herd milk were taken at irregular intervals over a 5-yr. period from the Montana State College Dairy Herd composed of purebred Holstein and Jersey cows. The samples were taken during periods when the cows were on all dry feed and during periods when they were grazing on green pasture grass supplemented with a small amount of dry grain concentrate. Determinations for vitamin A and carotene in the milk fat were made from which the vitamin A potency or International Units were estimated.

The average vitamin A potency generally was higher during the period when the cows were on green pasture than when the cows were getting all dry feed.

In general, the greatest increase in vitamin A potency was shortly after the cows were changed from all dry feed to pasture grass supplemented with a small amount of dry grain concentrate.

In some cases the highest vitamin A potency was found in the milk fat when the cows were on dry feed exclusively. This would indicate that under some dry feeding conditions milk with a high vitamin A potency can be produced.

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# THE EFFECT OF ANTIBIOTICS ON THE FERTILITY OF BULL SEMEN AND THEIR RELATIONSHIP TO THE ESTROUS CYCLE LENGTH OF DAIRY CATTLE FOLLOWING ARTIFICIAL INSEMINATION[1]

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Many bull studs producing semen for use in the artificial insemination of dairy cattle are routinely adding antibiotics to semen extenders. Questions relative to the results that may be expected from this practice, however, have not been answered completely.

Almquist (1, 2) found that penicillin, streptomycin or a combination of the two, when added at the rate of 500 to 1,000 units per milliliter, produced highly significant increases in the fertility of relatively infertile bulls. Almquist (5) and Mixner (8) found no significant improvement in the fertility when semen from high fertility bulls was treated with penicillin, while Easterbrooks *et al.* (6) found that the addition of 100 $\gamma$ of streptomycin per milliliter of diluted bull semen increased the fertility even of high fertility bulls to some degree. Foote and Bratton (7) reported that the addition of penicillin, streptomycin or a combination of these plus polymyxin and sulfanilamide may be expected to increase the over-all fertility level of bovine semen used for artificial breeding.

Almquist and Prince (4) found that streptomycin, penicillin plus streptomycin, and penicillin, streptomycin and sulfanilamide increased by 7 to 11 percentage units the fertility of bulls that were below average in the control samples. On the other hand, in the high fertility group no significant difference was found between the control samples and the treated samples.

Almquist *et al.* (3) found that a combination of penicillin and streptomycin in levels ranging from 100 to 1,000 units each per milliliter of diluted semen, did not affect the livability of bull spermatozoa during a 20-day storage period. Data comparing the relative decline in fertility of semen containing antibiotics and that not containing antibiotics when held for 1 to 3 days before being used, apparently have not been reported.

The extent to which infectious agents in the semen are capable of causing abortion and/or pyometra in the cow is a question of academic interest. A comparison of time intervals at which cows returned for second service after being bred with semen containing antibiotics and that not containing antibiotics might offer an incentive for further work in this connection.

## EXPERIMENTAL PROCEDURE

During the months of April and May, 1950, 184 semen samples were collected from 25 bulls. Each sample was divided into two equal portions and extended

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

with 2.9 per cent sodium citrate solution and fresh egg yolk (equal parts). The rate of extension averaged about 1:40. One portion was treated with 1,000 units of dihydrostreptomycin sulfate and 1,000 units of crystalline sodium penicillin G per milliliter, while the other portion was used as a control. This semen was shipped to 35 local cooperatives of the Kentucky Artificial Breeding Association, with each local receiving alternate shipments of semen containing antibiotics and that not containing antibiotics. Four shipments were made each week. There were 5,241 cows bred with control semen and 5,299 bred with semen containing antibiotics. Fertility was based on 120 to 150-day non-returns from first service.

## RESULTS

Of the cows bred with control semen, 68.4 per cent did not return for second service within 120 to 150 days, while cows bred with treated semen averaged 68.1

TABLE 1

*The effect of antibiotics on the fertility of bulls grouped according to their fertility (2 mo. combined)*

| Bull | Controls | | Antibiotics | | Difference |
|---|---|---|---|---|---|
| | Cows bred | % N-R | Cows bred | % N-R | |
| Royalist | 217 | 48.8 | 242 | 64.9 | +16.1 |
| F. Horse | 186 | 48.9 | 250 | 41.6 | − 7.3 |
| Admiral | 112 | 59.8 | 78 | 59.0 | − 0.8 |
| Marion | 234 | 61.5 | 240 | 65.4 | + 3.9 |
| Marco | 264 | 62.5 | 212 | 62.7 | + 0.2 |
| St. Albans | 215 | 64.2 | 238 | 76.5 | +12.3 |
| Av. | 1,228 | 57.9 | 1,260 | 61.8 | + 3.9 |
| Cavalier | 57 | 64.9 | 39 | 56.4 | − 8.5 |
| Bobo | 41 | 65.9 | 76 | 64.5 | − 1.4 |
| Masterpiece | 273 | 66.7 | 310 | 77.1 | +10.4 |
| Jeweler | 418 | 67.0 | 479 | 70.6 | + 3.6 |
| Canary | 218 | 68.8 | 259 | 66.0 | − 2.8 |
| Meredith | 27 | 70.4 | 30 | 60.0 | −10.4 |
| Av. | 1,034 | 67.2 | 1,193 | 70.2 | + 3.0 |
| Valiant | 111 | 71.2 | 103 | 67.0 | − 4.2 |
| Morocco | 543 | 71.6 | 516 | 75.8 | + 4.2 |
| Chadron | 136 | 72.1 | 160 | 68.8 | − 3.3 |
| Eli | 363 | 72.5 | 398 | 72.9 | + 0.4 |
| Roamer | 337 | 73.3 | 242 | 69.8 | − 3.5 |
| Victor | 116 | 73.3 | 119 | 66.4 | − 6.9 |
| Av. | 1,606 | 72.3 | 1,538 | 72.0 | − 0.3 |
| Forward | 240 | 75.0 | 264 | 66.7 | − 8.3 |
| Pabst | 263 | 75.3 | 254 | 70.9 | − 4.4 |
| Ted | 219 | 75.3 | 172 | 66.3 | − 9.0 |
| D. Wonder | 165 | 75.8 | 144 | 61.8 | −14.0 |
| Ranger | 274 | 75.9 | 274 | 74.8 | − 1.1 |
| Chieftain | 18 | 83.3 | 51 | 66.7 | −16.6 |
| Av. | 1,179 | 75.6 | 1,159 | 65.9 | − 9.7 |
| Total | 5,047 | 68.5 | 5,150 | 68.4 | − 0.1 |

per cent non-returns. The analysis of variance (9) indicated that neither the treatment difference nor the treatment × bull interaction were significant at the 5 per cent level of probability.

As another step in the analysis, 24 of the bulls[2] were arranged into groups of six each (table 1) according to the fertility of their semen when no antibiotics were added. Their over-all range in fertility was from 48.8 to 83.3 per cent. The lowest group of six bulls had a mean fertility of 57.9 per cent and the highest group, 75.6 per cent. When arranged in this manner, there was some evidence that the addition of antibiotics tended to improve the fertility of the low bulls and lower it for the high bulls. However, the analysis of variance showed that the addition of penicillin and streptomycin to the semen of these 24 bulls did not significantly affect their over-all fertility.

Only a small number of cows was bred with semen on the day that it was collected. On the first day of use most semen was 24 to 36 hr. old and when used on the second day it was 48 to 60 hr. old. Of the 3,143 cows bred with control semen during the first day of use, 70.5 per cent conceived (*i.e.*, did not return for service within 120 to 150 days), while among 3,134 cows bred with treated semen, 70.1 per cent conceived. During the second day of use, 65.1 per cent of the 1,978 cows bred with control semen conceived; similarly, 65.1 per cent of 2,119 cows bred with treated semen conceived. This was a decrease of 5.4 percentage units between the first and second day of use for the control semen and 5.0 percentage units for the treated semen. The difference in the rate of decline in fertility was not significant when tested by Chi-square.

The mean interval between first and second service for the 3,321 cows which did return for second service was 34.7 days. However, the modal interval was 21 days. Since this modal interval probably represents the center point for "normal" intervals, it was decided first to eliminate a sufficient number of long intervals from the data to reduce the mean to approximately 21 days and then to calculate the standard deviation. It was necessary to eliminate all intervals of 30 days or more. The standard deviation then was found to be 3.3 days. It was found that 81.6 per cent of the intervals which were less than 30 days were within the range of 18 to 24 days.

Attention next was given to the possible effect that semen treatment might have had on the percentage of cows having "normal" cycles following service. To measure such a possible effect, the distribution of returns for cows bred with control semen was compared to that for cows bred with treated semen (table 2). The 18- to 24-day group was considered "normal" and the 36- to 48-day group was intended to include all intervals consisting of two normal cycles (*i.e.*, cases in which heat periods may have been missed or calls for service were not made). As shown in table 2 the proportion of returns appearing in the 18- and 24-day group was 4.4 percentage units higher when treated semen was used for service at the previous heat period than when control semen was used. However, in the 25- to 35-day and 49-day or over groups the proportion of returns was 2.7 and 2.9 percentage units lower, respectively, if treated semen had been used. These

[2] One bull was omitted because he was not in service for the entire period.

differences were statistically highly significant and, therefore, would indicate that the addition of antibiotics to semen tends to decrease the number of cows returning to heat after abnormally long intervals. This evidence may lead one to suspect that certain infectious agents in the semen, which are controllable by

TABLE 2

*The time at which cows returned for second service after having been bred with either semen containing antibiotics or that not containing antibiotics*

| Days between 1st and 2nd service | Controls (1,625 cows) | Antibiotics (1,696 cows) |
|---|---|---|
| | (%) | (%) |
| 1–17 | 5.3 | 4.5 |
| 18–24 | 46.7 | 51.1 |
| 25–35 | 11.3 | 8.6 |
| 36–48 | 16.4 | 18.4 |
| 49 or more | 20.3 | 17.4 |

Chi-square = 29.6; $P = < 0.01$

penicillin and streptomycin, might cause early embryonic or fetal deaths and/or pyometritis, either of which might result in abnormally long intervals between heat periods. Of course, there may be other possible explanations and, in any case, further and more specific work should be done before conclusions are drawn.

## SUMMARY

Fertility data were obtained for 184 semen samples collected from 25 bulls. Each sample was split into two equal portions; one portion was used as a control and the other was treated with 1,000 units each of penicillin and streptomycin per milliliter. Of 5,241 cows bred with control semen, 68.4 per cent did not return for second service within 120 to 150 days, while of the 5,299 cows bred with treated semen, 68.1 per cent apparently conceived. The fertility of the bulls ranged from 48.8 to 83.3 per cent. The addition of penicillin and streptomycin to the semen of these bulls did not significantly affect their fertility. The trend, though not statistically significant, was for the antibiotics to improve the low fertility bulls and lower the bulls ranking high in fertility.

Most of the semen was 24 to 36 hr. old during its first day of use. There was a decrease of 5.4 percentage units between the first and second day of use for the control semen and 5.0 percentage units for the treated semen. The antibiotics did not reduce this decline to a significant degree.

Among 3,321 cows which did return for second service, the mean interval between first and second service was 34.7 days. However, the modal interval was 21 days. There were highly significant differences between the distribution of returns for cows bred with control semen and for cows bred with treated semen. Among cows bred with semen containing antibiotics, a larger percentage of the returns were at intervals approximating the expected 3- and 6-wk. intervals, whereas among the controls there was a larger percentage of the returns in the 25- to 35-day and 49-day or over groups.

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# THE EFFECTS OF INFERTILE INSEMINATION AND INDIVIDUALITY OF BULLS UPON THE SUBSEQUENT FERTILITY OF COWS RETURNING FOR SERVICE[1, 2]

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In a study on repeat-breeder cows, Tanabe and Casida (5) observed that Holsteins and Guernseys did not differ significantly in either fertilization rate or in percentage of cows with normal embryos at 34 days after breeding. In a later study, Christian *et al.* (1) found the fertilization rate to be somewhat lower (although not statistically significant on the small numbers involved) in the Guernsey than in the Holstein repeat-breeders when both were bred to bulls of their own breed. When Guernsey cows were bred to Holstein bulls, however, the percentage of cows with normal embryos at 34 days after breeding was significantly higher than in the Holstein cows bred to Holstein bulls.

It was thought that the difference observed in embryonic survival between the two breeds in the later study might have been the result of using a different breed of bull on the Guernsey cows than had been used to characterize them as repeat-breeders, whereas the Holstein cows again were bred to bulls of their own breed.

Later studies (2) have indicated that this difference probably was not due to breed-crossing *per se,* since low-fertility cows bred to other breeds of bulls in an experiment intentionally designed to study the problem did not show an increased conception rate.

Repeat-breeder cows actually constitute a highly selected population, a population of individuals each of which has failed in repeated attempts at fertility with small groups of bulls. A "lack of compatability," if such a phenomenon exists, might express itself under these conditions. As an illustration, bull *A* might be more fertile on the return cows from bull *X* than on those from bull *Y;* bull *B,* on the other hand, might be equally fertile when used on the return cows from either *X* or *Y* or even more fertile following bull *Y* than bull *X*. In addition to this, the return cows from particular first-service bulls might be harder for any bull to settle than return cows in general. Possibly this could result from selection by the first bull (because of a high fertility level on his part), so that only those cows would return that were particularly hard to settle. An-

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[2] The data for this study have been furnished by the Badger Breeders Coop., Shawano, Wis. and by the University of Wisconsin Department of Dairy Husbandry. The authors are indebted to W. H. Dreher and J. H. Webb of the Badger Breeders Coop. for their assistance in collecting a portion of the data.

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other possibility is that there is a general effect of the first bull on his return cows (such as a transmitted infection or embryonic death and a consequent susceptibility to infection) which might make his return cows particularly hard to settle.

The present study is an analysis of the variation in fertility of return cows when bred at second service. It is recognized that likely there would be bull differences in fertility when used on such cows, *i.e.*, bulls vary in general fertility level. The study was to determine if the bull used at first service had an effect on the fertility of his return cows at second service, regardless of the bull to which they were bred (general effect), and further, if the differences in fertility between bulls used for second-service varied significantly depending upon the bulls to which the cows had been bred for the first service (specific effects).

## EXPERIMENTAL

Fertility data were assembled on fifteen Holstein bulls in use at Badger Breeders Coop., Shawano, Wis., during the period from January 1 to June 30, 1949. Only those bulls were selected which, at the time the data were assembled (October, 1949) and on the basis of fertility and general health, appeared to have a good chance to remain in active service for at least 1 more yr. This was done to make experimental study possible in the event of suggestive results from the analysis. Approximately one-third of the inseminations included in this study were made with semen which contained penicillin in the diluter.

A 60-day non-return interval was used in calculating the fertility of an insemination; each cow that did not return for another breeding within 60 days from the date of service was assumed to be pregnant. The per cent fertility was calculated from all first services of the fifteen bulls and the interval between the first and second services was recorded for each cow that returned before 60 days. In computing the second-service fertility of the various bulls, only those services were used in which the first breeding was also to one of these bulls. These second breedings were classified by the bulls used on both first and second service to determine if there were any carry-over effects of the unsuccessful first bull upon the performance of the bull used on second service.

The average 60-day non-return percentage for the 15 bulls (table 1) was 68.3 on 18,217 first services and 64.0 on 3,631 second services, a difference of 4.3 per cent ($P < 0.01$). Although the lowest percentage of non-return for any bull was 54.2 (an acceptable level of fertility), there still were differences between bulls in conception rate at both first and second service ($P < 0.01$). The correlation between the non-return rates for first service and second service for the same bull was 0.73 ($P < 0.01$).

The general effects of the first-service bull were studied by grouping the return cows according to the bull used on them at first service and then determining if there were fertility differences between the groups at second service. All bulls in the study were used on each of these groups at second service. The fertility observed in these groups of cows ranged from 54.9 to 70.8 per cent (table 1, column 8) but when tested by Chi-square gave a probability of 0.10 to 0.20.

Thus, these data fail to indicate definitely that there was a general effect of a bull on the fertility of his return cows. Further, the association between the fertility of each bull on first service and the conception rate of his return cows was not significant ($r = 0.11$).

Determination of the specific effects involved the prediction of how a particular bull would perform on any one of these groups of return cows. This predicted performance for a bull was derived from his own average fertility on all of his second-service cows corrected for the deviation of the fertility of the particular group of cows from the average fertility for all second-service cows. For example: if bull *A* shows 50 per cent fertility on second service, and if the fertility

TABLE 1

*Fertility of Badger Breeders' Coop. Holstein bulls on first and second services (January 1,1949–June 30, 1949)*

| Bull no. | First services of this bull | | | Second services | | | |
|---|---|---|---|---|---|---|---|
| | | | | Of this bull following all bulls | | Of all bulls following this bull | |
| | No. | % non-return | Av. return interval | No. | % non-return | No. | % non-return |
| | | | (*d.*) | | | | |
| 1 | 1,665 | 68.9 | 27.3 | 302 | 61.6 | 304 | 68.1 |
| 2 | 842 | 64.7 | 26.9 | 197 | 59.9 | 166 | 69.9 |
| 3 | 1,116 | 72.7 | 27.5 | 201 | 66.7 | 240 | 62.9 |
| 4 | 2,183 | 66.3 | 27.8 | 478 | 66.7 | 481 | 60.7 |
| 5 | 423 | 68.3 | 27.9 | 57 | 66.7 | 109 | 68.8 |
| 6 | 727 | 65.5 | 27.1 | 153 | 54.2 | 139 | 63.3 |
| 7 | 1,987 | 70.0 | 28.2 | 427 | 63.0 | 414 | 61.8 |
| 8 | 1,331 | 57.8 | 27.9 | 240 | 55.4 | 367 | 59.9 |
| 9 | 1,112 | 71.3 | 29.1 | 237 | 64.6 | 209 | 63.2 |
| 10 | 1,242 | 73.0 | 28.8 | 205 | 69.3 | 221 | 64.3 |
| 11 | 640 | 67.8 | 31.3 | 126 | 69.8 | 102 | 54.9 |
| 12 | 1,476 | 70.1 | 28.9 | 283 | 64.7 | 270 | 66.3 |
| 13 | 1,523 | 69.7 | 28.9 | 303 | 68.0 | 295 | 64.4 |
| 14 | 778 | 72.1 | 27.6 | 195 | 70.3 | 122 | 67.2 |
| 15 | 1,172 | 66.0 | 26.9 | 227 | 58.6 | 192 | 70.8 |
| Total or unweighted mean | 18,217 | 68.3 | 28.1 | 3,631 | 64.0 | 3,631 | 64.4 |

of all second-service cows is 60 per cent, and if all return cows from bull *X* showed 40 per cent fertility at second service, the predicted fertility for bull *A* when he follows bull *X* would be $50-(60-40)$ or 30 per cent. A Chi-square then was calculated testing the actual performance of each bull when used on each "bull-of-first-service" group of cows against the predicted. The total of these Chi-squares for all 225 such comparisons was not significant, thus failing to offer evidence for specific "compatibility" between a second service bull and different groups of return cows.

The difference between bulls in average length of return-interval following first service (table 1, column 4) is significant ($P < 0.01$) indicating, as one possibility, that some bulls are having a larger proportion of cows returning because

of embryonic death than other bulls. In addition, there is a significant negative correlation ($r = -0.68$; $P < 0.01$) between the average return-interval of cows returning from different bulls and their conception rate at second service. If these longer return intervals should be indicative of embryonic death, then, even with bulls of relatively high fertility, some may be affecting the cows to which they are bred in such a way as to produce embryonic death at first service. This condition then makes the cows more difficult to settle at later services.

The failure to find a difference between bulls in general, in the fertility of their return cows may, therefore, have resulted from including only relatively fertile bulls in the study (perhaps those showing the least embryonic death). Also it may have been due to using a 60-day non-return interval as an index of fertility rather than a more reliable measure. It seemed desirable therefore, to investigate this problem in a breeding organization regularly employing pregnancy diagnosis and also not to restrict the study to bulls of high fertility.

Ten bulls, five Holsteins and five Guernseys, in use in the University of Wisconsin artificial insemination project during the period from April 1, 1947, to March 31, 1950, were selected for the second study. The same techniques were used in assembling the fertility data as above, except that those bulls selected had been in use for a period of 3 yr. Also, similar to the above, a portion (approximately one-third) of the inseminations were made with semen to which one or more of the following had been added: penicillin, sulfasuccinate, sulfanilamide or streptomycin.

All herds whose owners regularly used the "pregnancy-diagnosis" service were included in the study. Each cow in these herds that did not return to service within 35 to 49 days from the date of breeding was examined by experienced technicians to determine the presence and normality of an embryo (6). The fertility of each bull was calculated as the percentage of the total number of cows bred which were diagnosed as pregnant at 35 to 49 days after breeding. Long return intervals on cows diagnosed as non-pregnant were "chopped-off" at 60 days so as to make the data from the two studies comparable.

The percentage of cows diagnosed as pregnant at 35 to 49 days after first service (table 2) was 56.2 in the 5,186 cows bred to the five Holstein bulls and 56.4 in the 1,384 cows bred to the five Guernsey bulls. At second service the percentages for the Holsteins were 45.2 on 986 cows and for the Guernseys 49.7 on 288 cows.

The difference between bulls in the fertility of their return cows at second service (table 2) was significant ($P < 0.01$) in both breeds, thus demonstrating a general effect of the bull of first service. Further than this, no evidence was found for specific effects of different bull sequences; the Chi-square, when calculated as in the first study, was not significant within either breed.

One Holstein (H-5) and one Guernsey (G-5) bull were of particularly low fertility on both first and second service. In addition, the return cows from both of these bulls were difficult for other bulls to settle; all bulls following H-5 produced ony 31.9 per cent pregnancy and all bulls following G-5, 22.7 per cent. It appears, then, that these two bulls in particular were affecting the sample of cows

to which they were bred in such a way that their return cows were less likely to conceive at the next service than return cows in general.

The average interval between first and second services was 32.5 and 31.3 days for the Holstein and Guernsey bulls, respectively (table 2, column 4). As above and over both breeds, long return intervals are associated with low fertility of the return cows ($r = -0.58$); the apparent exception was Guernsey bull G-5 on which there was relatively little data.

