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STABILITY OF MILK AND ITS CONCENTRATES IN FROZEN STORAGE AT VARIOUS TEMPERATURES[1]

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The frozen state is the only form in which milk and its fluid concentrate can be preserved for relatively long periods without changing their characteristic flavor. For the most part, investigators (1, 2, 4, 5, 9) have worked with frozen milk of one concentration or milk stored at one temperature, but direct comparisons are needed covering the practical range of concentration at several temperatures below freezing. On this account and because of increasing interest in these products, the information on the stability of milk and its concentrates in frozen storage should be reexamined and further details should be made available.

Doan and Leeder (5) stored concentrated (3 to 1) milk at +5, −5 and −15 to −20° F. They did not compare its stability with that of milk of other solids content. Babcock *et al.* (1), working with milk of normal concentration only, held it at as low as −40° F. for 3 to 4 mo.

Bell (2) compared the keeping quality of milk of appproximately double solids concentration when it was stored at 32, 19 and 1° F. He found that samples which were stored at 1° F. developed an oxidized flavor sooner than samples of the same milk that were maintained at 19° F. and that deterioration in body began in 2 to 3 wk.

Doan and Warren (6) have investigated many phases of the frozen milk problem, among them being the use of heat to redisperse the flocculated protein which results from storage beyond the satisfactory life of the product.

Tracy *et al.* (8) found that fluid milk and approximately 3.5 to 1 milk, stored at a uniformly low temperature (−10 ± 5° F.), remained in satisfactory condition for at least 1 yr. However, in preparing each product, their milk of origin was held at 170° F. for 20 min.

EXPERIMENTAL METHODS

In this paper results are given on the body and flavor stability of frozen milks of various concentrations stored at different temperatures.

Fresh milk was heated in a stainless steel pasteurizer to 150° F., held for

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

30 min., cooled to 130° F. and homogenized at 2,500 lb. per in^2. Most of this homogenized milk was concentrated in a stainless steel pan under a vacuum of 28 in. to a 2 to 1 solids ratio or 25.4 per cent total solids content, a portion was withdrawn and the concentrating resumed to yield milks of 38.1 (3 to 1) and 50.7 (4 to 1) per cent solids content.

All samples were frozen in sealed cans of 160-ml. capacity in a room that was maintained at 1° F. The next morning some of the cans were placed in a room at −8° F. and some in a compartment at −17° F. Samples also were stored at 10 and −35° F. Although the temperature of the air in the 10° F. space fluctuated as much as ±3° F., that of the milk varied only a degree or two. This was determined by noting the temperature of alcohol in a small container among the canned samples. The temperature in each of the other four storage spaces was more nearly constant than that of the 10° F. compartment.

Since all samples were frozen under the same conditions, the rate of freezing was not a variable factor.

From time to time a sample of milk of each solids content and from each storage space was examined for body and flavor stability. The samples were prepared for examination by leaving them overnight in a room at 35° F. and the following morning warming them to 70° F. in a water bath and room at that temperature. Then a portion of each concentrate was diluted with distilled water to the solids concentration of the original milk and 50 ml. were centrifuged at 1,000 r.p.m. for 5 min. in a graduated tapered test tube. Fifty-ml. portions of the milk were centrifuged in the same manner. The centrifuge had a 15.5-in. head, as measured from the inside bottoms of opposite cups when the cups were in a horizontal position. Body stability is expressed as milliliters of deposit obtained from 50 ml. of milk.

Ascorbic acid (vitamin C), in the form of a freshly prepared concentrated water solution, was added to a duplicate set of samples (with the exception of the 4 to 1 milk) at the rate of 100 mg. per liter of product. These vitamin C-fortified samples were frozen and stored with the others.

RESULTS

Body. Inspection of figure 1 shows that, as would be expected, the physical stability of the 4 to 1 milk was the least and that of the normal milk was the greatest. However, there was little difference between the latter and the 2 to 1 product.

The addition of ascorbic acid at the rate of 100 mg. per liter of milk and of its 2 to 1 and 3 to 1 concentrates had only a slight effect on the body stability of the normal and the 2 to 1 products. In the milk of triple solids concentration, it substantially decreased this property. This was noticeable in the amount of deposit on the bottom of the cans as well as in the centrifuge tubes.