The results of the study to this point indicated that certain bulls used on first service may affect the conception rate of other bulls used on the return cows at later services. The lowered fertility produced in the cows by the first bull is more apparent for low-fertility bulls than for average-fertility bulls. Also, there is a

TABLE 2

*Fertility of University of Wisconsin bulls on first and second services (April 1, 1947–March 31, 1950)*

| Bull no. | First services of this bull | | | Second services | | | |
|---|---|---|---|---|---|---|---|
| | | | | Of this bull following all bulls | | Of all bulls following this bull | |
| | No. | Pregnant | Av. return interval | No. | Pregnant | No. | Pregnant |
| | | (%) | (*d.*) | | (%) | | (%) |
| H-1 | 1,342 | 63.0 | 31.6 | 222 | 51.4 | 203 | 48.8 |
| H-2 | 576 | 49.1 | 33.3 | 144 | 37.5 | 124 | 41.9 |
| H-3 | 1,238 | 60.3 | 32.0 | 226 | 45.6 | 215 | 57.7 |
| H-4 | 1,333 | 57.8 | 31.7 | 223 | 48.4 | 240 | 44.2 |
| H-5 | 697 | 38.9 | 33.8 | 171 | 39.2 | 204 | 31.9 |
| Total or av. | 5,186 | 56.2 | 32.5 | 986 | 45.2 | 986 | 45.2 |
| G-1 | 326 | 59.8 | 32.4 | 82 | 53.7 | 66 | 50.0 |
| G-2 | 384 | 59.6 | 32.3 | 59 | 49.2 | 70 | 38.6 |
| G-3 | 292 | 50.3 | 28.7 | 67 | 47.8 | 68 | 64.7 |
| G-4 | 285 | 58.9 | 32.2 | 66 | 51.5 | 62 | 54.8 |
| G-5 | 97 | 43.3 | 31.8 | 14 | 28.6 | 22 | 22.7 |
| Total or av. | 1,384 | 56.4 | 31.3 | 288 | 49.7 | 288 | 49.7 |

definite association between long return-intervals (by bulls) and low fertility of the cows at the next service.

Rottensten (3, 4) reported a decreased conception rate of the return cows following service to low fertility bulls. He has postulated that it is due to a "sterility-producing-factor" that is transmitted with the semen and which for a time makes the cow sterile. It is not known to what extent the presence of various antibiotics in the semen used in parts of this study has affected the return-interval length or the fertility of the return cows included in the present study. Rottensten (3) has suggested that the addition of sulfanilamide to the semen diluter lessens the effects of low-fertility bulls on the conception rate of their return cows. His data were not critical, however, because some of his low-fertility bulls were discarded at the time the change was made to a diluter containing sulfanilamide.

If there is a beneficial effect, it would tend to lessen the differences between bulls. Additional studies are needed to determine if the average return-interval length and the fertilty of return cows are affected by adding these substances to the semen diluter.

The records on the fertility of the bulls used to characterize the repeat-breeder cows in the two studies of Tanabe and Casida (5) and Christian *et al.* (1) were re-examined on the assumption that the difference in conception rate between the two samples of Guernsey cows and between the Guernseys and the Holsteins in the latter study may have been due to a difference in the fertility of the individual bulls that characterized them. It was found that the bulls used to characterize the Guernsey cows as repeat-breeders in the two studies were, on the average, of about the same fertility (49.4 and 49.6 per cent). However, the percentage of cows with at least one service to a bull of very low fertility (25 per cent 60- to 90-day non-return) was much lower in the study by Tanabe and Casida, where it was 8 per cent of 25 cows, than in the study by Christian *et al.*, where it was 40 per cent of 35 cows. Further, the percentage of Holstein cows in the latter study, characterized by the very poor bulls, was 0 per cent of 30 cows.

Rottensten (3, 4) states that the infertility produced in cows by the use of a low-fertility bull is only temporary and disappears after four or five additional infertile services. It seems highly possible, in retrospect, that this could account for the high conception rate observed in the Guernsey cows by Christian *et al.*, since in most cases an interval of at least 4 mo. had elapsed between the last service to one of the bulls of very low fertility and the experimental breeding.

It is apparent from the present study that only a portion of the infertility observed in repeat-breeder cows results from a persisting effect of low-fertility bulls on the conception rate of their return cows. None of the Holstein repeat-breeders in the latter study of Christian *et al.*, was characterized as such by bulls of very low fertility, yet they showed a high rate of embryonic death. The finding that the conception rate of return cows from bulls of average to high fertility is more closely associated with the average length of return-interval than with the actual fertility of the bull on first service would indicate that perhaps this fact should be considered in estimating the over-all "fertility-rating" of a bull.

## SUMMARY

Two studies were conducted to determine the effects of the bull used on first service on the fertility of his return cows at second service.

Certain bulls used on first service may affect the conception rate of other bulls used on his return cows at later services. The lowered fertility produced in the cows by the first bull is much more apparent for low-fertility bulls than for average-fertility bulls.

There is a definite association between the average length of the return-interval and the fertility of the return cows at second service, long return-intervals being associated with low fertility.

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# THE RESPONSE OF LOW-FERTILITY COWS TO CHLOROBUTANOL AND ASCORBIC ACID ADMINISTRATION[1, 2]

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The importance of infertility in dairy cattle has been emphasized by the increased attention being paid to accurate breeding records in connection with the rapid growth of artificial insemination. That this problem is of great importance to dairymen is shown by the findings of Barrett *et al.* (3) that 45.6 per cent of the cows were not pregnant after one service.

Tanabe and Casida (23) reported on the nature of the reproductive failure in repeat-breeder cows. Their results show a fertilization rate of 66.1 per cent in cows that had previously been bred four or more times without conception and which had no apparent genital abnormalities upon examination. By 34 days after breeding however, the percentage of cows with normal embryos had dropped to 23 per cent for a loss of 65.1 per cent of the embryos formed. The main problem in these cows appeared to be one of embryonic death. No therapy was attempted in any of the cows during the studies of these workers, the emphasis being placed instead on determining the stage at which the failure occurred.

Many treatments have been suggested for infertility but very few critical data are available on their effectiveness. One such treatment has been the administration of ascorbic acid. Phillips *et al.* (19), McIntosh (17) and Barker (2) report that ascorbic acid increases conception rate in low-fertility cows. None of these experiments was well controlled, however, so no conclusions can be drawn on the actual effectiveness of administration of ascorbic acid. Blood plasma ascorbic acid rises rapidly within a few minutes following its intravenous injection, but the level persists less than 2 hr. (12, 20, 9). Subcutaneous administration results in a slower rise in blood levels, the peak being reached in about 2 hr. The rate of excretion also is slower, the blood plasma level returning to normal in about 24 hr.

In addition to crystalline ascorbic acid, the oral administration of chlorobutanol (trichlorobutyl alcohol) has been observed to increase the blood plasma

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[3] Agent of the Bureau of Dairy Industry, U.S.D.A.

[4] A part of this work has been done under a cooperative agreement between the Wisconsin Agr. Expt. Station and the Bureau of Dairy Industry, U.S.D.A. The funds contributed by the Bureau of Dairy Industry came as an allotment from the Research and Marketing Act.

ascorbic acid levels in rats (14, 15, 22), sheep (7) and cattle (5, 6, 21, 16, 9, 10). The exact action of chlorobutanol in increasing the blood plasma ascorbic acid is not known. Apparently the rise is due to an increased synthesis, since blood and tissue levels are raised and at the same time there is a greater excretion of ascorbic acid in the urine. The effects of feeding chlorobutanol are not evidenced as rapidly as the administration of crystalline ascorbic acid, but the effects are more prolonged. The maximum blood plasma levels usually are not reached until the third or fourth day after feeding is started, but will persist with continued feeding.

The present experiment which was conducted in two different trials, was designed to determine in a controlled study the effects of increased ascorbic acid on embryonic survival in repeat-breeder cows. Since chlorobutanol appeared to give more satisfactory results than crystalline ascorbic acid in elevating and maintaining the blood plasma ascorbic acid levels and also because of the ease of administration, it was decided at the outset to use chlorobutanol as the therapeutic agent. While the study was in progress however, a change was made to include subcutaneous administration of crystalline ascorbic acid as well as feeding of chlorobutanol.

## TRIAL I

### *Materials and methods.*

A total of 94 cows, 52 Guernseys and 42 Holsteins, was studied at a farm operated by Badger Breeders Coop., Shawano, Wis., during the period from October 3, 1948, to February 25, 1949.

The cows were selected by the staff veterinarians of the Cooperative according to the following specifications: (a) bred at least four times previously without apparent conception; (b) produced at least one calf but not more than 10 yr. old; (c) no more than two cows from any one herd; (d) no purulent discharge or abnormality detectable upon manual palpation *per rectum;* and (e) estrual cycles of approximately normal length. Thus, an attempt was made to exclude any cows which had a condition that indicated treatment or obviously might prevent conception.

Only a few animals became available to the experiment at any one time and upon arrival at the research farm they were assigned to their experimental groups (usually alternate animals within the breed to treatment and control). Heat checks were made twice daily at 7 : 00 a.m. and 4 : 00 p.m.; the standing of a cow for other cows to mount was the criterion used to determine estrus. No cows were bred until after they had been in the barn for 10 days, an interval chosen so that the chlorobutanol, where fed, could have time to exercise its effects. All cows were artificially inseminated at the heat check following the appearance of estrus (second heat check) with 1 ml. of day-of-collection semen diluted with yolk-phosphate. This was obtained from the stud bulls regularly used in artificial breeding and was deposited in the cow between the second and third cervical rings. Most of the inseminations were made by one individual to minimize differences in technqiue so far as possible.

One-third of the cows within each experimental group was scheduled to be killed on the third day following the first experimental breeding to determine the fertilization rate. Cleavage of the egg is easily detected by this time and the ova are still in the oviducts, which facilitates their recovery. It would be difficult at a later time to distinguish between normally-cleaved and fragmenting-unfertilized ova. Each ovum was examined under 440× magnification and fertilization was determined by the presence of two or more blastomeres of approximately equal size.

The other two-thirds of the cows within each experimental group were scheduled to be killed on the 34th day to determine the normality of the embryos. The embryos are sufficiently large by this time to permit measurement and macroscopic examination to determine gross normality. Those cows scheduled to be slaughtered at 34 days which returned to estrus before that time, were rebred and slaughtered at 3 days following the second experimental breeding. Thus, they were used to furnish additional information on the fertilization rate.

One-half of the cows within each breed was treated and the other half served as controls. Each treated cow, under the original experimental plan, was fed 5 g. of chlorobutanol[5] daily with the evening feed, beginning with her arrival at the research farm. The plan was changed in the later stages of the experiment, however, so that each treated cow was injected subcutaneously with 3 g. of crystalline ascorbic acid[5] in 10 ml. of distilled water on alternate days from the day of heat until slaughter.

Ascorbic acid determinations were made, by the method of Mindlin and Butler (18), on the plasma of oxalated blood taken from each cow on the day of heat and 2 days after heat. Additional analyses were made on the day of heat and 2 days after heat on the plasma of those cows intended for slaughter at 34 days that returned to heat before that time. Duplicate determinations were made on each sample of blood, the ascorbic acid level being taken as the mean of the two. Duplicate analyses also were made on the anterior pituitary, corpus luteum, endometrium and embryo at the time of slaughter.

The reproductive tracts and pituitary glands from all cows were refrigerated immediately after slaughter and upon return to the laboratory, usually less than 1 hr. later, samples of the tissues to be analyzed were weighed, placed in 5 ml. of 3 per cent meta phosphoric acid and ground with acid-washed sand. Each sample then was filtered and the determinations made on the filtrate.

*Results.*

*General Level of Fertility.* Some difficulties encountered in the early stages of the experiment were interpreted at the time as making it desirable to change the mating plan for the Guernsey cows. The fertilization rate in the first 13 Guernseys was low (31 per cent). In addition, the three Guernsey cows bred to furnish information on the normality of the embryos at 34 days also returned to heat. During the same period, the fertilization rate in the Holstein repeat-breeder cows was satisfactory (86 per cent in seven cows).

[5] Furnished by Merck and Co., Rahway, N. J.

It had been a general opinion that the Guernsey bulls in use at that time were lower in fertility than the Holstein bulls when bred to first-service cows in the field. The 60- to 90-day rate of non-return to first service for the first 6 mo. of 1948 seemed to indicate this (46.4 per cent for the Guernsey bulls and 57.2 per cent for the Holstein bulls); the poor fertility in the first 16 Guernseys may have been due to the Guernsey bulls and for the study of embryonic survival as affected by ascorbic acid level to be effective it was deemed desirable to increase the fertilization rate in the Guernsey cows. Since there was apparently a high rate of fertilization in the Holstein repeat-breeder cows, it was decided to breed the Guernsey as well as the Holstein cows to Holstein bulls in an attempt to increase the fertilization rate.

With this change made, the percentage of cows with embryos at 3 days after experimental breeding was 88 in 26 Holsteins and 70 in 10 Guernseys, all bred to Holstein bulls; this difference is not significant.

A check was made later on the 60- to 90-day rate of non-return to first service in the field for the particular bulls during the exact period when they also were being used on the repeat-breeder cows in the experiment. It was thought that the field records would show that the Guernsey bulls which were used on the experimental Guernsey cows were of lower fertility than the Holstein bulls which were used on the experimental Guernsey cows. Instead, the records showed that these same Guernsey bulls which appeared to give a poor performance on the repeat-breeder Guernseys were performing in the field almost the same as the Holstein bulls which gave a satisfactory performance on the Guernseys (65.1 per cent non-return for these Guernsey bulls and 67.7 per cent non-return for these Holstein bulls). Thus, the differences observed in fertilization rate in the experimental Guernsey cows bred to Holstein bulls as compared to those bred to Guernsey bulls may have been due to chance.

The percentage of cows with normal embryos at 34 days after experimental breeding was 27 in 30 Holsteins and 56 in 36 Guernseys, all bred to Holstein bulls, a difference which is significant. The breed difference in embryonic death rate was a complicating factor in the study of the effects of the ascorbic acid level. The fact that the study was made within breeds, however, lessened this complication.

*Chlorobutanol.* The percentage of Guernsey cows with normal embryos at 34 days after breeding was 60 in 10 control cows and 43 in seven chlorobutanol-fed cows. The corresponding percentages in the Holstein breed were 17 in six control cows and 14 in seven treated cows. Thus, in each breed the percentage of normal embryos at 34 days is lower in the treated cows than in the control cows. These differences are not significant but they do fail to indicate that chlorobutanol is effective in increasing the percentage of normal embryos.

The daily feeding of 5 g. of chlorobutanol mixed with the feed appeared to make the mixture rather unpalatable for many of the cows. Most of the cows consumed all of the mixture but some only after considerable delay. A few of the treated cows appeared to lose their appetite and did not consume as much

feed as the control cows. In addition, it was found at the time of slaughter that many of the treated cows had an emphysema[6] of the omasal wall; this change was not observed in any of the control cows.

The average blood plasma ascorbic acid was 0.42 mg. per 100 ml. and 0.43 mg. per 100 ml. for the control Guernseys and control Holstein cows, respectively, on the day of heat and 0.43 mg. per 100 ml. and 0.40 mg. per 100 ml. 2 days after heat (table 1). In those cows fed 5 g. of chlorobutanol daily, the average on the day of heat was 0.63 mg. per 100 ml. of plasma for the Guernseys and 0.58 mg. per 100 ml. for the Holsteins; 2 days after heat it was 0.60 mg. per 100 ml. for the Guernseys and 0.58 mg. per 100 ml. for the Holsteins. The difference between breeds was not significant either on the day of heat or 2 days after heat

TABLE 1

*Blood plasma ascorbic acid levels, repeat-breeder cows, trial I*

| Treatment | Breed | Day of heat | | 2 d. after heat | |
|---|---|---|---|---|---|
| | | No. samples | mg./100 ml. | No. samples | mg./100 ml. |
| Control | Guernsey | 36 | 0.42 | 35 | 0.43 |
| | Holstein | 29 | 0.43 | 27 | 0.40 |
| Chlorobutanol (5 g. daily) | Guernsey | 18 | 0.63** | 19 | 0.60** |
| | Holstein | 16 | 0.58* | 16 | 0.58* |
| Ascorbic acid (3 g. every other day) | Guernsey | 10 | 0.34 | 10 | 0.39 |
| | Holstein | 9 | 0.33 | 10 | 0.47 |

** Indicates highly significant difference ($P < 0.01$) when compared with the controls of the same breed.

* Indicates significant difference ($P < 0.05$) when compared with the controls of the same breed.

nor was there a significant difference between days. There was a significant difference between the control cows and chlorobutanol-fed cows in both breeds on the day of heat and 2 days after heat.

[6] The following report was submitted by S. H. McNutt of the Veterinary Science Department who studied the histopathology of the omasal wall: Elongated or eliptical gas filled pockets up to 3 × 1.5 cm. in size were observed anywhere in the omasal wall—from the sub-epithelial inner lining outward to the serosal membrane but not bulging appreciably above the usual serosal surface. The omasal muscle was especially involved. That such pockets were not spherical but were elongated indicated that their shape was determined by the tissue structures in which they developed; the gas or gases formed or deposited in the omasal wall pushed the tissue structures apart. The large pockets predominated, small ones were few. Often as many as 100 or more were present—enough to cause the omasal wall to be double its normal thickness. The pockets were filled with a colorless, apparently odorless gas of undetermined nature. No inflammatory reaction was present. In so far as is known emphysema of the omasum has not been previously observed in any condition. The condition resembles mesenteric emphysema of swine to some extent except that the mesentery and serosa is involved in swine and the pockets of gas are usually spherical and smaller. Mesenteric emphysema of swine is also non-inflammatory. It has been produced in swine fed certain deficient diets. The pronounced effect of feeding chlorobutanol to certain of these cows suggests that the omasum may have a very important function and that chlorobutanol might be employed as a tool in the study of omasal physiology.

When both breeds of cows are grouped together, the average blood plasma ascorbic acid level in the control cows was 0.42 mg. per 100 ml., which is in good agreement with the average value for normal cows as reported by Phillips *et al.* (19) (0.39 mg. per 100 ml.) and Bortree *et al.* (4) (0.44 mg. per 100 ml.). This would not indicate that the level in these repeat-breeder cows was sub-normal, which was suggested as a cause of the infertility of repeat-breeder cows by Phillips *et al.* These same workers also reported a distinct rise at the time of heat in plasma ascorbic acid; no such rise was detected in these cows. Just why there should be a rise at the time of heat is difficult to understand since both estrogen and gonadotropin have been reported to decrease ascorbic acid levels (8, 1, 13) and both of these hormones are thought to be particularly high during and immediately after heat.

There were no significant differences between breeds in tissue-ascorbic acid levels and the results from the two breeds have been combined (table 2). The

TABLE 2

*Tissue ascorbic acid levels of repeat-breeder cows, trial I*

| | | At time of slaughter (mg./g.) | | | | |
|---|---|---|---|---|---|---|
| | | Anterior pituitary | 3-d. corpus luteum | 34-d. corpus luteum | Endo-metrium | Embryo |
| Control | Guernsey | 1.853 (26)[a] | 0.669 (15) | 2.360 (11) | 0.158 (26) | 0.058 (9) |
| | Holstein | 1.988 (20) | 0.643 (14) | 2.430 (4) | 0.156 (20) | 0.053 (3) |
| Chlorobutanol | Guernsey | 2.133* (14) | 0.748 (11) | 2.695 (3) | 0.188* (14) | 0.077 (3) |
| | Holstein | 2.046 (11) | 0.785** (9) | 2.717 (2) | 0.194** (11) | 0.072 (2) |
| Ascorbic acid | Guernsey | 1.816 (11) | 0.656 (4) | 2.259 (7) | 0.148 (10) | 0.051 (6) |
| | Holstein | 1.798 (10) | 0.564 (6) | 2.342 (4) | 0.143 (10) | 0.048 (4) |

[a] Numbers in parenthesis indicate number of duplicate determinations.

* Indicates significant difference ($P < 0.05$) when compared with the controls of the same breed.

** Indicates highly significant difference ($P < 0.01$) when compared with the controls of the same breed.

levels in the anterior pituitary, 3-day corpus luteum and endometrium were significantly higher in the chlorobutanol-fed cows than in the control cows. The levels in the 34-day corpus luteum and embryos, although distinctly higher in the chlorobutanol-treated cows, were not significantly different from the controls, mainly, it is thought, because of the few chlorobutanol-fed cows available for the comparisons. It appears that this dosage of chlorobutanol was effective in increasing the tissue and blood ascorbic acid levels.

Because chlorobutanol appeared to be unpalatable and may have induced certain pathological changes, making it undesirable as a practical treatment, this treatment was discontinued and the emphasis was placed on a more direct method of treatment, *viz.*, subcutaneous ascorbic acid administration.

*Ascorbic acid.* The average ascorbic acid concentration in the blood plasma, anterior pituitary, 3-day corpus luteum, 34-day corpus luteum, endometrium and embryo of these cows receiving 3 g. of ascorbic acid every other day from the

day of heat until slaughter, were not significantly different from the levels for corresponding tissues in the controls (tables 1 and 2). This dosage of ascorbic acid might not be expected to maintain increased levels however, since later work incidental to the study showed that approximately 6 g. daily are required to maintain materially increased blood levels in heifers. It is possible that there was a temporary increase in ascorbic acid levels following injection, but it was not detected because most of the tissue samples were not obtained for the determinations until 24 to 36 hr. later and Erb *et al.* (9) have indicated that blood levels return to normal within 24 hr. after injection.

There were normal embryos at 34 days after breeding in 52 per cent of the 21 cows of both breeds that received 3 g. of ascorbic acid every other day from the day of heat until slaughter and in 40 per cent of the 15 control cows. This difference of 12 per cent in favor of ascorbic acid therapy is not statistically significant. Within the Guernsey breed there were normal embryos at 34 days in 64 per cent of 11 treated cows and in 50 per cent of 8 control cows, a difference of 14 per cent in favor of ascorbic acid treatment. In the Holstein breed there were normal embryos at 34 days in 40 per cent of 10 treated cows and in 29 per cent of 7 control cows, a difference of 11 per cent, also in favor of ascorbic acid treatment. Neither of these differences is significant.

Ascorbic acid administration did not maintain increased ascorbic acid levels in the blood or in any of the tissues studied, but it did increase the percentage of normal embryos at 34 days slightly in both breeds in this particular trial.

## TRIAL II[7]

The point of view was adopted arbitrarily at the beginning of this trial that continuance of the work would depend on one additional test. A trial involving control and treated groups, 10 to 12 cows each, would be run and a difference of 20 per cent in favor of the treated cows would be required if the study was to be continued.

### *Materials and Methods.*

This portion of the study was conducted at the experiment station during the period from January 17, to July 12, 1950, and included 11 Guernseys and 11 Holsteins. The cows were procured through various artificial breeding organizations in the state of Wisconsin and were examined and purchased by the authors according to the same specifications as for Trial I.

The experimental design was the same as in the earlier trial except that the cows were not assigned to their treatment groups until they came in heat. At that time alternate cows within the breed were assigned to the treated and control groups. The treated cows received the same dosage of crystalline ascorbic acid as before. Since the main objective was to determine the effects of ascorbic

[7] The help of the following organizations in obtaining experimental animals for this portion of the study is greatly appreciated: Dane County Cooperative, D.H.I.A., Madison; Badger Breeders Cooperative, Shawano; Tri-State Breeders Cooperative, Westby; Rock County Breeders Cooperative, Janesville.

acid therapy on embryonic survival, all cows were scheduled to be slaughtered at 34 days after breeding. The cows that returned to heat before that time were rebred and slaughtered at 3 days after the second experimental breeding to furnish information on the fertilization rate.

*Results.*

The general fertility was very low in both breeds of repeat-breeder cows in this sample, as evidenced by the low fertilization rate (23 per cent in 13 "return" cows). The injection of 3 g. of ascorbic acid on alternate days, from the day of heat until slaughter, did not appear to increase the conception rate materially; the percentage of cows having normal embryos at 34 days was 0 in 12 control cows and 10 in 10 ascorbic-acid treated cows.

As indicated above, continuance of the work was contingent upon at least 20 per cent more of the treated cows having normal embryos than of the control

TABLE 3

*Effect of chlorobutanol and ascorbic acid administration on repeat breeding*

| Trial | Breed | Treatment | Control | | Experimental | |
|---|---|---|---|---|---|---|
| | | | No. cows | % with normal embryos | No. cows | % with normal embryos |
| I | Guernsey | Chlorobutanol | 10 | 60 | 7 | 43 |
| | | Ascorbic acid | 8 | 50 | 11 | 64 |
| | Holstein | Chlorobutanol | 6 | 17 | 7 | 14 |
| | | Ascorbic acid | 7 | 29 | 10 | 40 |
| II | Guernsey | Ascorbic acid | 6 | 0 | 5 | 20 |
| | Holstein | Ascorbic acid | 6 | 0 | 5 | 0 |
| Total or unweighted mean | | | 43 | 26 | 45 | 30 |

cows at 34 days after breeding. Therefore, it was decided to explore other possibilities for preventing embryonic death.

## DISCUSSION

In these studies, 5 g. of chlorobutanol fed daily appeared to increase blood plasma and tissue ascorbic acid levels materially. It has been suggested that the elevated ascorbic acid levels found when chlorobutanol is fed are due to an increased synthesis of this vitamin within the body. Longenecker *et al.* (14) observed that when rats are fed 20 mg. of chlorobutanol daily there is a marked increase in ascorbic acid excretion; this high rate of excretion can be maintained for as long as 3 mo. with continued feeding. No deficiency symptoms were noted by these workers during this period. That at least some synthesis takes place while chlorobutanol is fed has been indicated by Jackel *et al.* (11). They found that approximately 0.30 per cent of radioactive carbon fed as glucose is present in excreted ascorbic acid within 24 hr.

No studies were made on ascorbic acid excretion in these cows and it is not known if it was affected. No evidence was noted in the present studies, how-

ever, that the utilization of this vitamin was in any way decreased, since no clinical symptoms of a vitamin C deficiency were noted even after 2 mo. of chlorobutanol feeding. Chlorobutanol was assumed to increase ascorbic acid synthesis and provide for maximum utilization. The results from the study on chlorobutanol feeding, consequently, have been combined with the two studies on ascorbic acid injection.

When the results of the three studies are combined (table 3), the percentage of cows with normal embryos at 34 days after breeding becomes 26 in 43 control and 30 in 45 treated cows. The very slight advantage in favor of treatment did not give evidence of ascorbic acid deficiency being a major factor in the repeater-cow problem.

## SUMMARY

This study covers 116 cows, 53 Holsteins and 63 Guernseys, each of which had been bred four or more times previously without apparent conception.

Five g. of chlorobutanol fed daily increased the ascorbic acid concentration of the blood plasma on the day of heat and 2 days after heat and also increased the ascorbic acid concentration of the anterior pituitary, 3-day corpus luteum and endometrium as determined at the time of slaughter. The feeding of chlorobutanol with the feed made the mixture unpalatable to many of the cows and apparently induced an emphysema of the omasal wall.

Three g. of ascorbic acid injected subcutaneously every other day from the day of heat until slaughter failed to maintain an increased ascorbic acid content of the blood plasma, 3-day corpus luteum, 34-day corpus luteum, endometrium or embryo.

When the results of the studies with chlorobutanol and ascorbic acid are combined, the percentage of cows with normal embryos at 34 days after breeding is 26 in 43 control cows and 30 in 45 treated cows.

There is no evidence from these studies that ascorbic acid stimulation is significantly beneficial in increasing conception rate in repeat-breeder cows.

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# THE RESPONSE OF LOW-FERTILITY COWS TO INSEMINATION WITH SEMEN FROM BULLS OF ANOTHER BREED[1, 2, 3]

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Early embryonic mortality is a major cause of infertility in farm animals. Warnick *et al.* (5) reported that embryonic death in the first 25 days of gestation is a major cause for repeat-breeding in sows (67.4 per cent). Laing (3) estimates the incidence of embryonic mortality in commercial cattle at approximately 30 per cent. In a study on the nature of the reproductive failure in cows of low fertility, Tanabe and Casida (4) found a fertilization rate of 66.1 per cent but by 34 days after breeding the percentage of cows with normal embryos had dropped to 23.1 for a death rate of 65.1 per cent of the embryos.

It does not appear likely that much of the embryonic mortality observed in artificial insemination is due to early genetic lethals, since several different bulls may be used at the various services on any one "repeat-breeder" cow and it is unlikely that a very high proportion of the bulls is carrying the same lethal gene. In addition, repeat-breeding in two herds studied by Casida (1) appeared to be only 14 to 18 per cent repeatable in immediately succeeding service periods of the same cow and only 8 to 11 per cent repeatable from dam to daughter in comparable service periods. It would appear that the major amount of variations in number of services required for conception is due to temporary environmental factors.

It is a general opinion among dairy farmers, however, that breeding a "repeater" cow to a bull of another breed increases the probability of her conceiving; such a plan of inseminating is commonly practiced, especially in commercial herds.

In a previous study on repeat-breeding in dairy cattle (2), it was noted that there was a higher percentage of normal embryos at 34 days after breeding in the Guernsey cows bred to Holstein bulls than in the Holstein cows bred to Holstein bulls. It could not be determined, however, if the difference in embryonic death was due to using a different breed of bull (crossbreeding) because the experiment did not include reciprocal matings.

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[3] The authors are indebted to the following for aid in locating experimental animals for this study: Badger Breeders Coop., Shawano, Wis.; Tri-State Breeders Coop., Westby, Wis.; Dane County Coop., D.H.I.A., Madison, Wis.; Rock County Breeders Coop., Janesville, Wis.; Land O'Lakes Creameries, Herd Improvement Division, Minneapolis, Minn.

[4] Agent of the Bureau of Dairy Industry, U.S.D.A.

[5] This work was done under a cooperative agreement between the Wisconsin Agricultural Experiment Station and the Bureau of Dairy Industry, U.S.D.A. The funds contributed by the Bureau of Dairy Industry came as an allotment from the Research and Marketing Act.

The present study was designed to determine the effects of crossbreeding on embryonic survival in cows bred four or more times previously and which showed no detectable genital abnormalities.

## MATERIALS AND METHODS

This study was conducted in two parts. The first was at the Experiment Station during the period from April 20, 1950, to October 28, 1950, and included 27 cows, 13 Guernseys and 14 Holsteins. The second part was a field study conducted on nine cooperating farms located in six Wisconsin counties and involved 39 cows, 13 Guernseys and 26 Holsteins.

*Experiment Station study.* Details on the selection of the cows, management and procedures followed have been described previously (2). The animals were assigned to their experimental groups at the time of heat; alternate cows were bred to bulls of their own breed (control group) and to bulls of the other breed (crossbred group). As before, all cows were scheduled to be killed at 34 days after the experimental breeding. Those cows that returned to heat before that time were rebred and slaughtered at 3 days after the second experimental breeding to furnish information on the fertilization rate.

*Field study.* Each of the nine farmers involved agreed to permit any cow in his herd that returned for a fifth service to be used in the study. The same criteria were used to select the cows as above, except that the number of cows from any one herd was not limited to two. The herd inseminator examined each cow at the time of heat and made the decision as to her suitability. Each cow that met all requirements according to instructions given the inseminator then was assigned to the proper experimental group; alternate cows within each herd were bred to a bull of the same breed (control group) and to a bull of another breed (crossmated group). A cow was used only once in the experiment whether or not she became pregnant to the experimental breeding. All cows that did not return to heat after the experimental breeding were examined for pregnancy by the authors 35 to 70 days after breeding by the method of Wisnicky and Casida (6).

## RESULTS

*Experiment Station study.* Normal embryos were found at 34 days after breeding in only two cows from the 14 control matings of both breeds (table 1). The 13 cross-matings also yielded only two normal embryos. A distinct difference was observed in the incidence of normal embryos between the two breeds of cows; the 14 Holsteins yielded six embryos, whereas the 13 Guernseys yielded none. The fertilization rate was determined in four Holsteins that returned in heat before 34 days and in seven Guernseys; two of the former had fertilized eggs and five of the latter.

*Field study.* No estimates could be made of the fertilization rate and normality of the embryos in the various cows involved in this study because the animals were not slaughtered. The embryos were considered normal however, if the quantity of chorionic fluid and size and consistency of the amniotic vesicle were, by palpation, determined to be approximately normal.

TABLE 1

*Effects of crossbreeding upon percentage of cows with normal embryos (Experiment Station study)*

| Mating | 34 d. after first experimental breeding | | 3 d. after second experimental breeding | |
|---|---|---|---|---|
| | No. cows | % with normal embryos | No. cows | % with fertilized eggs |
| Guernsey ♀♀ × Guernsey ♂♂ | 7 | 0 | 3 | 100 |
| Holstein ♀♀ × Holstein ♂♂ | 7 | 57 | 1 | 100 |
| Guernsey ♀♀ × Holstein ♂♂ | 6 | 0 | 4 | 50 |
| Holstein ♀♀ × Guernsey ♂♂ | 7 | 29 | 3 | 33 |

Of the 39 cows involved in this study, 10 became pregnant to the experimental breeding. There was a higher conception rate in the Holstein cows (eight of 26) than in the Guernsey cows (two of 13).

In the 13 Holstein cows bred to Holstein bulls, five were diagnosed as pregnant and in the 13 Holstein cows bred to other breeds of bulls (Guernsey and Angus), three were diagnosed as pregnant (table 2). One of the six Guernsey cows bred to Guernsey bulls and one of the seven Guernsey cows bred to other breeds of bulls (Holstein and Jersey) became pregnant to the experimental breeding. When both breeds of cows are combined the number of cows diagnosed pregnant becomes six of the 19 control and four of the 20 cross-mated.

## DISCUSSION

When the results from both the Experiment Station and the field study are combined, the unweighted percentage of normal embryos is 28 in the 33 control matings and 16 in the 33 crossbred matings.

It was noted above that a previous study of repeat-breeding cows gave evidence of a lower incidence of embryonic death in the Guernseys bred to Holstein bulls than in the Holsteins bred to Holstein bulls (2). It was thought that the low embryonic death rate in the Guernseys might have been the result of using a different breed of bull than was used to characterize them as repeat-breeders. The results of the present study fail to furnish support for this hypothesis; the percentage of cows with normal embryos being the same (8 per cent) whether the repeat-breeding Guernseys were bred to Guernsey bulls or to other breeds of bulls.

TABLE 2

*Effects of crossbreeding upon percentage of cows pregnant (Field experiment)*

| Breed of cow | Breed of bull | No. of Matings | Percent pregnant |
|---|---|---|---|
| Holstein | Holstein | 13 | 38 |
| Holstein | Other than Holstein | 13 | 23 |
| Guernsey | Guernsey | 6 | 17 |
| Guernsey | Other than Guernsey | 7 | 14 |

SUMMARY

This study included 66 cows each of which had been bred four or more times previously without apparent conception. There was a distinct breed difference in conception rates; 35 per cent of the Holsteins and 8 per cent of the Guernseys had normal embryos.

There is no evidence from this study that inseminating with semen from bulls of another breed is beneficial in increasing conception rate in repeat-breeder cows. The unweighted percentage of cows with normal embryos was 28 in the control cows and 16 in the cross-mated cows.

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# MANGANESE, TRYPSIN, MILK PROTEINS AND THE SUSCEPTIBILITY OF MILK TO OXIDIZED FLAVOR DEVELOPMENT[1, 2]

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Much has been learned regarding those factors which promote and those which inhibit development of an oxidized flavor in market milk. For a complete study of this information the reader is referred to the reviews by Brown and Thurston (5) and Greenbank (12). The chemical mechanism by which these practical factors function have been studied less thoroughly. Information about such chemical mechanisms will broaden our field of knowledge and eventually may lead to more suitable methods of control.

The relationship between oxidized flavor development and the ascorbic acid content of milk has received much attention (5, 12). Kende (17) noted that addition of ascorbic acid to milk would give a marked degree of protection. Dahle and Palmer (7) confirmed this finding and noted, in addition, that by the time an oxidized flavor had developed, the ascorbic acid had decreased to a low level. Since such a decrease also occurred in milk which failed to develop an oxidized flavor, they concluded that the factor responsible for development of the off-flavor was not necessarily responsible for the reduction in ascorbic acid. Sharp *et al.* (23) found a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of oxidized flavor. Olson and Brown (20) attributed oxidized flavor development to the presence of $H_2O_2$ produced as a result of oxidation of ascorbic acid with $Cu^{++}$ acting as catalyst. In a later paper (21) they stated, with reference to oxidized flavor, that ". . . it is readily seen that it can be prevented . . . by the removal of copper ions so that the ascorbic acid is not oxidized." A number of observations do not conform with this view. Garrett (9) found that addition of $Mn^{++}$ to milk containing added $Cu^{++}$ would prevent flavor development yet had no effect on the rate of oxidation of ascorbic acid. Later, Hartman and Garrett (13) concluded that the ascorbic acid of milk acts as an antioxidant in that its presence delays onset of the reaction which leads to an oxidized flavor. A different view has been taken by Krukovsky and Guthrie (18, 19). They observed that complete oxidation of ascorbic acid to dehydroascorbic acid, prior to pasteurization of the milk, would prevent development of the flavor in the presence of $Cu^{++}$ and that the reaction, by which the flavor was produced, could be induced again by addition of ascorbic acid to milk so treated. They concluded (19) that development of oxidized flavor took place most readily when the ascorbic-dehydroascorbic acid ratio was less than or

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approaching one. They attributed the protective action of large amounts of added ascorbic acid to exhaustion of the dissolved oxygen prior to establishment of this favorable ratio between the two forms of vitamin C. Such a mechanism, however, does not explain why the addition of similar amounts of ascorbic acid to washed cream causes rapid development of the flavor (20).

Kende (16, 17) suggested that the sensitivity of milk to metal-induced oxidized flavor depended upon the relative amounts of unknown protective substances, concluding that these inhibiting substances were actually reducing substances in a chemical sense and explaining the protective effects of high heat treatments and bacterial growth in terms of the production of such reducing substances. Other observations in the literature support his stand. Stebnitz and Sommer (24), for instance, found that milk fat produced in summer was more readily oxidizable than that produced in winter, yet winter milk was more susceptible to oxidized flavor development. They believed that summer milk must, therefore, contain a higher concentration of protective substances in the serum. Gould and Sommer (11) and Gould (10) attributed the protective action of high heat treatments to the liberation of sulfides.

The addition of $Mn^{++}$ to milk affords protection against oxidized flavor development (9) but has no such effect when added to washed cream containing ascorbic acid and Cu (21). Ascorbic acid additions to milk also afford protection but such additions to washed cream promote flavor development (20). Heating milk at 180° F. for 5 min. previous to separating and the preparation of washed cream does not reduce the susceptibility of the cream to oxidized flavor development when ascorbic acid and Cu were added (4). These observations confirm the earlier belief of Kende (16), namely, that reducing substances in the serum are involved in the protective action. What these reducing substances are and their source has not been definitely established. Gould and Sommer (11) attributed the sulfides obtained by high heat treatments to a protein which was closely associated with the fat. The observation that a pancreatic enzyme treatment affords protection against oxidized flavor development (1, 2, 6) also suggests that milk proteins may be the source of these reducing substances.

The work reported herein is in substantial agreement with earlier findings and offers additional evidence that some constituent of milk serum must be involved in determining the susceptibility of milk to oxidized flavor development.

## EXPERIMENTAL

*The protective action of manganese.*

(a) *Addition of manganese sulfate to milk.* Garrett (9) showed that additions of $MnSO_4$ to susceptible (26) milk contaminated with Cu would inhibit or greatly retard development of an oxidized flavor. No observations were made with spontaneous (26) milk, however. The experiment described below was undertaken to investigate this point.

A quart of milk was obtained from each of two cows (no. 19 and 20) known to have produced spontaneous milk in the immediate past. Precautions were taken to guard against Cu and Fe contamination. Brought to the laboratory

immediately after collection, these samples were cooled to 50° F., and eight 100-ml. portions from each sample were set up on clean 0.5-pt. milk bottles. In each case, four of these portions were used for a raw sample series, and four for a pasteurized sample series. For the latter purpose, the samples were pasteurized in the bottles at 143 to 145° F. for 30 min., and then cooled to 50° F. The four samples in each series then were prepared by the addition of $MnSO_4$ solution to represent $Mn^{++}$ concentrations of O (Control), 0.001, 0.005 and 0.01 moles per liter, respectively. When the samples were examined after 4-days storage at 40° F., the milk from cow 19 showed no oxidized flavor in the raw control, and only a slight oxidized flavor in the pasteurized control; while the milk from cow 20 showed a pronounced oxidized flavor in the raw control and a very pronounced oxidized flavor in the pasteurized control. No oxidized flavor could be detected by the judges in any of the samples containing added $Mn^{++}$.

$Cu^{++}$ added to milk to induce oxidized flavor is rendered less effective by subsequent pasteurization. An experiment was conducted to determine whether pasteurization might similarly influence the effectiveness of $Mn^{++}$ in preventing development of the flavor. A quantity of susceptible (26) milk was obtained and five 100-ml. portions were set up in 0.5-pt. milk bottles. These samples then were pasteurized in the bottles, as in the previous experiment. Sample 1 served as the control; to samples 2 and 3 $Mn^{++}$ was added, at the rate of $10^{-4}$ mole per liter, before pasteurization; to samples 4 and 5 the same addition was made after pasteurization. $Cu^{++}$ was added at the rate of $10^{-5}$ mole per liter to all of the samples after pasteurization. When the samples were examined after 60-hr. storage at 40° F., only the control showed an oxidized flavor, and it was pronounced. Apparently, pasteurization after the $Mn^{++}$ addition does not impair the effectiveness of the $Mn^{++}$ in preventing the development of the oxidized flavor in $Cu^{++}$ contaminated milk.

(b) *Influence of added $Mn^{++}$ on the rate of disappearance of ascorbic acid.* An experiment was undertaken to test further the report (9) that addition of $Mn^{++}$ to milk containing added $Cu^{++}$ did not influence the rate of disappearance of ascorbic acid. One qt. of fresh milk was pasteurized at 143 to 145° F. for 30 min., cooled rapidly to 56° F. and the ascorbic acid content was estimated by the method of Sharp (22). The ascorbic acid content was 17 mg. per liter. From this pasteurized milk, four portions of 200 ml. were set up in 0.5-pt. milk bottles to represent: (a) No added $Cu^{++}$ or $Mn^{++}$; (b) added $Cu^{++}$, but no added $Mn^{++}$; (c) no added $Cu^{++}$, but added $Mn^{++}$; (d) both added $Cu^{++}$ and $Mn^{++}$. The $Cu^{++}$ additions were $10^{-5}$ mole per liter; the $M^{++}$ additions were $10^{-4}$ mole per liter. After 24 hr. of storage at 40° F., the ascorbic acid content of samples 1 and 3 was 10.2 and 10.0 mg. per liter, respectively; it was 0.56 and 1.2 mg. per liter in samples 2 and 4, respectively. Sample 1 showed a slight, and sample 2 showed a pronounced oxidized flavor in 48 hr.; samples 3 and 4 did not show any oxidized flavor. The presence of the added $Mn^{++}$ did prevent the oxidized flavor, even though there was no significant effect on the rate of disappearance of ascorbic acid.

(c) *Addition of $MnSO_4$ to washed cream.* The experiments described in the previous sections, together with the work of Garrett (9), clearly demonstrate that the addition of $Mn^{++}$ to susceptible or spontaneous milk will prevent or greatly retard development of an oxidized flavor. Olson and Brown (21) have reported that such addition of $Mn^{++}$ to washed cream containing added ascorbic acid and $Cu^{++}$ is much less effective in retarding development of this flavor. The following experiment was undertaken to observe the protective action of $Mn^{++}$ in washed cream.

Twenty-five lb. of fresh raw milk were warmed to 100 to 105° F. and separated. The cream obtained was washed, by dilution and reseparation, four times with distilled water at a temperature of 100 to 105° F. During separation of the milk and washing the cream, the product came in contact only with the stainless steel of the separator and the tin of well-tinned milk cans. This washed cream then was used to set up the 16 samples described in table 1. $Cu^{++}$

TABLE 1

*Influence of added $Mn^{++}$, $Cu^{++}$ and ascorbic acid on the development of oxidized flavor in washed cream*

| Sample no. | $Cu^{++}$ conc. | $Mn^{++}$ conc. | Ascorbic acid conc. | Oxidized flavor intensity[a] |
|---|---|---|---|---|
| | *(moles/l.)* | *(moles/l.)* | *(mg./l.)* | |
| 1 | 0 | 0 | 0 | − |
| 2 | 0 | 0 | 0 | − |
| 3 | $10^{-5}$ | 0 | 0 | − |
| 4 | $10^{-5}$ | 0 | 0 | − |
| 5 | 0 | 0 | 100 | + |
| 6 | 0 | 0 | 100 | + |
| 7 | 0 | $10^{-4}$ | 0 | − |
| 8 | 0 | $10^{-4}$ | 0 | − |
| 9 | $10^{-5}$ | 0 | 100 | + |
| 10 | $10^{-5}$ | 0 | 100 | + |
| 11 | $10^{-5}$ | $10^{-4}$ | 0 | − |
| 12 | $10^{-5}$ | $10^{-4}$ | 0 | − |
| 13 | 0 | $10^{-4}$ | 100 | + |
| 14 | 0 | $10^{-4}$ | 100 | + |
| 15 | $10^{-5}$ | $10^{-4}$ | 100 | + |
| 16 | $10^{-5}$ | $10^{-4}$ | 100 | + |

[a] − = none, + = slight oxidized flavor after 48-hr. storage at 40° F.

was added from a $10^{-3}$ molar solution of $CuSO_4$, $Mn^{++}$ from a 0.1 molar solution of $MnSO_4$ and ascorbic acid from a freshly prepared solution containing 10 mg. of ascorbic acid per milliliter. Samples were stored for 48 hr. at 40° F. previous to examination for oxidized flavor.

The results presented in table 1 reveal that an oxidized flavor developed in every sample to which ascorbic acid was added. $Mn^{++}$ had no influence in retarding development of the flavor and $Cu^{++}$ showed no tendency to catalyze flavor development. An oxidized flavor developed in those samples containing ascorbic acid, whether or not $Cu^{++}$ and/or $Mn^{++}$ also was present.

*Prevention of oxidized flavor development by action of trypsin.*

Anderson (1, 2) and others (6) have shown that the development of an oxidized flavor in pasteurized milk may be prevented by treating the milk, before

pasteurization, with a very low concentration of a pancreatic enzyme preparation. Undoubtedly this preparation consists of a mixture of several pancreatic enzymes. Which of these enzymes is responsible for the prevention of oxidized flavor has not been determined, so far as the writers are aware. The three trials described below were undertaken to determine if the action of pure pancreatic trypsin would prevent flavor development.