As indicated in figure 1, 1° F. was not sufficiently cold to preserve satisfactorily the body of the milks, nor was −8° F. cold enough to prevent deterioration before the end of 12 wk. On the other hand, all concentrations except the 4 to 1 still had a satisfactory body after 12 wk. at −17° F.

Stability data for milk of normal and double solids concentration at – 17° F. are not shown because no precipitate formed in the graduated tubes after the usual centrifuging.

Milk of triple solids content that was stored at – 17 and – 35° F. for more than 6 mo. showed no physical deterioration in its thawed product.

This experiment has been performed repeatedly during the course of the last 2 yr. The results were similar and they confirm the conclusion that 1° F. is not cold enough to insure homogeniety in thawed triple solids milk for more than

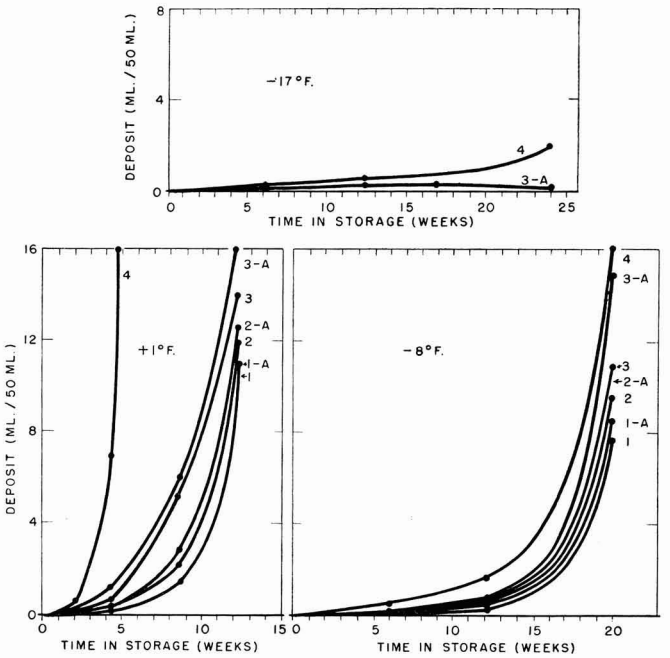

FIG. 1. Stability of normal and multiple solids milks at various storage temperatures. Nos. 1, 2, 3, and 4 associated with the curves refer to the solids ratios to which the milk was concentrated. The letter A indicates addition of ascorbic acid at the rate of 100 mg. per liter of product.

2 to 3 wk. or in double solids milk for more than 3 to 4 wk. Most of the thawed 4 to 1 milks were semisolid even after storage at – 17° F. On being held at 14° F. for a few days before they were thawed, the 4 to 1 milks became grainy due to crystallization of lactose. For these reasons milk that is to be stored for a long period in a frozen state should not be concentrated more than about 3.5 to 1, *i.e.*, it should not contain more than about 45 per cent total solids.

Figure 2 shows in a different manner the relationship between the solids con-

tent and the body stability of milk stored at 10 and 1° F. In plotting these curves an arbitrary value of 0.5 ml. of deposit in the centrifuge tubes was taken to represent destabilization of the physical system. According to these data there is but little advantage, even in the case of milk, in storing at 1 instead of 10° F. Since body stability in triple solids milk was not measurably diminished after 1 yr. at −17° F., no curve for this storage temperature is shown.

Flavor. Except for the old and stale flavors which tend to develop slowly in all frozen milk of initial beverage quality, an oxidized (cardboard) off-flavor defect in normal, or single strength milk is the one most likely to develop (3). After 12 wk. storage at 1° F., only the unfortified unconcentrated milk (curve 1) of figure 1 was oxidized. It was not until these samples had been in storage for 19 wk. that this milk was described as strongly oxidized. At this time 2 to 1

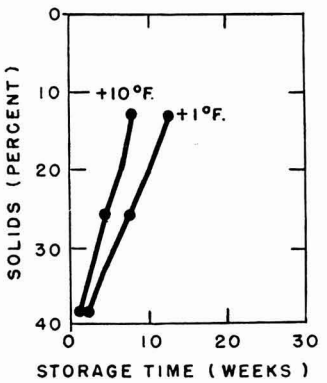

FIG. 2. Dependence of body stability on solids content and storage time for milk stored at 10 and 1° F. Sample was considered unstable if 0.5 ml. of deposit formed on centrifuging 50 ml. of thawed milk.