Milk for these trials was obtained from cows 19 and 20 which previously had been shown to produce spontaneous milk (26). At the time these trials were conducted, however, cow 19 was producing susceptible rather than spontaneous milk. Brought to the laboratory immediately after collection, the milk to be used was cooled to 50° F. and the desired number of 100-ml. samples set up in 0.5-pt. milk bottles. All samples then were brought to a temperature of 100° F.

TABLE 2

*Concentration of trypsin required to prevent development of oxidized flavor in spontaneous milk*

| Sample no. | 0.015 *M* phosphate added | Trypsin solution added | Trypsin conc. | Oxidized flavor intensity[a] | Remarks |
|---|---|---|---|---|---|
| | (*ml.*) | (*ml.*) | (*mg./100 ml.*) | | |
| 1–19 | 0.1 | | | – | |
| 2–19 | 1.0 | | | – | |
| 3–19 | 10.0 | | | – | |
| 4–19 | | 0.1 | 0.05 | – | |
| 5–19 | | 1.0 | 0.5 | ? | Very bitter |
| 6–19 | | 10.0 | 5.0 | ? | Very bitter |
| 1–20 | 0.1 | | | + | |
| 2–20 | 1.0 | | | + | |
| 3–20 | 10.0 | | | + | |
| 4–20 | | 0.1 | 0.05 | – | |
| 5–20 | | 1.0 | 0.5 | ? | Very bitter |
| 6–20 | | 10.0 | 5.0 | ? | Very bitter |

[a] – = none, + = slight after 4-d. storage at 40° F., ? = too bitter to distinguish any other flavor.

in a water bath and trypsin solution added to the desired samples. The trypsin solution used was prepared by dissolving 50 mg. of crystalline trypsin in 50 ml. of 0.015 *M* phosphate buffer (pH 6.8). The dry crystalline trypsin contained approximately 50 per cent $MgSO_4$; hence, the solution contained 0.5 mg. trypsin per milliliter. Following addition of trypsin solution, all samples were incubated at 100° F. for 15 min., after which they were brought quickly (*ca.* 15 min.) to the pasteurizing temperature. Pasteurization was carried out at 143 to 145° F. for 30 min. and was followed by rapid cooling to 50° F. $Cu^{++}$ then was added in those cases where Cu was used and the samples stored at 40° F. for flavor development.

Table 2 shows the setup of the samples and results of the first trial in which trypsin was added. This trial was composed of two groups of samples, six

samples to each group. The first group of six samples was prepared from the milk of cow 19 and the second group from the milk of cow 20. Control samples 1–19, 2–19 and 3–19 failed to develop an oxidized flavor; hence, the absence of the flavor in 4–19 had no significance. Samples 1–20, 2–20 and 3–20, control samples for the second group, all developed an oxidized flavor, whereas sample 4–20, to which 0.05 mg. of trypsin had been added, failed to develop the flavor. The action of 0.05 mg. of trypsin per 100 ml. of milk was sufficient to prevent development of an oxidized flavor in this case. Samples 5–19, 6–19, 5–20 and 6–20 were intensely bitter indicating that too high a concentration of trypsin was used in these samples.

Table 3 shows the concentration of trypsin added to samples prepared in the second trial and the intensity of oxidized flavor which developed. Test 1 samples were prepared from the milk of cow 19 and test 2 samples from the milk of cow 20. $Cu^{++}$ at a concentration of $10^{-5}$ moles per liter was added to these

TABLE 3

*Influence of various concentrations of trypsin in retarding development of $Cu^{++}$ induced oxidized flavor*[a]

| Trypsin conc. | Oxidized flavor intensity[b] | |
|---|---|---|
| | Test 1 | Test 2 |
| *(mg./100 ml.)* | | |
| 0.0 | + | ++ |
| 0.0 | + | ++ |
| 0.001 | ± | ++ |
| 0.001 | ± | ++ |
| 0.01 | − | ++ |
| 0.01 | ± | + |
| 0.1 | − | + |
| 0.1 | − | + |

[a] Added $Cu^{++}$ conc. was $10^{-5}$ moles/l.

[b] − = none, ± = doubtful, + = slight, and ++ = pronounced oxidized flavor after 60 hr. storage at 40° F.

samples to render them more susceptible to oxidized flavor development. Results presented in table 3 show that the action of 0.1 mg. of trypsin per 100 ml. of milk was sufficient to prevent development of an oxidized flavor in the milk used in test 1. This same concentration of trypsin reduced the intensity of the flavor which developed in the milk of test 2.

The third trial was carried out with the milk of cow 20 only. Three portions were prepared to represent trypsin additions of 0 (control), 0.1 and 0.2 mg. per 100 ml., respectively. These portions were incubated, pasteurized, $Cu^{++}$ added and stored at 40° F. as previously described. After 60 hr. of storage at 40° F. the control showed a pronounced oxidized flavor, but the 0.1 mg. trypsin sample showed only a slight flavor, and 0.2 mg. trypsin sufficed to prevent the off-flavor entirely.

The three trials described above, in which milk samples were treated with various concentrations of pure trypsin, are in the nature of preliminary experiments. However, the results of these preliminary trials indicate that the action

of a low concentration of pure trypsin will prevent the development of an oxidized flavor in milk. Judging from these results, it is entirely possible that the beneficial effects obtained when milk is treated with one of the commercial pancreatic enzyme preparations is due to the trypsin contained therein.

## DISCUSSION

The findings of Swanson and Sommer (25), supported by the observations of others (27, 28, 3), indicate that the development of an oxidized flavor in milk is due to, or accompanied by, oxidation of the phospholipids. Though there is no real evidence that the flavorsome materials arise from the phospholipids, the ready development of the flavor in washed cream indicates that these materials must, almost certainly, originate from some component of the fat phase. Without attempting to designate the actual compound from which the flavor arises, it is assumed, for purposes of the following discussion, that oxidation of lipid material takes place when an oxidized flavor develops in milk.

It has been shown (table 1) that washed cream and washed cream plus $Cu^{++}$ failed to develop an oxidized flavor after 48 hr. of storage at 40° F., whereas the flavor developed in similar samples to which 100 mg. per liter of ascorbic acid had been added. This suggests that lipid material reacts but slowly, if at all, with molecular oxygen and that $Cu^{++}$, at the concentration used ($10^{-5}$ molar or 0.64 mg. per liter), is not a sufficiently active catalyst to account for development of the flavor. Apparently presence of an intermediary compound, a hydrogen carrier, is essential to the system and ascorbic acid acts in this capacity. Additional support to this view is provided by the observations of Krukovsky and Guthrie (18). They found that complete oxidation of the ascorbic acid of milk, prior to pasteurization, would prevent development of the flavor and that the addition of ascorbic acid to milk so treated would provide conditions such that the flavor was again able to develop.

Though the addition of ascorbic acid to washed cream induces development of an oxidized flavor, a similar addition to susceptible milk will retard or prevent flavor development. Two possible explanations might be advanced to account for this variable action. Assuming that ascorbic acid acts as a hydrogen carrier, the excess amount added to milk may simply be acting as an antioxidant after the manner suggested by Krukovsky and Guthrie (19). Another possibility is that the added ascorbic acid reacts with some constituents of the serum, being oxidized itself while the other constituent is reduced. Perhaps, when ascorbic acid is added to milk, it reduces certain reactive groups of the milk proteins with the result that protein oxidation is favored and the product is rendered less susceptible to oxidized flavor development. A mechanism for such a reaction is suggested in a later paragraph.

Garrett (9) showed that the addition of divalent manganese ($Mn^{++}$) to susceptible milk would prevent development of the flavor. Trials reported herein corroborate this observation and indicate that added $Mn^{++}$ also will prevent development of the flavor in spontaneous milk. The data of table 1, however, show that similar additions of $Mn^{++}$ to washed cream have no such protective action.

This suggests that the protective action of $Mn^{++}$ is not due to the presence of these ions alone but must involve some other components of the milk serum as well. Perhaps a mechanism similar to that suggested by Dickens (8) is involved in this protective action. He found that the respiration of slices of brain cortex was protected against the toxic effect of high $O_2$ pressure by the presence of $Mn^{++}$. He suggested that high $O_2$ pressures probably poisoned the pyruvate oxidase system of the tissue by attacking the essential – SH groups of this enzyme and that these – SH groups were both activated and protected, against high oxygen pressure, by the presence of $Mn^{++}$. If such a mechanism is involved when manganese is added to spontaneous or susceptible milk, the proteins and particularly the serum proteins, which are relatively high in cystine, must play a fundamental role in protecting milk against oxidized flavor development.

The results presented in tables 2 and 3 show that the action of pure trypsin will retard or prevent development of an oxidized flavor in spontaneous and susceptible milk. Trypsin is a proteinase enzyme; hence, its protective action is most likely associated with the hydrolytic cleavage of milk proteins. This information is additional evidence that milk proteins may be involved in determining the susceptibility of milk toward development of an oxidized flavor.

The preceding discussion has suggested the possibility that milk proteins play a significant part in determining the susceptibility of milk toward oxidized flavor development. Other evidence in the literature supports this view. Gould and Sommer (11) attributed the protective action of high heat treatments to the liberation of sulfides from a protein closely associated with milk fat. Perhaps milk proteins constitute the reducing substances discussed by Kende (17) and the protective substances suggested by Stebnitz and Sommer (24). If milk proteins can function in a manner such as to prevent oxidized flavor development, then the question of the mechanism by which they function becomes of interest.

The work of Hopkins (14) suggests a possible mechanism. He showed, among other things, that (a) oxidation of protein, in the presence of glutathione, could not proceed in the absence of "fixed" or reactive – SH groups in the protein; (b) blood serum protein showed no oxygen uptake and no "fixed" – SH groups until such protein had been denatured; (c) provided these – SH groups were present, oxidation of protein proceeded rapidly at pH 7 or slightly higher but not at all at pH 3 to 4; (d) the oxygen uptake of the protein amounted to as much as ten times that required to oxidize the – SH groups which he could measure; and (e) in the system fat plus protein plus glutathione, oxidation of protein only took place at pH 7.6, oxidation of fat only at pH 3.8 and oxidation of both fat and protein at pH 6.0.

Milk may be considered to be similar in some respects to the fat-protein systems studied by Hopkins (14). The pH of milk is such that oxidation of protein would be favored provided a suitable oxidative pathway existed. It has been suggested earlier in this discussion that ascorbic acid may be able to act as a hydrogen carrier in the oxidation of lipids and perhaps it can act in a similar

capacity in the oxidation of protein. In addition to a suitable hydrogen carrier, reactive – SH groups would have to be present in the protein before protein oxidation could proceed. However, assuming the presence of both a suitable hydrogen carrier and reactive – SH groups, one would predict, from the work of Hopkins, that protein oxidation would proceed at a fairly rapid rate. Since the $O_2$ supply would be limited, essentially, to that dissolved in the milk, oxidation of protein would soon exhaust the available $O_2$, thereby preventing lipid oxidation with its accompanying oxidized flavor development.

Hopkins (14) used the nitroprusside reaction to test for the presence of reactive – SH groups in the proteins he worked with. When the reaction was negative his protein showed no oxygen uptake. Jackson (15) and others (11) have shown that the nitroprusside reaction of fresh milk is invariably negative, hence it must be assumed that the number of reactive – SH groups available for protein oxidation is limited. Perhaps, however, relatively few such groups are necessary in order to provide for sufficient protein oxidation to protect milk against flavor development. If such be the case, perhaps the differences in susceptibility between various samples of milk may be explained on the basis of differences in the number of these reactive – SH groups or in their state of oxidation.

On the basis of the above theory, one would expect that any treatment of milk which tended to cause protein hydrolysis or denaturation would expose (by splitting or uncoiling of the protein molecule) more – SH groups for the oxidation of protein. This, in turn, would tend to retard or prevent lipid oxidation and, hence, oxidized flavor development. The action of some of the practical factors which influence flavor development may well be explained in terms of their effect on the number of available – SH groups. Bacterial growth, high heat treatments (170 to 180° F. for 5 to 10 min.), homogenization and the action of trypsin all tend to retard or prevent development of an oxidized flavor in milk. These same factors also must be expected to cause some degree of protein hydrolysis or denaturation.

The action of $Cu^{++}$ and $Mn^{++}$ must be explained on the basis of some effect other than protein hydrolysis or denaturation. Reference has already been made in this discussion to a possible means by which $Mn^{++}$ may act to retard oxidized flavor development. It has been suggested that $Mn^{++}$ acts by activating the essential – SH groups of milk proteins in the manner postulated by Dickens (8). $Cu^{++}$, on the other hand, is considered by most investigators in this field to act as a catalyst in the oxidation of lipid material. However, certain observations suggest that the action of $Cu^{++}$ may not be so readily explained. Spontaneous milk will develop an oxidized flavor in the absence of added $Cu^{++}$. Also, the concentration of added $Cu^{++}$ required to produce an oxidized flavor in susceptible milk varies greatly from one milk to another. If the action of $Cu^{++}$ were strictly a matter of catalysis, one would not expect to find such large differences in the concentration required to produce an oxidized flavor in different milks. Perhaps $Cu^{++}$ promotes oxidized flavor development by, in some way,

blocking the reactive – SH groups so that protein oxidation cannot proceed. If this be the case, then the concentration of added $Cu^{++}$ required to initiate development of the flavor would be related quantitatively to the number of reactive – SH groups in the milk protein. This, in turn, would explain why the concentration of $Cu^{++}$ which must be added to produce an oxidized flavor varies so greatly from one milk to another.

Other factors known to influence oxidized flavor development might also be discussed in relation to their effects on the "fixed" or reactive – SH groups of milk proteins. The foregoing outline, however, will suffice to illustrate a possible mechanism by which these proteins, particularly the serum proteins high in cystine, may have a decided influence on the susceptibility of milk toward oxidized flavor development.

## SUMMARY

A concentration of $10^{-4}$ moles per liter of added $MnSO_4$ was found to prevent development of an oxidized flavor in spontaneous milk and in susceptible milk containing $10^{-5}$ moles per liter of added $CuSO_4$. Addition of the Mn salt either before or after pasteurization was equally effective. Such addition, however, had no effect on the rate of oxidation of ascorbic acid.

The addition of ascorbic acid (100 mg. per liter) to washed cream induced development of an oxidized flavor during 48-hr. storage at 40° F. The presence of added $CuSO_4$ ($10^{-5}$ moles per liter) showed no tendency to catalyze development of the flavor in washed cream. $MnSO_4$, when added to washed cream containing ascorbic acid, did not prevent oxidized flavor development.

The action of pure trypsin retarded or prevented development of an oxidized flavor in spontaneous and susceptible milk. The suggestion is made that the protective action obtained by treating milk with the pancreatic enzyme preparations presently being marketed is due to the trypsin contained therein.

A possible mechanism by which milk proteins may function as reducing agents, through their "fixed" or reactive – SH groups, is discussed. The suggestion is made that these – SH groups in milk proteins may have a pronounced influence on the susceptibility of milk toward oxidized flavor development.

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# THE PROTEIN AND NON-PROTEIN NITROGEN FRACTIONS IN MILK. I. METHODS OF ANALYSIS[1]

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While the major part of the nitrogen in milk is accounted by casein, albumin, globulin and proteoses-peptones, milk also contains nitrogen in the form of ammonia, urea, creatinine, creatine, uric acid and amino acids, and mere traces in the form of vitamins, enzymes, phospholipids and cerebrosides. The non-protein nitrogen components of milk have not been studied extensively. They have not been studied adequately with respect to their constancy or variability in milk, their role in the behavior or properties of milk and their possible significance in accounting for or measuring changes produced in milk by various processing treatments.

While the few reasonably complete analyses that have been reported account for the total nitrogen content of milk so completely as to leave little room for any other forms of nitrogen, this subject has received so little attention that from time to time the existence in milk of proteins other than those included in casein and albumin has been postulated. Denis *et al.* (3) in 1919, and Kieferle and Gloetzl (6) in 1930, were among the first workers who reported such non-protein nitrogen fractions in milk and have offered some evidence to show that their content in milk parallels their content in the blood from which the milk stemmed.

Not much information is available about the significance of non-protein nitrogen constituents in the physiology and abnormalities of milk secretion. Perkins (8) reported that there are indications that dietary and other factors influencing milk secretion may be reflected significantly in the non-protein nitrogen fractions, rather than in gross composition. Milk from the cows maintained on high protein diet contains high non-protein nitrogen.

The methods for determining the non-protein nitrogen fractions as used by various workers in studying milk generally have been adapted from blood and urine analyses. The need for modification of such methods arises from the presence of lactose, fat and proteins in milk, and from the characteristic properties of the milk proteins. Work was started to test the applicability of the methods described by previous workers. It was found that the methods for some of the fractions were too lengthy and laborious. Hence, extensive changes were introduced in determining globulin and uric acid, and a few modifications were introduced in other methods to make them more suitable for our purpose.

## EXPERIMENTAL METHODS

### *Protein fractions.*

Total nitrogen, non-casein nitrogen, proteose-peptone nitrogen and non-pro-

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tein nitrogen were determied by semi-micro Kjeldahl method as recommended by Menefee *et al.* (7) and Rowland (11).

(a) *Total nitrogen.* Five ml. of milk are diluted with water to the mark in a 100-ml. flask and a 5-ml. aliquot is taken in a 100-ml. Kjeldahl flask for nitrogen determination. After adding approximately 1 g. of catalyst mixture (14 parts $Na_2SO_4$ and 1 part HgO) and 3 ml. concentrated $H_2SO_4$, the mixture is digested until it is clear. After cooling, the sides of the Kjeldahl flask are rinsed down with a minimum amount of water and the flask contents are redigested for 30 min. When cool, 25 ml. of water and approximately 8 ml. of 50 per cent of NaOH containing $Na_2S_2O_3$ are added. By steam distillation, $NH_3$ is received in a flask containing about 20 ml. of 2 per cent of boric acid and a few drops of methyl red and methylene blue indicator. $NH_3$ is completely distilled off in about 6 min. and the receiving flask is disconnected and titrated from a microburette with 0.03 to 0.04 *N* standard HCl.

(b) *Non-casein nitrogen.* In a 100-ml. flask, 10 ml. of milk are introduced and diluted with 70 to 80 ml. of water. The contents are brought to 40° C. and then acidified with 1.0 ml. of 10 per cent acetic acid. After holding for 5 to 10 min. at 40° C., 1.0 ml. of normal sodium acetate is added. The contents then are cooled and the volume is made up to the mark with water. The pH of the solution is determined and whenever necessary the pH is adjusted to 4.6 to 4.7 by adding a few drops of 10 per cent acetic acid. Casein-free serum is obtained by filtering through a dry, pleated no. 42 Whatman filter paper. Ten ml. of the serum are used for determining non-casein nitrogen, using the same Kjeldahl technique as above.

(c) *Proteose-peptone plus non-protein nitrogen.* Ten ml. of milk are measured in a 100-ml. flask and placed in a boiling water bath for 30 to 40 min., to ensure complete denaturation of albumin and globulin. Precipitation of albumin and globulin is carried out along with casein by the same method as described in non-casein nitrogen. Ten ml. of the serum are used for nitrogen determination by the semi-micro Kjeldahl method.

(d) *Globulin nitrogen.* Twenty ml. of non-casein filtrate are cooled to 0 to 5° C. in a 50-ml. beaker, and 7 ml. of absolute methanol are added. The beaker is kept at 0 to 5° C. for 40 min. and precipitated globulins are filtered. To transfer the precipitate quantitatively, the beaker is rinsed twice with chilled 40 per cent methanol. After thorough washing, the filter paper together with the precipitate is subjected to nitrogen determination by the semi-micro Kjeldahl method. This procedure is adapted from a method reported by Pillemer and Hutchinson (10) for globulin determination in blood and later modified by Kemp (5) for milk analysis.

(e) *Non-protein nitrogen.* In a 50-ml. flask, 10 ml. of milk are diluted up to the mark with 15 per cent trichloracetic acid. The contents are mixed well and after 10 min. filtered through a dry filter paper. A 20-ml. aliquot is used for nitrogen determination.

*Calculation of nitrogen in the various protein fractions.* (a) Total nitrogen *i.e.,* casein, albumin, globulin, proteose-peptone and non-protein nitrogen. (b)

Non-casein nitrogen, *i.e.,* albumin, globulin, proteose-peptone and non-protein nitrogen. (c) Proteose-peptone plus non-protein nitrogen. (d) Globulin nitrogen. (e) Non-protein nitrogen. (f) Casein nitrogen = total minus non-casein nitrogen $(a-b)$. (g) Albumin nitrogen = Non-casein nitrogen minus $(c+d)$. (h) Proteose-peptone nitrogen = $(c-e)$. Thus the total globulin and non-protein nitrogen are determined directly and the other constituents are calculated by difference.

*Non-protein nitrogen fraction.*

*Ammonia.* One hundred ml. of milk are treated with 20 g. of anhydrous $MgSO_4$ in a 500-ml. flask and the volume is made up with 95 per cent alcohol with one intermediate shaking. It is kept for 10 min. and then filtered through dry pleated no. 42 Whatman filter paper. A 100-ml. aliquot of the filtrate representing 20 ml. milk is introduced in a 300-ml. Kjeldahl flask and alkalinated with about 1 to 2 g. of MgO. The flask then is quickly fixed to a steam distillation rack and $NH_3$ is received in a flask containing about 20 ml. of 2 per cent boric acid and a few drops of methyl red and methylene blue indicator. Distillation is carried out until about 50 to 60 ml. distillate is received. The receiving flask then is removed and the contents are titrated with a very dilute standard HCl solution. After deducting the proper blank value, the $NH_3$ content is calculated. This is the procedure of Perkins (9) with slight modifications.

*Urea.* A 20-ml. aliquot of the above alcoholic $MgSO_4$ filtrate, equivalent to 4 ml. milk, is treated with one crushed, commercial, Arlington urease tablet (0.1 g.) in a 300-ml. Kjeldahl flask. The mixture is diluted with 20 ml. water and the Kjeldahl flask is incubated at 40° C. for 8 hr. to hydrolyze urea into ammonia. (Usually the Kjeldahl flask was allowed to stand overnight in a thermostatically controlled water bath at 40° C.). After the incubation, the mixture is further diluted with 50 to 60 ml. of water, 1 g. of MgO is added and $NH_3$ is distilled off and titrated in a similar manner as in the case of the $NH_3$ determination. This represents the $NH_3$ and urea nitrogen; hence, after deducting ammonia nitrogen, the rest is reported as urea nitrogen.

*Preformed creatinine.* In a 100-ml. volumetric flask, 20 ml. of milk, 10 ml. of 20 per cent $CuSO_4$ solution and 30 ml. of 10 per cent CaO suspension are introduced. The mixture is shaken and made up to the mark with water. After allowing it to stand for 30 min., it is filtered through a small dry filter paper. The filtrate should be absolutely clear and colorless and free from all proteins, fat and lactose. In certain cases, the filtrate may have a slight bluish tinge, but that doesn't seem to interfere with the determination.

Preformed creatinine is determined by transferring 10 ml. of the filtrate to a 25-ml. flask. To this, two drops of normal HCl, 10 ml. of saturated picric acid and 1 ml. of 10 per cent NaOH are added. After 10 min. the contents are made up to the mark with water and filtered to remove the cloudiness caused by the precipitation of $Ca(OH)_2$. The color of the filtrate is measured in an Evelyn Photoelectric Colorimeter using a 520 m$\mu$ filter, the instrument having first been adjusted to 100 per cent transmission with a blank solution. The reading so

obtained is expressed as creatinine by comparison with the reading similarly made on a standard creatinine solution. For normal milk a standard containing 0.03 mg. of creatinine in 10 ml. of water, 10 ml. of saturated picric acid and 1 ml. of 10 per cent NaOH, made up to the mark, is most convenient. The blank is prepared in the same way, except water is used in place of milk serum or standard creatinine solution. This is the method of Denis and Minot (2) with slight modifications.

*Total creatinine.* Ten ml. of the above milk serum in a 50-ml. volumetric flask are treated with three drops of normal HCl and 10 ml. of saturated picric acid. The flask is autoclaved for 30 min. at 248° F. When cool, the mixture is treated with 1 ml. of 10 per cent NaOH and after standing for 30 min. is made up to volume, filtered directly into a colorimeter tube and compared against a similarly prepared standard. Normally, a standard is made to contain 0.06 mg. of creatinine, 10 ml. of saturated picric acid and 1 ml. of 10 per cent NaOH made up to volume in a 50-ml. flask. The filtration is carried out if there is any precipitation of $Ca(OH)_2$ caused by addition of NaOH.

*Uric acid.* To 5 ml. of milk in a 50-ml. flask, 40 ml. of 0.083*N* $H_2SO_4$ and 5 ml. of 10 per cent $Na_2WO_4$ are added. The contents are shaken and filtered through a dry filter paper. Ten ml. of filtrate are pipetted into a 50-ml. volumetric flask and treated with 5 ml. of 5 per cent NaCN solution from a burette, followed by 1 ml. of arsenophosphotungstic acid reagent. The flask is shaken gently and after standing for 10 min. the volume is made up with water. The mixture then is centrifuged at 2,000 r.p.m. for about 10 min. and the supernatant liquid is taken in a colorimeter tube and compared with a similarly prepared standard in an Evelyn Photoelectric Colorimeter using 520 m$\mu$ filter. The colorimeter first is set at zero density with a blank solution. A suitable standard is prepared to contain 0.02 mg. of uric acid, in 10 ml. of buffer solution, 5 ml. of 5 per cent NaCN and 1 ml. of arsenophosphotungstic acid in a 50-ml. volumetric flask.

The above procedure follows the technique of Haden (4) for the deproteinizing and Benedicts' procedure (1) for color development. This adaptation was made in the interest of shortening the procedure. Recovery experiments showed that the method as described gave consistent and reproducible results.

*Alpha-amino nitrogen.* In a 200-ml. volumetric flask, 20 ml. of milk are diluted with 40 ml. of 0.01 *N* acetic acid, 10 ml. of 5 per cent sodium acetate and 50 to 60 ml. of water. The flask is kept in a boiling water bath for about 20 to 30 min. At the end of this heating period, 1 ml. of 15 per cent $K_2C_2O_4$ is added, and the mixture cooled, made to volume and filtered through a dry filter paper. To the filtrate is added about 0.5 g. of $K_2C_2O_4$ and after shaking the flask for 1 to 2 min., the precipitate is removed by centrifuging at 1,500 r.p.m. for 10 min. All the supernatant liquid is filtered into a 250-ml. beaker.

For amino nitrogen determination, all the filtrate is evaporated to a volume of less than 50 ml. by placing the beaker on a steam bath. The condensed filtrate then is transferred quantitatively to a 50-ml. volumetric flask and filled

up to a mark. A 10-ml. aliquot is used for the determination of amino nitrogen by the Van Slyke apparatus. This method was adapted from a procedure recommended by Denis and Minot (2).

*Unaccounted nitrogen* is the difference between the total non-protein nitrogen and the sum of the nitrogen accounted by ammonia, urea, creatinine, creatine, uric acid and *alpha*-amino nitrogen fractions.

## EXPERIMENTAL

The above methods were selected and adopted to expedite the comprehensive program of analysis which was to be applied to milk samples. Even so, it was impossible to complete the analyses in such a short time as to preclude the possibility of changes occurring in the milk. Accordingly, experimental observations were undertaken to determine whether any changes occur in the protein and non-protein nitrogen fractions during storage of milk at 0 to 5° C.

Fresh, raw, mixed milk samples were obtained from the commercial supply of the Department of Dairy Industry as soon as milk was received in the morning. After thorough mixing, each sample was divided into two lots. One lot was analyzed as rapidly as possible for all the fractions and the other lot was stored in glass quart bottles at 0 to 5° C. with toluene added as a preservative at a rate of 10 drops per quart. At the end of 10 days, the samples were brought to room temperature and analyzed.

Table 1 gives the data for three mixed milk samples, fresh and 10 days old. Aging in the cold produced no significant changes in the nitrogen distribution, except that there were indications of a slight shift in albumin, globulin and $NH_3$ nitrogen. Albumin nitrogen increased slightly, whereas globulin nitrogen decreased to the same extent. $NH_3$ nitrogen also increased slightly. Though the changes involved were not of appreciable magnitude, nevertheless it was decided that milk samples should be analyzed as fresh as possible. Since the complete analyses for all fractions required several days, it was decided in subsequent work to follow a program of prompt preparation of all the sera required for the different fractions, and storing the sera in a refrigerator rather than storing the milk itself. Analyses then were completed from the sera as rapidly as possible. Even when a number of samples were started at the same time, the analyses were in all cases completed in 5 to 7 days. Since all the sera contained high concentrations of either acids or strong protein precipitants, no bacterial development should be expected.

## SUMMARY

Methods have been described for determination of total nitrogen, casein, albumin, globulin, proteoses-peptones, non-protein nitrogen, ammonia, urea, creatinine, creatine, uric acid and *alpha*-amino nitrogen in milk.

Bulk milk samples were analyzed fresh and after holding at 0 to 5° C. for 10 days (toluene preserved). No significant change was observed in the nitrogen distribution during this aging.

TABLE 1

*Effect of aging (0–5° C.) on nitrogen distribution in milk (preserved with toluene)*
*(Mg./100 ml. milk)*

| Sample | Total N | Casein N | Albumin N | Globulin N | Proteoses Pep. N | N.P. N | Ammonia N | Urea N | Creatinine | Creatine | Uric Acid | α-Amino N | Unaccounted N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fresh | 487.8 | 379.0 | 48.2 | 17.9 | 13.2 | 29.7 | 0.88 | 8.16 | 0.45 | 3.60 | 3.00 | 3.83 | 14.45 |
| Aged | 487.9 | 380.5 | 47.2 | 16.3 | 11.5 | 32.4 | 0.85 | 8.07 | 0.40 | 3.80 | 2.60 | 4.10 | 17.13 |
| Fresh | 506.8 | 398.9 | 33.9 | 24.1 | 24.1 | 25.7 | 0.41 | 10.43 | 0.65 | 3.60 | 2.20 | 2.60 | 10.18 |
| Aged | 506.8 | 395.7 | 46.6 | 17.4 | 24.4 | 22.8 | 0.67 | 9.36 | 0.65 | 3.75 | 2.00 | 2.74 | 7.88 |
| Fresh | 533.4 | 419.6 | 51.5 | 22.2 | 14.2 | 25.9 | 0.51 | 9.63 | 0.65 | 4.30 | 2.70 | 3.09 | 10.13 |
| Aged | 537.7 | 425.0 | 52.6 | 18.4 | 16.4 | 25.3 | 0.84 | 9.72 | 0.65 | 4.05 | 2.60 | 3.30 | 9.07 |
| Ave.: | | | | | | | | | | | | | |
| Fresh | 509.3 | 399.1 | 44.5 | 21.4 | 17.2 | 27.1 | 0.60 | 9.41 | 0.58 | 3.83 | 2.63 | 3.17 | 11.57 |
| Aged | 510.8 | 400.4 | 48.8 | 17.4 | 17.4 | 26.8 | 0.79 | 9.05 | 0.57 | 3.87 | 2.40 | 3.83 | 11.57 |

Reported as such, and unaccounted N calculated on N basis.

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# THE PROTEIN AND NON-PROTEIN NITROGEN FRACTIONS IN MILK.[1] II. THEIR CONTENT IN FRESH RAW MILK

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Using the methods described in a previous paper (10), the protein and non-protein nitrogen fractions were studied in fresh raw milk from individual cows and from a pooled milk supply.

## EXPERIMENTAL RESULTS

Table 1 presents the analyses of six milk samples from individual cows. All the cows were young, in good health, and in the general middle range of the lactation period. Their milk yield ranged from 11 to 54 lb. per day. The samples were correct composites of evening and morning milkings.

In addition to the individual cow samples of table 1, samples from single milkings from eight other cows were analyzed incidental to other phases of the general project. Table 2 gives the range and average for all of the nitrogen fractions in milk as found in all of the individual cow samples.

Fresh mixed milk samples, taken from the supply as received commercially by the Department of Dairy Industry, were analyzed in various phases of the work. Table 3 gives the range and average values for the nitrogen fractions as found in 14 such milk samples.

The values show variations of considerable magnitude. The average figures for casein nitrogen as percentage of the total nitrogen are in good agreement with the figures reported in the literature. Davies (2) found casein nitrogen to account for 76.7 per cent of the total nitrogen, Golding *et al.* (5) have reported 76.5 per cent, and Rowland (9) 78.7 per cent in the individual and 78.3 per cent in bulk milk samples. In the present study casein nitrogen accounts for 76.6 per cent of the total nitrogen in the individual and 78.9 per cent in the mixed milk samples.

The average albumin content was 0.23 per cent in the individual and mixed milk samples and the globulin content was 0.21 and 0.14 per cent in the individual and mixed milk samples, respectively. The combined albumin and globulin content was 0.44 per cent in the individual and 0.37 per cent in the mixed milk samples, as compared to 0.55 to 0.70 per cent heat coagulable proteins as frequently reported in the literature. This difference likely is due to methods employed. The higher values probably are the result of reporting as "albumin," all of the heat-coagulable proteins obtainable from the serum after removal of casein. It is probable that proteoses are included to some extent. The values for albumin and globulin reported in this study, however, are in harmony with

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TABLE 1

*Nitrogen distribution in the individual milk samples*
*(mg. per 100 ml. milk)*

| Sample | Total N | Casein N | Albumin N | Globulin N | Proteoses-pep. N | N.P.N. | Ammonia N | Urea N | Creatinine | Creatine[a] | Uric acid[a] | α Amino N | Unaccounted N[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (*mg./100 ml. milk*) | | | | | | | |
| 1 (Brown Swiss) | 549.8 | 440.3 | 37.1 | 20.5 | 21.5 | 30.3 | 0.66 | 15.0 | 0.96 | 3.08 | 1.62 | 4.46 | 8.31 |
| 2 (Holstein) | 492.4 | 379.5 | 26.9 | 25.2 | 29.5 | 31.3 | 0.56 | 12.5 | 1.00 | 2.45 | 1.40 | 3.70 | 12.88 |
| 3 (Holstein) | 505.8 | 397.8 | 36.4 | 29.6 | 17.1 | 24.9 | 0.71 | 6.1 | 0.61 | 3.89 | 1.13 | 4.01 | 12.21 |
| 4 (Guernsey) | 470.4 | 366.3 | 28.3 | 25.9 | 17.6 | 32.3 | 0.98 | 20.4 | 0.96 | 2.54 | 1.80 | 3.74 | 5.36 |
| 5 (Guernsey) | 518.0 | 412.0 | 36.4 | 26.6 | 13.2 | 29.8 | 0.64 | 17.4 | 1.03 | 2.55 | 1.59 | 4.75 | 5.25 |
| 6 (Guernsey) | 480.1 | 380.9 | 36.7 | 22.0 | 9.3 | 31.3 | 0.83 | 15.5 | 0.78 | 3.30 | 1.73 | 6.23 | 6.75 |
| Av. | 502.7 | 396.1 | 33.6 | 25.0 | 18.1 | 30.0 | 0.73 | 14.5 | 0.89 | 2.97 | 1.54 | 4.48 | 8.47 |

[a] Reported as such. Unaccounted N calculated on the basis of N only.

TABLE 2

*Nitrogen distribution in 14 individual milk samples*

| Component | Low | High | Average |
|---|---|---|---|
| | | (*mg./100 ml. milk*) | |
| Total N | 470 | 577 | 512 |
| Casein N | 366 | 440 | 392 |
| Albumin N | 26.9 | 46.1 | 36.0 |
| Globulin N | 20.5 | 59.2 | 32.7 |
| Proteoses-peptones N | 9.28 | 36.2 | 23.1 |
| Non-protein N | 24.3 | 32.3 | 28.1 |
| Ammonia N | 0.29 | 0.98 | 0.59 |
| Urea N | 6.13 | 20.40 | 13.1 |
| Creatinine[a] | 0.40 | 1.22 | 0.87 |
| Creatine[a] | 2.45 | 5.62 | 3.72 |
| Uric acid[a] | 1.13 | 3.69 | 2.32 |
| *Alpha* Amino N | 3.70 | 6.23 | 4.82 |
| Unaccounted N | 5.07 | 12.9 | 7.41 |

[a] See table 1.

observations of Rowland (9) who reported 0.42 per cent as total albumin and globulin.

TABLE 3

*Nitrogen distribution in fresh, raw, mixed milk samples*

| Component | Low | High | Average |
|---|---|---|---|
| | | (*mg./100 ml. milk*) | |
| Total N | 442 | 533 | 476 |
| Casein N | 343 | 420 | 376 |
| Albumin N | 22.6 | 51.5 | 36.7 |
| Globulin N | 10.81 | 28.5 | 22.3 |
| Proteoses-peptones N | 13.15 | 24.1 | 17.2 |
| Non-protein N | 18.1 | 28.7 | 23.8 |
| Ammonia N | 0.17 | 1.19 | 0.67 |
| Urea N | 6.54 | 10.85 | 8.38 |
| Creatinine[a] | 0.19 | 0.65 | 0.49 |
| Creatine[a] | 3.55 | 4.51 | 3.93 |
| Uric acid[a] | 1.55 | 2.70 | 2.28 |
| *Alpha* Amino N | 2.20 | 5.18 | 3.74 |
| Unaccounted N | 5.63 | 14.45 | 8.81 |

[a] See table 1.

Following are the values observed for albumin and globulin nitrogen (percentage of the total nitrogen) as compared to Rowland (9) and Davies (1 and 2):

| | Present | | Rowland | | Davies | |
|---|---|---|---|---|---|---|
| | Individual | Mixed | Individual | Bulk | 1932 | 1935 |
| Albumin | 7.0 | 7.7 | 9.3 | 9.3 | 12.6 | 13.4 |
| Globulin | 6.3 | 4.7 | 3.1 | 3.5 | 6.3 | 4.6 |

Davies (3) has reported that globulin content is about half as high as the albumin content, whereas in the present study, the globulin content was 90.8 and 61.0 per cent as high as albumin content in the individual and mixed milk, respectively.

Non-protein nitrogen was found to account for 5.5 per cent of the total nitrogen in the individual and 5.0 per cent in the mixed milk, as compared to 6.0 per cent reported by Davies (1) and 5.0 per cent reported by Rowland (9).

Individual non-protein nitrogen fraction showed great variations. Among those, urea, and unaccounted nitrogen showed the greatest variations. Urea ranged from 6.13 to 20.4 with an average of 13.1 mg. per 100 ml. of individual milk, whereas in case of fresh raw mixed milk, it ranged from 6.54 to 10.85 with an average of 8.38 mg. per 100 ml. Though the variation was quite great, similar observations have been reported by Perkins (8).

Unaccounted nitrogen ranged from 5.07 to 12.9 mg. per 100 ml. with an average of 7.41 mg. per 100 ml. individual milk, whereas, in fresh raw mixed milk, it ranged from 5.63 to 14.45 with an average of 8.81 mg. per 100 ml. On the whole, values obtained in these experiments are in harmony with the figures reported by Kieferle and Gloetzl (6), Denis and Minot (4), Rowland (9) and Menefee *et al.* (7).

## SUMMARY

A detailed study of the protein and non-protein nitrogen fractions in individual, single-milking and mixed-milk samples has been made. Great variations were observed among individual fractions.

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# THE EFFECT OF FEEDING THYROPROTEIN TO DAIRY COWS DURING THE DECLINE OF LACTATION IN SUCCESSIVE LACTATIONS

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The milk secretion stimulating properties of substances containing thyroxine have been amply demonstrated in numerous experiments of relatively short duration. The results, conclusions and problems which these experiments have emphasized have been reviewed by Blaxter *et al.* (2). In the general application of feeding thyroprotein, feeding periods are for several months rather than the weeks covered by most reported experiments. This is necessary to prevent a serious decline in lactation following removal of thyroprotein from the ration. The possibility of harmful effects following prolonged stimulation with thyroxine has prompted some investigations covering extended periods and successive lactations.

Van Landingham *et al.* (15), after feeding thyroprotein for extended periods in two successive lactations, mentioned possible interference with reproductive efficiency and loss of body weight as undesirable effects. Reece (7) reported no undesirable effects except loss of body weight when thyroprotein was fed for 3 to 17 mo. to nine cows. After a few months the cows regained the lost weight even though still receiving the thyroprotein. Similar observations were reported by Reece (8) in a later report. Thomas and Moore (12) found that cows fed thyroprotein from 50 days postpartum to 90 days before next parturition in successive lactations produced at a subnormal rate and had subnormal fat tests. Response to thyroprotein feeding was obtained in each lactation. Thomas *et al.* (13) reported on 11 of these cows for their first lactation during which thyroprotein was fed an average of 301 days. The feeding regimes were varied. Eight of the cows fed at the same rate as a control group gave an initial increase in production following thyroprotein feeding, but in about 100 days had declined below the controls unless extra feed was provided. During this time, they had lost over 100 lb. body weight. One group given 25 per cent extra feed throughout the thyroprotein feeding period continued to produce at a high rate, and the body weights paralleled those of the control group. A gain in body weight near the end of lactation occurred in all groups. The calculated energetic efficiency of milk production was not altered by thyroprotein feeding. Gardner and Millen (4) found that thyroprotein fed to high-producing cows in mid-summer increased production but at a lower-than-normal efficiency because of the large decreases in body weight.

The purpose of this investigation was to secure additional information upon the effect of feeding thyroprotein to cows for successive lactations. Since Herman *et al.* (5) had shown that thyroid stimulation of cows at the peak of lacta-

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tion caused possible decreases and no increases in production, thyroprotein feeding was not planned until the cows were definitely in the declining phase of lactation.

## MATERIALS AND METHODS

Twelve cows (six Jersey and six Holstein-Friesian) were selected from the University of Tennessee herd in the fall of 1947, and paired as closely as possible on the basis of previous lactations to give equal production and similar size, temperament and calving dates. Each member of the pairs then was assigned at random to the control (I) or thyroprotein (II) group. At the end of the first experimental lactation it was necessary to substitute for two of the control cows because of failure to breed. One of the thyroprotein-fed cows lost a quarter following a teat laceration near the end of its lactation. Another pair of cows was used to replace this injured cow and its mate for the next lactation. This experiment then presents data from five cows fed thyroprotein for two successive lactations and two cows fed thyroprotein for only one lactation, along with their respective paired controls.

Thyroprotein[1] was introduced into the ration of the cows in group II on an individual basis after their lactation curves showed a definite decline in milk production for 4 to 8 wk. The average time of starting thyroprotein in 1948 was 122 days in lactation and in 1949 it was 142 days. Thyroprotein was fed at the rate of 15 g. per day to all cows, with the exception of one small Jersey which was started at 12 g. and later changed to 15 g. The thyroprotein was mixed with the evening concentrate feed and fed daily until the end of lactation. The average period thyroprotein was fed to the five cows used in two lactations was 202 days in 1948 and 174 days in 1949. Each cow was dried off 60 days before expected parturition. No concentrates were fed during the dry period. Total feed intake was not determined because the cows were pastured and fed roughage *ad lib.* in groups. An attempt was made to maintain uniform physical condition of all cows and groups by adjustments in concentrate feeding which was recorded. Some cows which were poor feeders were fed heavier ratios of grain to milk than others which had better appetites for roughage or gave less milk. Concentrate feeding was purposely increased for the cows fed thyroprotein compared to the controls on an individual basis according to apparent needs either by increasing the ration over the pretreatment level or by decreasing it at a slower rate than that of the controls as lactation advanced. Because of differences in quality of roughage, the concentrate feeding schedule was not as high during the second experimental lactation as the first.

Milk weights were recorded each milking. Fat tests were determined from 2-day composite samples each week. On a typical day each week the pulse rate, respiration rate and rectal temperature of each of the cows were determined along with the barn temperature at the time of these observations. These were determined at the evening milking time, about 3:30 p.m. The cows were weighed following the morning milking on 2 successive days each month.

[1] Standardized "*Protamone*" was furnished for this experiment through the courtesy of W. R. Graham, Jr., Cerophyl Laboratories, Kansas City, Mo.

## RESULTS

The experimental results have been analyzed on two bases, (a) the short-term changes occurring during the stimulation period and (b) the changes occurring over the full lactation. In the former, each cow is compared with its control at the same day of the year regardless of day of lactation because the effect of day-to-day variations in atmospheric temperature upon the measurements taken was

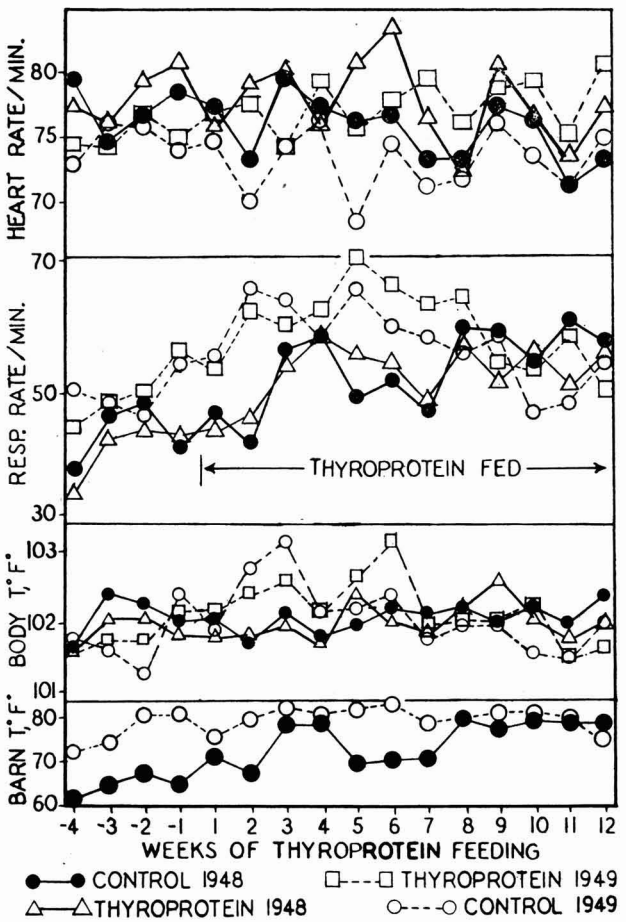



FIG. 1. Heart rate, respiration rate and body temperature of cows fed thyroprotein in 2 successive lactations compared with normally fed controls (each group average of 6 cows).

so important. In the full lactation comparisons, the lactation curves have been plotted without regard to any effect due to the difference in calving dates between pair-mates. Since no carry-over effect of feeding thyroprotein during the previous lactation was noted, data for the pair used only one lactation each year were averaged with the others where it could be used.