(curve 2) as well as the control (curve 1) samples that had been held at −8° F. were strongly oxidized. At 19 wk. of age the flavor of a set of −8° F. samples was judged not as good as a similar set that had been maintained at 1° F. In other experiments the opposite result has been obtained.

At the end of 9 wk. at −17° F. the unfortified, unconcentrated milk was slightly oxidized. However, 15 wk. later no increase in the intensity of this off-flavor could be detected. There was less deterioration in flavor in samples stored at −17° F. than in those held at the higher temperatures.

Vitamin C (ascorbic acid) fortification at the rate of 100 mg. per liter of product prevented the development of an oxidized flavor in the unconcentrated and 2 to 1 milk. In no instance, with or without added ascorbic acid, did an oxidized flavor develop in the 3 to 1 or the 4 to 1 samples. Fortification of the milk in this solids range with ascorbic acid in order to prevent an oxidized flavor was unnecessary. Seldom in the course of numerous experiments has any

semblance of an oxidized flavor in thawed and reconstituted 3 to 1 and 4 to 1 milk been detected.

CONCLUSIONS

The temperature range for storing frozen milk and its concentrate up to approximately 45 per cent solids content without the early development of flakiness begins below $-8°$ F. and extends to below $-17°$ F. Data presented here indicate that the storage temperature should be lower than $-10°$ F. but need not be lower than $-20°$ F. This is a narrow temperature range above which neither milk nor its concentrate should be stored if prolonged preservation of a satisfactory body is desired and below which little additional stability is to be attained.

The effects of temperature and duration of storage on body stability are similar for milks of equal solids content at different times of the year.

Storage at $-8°$ F. may or may not cause a milk to retain a somewhat better flavor than at $1°$ F. However, milk held at $-17°$ F. is less likely to develop an oxidized (cardboard) flavor than it is at -8 or $1°$ F. and it is otherwise more stable in flavor.

The greater the concentration of solids up to a 4 to 1 product the less susceptible a milk is during frozen storage to the development of an oxidized flavor and the less beneficial is fortification with ascorbic acid. Fortification with ascorbic acid at the rate of 100 mg. per liter of product appreciably destabilizes the body of milk of triple solids but not that of double or single solids milk.

Retention of a fresh milk flavor is the limiting factor in the preservation in a frozen state of milk and its concentrates up to about 45 per cent solids content.

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THE EFFECT OF DDT UPON THE DIGESTION AND UTILIZATION OF CERTAIN NUTRIENTS BY DAIRY CALVES[1]

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DDT has been shown to be an effective insecticide for controlling alfalfa weevil and lygus bugs that impair the production of alfalfa seed (9, 10, 14), but little work has been done to determine the effect ingestion of DDT has upon the digestion and utilization of different nutrients in a ration.

Harris *et al.* (6) conducted balance and digestion trials with four sheep, using a Latin square design in which each sheep was fed each of four alfalfa hays. The alfalfa had previously been dusted with 0, 1, 2 and 4 lb. of technical DDT per acre. These alfalfa hays had residues of 0, 15, 22 and 44 ppm. DDT, respectively. The nitrogen balance of the sheep decreased with each increase of DDT on the hay. No difference existed among the digestion coefficients for protein, ether extract, crude fiber, nitrogen free extract and total digestible nutrients. Since micro-organisms play an important role in the synthesis of protein from nitrogeneous non-protein compounds and also aid in the digestibility of many complex carbohydrates, it appeared that DDT might interfere with the action of these organisms.

The purpose of this study was to determine the effect of ingestion of DDT upon the digestion and utilization of certain nutrients by dairy calves.

PROCEDURE

Digestion and balance studies were conducted with 16 3- to 6-mo.-old Holstein bull calves weighing initially 200 lb. They were fed different levels of DDT and protein equivalent in the diet. Each calf received one level of DDT throughout the experiment and four protein equivalent levels. The protein equivalent levels were arranged in an orthogonal manner to take into account the time the protein level was fed in relation to growth of the calves. The protein equivalent levels (dry basis) were 10.2, 12.4, 14.5 and 16.5 per cent, respectively.