The effects of thyroprotein feeding upon the heart rate, respiration and body temperature in each of the 2 yr. are shown in figure 1. A definite increase in heart rate occurred each year following thyroprotein feeding, with heart rates in 1949 being generally lower than those in 1948 in both groups. This result was undoubtedly due to the atmospheric temperature differences indicated in figure 1. These temperature differences also caused higher respiration rates and rectal temperatures in 1949 than in 1948. These values for both groups generally rose and fell together according to weekly climatic changes with slightly higher respiration rates and rectal temperatures in the thyroprotein group at the peak observations. These data indicate no harmful effect due to the thyroprotein feeding under the conditions of this experiment. The values for heart, respira-

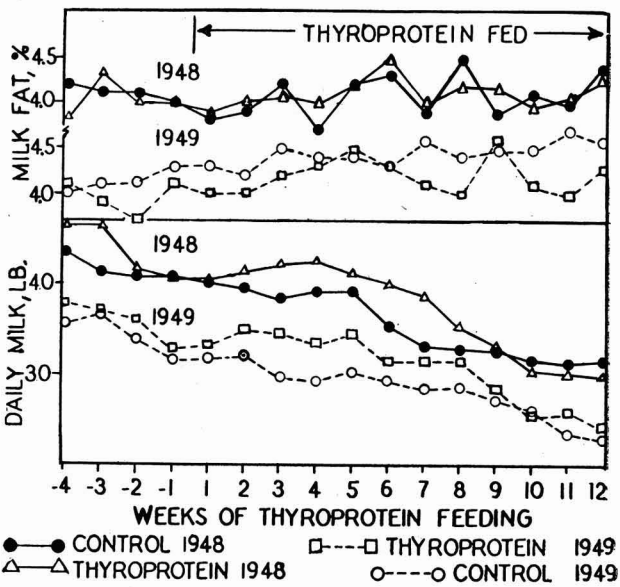



FIG. 2. Weekly average fat test and milk yield of cows fed thyroprotein in 2 successive lactations compared with normally fed control cows (each group average of 6 cows).

tion and temperature before and after the period covered by figure 1 were nearly identical for the two groups.

The daily average milk production and the fat test of the milk during the first 12 wk. of thyroprotein feeding and the 4 wk. before are presented in figure 2. Although the groups were not at strictly equal stages of lactation in this comparison, the stimulus to milk production due to feeding thyroprotein is clearly shown. Contrary to many previous reports, a change in the fat test was not caused by feeding thyroprotein in this experiment. The peak milk production response was noted in the third and fourth weeks in both years. Daily average milk yield of the group II cows had declined to the level of the control group by the tenth week of feeding thyroprotein in both years. Differences between

the groups thereafter were not important, even though the group II cows continued to receive thyroprotein and extra concentrate feed.

The daily average consumption of concentrate feed by the two groups in 1948 and 1949 is shown in figure 3 along with the milk production per pound of concentrates from 4 wk. before to 12 wk. after the start of feeding thyroprotein. In spite of increased concentrate feeding, the cows fed thyroprotein returned more milk per pound of feed until the eighth week. After this point they apparently were less efficient in use of their feed for milk production than were the cows of the control groups. Since total feed consumption was not recorded, the gross efficiencies of the groups cannot be compared accurately.

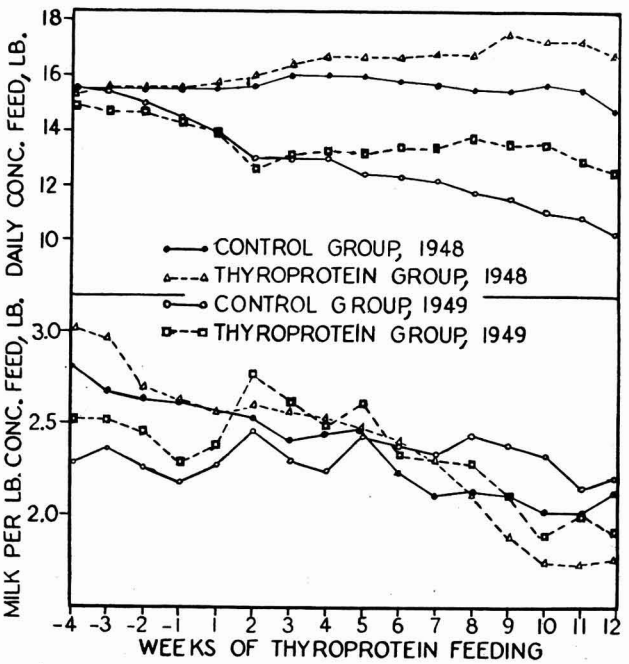



FIG. 3. Concentrate feed fed and the return of milk per lb. concentrates from thyroprotein-fed and normally fed cows in two successive lactations (each group average of 6 cows).

Even though the cows fed thyroprotein were given more feed, they lost body weight during the first 2 mo. of feeding thyroprotein as shown in figure 4. It seems significant that the point at which weight gains were resumed also was the point of diminution of milk production stimulus (figure 2), the point of decreased efficiency of feed utilization for milk production (figure 3) and the point of return to normal heart and respiration rates (figure 1). These coincidental changes indicate that the hyperthyroid condition elicited by feeding 15 g. thyroprotein per day was completely counteracted by the eighth to tenth week.

The physiology of this counteraction effect presumably depends upon the relationship of pituitary thyrotrophic hormone to circulating thyroxine. The

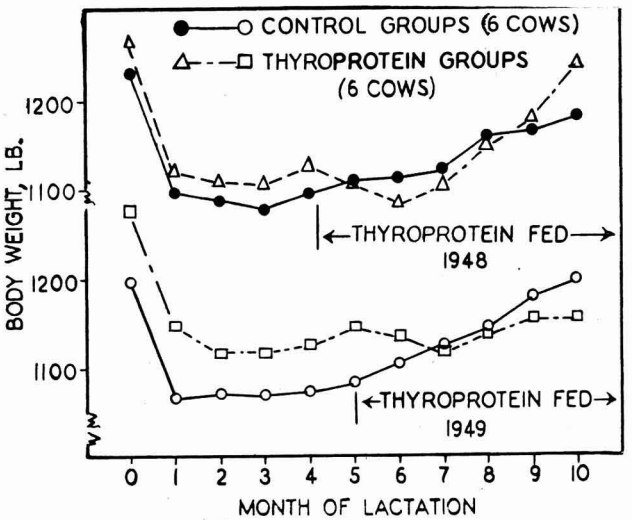
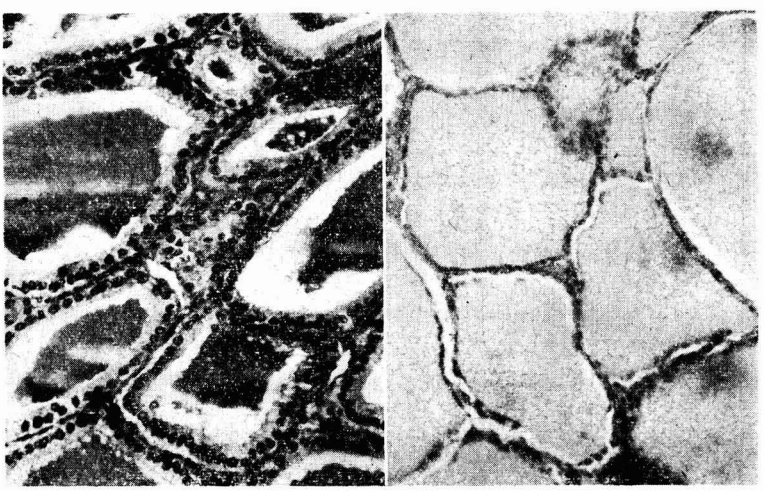



FIG. 4. Body weight changes of cows fed thyroprotein in 2 successive lactations compared with normally fed control cows (each group average of 6 cows). Precalving weight at 0 mo.

effect of thyroprotein feeding upon the histology of the thyroid gland is shown in figure 5. The normal active condition of the thyroid secretory epithelium in a lactating cow is shown in figure 5a. Contrast this with the inactive condition of the gland in figure 5b, which photomicrograph was secured from thyroid tissue of one of the group II cows which had been fed thyroprotein for 150 days. The same condition was noted in thyroids of two other cows not included in the pro-



a b

FIG. 5. Comparable sections of thyroid gland from a normal lactating cow (a) and a cow fed thyroprotein 150 days (b).

TABLE 1

*Reproduction summary of cows fed thyroprotein for two successive lactations compared with control cows*

| | Group I Control | Group II Thyroprotein |
|---|---|---|
| Cow years (*no.*) | 12 | 12 |
| Pregnancies (*no.*) | 11 | 11 |
| Total services (*no.*) | 40 | 45 |
| Services per conception (*no.*) | 3.6 | 4.1 |
| Live calves (*no.*) | 11 | 10 |
| Av. birth weight of Holsteins (*lb.*) | 86 | 86 |
| Av. birth weight of Jerseys (*lb.*) | 55 | 56 |
| Calves died in 6 wk. (*no.*) | 3 | 2 |
| Av. gain in weight in 6 wk. (*lb.*) | 30 | 38 |

duction experiment that were slaughtered after receiving 15 g. thyroprotein daily for 120 and 97 days, respectively.

Harmful physiological effects on a long-time basis may be expressed as interferences with normal reproduction and lactation. Although most of the cows had conceived before they were fed thyroprotein, a general low breeding efficiency in the herd resulted in several services after thyroprotein feeding. Cows so bred had failed to conceive on previous services and were, in general, difficult breeders. There was no evidence that feeding thyroprotein either improved or decreased the breeding efficiency. One cow in each group was finally eliminated for failure to breed. A summary of the reproduction data is given in table 1. The differences are not considered significant. The health of the calves was not affected.

The influence of feeding thyroprotein in successive lactations upon lactational variations is demonstrated by the lactation curves in figures 6, 7, and 8. These

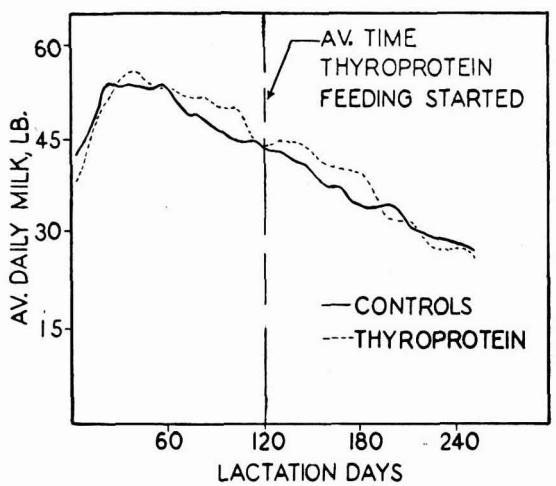



FIG. 6. Average lactation curves of control and thyroprotein-fed cows in 1st experimental lactation, 1948 (each group average of 5 cows).

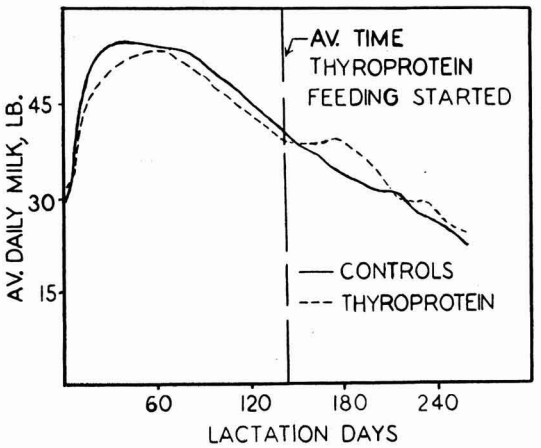



FIG. 7. Average lactation curves of control and thyroprotein-fed cows in 2nd experimental lactation, 1949 (each group average of 5 cows).

curves represent the average daily milk yield of the five cows which were used in two successive experimental lactations compared with their respective paired control cows and with their pre-experimental lactation. The curves were plotted only to 250 days in order to minimize the effect of unequal stages of gestation which affected the production of cows in both groups after that time. Figures 6 and 7 show that the response to feeding thyroprotein lasted about 70 days, that it was of about equal magnitude in both lactations and that the total lactational benefit was relatively small. The actual average lactation totals to 250 days for

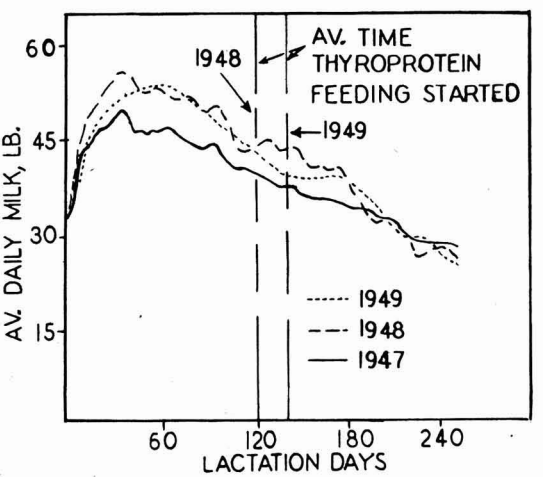



FIG. 8. Average lactation curves of 5 cows in the pre-experimental lactation compared with the 2 successive lactations in which thyroprotein was fed.

groups I and II, respectively, were 10,320 and 10,378 lb. in 1948, and 9,971 and 9,975 lb. in 1949.

The two lactations in which thyroprotein was fed compared with the pre-experimental lactation of the same cows (figure 8) gives a suggestion of a slight depression effect of feeding thyroprotein upon the early part of the next lactation. However, two of the group II cows had difficulty at calving and retained placentas in the 1949 lactation, while none of the group I cows was so affected. It is not believed that thyroprotein feeding was responsible for this trouble as it also was found in many other cows in the herd not on the experiment. Because of individual health and breeding problems, it was not possible to compare the group I and II cows in 1950 following the second lactation of feeding thyroprotein.

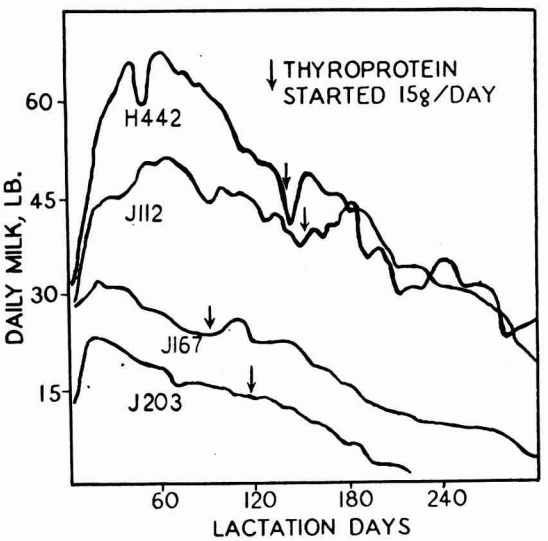



FIG. 9. Lactation curves of selected cows fed thyroprotein, showing the wide variation in response.

The response of individual cows to feeding thyroprotein was quite variable, as shown by four typical lactation curves in figure 9. These represent the cows which responded the least and the most. Cows which responded well in one lactation repeated the good response the next lactation. The response from low-producing cows was very slight.

## DISCUSSION

One objection frequently made to extended use of thyroprotein for dairy cows has been the possibility of physiological harm to the cow. Adverse physiological results of feeding thyroprotein may occur because of hyperthyroidism due to an abnormally high level of circulating thyroxine or because of interferences with

normal thyroid function following the thyroprotein feeding period. At the 15-g. level employed in this experiment serious effects due to hyperthyroidism were not evident. The stimulation achieved by thyroprotein feeding apparently was no greater than that experienced naturally by high-producing cows at the peak of lactation. On only the most extremely hot days did the thyroprotein-fed cows react differently than the control cows. If higher levels of thyroprotein had been fed, the degree of hyperthyroidism might have been excessive. However, the results of this experiment are not greatly different from those of Gardner and Millen (4) who fed up to 2 g. thyroprotein per 100 lb. body weight in midsummer. On the other hand, more extended periods of high temperature may have made the hyperthyroidism more critical (11).

Relative hyperthyroidism would depend upon how the amount of thyroprotein fed compares with the natural daily secretion rate of thyroxine. The natural level of circulating thyroxine has not been determined accurately in cows at different stages of the lactation. Schultze and Turner (10) found that the thyroxine secretion rate in lactating goats paralleled the level of milk production. They also estimated that one Jersey and one Holstein each was secreting slightly more than 10 mg. DL-thyroxine daily. On the basis of recent evaluation of the L-thyroxine content of thyroprotein (9), it has been estimated that cows receiving 15 g. of thyroprotein actually received only about 0.1 g. L-thyroxine daily. Because of losses in digestion and assimilation (14) this would produce activity equal to only 5 mg. L-thyroxine. Similar estimates of the daily L-thyroxine equivalent of 15 g. thyroprotein have been arrived at by another method (1). This amount practically is equal to the normal daily thyroxine secreted in the cow.

An estimated 100 per cent increase in the daily thyroxine supply for lactating cows would seem unduly large. However, because of the effect of thyroxine upon the thyrotrophic hormone (3), as thyroxine increases in the blood above normal, the thyroid gland is depressed. The effect of this counteraction was clearly shown in the histological sections of thyroids from cows fed thyroprotein. It is reasonable to assume that with a regular daily intake of thyroxine equal to requirement, the cow's own thyroid becomes almost inactive. Moreover, thyroxine elimination processes are increased in the presence of excess thyroxine (6). Thus, feeding no more than 15 g. thyroprotein daily should not produce an extensive period of hyperthyroidism, and no danger from such effect was noted or should be expected.

The combined data here presented indicate that the cow has balanced thyroid activity against thyroxine intake after 8 to 10 wk., and this agrees with data presented by Reece (8). The cow at this stage is more nearly normal than later when thyroprotein is withdrawn from the ration and mild hypothyroidism exists due to an inactive thyroid gland.

Possible undesirable effects due to a depression of thyroid activity following removal of thyroprotein from the ration have not been demonstrated clearly. Cows in this experiment lactated normally following 60-day dry periods in which no thyroprotein or grain was fed, yet cows in the experiment by Thomas and

Moore (12) were reported still to be hypothyroid after 90 days without thyroprotein. The latter cows were fed thyroprotein from 50 days postpartum as compared with an average of 122 days in this experiment. More information is needed concerning the factors affecting resumption of normal thyroid function. Other factors than the period of thyroprotein feeding, such as weight and condition of the cow, also may have a large influence upon the next lactation.

The attempt to prevent weight losses by increasing the concentrate ration was not successful. It is probable that higher levels of feeding would have been more effective, but some of the cows refused to eat more feed than was offered. The loss in weight was not entirely associated with increased milk secretion because cows that gave only small responses in milk yield lost body weight, although not as much as the others, and their losses were more quickly regained.

## SUMMARY

Five cows were fed 15 g. thyroprotein daily during the declining phase of two successive lactations, and two other cows were fed thyroprotein in single lactations. Each of the cows fed thyroprotein was paired with a suitable control cow. Thyroprotein produced an increase in milk yield and heart rate and slight to insignificant changes in respiration, body temperature and milk fat test, as well as a loss of body weight. Body weight gains were resumed in 2 mo. following introduction of the thyroprotein and, at the same time, the average milk production and other physiological characteristics indicated that the treated cows again were functioning similarly to the controls. The histology of the thyroid gland of the treated cows indicated that it was almost inactive at this time. Following removal of thyroprotein when the cows were dried off, the cows continued to regain weight during 60-day dry periods and freshened apparently in normal condition. No harmful effect could be attributed to this moderate thyroprotein feeding either because of stimulation or of depression.

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# MOLYBDENUM IN COWS' MILK[1]

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This paper is the sixth in a series on the mineral elements of cows' milk (1, 2, 3, 4, 5). Molybdenum is of interest here because, although apparently essential for certain plant species (6, 11, 16), notably for nitrogen fixation (10, 18, 20), it has been shown to be toxic to animals (7, 9, 15, 19).

Previous investigators are not in good agreement regarding the presence and amount of molybdenum in milk. Blumberg and Rask (8) were unable to detect it; Dingle and Sheldon (12) state that in minute traces it is possibly a normal constituent. Drea (13) reports it qualitatively in all samples of cows' milk examined, but not in goats' milk. ter Meulen (21) states that it is widely distributed in tissues and reports levels in blood, bile, milk and eggs of 30 to 140 $\gamma$ per kilogram. Teresi *et al.* (22) report an average concentration of 47.5 $\gamma$ per liter of cows' milk, but only 13.5 $\gamma$ in goats' milk.

## EXPERIMENTAL

A description of the procedure appeared in an earlier paper (1). The work was carried on during the winter of 1950–51, with eight cows divided into two sub-groups of four each, representing the Ayrshire, Holstein, Guernsey and milking Shorthorn breeds. Each breed pair was matched as closely as possible with respect to stage of lactation. The supplement fed was ammonium molybdate $[(NH_4)_6MO_7\ O_{24} \cdot 4H_{20}]$ in an approximate daily amount of 500 mg. equivalent to 272 mg. of elemental molybdenum. One group received the supplement during November and December, the other during January and February.

Composite 2-day milk samples of 2 l. each were taken from each cow once a month. Triplicate 500-ml., aliquots were evaporated to dryness and ashed in an electric furnace, the ash then being dissolved in 4*N* HCl. Molybdenum was determined photometrically[2] by the method of Ellis and Olson (14).

## RESULTS

The values obtained are summarized in table 1. The amounts of molybdenum in the milk from the control cows varied considerably from month to month and between individuals, the average being 73 $\gamma$ per liter of milk (range: 18 to 147). Without exception, the milks contained strikingly more molybdenum when the supplement was fed, the average increase being fivefold. The least increase (cow 447) was better than twofold and the greatest (cow 638) was nearly twelvefold. The average difference ($371 - 73 = 298$) was statistically highly significant, being nearly nine times its standard error ($S.E._D = 34$).

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[1] Contribution no. 805 of the Massachusetts Agricultural Experiment Station.

[2] The instrument used was a Model DU Beckman spectrophotometer. Measurements were made at a wave length of 420 mμ, and a slit width of 0.085 mμ.

TABLE 1

*Effect on molybdenum content of milk of feeding cows ammonium molybdate*

| | Cows on control ration | | | | | Cows receiving supplemental molybdenum | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st half of season | | | | | | | | | |
| | A258[a] | G645 | H447 | S112 | Av. | A246 | G638 | H444 | S74 | Av. |
| | ($\gamma$/*l. of milk*) | | | | | | | | | |
| November | 75 | 63 | 140 | 70 | 87 | 445 | 690 | 383 | 540 | 515 |
| December | 45 | 32 | 147 | 107 | 83 | 225 | 332 | 285 | 263 | 276 |
| Av.—1st half | 60 | 48 | 144 | 89 | 85 | 335 | 511 | 334 | 402 | 396 |
| | 2nd half of season | | | | | | | | | |
| | A246 | G638 | H444 | S74 | Av. | A258 | G645 | H447 | S112 | Av. |
| January | 49 | 59 | 35 | 18 | 40 | 475 | 330 | 268 | 317 | 348 |
| February | 89 | 30 | 8[b] | 120 | 80 | 395 | 200 | 390 | 377 | 341 |
| Av. 2nd half | 69 | 44 | 35 | 69 | 60 | 435 | 265 | 329 | 347 | 345 |
| Av. entire season | 65 | 46 | 107 | 79 | 73 | 385 | 388 | 332 | 374 | 371 |

[a] The initial letter prefixed to each cow's no. indicates the breed.
[b] Not included in the average—sample overheated in the ashing process.

In comparison with most of the other trace elements investigated in this laboratory, the amount of molybdenum in milk, either from natural sources or as a result of feeding a specific supplement, is relatively high, and there is a larger average increase due to feeding the supplement. Table 2 shows the contrasts; zinc is the one exception to the above statement, but it did not show the large relative increase due to the supplement that molybdenum has.

Recalling experience with nickel in cows' milk (4), it was ascertained that the amounts of molybdenum found in the milk when the cows were on the control ration did not come from the milking machine. This was done at the end of the trial by milking directly into a glass jar a large composite sample from the four cows that had been on the control ration for a period of 2 mo. The average molybdenum content of this sample was 42 $\gamma$ per liter, a value similar to that reported by Teresi *et al.* (22); this is a rational finding. Unlike nickel, molybdenum when present in steel alloys would tend to be distributed through the mass rather than concentrated in an exposed surface plating, so that the possibility of contamination due to contact with the milking machine would seem to be much less.

Partition of the molybdenum between fat and solids-not-fat of the milk also was investigated. Composite 2-day samples were taken from both groups of

TABLE 2

*Recapitulation of average amounts of various trace elements in cows' milk*

| Element | "Control" milks | "Supplement" milks | Increase due to feeding of specific supplements |
|---|---|---|---|
| | ($\gamma$/*l. of milk*) | ($\gamma$/*l. of milk*) | |
| Manganese | 22 | 65 | Approx. 3-fold |
| Zinc | 3900 | 5100 | Approx. 1.3-fold |
| Cobalt | 0.6 | 2.4 | 4-fold |
| Nickel | none | none | none |
| Molybdenum | 73 | 371 | Approx. 5-fold |

cows after the trial had been in progress about 3 mo. The cream was separated centrifugally, and molybdenum was determined in cream and skimmilk in the same manner as the regular samples. Cream from the cows on the control ration contained 263 γ per liter, from those receiving the molybdenum 505 γ per liter; the amounts in the skimmilk were 45 γ and 193 γ per liter, respectively.

The possible significance of these results seems evident. It has been shown (15) that molybdenum is not only toxic to cattle if its concentration in forage exceeds 30 ppm., but it also markedly retards the growth of rats when added to their diet (16). Because molybdenum is an essential trace element for some plant species, small amounts of it are being added to mixed fertilizers by some manufacturers. This work has shown that cows pass into their milk substantial amounts of supplemental molybdenum. It is, therefore, an open question whether cows eating forage grown with fertilizers containing added molybdenum might pass into their milk enough of the element to be toxic to calves, other young animals, or humans.

## SUMMARY

Molybdenum appears to be a natural constituent of cows' milk, the amount varying in different individuals but of the general order of 40 to 70 γ per liter of whole milk.

Feeding 500 mg. daily of ammonium molybdate to eight cows for periods of 2 mo. increased the average amount of molybdenum in their milk about fivefold.

The level of molybdenum in these milks was higher than that of manganese and much higher than the level of cobalt. The response to feeding a supplement of the element, as indicated by increased levels in the milk, was greater than for any other trace element studied thus far.

Milk samples from control cows milked directly into glass showed similar levels of molybdenum as those obtained *via* the milking machine, thus eliminating the possibility that any of the molybdenum in the milks was due to metallic contamination.

Analysis of composite samples of cream and skimmilk showed that most of the molybdenum in "control" milks or in those from cows receiving a molybdenum supplement was concentrated in the cream fraction.

The possible significance of these findings from the standpoint of toxicology is discussed briefly.

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# INFLUENCE OF PENICILLIN ON THE LACTIC ACID PRODUCTION OF CERTAIN LACTOBACILLI[1]

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Penicillin is used widely in the treatment of mastitis of dairy cows. Foley *et al.* (1) reported that penicillin was effective in eliminating the infection when used against streptococci and staphylococci (micrococci) associated with bovine mastitis. After infusion of an ointment base penicillin preparation into dairy cows' udders (up to 100,000 units per quarter), it was found (4) that the milk produced for the next several days contained sufficient concentrations of the antibiotic to retard significantly the growth of the lactic acid-producing streptococci of commercial butter and cheese starters. The critical concentration was established at 0.1 unit of penicillin per milliliter of milk, above which insufficient acid developed in starters resulting in improper coagulation of milk during cheese manufacture. Hunter (2) reported that 10 strains of starter streptococci were found to be susceptible to penicillin. *Streptococcus cremoris* strains were markedly inhibited under the conditions employed by doses of 0.1 unit per milliliter. *Streptococcus lactis* strains were not inhibited to the same degree, unless 0.25 to 0.3 unit per milliliter was present in the milk. Katznelson and Hood (3) reported that all 45 strains of lactic streptococci isolated from starter cultures were completely inhibited by penicillin in skimmilk in amounts ranging from 0.2 to 0.4 unit per milliliter. These investigations (2, 3, 4) were not concerned with the effects of penicillin on lactic lactobacilli. Hargrove *et al.* (5) reported that growth of *Lactobacillus bulgaricus* in a Swiss cheese starter was inhibited by 0.1 unit of penicillin.

Since some species of lactic acid-producing lactobacilli also are used in the manufacture of fermented dairy products, it appeared that information regarding the susceptibility of these lactobacilli to the action of penicillin would be of value. Experiments were conducted to determine the concentrations of penicillin required to influence the lactic acid production of three lactobacillus species commonly associated with dairy products. Those selected were *Lactobacillus acidophilus* and *Lactobacillus bulgaricus,* which are used in the manufacture of fermented milk drinks, and *Lactobacillus casei,* which generally is present during the aging process of cheddar cheese and may contribute, in part, to the ripening of the cheese.

## EXPERIMENTAL

Cultures of each lactobacillus species were transferred daily in skimmilk for at least 1 wk. prior to their use in the study. The milk from which the skimmilk was separated had been obtained from a herd known not to have been treated

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TABLE 1

*Influence of penicillin on lactic acid development of Lactobacillus bulgaricus*
*(Figures expressed as per cent lactic acid)*

| Concentration of penicillin | Titratable acidity values at various hours of incubation at 35° C. | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 6 | 8 |
| (*Units/ml.*) | (%) | (%) | (%) | (%) | (%) |
| 0.0 | 0.23 | 0.25 | 0.27 | 0.42 | 0.71 |
| 0.1 | 0.23 | 0.26 | 0.28 | 0.43 | 0.72 |
| 0.3 | 0.23 | 0.26 | 0.28 | 0.45 | 0.73 |
| 0.6 | 0.23 | 0.26 | 0.27 | 0.38 | 0.45 |
| 1.0 | 0.24 | 0.25 | 0.26 | 0.29 | 0.30 |
| 2.0 | 0.22 | 0.23 | 0.24 | 0.24 | 0.24 |
| 5.0 | 0.22 | 0.23 | 0.23 | 0.24 | 0.24 |

with penicillin or other antibacterial agents for several months. Various desired concentrations of penicillin were added to the skimmilk. Aqueous dilutions of crystalline sodium penicillin "G" were made so that 1 ml. of diluted material was added to 99 ml. of skimmilk. Thus, various desired penicillin concentrations were obtained in the medium without a dilution variable. The prepared samples were sterilized by autoclaving for 10 min. at 15 lb. pressure. After adjustment of the temperature to 35° C., each sample was inoculated with a 1 per cent culture, followed by thorough mixing and dispensing into sterile glass test tubes in 17.5-ml. quantities. The samples were incubated at 35° C. for various time intervals selected to yield differences in lactic acid production. Titratable acidities were determined using the entire contents of a tube of material twice rinsed with 9-ml. portions of distilled water. Simultaneous pH determinations also were made.

At least three trials were made with each culture species. Results of one trial with *L. bulgaricus* are shown in table 1, including concentrations of penicillin and the time intervals used for the particular trial. By the end of 8 hr. of incubation, the culture had not reached its maximum acidity but the trial had progressed sufficiently to show that this culture was susceptible to the action of penicillin at certain concentrations. It was found that 0.3 unit of penicillin per milliliter of milk did not retard acid development, while 0.6 unit per milliliter did retard acid production as compared to the penicillin-free control sample.

TABLE 2

*Influence of penicillin on certain lactobacillus species*
*(Figures are concentrations of penicillin)*

| Organisms | Lactic acid production at 35° C. up to 48 hr. duration | | |
|---|---|---|---|
| | Normal | Retarded | None |
| | (*Units/ml.*) | (*Units/ml.*) | (*Units/ml.*) |
| *Lactobacillus bulgaricus* | 0.1–0.3 | 0.3–0.6 | 2.0 |
| *Lactobacillus acidophilus* | 0.1 | 0.1–0.3 | 1.0 |
| *Lactobacillus casei* #1 | 0.1–0.3 | 0.3–0.6 | 2.0 |
| *Lactobacillus casei* #2* | 0.1–0.3 | 0.3–0.6 | 2.0 |

* American Type Culture Collection no. 7469.

A concentration of 2.0 units per milliliter was sufficient to practically stop acid development during 8 hr. of incubation. The results of similar trials with *L. acidophilus* and two strains of *L. casei* are summarized in table 2.

Of the three species of lactobacilli used, *L. bulgaricus* culture produced lactic acid the most rapidly as well as in the largest quantities. After 12 and 24 hr, penicillin-free control cultures of *L. bulgaricus* developed 0.76 and 1.87 per cent acidity, respectively. The *L. acidophilus* control culture developed 1.05 per cent acidity after 24 hr. The *L. casei* no. 1 control culture required 48 hr. to develop 0.90 per cent acidity and the *L. casei* no. 2 control culture required 48 hr. to develop 1.08 per cent acidity. With rapidly growing cultures such as *L. bulgaricus* and *L. acidophilus,* significant differences in acid production could be obtained within 8 to 12 hr., which could be reliably attributed to the action of varying concentrations of penicillin. In experiments using the two different *L. casei* strains it generally was necessary to extend the period of incubation before titration up to as much as 36 hr. in some instances in order to obtain significant differences in acid production which were due to penicillin activity.

## DISCUSSION

The results showed that the three species of lactobacilli were of approximately the same level of sensitivity to penicillin. Essentially the same results were obtained from triplicate trials made with the same culture on different days. A decreased rate of lactic acid development by these organisms occurs in the range of 0.3 to 0.6 unit of penicillin per milliliter. When sufficient penicillin was present to stop acid development, no evidence was found to indicate that the cultures were completely inactivated. Some penicillin-resistant organisms were present resulting in a delayed acid development which was demonstrated by extending the period of incubation to 3 or 4 days. This delayed growth of penicillin-resistant organisms would be of little practical significance in the case of fermented buttermilks, but might be of more importance in the case of cheddar cheese ripening. In these studies the influence of penicillin on *L. casei* was demonstrated in skimmilk at 35° C., which permits comparisons with other lactobacillus species and streptococci studied under similar conditions. Whether similar effects occur in a cheese medium at lower incubation temperatures remains to be determined and would be useful information. It has not been determined whether milk containing penicillin in small enough quantities to permit cheddar cheese manufacture would result in cheese containing penicillin in sufficient concentration, due to a concentrating effect in the maufacturing process, to affect the growth of the lactobacilli. The *L. bulgaricus* culture used by Hargrove *et al.* (5) was somewhat more sensitive to penicillin than the culture used in this study, which suggests strain differences occur with respect to penicillin sensitivity.

## SUMMARY

The influence of penicillin in skimmilk on the lactic acid-producing ability of *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus casei* was

determined. Less than 0.3 unit per milliliter of penicillin in milk did not significantly retard acid development. A decreased amount of lactic acid production by these three species of microorganisms occurred in the range of 0.3 to 0.6 unit of penicillin per milliliter. In concentrations of 2.0 units per milliliter, no acid production was observed in 48 hr. at 35° C. In general, these species of lactobacilli were sensitive to concentrations of penicillin only slightly greater (0.3 to 0.6 unit per milliliter) than the levels that have been previously reported for the lactic acid streptococci (0.1 to 0.4 unit per milliliter) commonly used in dairy starters.

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## ABSTRACTS OF LITERATURE

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### BUTTER

O. F. HUNZIKER, SECTION EDITOR

**549. Relation of the essential oils of Coronopus didymus to the tainting of butter.** D. A. FORSS. Nature, **167,** 4253: 733. 1951.

Milk, cream and butter from cows which have pastured on this weed have a burnt or scorched flavor, which is intensified by heating. The flavor was shown to be due to benzyl mercaptan and disulphide, which are fat-soluble. Removing cows from pasture prior to milking and dairy plant processing steps did not control the objectionable flavor. R. Whitaker

**550. Butter cutter.** W. GROCOFF. U. S. Patent 2,563,237. 7 claims. August 7, 1951. Official Gaz. U. S. Pat. Office, **649,** 1: 159. 1951.

A device for cutting small blocks of butter into pats for table use is described. R. Whitaker

### CHEESE

A. C. DAHLBERG, SECTION EDITOR

**551. Ueber einige Versuche auf dem Gebiete der Kaserei. (Concerning some experiments in the sphere of the cheese factory.)** G. SCHWARZ and H. MUMM. Molkerei Zeitung, **5,** 29: 786–788. July 19, 1951.

In making half-fat Tilsit cheese, the yield of ripened cheese and the fat content of the dry matter both were higher when the curd was cut in larger particles than normal and considerably reduced when the curd was in abnormally small particles. Adding the fine particles from the whey back to the Tilsit cheese during subsequent operations increased the yield slightly but the resulting cheese was sour and bitter and the body was chalky and showed numerous small white spots of incompletely broken-down particles. A commercial preparation (Antibut) added at the rate of 65 g./100 l. of milk suppressed butyric acid fermentation in cheese from inoculated milk; however, cost and certain legal aspects might prevent use of the product. Immersion of Edam and Gouda cheese in a 15% alcoholic solution of "Nipagin M" prevented mold growth for 6–7 wk. without affecting taste or rind formation. F. E. Nelson

**552. Processed cheese and method of making the same.** C. T. ROLAND (assignor to Hall Laboratories, Inc.). U. S. Patent 2,564,374. 8 claims. Aug. 14, 1951. Official Gaz. U. S. Pat. Office, **649,** 2: 529. 1951.

A combination of an alkali-metal metaphosphate, such as Na metaphosphate, and a cheese-solubilizing, water-soluble alkali-metal (Ca or Mg salt) is used as a stabilizer-emulsifier for processed cheese. R. Whitaker

### CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**553. It's here—frozen concentrated milk.** Food Eng. Staff Food Eng., **23,** 7: 35, 121. July, 1951.

Gould Milk and Cream Co., Minneapolis, is using the patented system of the Milk Concentrates Corp. for making a 4:1 dairy product. Various advantages of the product are given. The method of manufacture was developed by J. A. Lewis of the University of Miami as an outgrowth of frozen concentrated citrus juice. Although all manufacturing details are not disclosed, the processing generally is similar to the 3:1 operation now employed by other dairies. The key factors are the special falling film-type evaporator and the method of pasteurizing. The initial output has been packed in 1-qt. cartons for military consumption. T. J. Claydon

**554. Manufacture of casein.** P. F. SHARP and J. B. SHIELDS (assignors to Golden State Co., Ltd.). U. S. Patent 2,562,646. 19 claims. July 31, 1951. Official Gaz. U. S. Pat. Office, **648,** 5: 1480. 1951.

Acid-coagulated casein, whipped to form a foam, is drained free of whey and water by continuously feeding the foam into a series of several tanks, each one set at a lower level. The foam progresses from tank to tank by crowding and overflowing, the liquid drained from the foam being removed from the bottom of the tanks. R. Whitaker

**555. Meat substitute and process for making same.** C. L. WRENSHALL. U. S. Patent 2,560,-621. 13 claims. July 17, 1951. Official Gaz. U. S. Pat. Office, **648,** 3: 760. 1951.

Milk solids are gelled with a texturizing agent to yield a product having the fibrous texture of comminuted meats. R. Whitaker

Also see abs. no. 564, 565.

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**556. Spore-forming thermophiles in sterilized milk.** L. F. L. CLEGG, Natl. Agr. Advisory Service, Woodthorne, Tettenhall, Staffordshire. J. Soc. Dairy Technol., **3,** 4: 238–249. July, 1950.

An outbreak of spoilage caused by thermophilic sporeforming organisms in bottled sterilized milk is described. The organisms were believed to come from the farms, since a considerable proportion of the raw milks tested contained thermophiles. These were detected by autoclaving the milk for 30 min., then incubating at 63° C. for at least 1 wk.

The main organism involved grew at 63° C. but not at 37° C. It grew poorly on ordinary laboratory media but grew well in yeastrel milk agar containing blood serum. With a few minor exceptions, the characteristics of the organism were the same as those of *Bacillus calidolactis.*

The time and temperature combination necessary to destroy spores of the organism in milk caused severe browning of the milk. The authors think that excessive caramelization can be avoided by rapid cooling of the milk, which would in turn help to prevent growth of thermophiles during the usual slow cooling of large stacks of bottles in cases. They suggest that it might be possible to control spoilage of sterilized milk by all sporeformers if the milk is first flash heated in bulk to 125–130° C. for 1 min. or less, cooled immediately to homogenizing temperature, bottled and sterilized at low pressure. Because of the wide distribution of spores of thermophiles in nature the authors think there is little hope that milk free of these organisms can be produced under practical conditions. E. M. Foster

**557. Heat resistant bacteria in raw milk. Part III. Occurrence of thermoduric bacteria in farm milk supplies.** S. B. THOMAS, D. G. GRIFFITHS, B. F. THOMAS, M. HUMPHREYS and D. ELLISON, Natl. Agr. Advisory Service, Trawscoed, Aberystwyth, and Natl. Milk Testing Service, Brynawel, Aberystwyth. J. Soc. Dairy Technol., **4,** 1: 51–60. Oct., 1950.

Samples of farm milk were held at atmospheric shade temperature until 24–28 hr. after milking. They were pasteurized in the laboratory for 35 min. at 63.5° C. and plated on yeastrel milk agar. The plates were incubated for 4 d. at 30° C. Samples were taken from farms using one of the following methods of treating the utensils: (a) sterilization in a steam chest, (b) immersion in boiling water, (c) sterilization with sodium hypochlorite or (d) washing in warm water.

As might be expected, milk with low numbers of thermoduric organisms was produced when any of the first 3 methods of utensil treatment was used properly. Thermoduric counts increased rapidly when the sterilization treatment was used only intermittently, *e.g.,* twice weekly. Under the practical conditions studied, the hypochlorite treatment was as effective as steaming only if the utensils were thoroughly cleaned and had smooth surfaces. The value of weekly or bi-weekly steaming and replacing worn, rusty utensils was stressed for farmers using hypochlorite sterilization. Immersion of utensils for at least 2 min. in boiling water gave results as satisfactory as steaming, but under practical conditions hot water treatment often did not give results comparable to those from steaming. Excessive thermoduric counts were found in a very high proportion of the milk samples from farms on which the utensils were merely washed in warm water without a sterilization treatment.

Evidence is presented to show that the majority of thermoduric organisms get into milk from the utensils. E. M. Foster

**558. Heat resistant bacteria in raw milk. Part IV. Further observations on the occurrence of thermoduric bacteria in different types of raw milk supplies.** S. B. THOMAS, P. M. HOBSON, D. G. GRIFFITHS, G. GEORGE and E. JENKINS, Natl. Agr. Advisory Service, Trawscoed, Aberystwyth, and Natl. Milk Testing Service, Brynawel, Aberystwyth. J. Soc. Dairy Technol., **4,** 3: 177–183. Apr., 1951.

Samples of farm milk were held at atmospheric shade temperature until 24–28 hr. after milking. Bulk tanker milk was held at 3–10° C. until 10 a.m. on the day after sampling. The samples were pasteurized in the laboratory for 35 min. at 63.5° C. and plated on yeastrel milk agar. The plates were incubated at 30° C. for 4 d. Numbers of thermoduric bacteria were determined in tuberculin-tested milk, milk from farms using milking machines and bulk raw tanker milk at country creameries. In addition, a comparison was made between the numbers of thermoduric bacteria in morning and evening milk from farms.

Tuberculin-tested milk generally contained low numbers of thermoduric organisms in areas where strict supervision and rigorous bacteriological control had been applied routinely. The thermoduric content of milk from farms using milking machines varied widely, depending on the sterilization treatment given the machines. A large proportion of the samples of bulk tank milk showed high counts of thermoduric organisms. There was no significant difference in the thermoduric counts of morning and evening milk.

E. M. Foster

**559. The use of broth for sterility tests on milk bottles and the effect of ageing the rinse.** L. A. E. BAKER, J. Soc. Dairy Technol., **4,** 1: 47–49. Oct., 1950.

In an attempt to determine whether the organisms surviving bottle washing would grow in milk before it was consumed, freshly washed bottles were rinsed with a nutrient broth containing 5 g. peptone, 3 g. yeastrel, 10 ml. whole milk and 1,000 ml. distilled water and adjusted to pH 7.4. The rinse was plated immediately, then stored in the refrigerator until 9 a.m. of the following day, after which it was held for 24 hr. at 18 ± 2° C. The conditions chosen for aging were intended to simulate those to which milk is exposed before

delivery and during the first 24 hr. in the consumer's possession.

The majority of the bottles (81%) showed counts of less than 200 immediately after washing. After the refrigerated storage, however, the broth rinse from 20% of these "satisfactory" bottles showed counts in excess of 20,000/pt. bottle, and an additional 15% showed counts between 2,000–20,000. It is suggested that a low initial count does not always mean a bottle that will be entirely satisfactory for storing milk.

E. M. Foster

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**560. Composition and function of colostrum and regression milk.** R. A. McCance and E. M. Widdowson. Nature, **167,** 4253: 722. 1951.

Regression milk, which is the fluid produced by the mammary gland during its involution, and colostrum are both higher in protein and acid and alkaline phosphatase than normal milk.

R. Whitaker

**561. $C_{20-22}$ unsaturated acids of butterfat.** F. B. Shorland and D. L. Johannesson. Nature, **168,** 4263: 75. 1951.

This is a preliminary report in which unsaturated fatty acids, ranging in I values from 157 to 228, were prepared by high-vacuum distillation. Data are given on the composition of the $C_{20-22}$ fatty acid series. The presence of monoene, diene, triene, tetraene and pentaene acids was shown by means of chromatographic absorption.

R. Whitaker

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**562. Die Beeinflussung Des Homogenisiereffektes durch die Art und Anordnung des Homogenisierspaltes. (The influence of the type and arrangement of the homogenizer valve upon homogenization).** English summary. W. Schulz. Die Milchwissenschaft, **5,** 10: 349–353. Oct., 1950.

The process of homogenization is regulated mainly by the amount of pressure used, provided the change in pressure takes place in a thin, uniform layer of milk. Time and path of flow are of minor importance only, as is the use of a 2-stage valve in a homogenizer.

I. Peters

**563. Test units nip costly shutdowns.** W. O. Whitney, Creamery Package Mfg. Co., Chicago. Food Eng., **23,** 6: 75, 154, 155, 156. June, 1951.

Bowman Dairy Co., Chicago, maintains laboratory test units for checking its temperature recorders and control instruments. Personnel trained at instrument company schools handles maintenance and repairs of this equipment. Spare flow diversion valves and spare instruments of each type are carried for emergencies. A stock of parts is maintained at the instrument testing laboratory. With these facilities, necessary repairs can be made quickly. Control instruments are inspected and cleaned monthly. Among the benefits of the program are less lost time in plants, better operation and increased life of instruments. The test laboratory also has stimulated the training of operators and mechanics.

T. J. Claydon

**564. Evaporating apparatus.** J. V. M. Risberg (assignor to A. K. Ticbolaget Separator Corp.). U. S. Patent 2,562,739. 5 claims. July 31, 1951. Official Gaz. U. S. Pat. Office, **648,** 5: 1506. 1951.

A continuous evaporator, designed for nutritious liquids, especially whey, consists of a horizontal closed tank where the evaporation takes place directly above a vertical plate-type heater. The whey circulates from the tank to the bottom of the plates and thence upward to the tank. The hot vapors from the evaporator, with additional steam, provide the heating medium in the alternate plates.

R. Whitaker

**565. Spray drying apparatus.** J. J. Mojonnier (assignor to Mojonnier Bros. Co.). U. S. Patent 2,562,473. 5 claims. July 31, 1951. Official Gaz. U. S. Pat. Office, **648,** 5: 1434. 1951.

The main feature of this drier is the design of the hot air ducts which direct the air stream in a downward circular motion around the nozzle and cone-shaped spray of product produced therefrom.

R. Whitaker

**566. Contents gauge for milk tanks.** C. A. deGiers (assignor to The Liquidometer Corp.). U. S. Patent 2,562,529. 3 claims. July 31, 1951. Official Gaz. U. S. Patent Office, **648,** 5: 1449. 1951.

A float-actuated device attached to the inside wall of milk tanks for indicating on the outside the volume of liquid within the tank is described.

R. Whitaker

**567. Milk cooling by direct-expansion and flooded systems of ammonia.** B. Norman. J. Soc. Dairy Technol., **4,** 1: 33–34. Oct., 1950.

The principle and the advantages of direct-expansion cooling systems are discussed.

E. M. Foster

**568. Chilled water and brine for liquid milk cooling.** W. Milligan. J. Soc. Dairy Technol., **4,** 1: 30–32. Oct., 1950.

The merits and demerits of water and brine systems for milk cooling are discussed.

E. M. Foster

**569. Refrigerating retail and wholesale trucks.** E. L. White, White Ice Cream & Milk Co., Wilmington, N. C. Sou. Dairy Prod. J., **49,** 3: 28, 29, 106, 107, 116. Mar., 1951.

Experience with mechanical refrigeration of 11 trucks in 1947 to a total of 48, an entire fleet, in 1950 is reported. Installations included complete insulation, smaller doors and the refrigeration units consisting of plates and piping connections or individual mechanical units. The costs ranged from an average of $450/small truck to $2,000 for a trailer. For individual compressors $250–$350/unit additional cost was involved. Temperatures below 40° F. were maintained for

6–8 hr. The advantages of mechanical refrigeration of the trucks include elimination of a large ice bill and ice storage, reduced total cost with very little upkeep, cleaner trucks and workers, lower and more uniform temperatures, the use of trucks for storage space, increased efficiency of plant operations and improved service to customers. F. W. Bennett

Also see abs. no. 586.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**570. Simplified work simplification.** R. A. BAER, Bowman Dairy Co., Chicago. Food Eng., **23,** 7: 76, 77, 119. July, 1951.

Work simplification at Bowman Dairy Co. involves a philosophy and attitude among workers of seeking and developing more efficient methods of performing various operations. Special training helps employees to develop an awareness of wasted actions and then devise ways of eliminating them. The program has demonstrated that the man on the job can contribute valuable ideas toward improved plant efficiency. T. J. Claydon

**571. Basic requirements for a profitable business.** J. W. POST, Armour and Co., Chicago. Sou. Dairy Prod. J., **48,** 6: 80–82. Dec., 1950.

Six basic requirements for a profitable business are discussed: a sufficient potential demand for goods; trained, intelligent and energetic personnell; plans for economical location, suitable raw material and a uniform satisfactory standard of products; proper legal counsel and compliance with all laws; operation of an adequate accounting system; and availability of adequate finances. F. W. Bennett

**572. Pushbutton truck door.** Anon. Food Eng., **23,** 7: 115, 117. July, 1951.

An automatic door closing device installed in retail delivery trucks of a west coast dairy saves as much as 1 hr./day/truck and spares drivers many "lost" motions. Installation costs were approx. $250/truck. T. J. Claydon

Also see abs. no. 559, 583.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**573. The estimation of lactic acid in silage.** (Abs.) A. J. G. BARNETT, Univ. of Aberdeen Biochem. J., **48,** 4: lvii–lviii. 1951.

A modification of a method whereby lactic acid is oxidized to acetaldehyde which gives a red color with p-hydroxydiphenyl in the presence of Cu is mentioned as being satisfactory for estimating lactic acid in silage. No details are given. A table shows lactic acid values of about 8% (dry basis) at pH 3.8 decreasing to 0.5% at pH 5.6. A. O. Call

**574. Feed for ruminant animals.** C. W. TURNER (assignor to American Dairies and Quaker Oats Co.). U. S. Patent 2,560,830. 7 claims. July 17, 1951. Official Gaz. U. S. Pat. Office, **648, 3:** 817. 1951.

A feed for ruminating animals is described consisting of coated urea, ground grain, a bacterial growth-stimulating material of the B-vitamin complex, proteins, essential amino acid compounds and cultures of rumen microflora; it is designed to improve the utilization of non-protein-nitrogen feeds and cellulose. R. Whitaker

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**575. Milking system.** G. W. BERRY. U. S. Patent 2,564,620. 5 claims. Aug. 14, 1951. Official Gaz. U. S. Pat Office, **649,** 2: 594. 1951.

A device for allowing a milking machine to operate continuously is described. Two milk receivers are mounted on a scale beam. When 1 receiver is full, the weight causes the beam to tip, causing the incoming milk to enter the other receiver which is under vacuum and releases the vacuum on the full one so that it drains at atmospheric pressure. When the second receiver is full, the beam tips in the other direction, reversing the mechansim. R. Whitaker

**576. Milking parlor stall.** H. B. BABSON and C. A. THOMAS (assignors to Babson Bros. Co.). U. S. Patent 2,564,047. 5 claims. Aug. 14, 1951. Official Gaz. U. S. Pat. Office, **649,** 2: 439. 1951.

A stall designed especially for cows being milked is described. R. Whitaker

**577. Can hoist.** W. A. SCOTT and J. E. COOK (assignors to DeLaval Separator Co.). U. S. Patent 2,562,066. 7 claims. July 24, 1951. Official Gaz. U. S. Pat. Office, **648,** 4: 1237. 1951.

A hoist designed especially for lifting cans of milk in and out of a cooling tank is described. R. Whitaker

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**578. Confectionery article.** K. A. BEVINGTON. U. S. Patent 2,564,049. 10 claims. Aug. 14, 1951. Official Gaz. U. S. Pat. Office, **649,** 2: 440. 1951.

The mold for a frozen stick novelty is so shaped that 2 wafers or cookies may be inserted. The mix is injected between the cookies and the confection frozen. Small holes are provided in the cookies into which the mix flows and hardens, thus holding the piece together when being consumed. R. Whitaker

**579. Apparatus and method for forming frozen confections.** I. A. RUMMEL and J. D. WELCH (assignors to Henningsen Produce Co.). U. S. Patent 2,563,278. 10 claims. Aug. 7, 1951. Official Gaz. U. S. Pat. Office, **649,** 1: 170. 1951.

Semi-frozen mix is filled into tubular flexible casings in a pulsating stream, each pulsation forming an individual metered portion of finished product, the casing being sealed between each portion. R. Whitaker

**580. Confection coating machine.** E. J. OTKIN (assignor to Good Humor Corp.). U. S. Patent 2,562,059. 4 claims. July 24, 1951. Official Gaz. U. S. Pat. Office, **648,** 4: 1236. 1951.

A rotating drum device for coating frozen stick novelties and confections with nuts, crumbs, candy, and the like is described.

R. Whitaker

**581. Custard dispenser.** J. H. SAMMY. U.S. Patent 2,560,664. 1 claim. July 17, 1951. Official Gaz. U. S. Pat. Office, **648,** 3: 771. 1951.

A freezer is described for making frozen custard, soft ice cream, etc., consisting of a horizontal freezing chamber and having a pump which automatically introduces mix into the freezer through a ball-type valve. R. Whitaker

**582. Vending machine.** L. W. SPRINGSTEERS (assignor to J. H. Hardy and M. L. Quinn). U. S. Patent 2,561,828. 5 claims. July 24, 1951. Official Gaz. U. S. Pat. Office, **648,** 4: 1173. 1951.

A vending machine dispenses factory-filled ice cream cones one at a time. R. Whitaker

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**583. They call it the world's most modern dairy.** A. V. GEMMILL, Asst. Ed. Food Eng., **23,** 7: 60–71. July, 1951.

A detailed description is given of the layout, construction, equipment and processing operations of the new Supplee-Willis-Jones Milk Co. plant in Philadelphia. A combination of latest developments and innovations has been utilized to achieve efficiency and economy of operation. Functional design has been emphasized with a straight-through processing line. The most outstanding feature is the huge storage room for bottled milk. Here palletized handling is utilized for loading and unloading of trucks, resulting in a 75% saving in time involved. Truck bodies were redesigned for the purpose. Recent advances in automatic control equipment have been employed throughout and offices and processing rooms are air conditioned. Many other special features are described. T. J. Claydon

**584. Canned fresh milk now a reality.** R. BLOOMBERG and F. E. HESSEY. Food Eng., **23,** 71–74. June, 1951.

The Graves and Stambaugh method, together with the Martin aseptic canning process, now is being used by Med-O-Milk, Inc., at East Stanwood, Wash. to produce canned whole milk that will keep 4–6 mo. without refrigeration. Because of the special flash sterilizing procedure employed, the product does not exhibit a cooked flavor. The present output is being shipped to Alaska, Japan and the armed forces.

The method involves the collection of milk on the farm under vacuum in 100- and 200-gal. stainless steel tanks which are trucked directly to the processing plant. At the plant the milk is homogenized, sterilized and cooled without contact with air. In the aseptic canning machine the milk is filled into sterile no. 10 cans, which then are sealed with sterile lids. The cans are cooled under a cold water spray and packed. The processing is completely controlled by a regulator system. T. J. Claydon

**585. Southern milk for arctic markets.** R. E. GRAVES, International Milk Processors, Inc., Chicago, and JACK MEYER, Minneapolis-Honeywell Regulator Co., Philadelphia. Sou. Dairy Prod. J., **50,** 2: 34, 35, 111–114. Aug., 1951.

A process for canning whole milk which recently has been adopted commercially in E. Stanwood, Wash., is described. The success of the operation depends upon an adequate supply of high quality milk. Each farm supplying milk must install a specially-designed milking parlor. The milking is done by machine and the milk is carried through sanitary pipe lines to a stainless steel tank where it is maintained under vacuum. The tank and milk are picked up by truck shortly after milking. The tank is hoisted in the plant and carried by overhead support to a position over preheating tanks into which the milk is allowed to flow by gravity. The larger tanks also are maintained under vacuum. Milk is standardized with skimmilk or cream handled to avoid exposure to air, homogenized, forced through a special heat exchanger to bring the milk to almost 300° F. for a few sec. to completely sterilize and finally aseptically canned. The product has a storage life without refrigeration of about 6 mo. or under tropical conditions, 4 mo.

F. W. Bennett

**586. Refrigerated trucks speed canned-whip to market.** Anon. Food Eng., **23,** 6: 114. June, 1951.

A description is given of the construction and operation of the refrigerated trucks used by Reddiwip Mfg. Co., Inc., for distribution of its pressure-can whipped cream from distributing plants to retail stores. T. J. Claydon

**587. Sterilized milk—a broad outline.** P. G. KEELING. J. Soc. Dairy Technol., **3,** 4: 264–268. July, 1950.

The processing of sterilized milk is discussed in general terms. The product is defined (in essence) as milk that has been heated at not less than 212° F. for sufficient time to insure that it will give a negative turbidity test (See abs. no. 589). E. M. Foster

**588. The production of sterilized milk.** F. PROCTOR. J. Soc. Dairy Technol., **4,** 2: 107–109. Jan., 1951.

Modern methods of producing sterilized milk are described. These involve both continuous and batch methods of heating. The advantages of sterilized milk over other forms of milk are mentioned. Among the advantages listed are freedom from post-sterilization contamination, economy in use, ease of digestibility and long keeping quality. E. M. Foster

**589. A simple turbidity test for sterilized milk.** R. ASCHAFFENBURG, Natl. Inst. for Research in

Dairying, Univ. of Reading. J. Soc. Dairy Technol., **3,** 4: 236–237. July, 1950.

The test is used to determine whether milk has been heated to sterilizing temperatures, *i.e.,* at least to boiling for several minutes. Twenty ml. of milk are mixed with 4 g. of $(NH_4)_2SO_4$. The material is filtered through paper and a sample of the filtrate is placed in boiling water for 5 min. Absence of turbidity after cooling the filtrate indicates that the milk had been heated to sterilizing temperatures.

The test detected as little as 0.6–0.8% raw milk added to sterilized milk. All 24 samples of commercially sterilized milk gave negative tests. It is suggested that the test provides an easy way to determine whether a sample of milk has been effectively heated to sterilization temperatures much in the way the phosphatase test is used to show adequate pasteurization. E. M. Foster

**590. Laboratory control.** A. L. Provan, Milk Mktg. Board. J. Soc. Dairy Technol., **4,** 2: 115–121. Jan., 1951.

The function of the laboratory in the control of milk supplies is discussed. Different types of laboratory equipment are described with precautions on calibrating them and checking their accuracy. Methods of sampling, butter fat and total solids determination, dye reduction tests and tests for adulteration are discussed. Precautions are given on interpretation and application of results obtained with these methods. E. M. Foster

**591. Laboratory control of processing and manufacture.** J. G. Davis and T. R. Ashton, Express Dairy Co., Ltd. J. Soc. Dairy Technol., **4,** 2: 78–99. Jan., 1951.

The role of the laboratory in the dairy plant is discussed in detail. A few fundamental considerations are mentioned before the laboratory work connected with all types of milk and milk products is discussed. Consideration is given to raw, pasteurized, sterilized, sweetened condensed, evaporated, dried and cultured milks, butter, cheese, cream and ice cream. Each of these is discussed from the standpoint of methods of testing used and problems involved with the methods. Bacteriological, chemical and physical tests and analytical methods are discussed where applicable. These include tests applied to detergents, chlorine solutions and water supplies.

E. M. Foster

Also see abs. no. 556.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**592. Utilization of acetate for milk-fat synthesis in the lactating goat.** G. Popjak, Natl. Inst. for Med. Research, London, and T. H. French and S. J. Folley, Univ. of Reading. Biochem. J., **48,** 4: 411–416. 1951.

A lactating goat was injected intravenously with radioactive Na acetate. The respired $CO_2$ was measured and milk samples were drawn at regular intervals for a 48-hr. period after which the goat was killed. The lipids from the milk, from the plasma and also from the dried viscera were fractionated and tested for radioactivity. Of the total radioactivity, 80% appeared in the respired $CO_2$ and of that which remained in the body, half appeared in the milk fat. By comparison of the various fatty acid fractions it was shown that the short-chain (steam-volatile) group had a higher activity than the non-volatile, long-chain fatty acids, indicating their independent synthesis from acetate rather than their formation by degradation of long-chain acids. The milk fatty acids showed greater activity than the plasma fatty acids, indicating synthesis of the former in the udder rather than coming from plasma fatty acids. It also was shown that milk cholesterol is synthesized in the udder rather than originating from blood cholesterol. (See abs. 58, Jan., 1951.)

A. O. Call

**593. Further observations on the stimulation by insulin of fat synthesis by lactating mammary gland slices.** (Abs.) J. H. Balman and S. J. Folley, Univ. of Reading. Biochem. J., **48,** 1: i–ii. 1951.

Insulin had no effect on the rate of fat synthesis in rat mammary tissues when the tissues were taken shortly before parturition and after weaning, but when the tissues were taken at the lactating stage insulin did stimulate fat synthesis. No such insulin stimulation was noticed using sheep mammary tissue. (Also see abs. 57, Jan., 1951.)

A. O. Call

**594. 2,4-Dinitrophenol in the study of pathways of pyruvate metabolism in lactating mammary tissue.** C. Terner, Univ. of Reading. (Abs.) Biochem. J., **47,** 4: xlix. 1950.

Use of the Warburg apparatus demonstrated that $Q_{O_2}$ of mammary tissue, using pyruvate as the substrate, was increased by the addition of DNP. A. O. Call

**595. Studies of the synthesis of milk fat by the perfused, isolated bovine udder.** (Abs.) A. T. Cowie, W. G. Duncombe, S. J. Folley, T. H. French and R. F. Glascock, Univ. of Reading, L. Massart and G. Peeters, Univ. of Ghent and G. Popjak, Natl. Inst. for Med. Research, London. Biochem. J., **48,** 3: xxxix–xl. 1951.

One-half of an isolated lactating bovine udder was perfused with heparinized blood containing, among other things, $CH_3{}^{14}COONa$. The other half was perfused similarly except the acetate was replaced by $NaH^{14}CO_3$. It was shown that the mammary gland itself utilizes acetate for the synthesis of milk fat. A. O. Call

## PHYSIOLOGY

R. P. REECE, SECTION EDITOR

**586. Heat tolerance of cows and buffaloes in Egypt.** A. L. Badreldin, M. M. Oloufa and M. A. Ghany. Nature, **167,** 4256: 856. 1951.

The body temperature (**I**) and respiration rate (**II**) of Shorthorn, Jersey, native cows and buffaloes decreased in this order. The pulse rate (**III**) of the buffaloes was lower than the 3

breeds of cows. **I, II** and **III** decreased as the age of the animals increased. **I** and **II** increased and **III** decreased in all animals with increase in air temperature. An increase in relative humidity caused an increase in **I** and **III** but had no effect on **II** except the Shorthorns which increased.
R. Whitaker

**597. Technique and use of liver biopsy in cattle.** R. M. Loosmore and Ruth Allcroft. Vet. Record, **63:** 24: 414–416. June, 1951.

A modification of previous techniques of liver biopsy in cattle is described. Illustrations show instruments used and steps involved in the operation. A wider bore cannula is used and the site of puncture is further forward into a thicker part of the liver than in other techniques. Authors used this biopsy in studies of the Cu status of cattle. Data on Cu levels of the blood and liver are given and indicate that the liver Cu content is the most reliable index. In other than extremely low levels, blood Cu levels are a poor guide for liver reserves. R. P. Niedermeier

## SANITATION AND CLEANSING

K. G. Weckel, section editor

**598. Selling management on plant sanitation.** C. W. Griffin, Jr., California Packing Corp., San Francisco. Food Eng., **23,** 6: 76–79. June, 1951.

The author presents arguments for the organization of a sanitation department in food plants. A sanitarian is needed because: (a) Sanitation improves the quality of a food product; (b) the food industry must choose between self regulation and regulation by a govt. agency. A trained full-time sanitarian can keep informed on the thinking and viewpoints of the F. & D. A. and can help interpret rules and regulations for the food plant. The activities of F. & D. A. and also non-official critics will increase and a competent sanitation dept. is the best insurance against an occasional "off color" product which can cause costly grief. The possible losses from seizures could pay the cost of a sanitation program for a long time. A number of specific illustrations are given to show ways of improving sanitation and at the same time giving more efficient and economical operation. The sanitation department must be represented at the top management level and cooperation must exist throughout the organization. T. J. Claydon

**599. The effect of omitting warm rinses in bottle washing.** L. A. E. Baker and T. Goodale. J. Soc. Dairy Technol., **4,** 1: 43–47. Oct., 1950.

The suggestion of Wilson (J. Hyg., **43,** 2. 1943.) that "warm" recirculating rinses should be eliminated from the bottle washing process because they were a serious source of recontamination was reviewed. The average rinse count of pt. bottles was 195 when the warm recirculating rinse was omitted and 304 when it was used. The improvement was more marked in summer than in winter. The cold water rinse received by the experimental bottles was as effective in removing the detergent as was the warm rinse followed by a cold rinse. E. M. Foster

**600. Probleme des Milchhygiene-Meisters (Problems of the milk sanitarian).** English summary. M. E. Schulz. Die Milchwissenschaft, **5,** 10: 338–349. Oct., 1950.

Author recommends the phosphatase test as check test for proper pasteurization of milk and the coliform test as check test for possible recontamination of otherwise properly pasteurized milk. Heating milk to a temperature just high enough to result in a negative phosphatase test is more desirable than overheating the milk, even though the latter could result in a lower bacterial plate count. The following recommendations are made: (a) Manufacture of a special milk for infant feeding containing less than 1 coliform ml. of milk; (b) coliform test to be made 1 hr. after pasteurization of milk; (c) milk ordinances to be formulated by local authorities; (d) installation of milk sanitarians whose duty it shall be to supervise the sanitary quality of the outgoing consumers milk from each plant. I. Peters

**601. Recent court decisions on municipal milk inspection.** M. Stein and I. L. Sonenshein, Federal Security Agency, Pub. Health Div. Public Health Reports, **66,** 28: 898–902. July 13, 1951.

A summary is presented of litigation regarding the validity of requirements established by municipal ordinances regarding the location of milk production and processing facilities for the milk supply of that city. The right of a municipality to regulate the production, processing and handling of milk, under validly delegated power from a state, as a health protection measure, has been well settled. The courts, however, have held that a municipality legally cannot curtail interstate commerce by creating a limited inspection area within which milk must be processed as a condition precedent to inspection, if reasonably nondiscriminatory alternatives adequate for the protection of local health interests are available. Such alternatives include either inspection by the municipal officials of the distant milk source or adoption of section 11 of the USPHS model milk ordinance which would exclude milk that does not conform to standards of production and pasteurization as high as those enforced by the receiving municipality. D. D. Deane

Also see abs. no. 557, 558, 559.

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