The basal diet was composed of 40 per cent chopped meadow hay, 20 per cent ground yellow corn, 10 per cent rolled oats, 5 per cent dried whey, 5 per cent beet molasses, 1.35 per cent steamed bonemeal, 0.5 per cent salt and 8.15 per cent dextrinized starch. The higher levels of protein equivalent ($N \times 6.25$) were made by replacing equal parts of dextrinized starch with urea. Technical DDT was dissolved in corn oil and an aliquot was spread over the diet at each feeding to supply DDT at levels of 0, 25, 50 and 75 ppm. of the diet (dry basis). Corn oil was added to the diets of those calves receiving no DDT so that all diets had the same fat content. Each diet was fed for a preliminary period and a collection

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period of 10 days each. The nitrogen, Ca and P balance and the digestibility of organic matter, crude protein ($N \times 6.25$), ether extract, crude fiber, nitrogen-free extract, cellulose and lignin were determined.

Nitrogen, ether extract, crude fiber, dry matter and ash were determined by AOAC (1) methods. Lignin was determined by the method of Ellis *et al.* (3); cellulose by the method of Matrone *et al.* (11). Calcium was determined by the AOAC micro-method (1), except the samples were digested by the method of Gerritz (4). Phosphorus was determined on the same digested samples by the colorimetric method of Koenig and Johnson (8).

RESULTS AND DISCUSSION

Health of animals and liveweight gains. All calves were in good health and there were no apparent skin disturbances of any kind at the beginning of the experiment.

FIG. 1. Head and neck of calf K-405 fed 0 ppm. of DDT in the diet. The hair and skin of this calf are normal. Note bright eye and absence of a watery discharge from nose.

The calves in replications I and II were started on experiment on July 12, 1949. During September, it was noticed that some of the calves fed DDT had marked wrinkling on the side of the neck, side of the jaw and top of the withers. This wrinkling was accompanied by thickening of the skin, profuse scaling and thinning of the hair in these same areas. In addition, some of the calves' skin had small proliferations and a lack of pliability. Frequently there was a lacrimary discharge from the eyes and a watery discharge from the nostrils. The calves receiving no DDT were normal. These observations are shown in figures 1, 2 and 3. These results are somewhat similar to those described by Nelson *et al.* (12) when they included DDT in the rations of rabbits.

The calves in replications III and IV were started on experiment on November 22, 1949. On December 12, it was noticed that calf K-411, receiving 75 ppm. of

FIG. 2. Head and neck of calf K-395 fed 25 ppm. of DDT in the diet. Note dull eye and lacrimation.

FIG. 3. Head and neck of calf K-399 fed 75 ppm. of DDT in the diet. Note thinning of hair on top of neck, marked wrinkling on top and side of neck accompanied by thickening of the skin, profuse scaling, small proliferations and a lack of pliability of the skin.

DDT, had severe tremors in the thigh and rump. These tremors continued for several days and then disappeared. No tremors were noticed with any of the other calves. These tremors were similar to those described by Orr and Mott

(13) when they fed 100 to 200 mg. of DDT, in powder form, per kilogram body weight to cattle. However, this calf was receiving only 2.2 mg. of DDT per kilogram of body weight. This high degree of toxicity may be attributed to the fact that 50 to 90 per cent of DDT is absorbed from the gastro-intestinal tract when fed in oil, but the amount absorbed is extremely variable when supplied in the form of powder, according to Kanegis and Roepke (7). The general observation of the authors seems to justify the deduction that the protein sequence may have influenced the toxicity of DDT, because all the calves fed DDT did not develop skin disturbances.

During the collection period, no significant differences existed among the liveweight gains of calves fed the different levels of DDT and protein equivalent.

Balance studies. As the DDT in the diets increased, the amount of nitrogen stored by the calves decreased (table 1). The average amounts stored per 100 lb.

TABLE 1

Nitrogen stored daily per 100 lb. body weight when calves are fed varying levels of DDT and varying levels of protein[a]

Protein levels (dry basis)	DDT levels, ppm. of diet (dry basis)				Grand av.
	0	25	50	75	
(%)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
10.2	6.2	4.5	5.8	7.3	6.0
12.4	7.0	7.5	7.5	4.9	6.7
14.5	7.3	8.2	9.0	6.9	7.8
16.5	11.0	8.6	6.5	5.4	7.9
Grand av.	7.9	7.2	7.2	6.1	7.1

[a] Each value in the body of the table is the average for 4 calves.

body weight were 7.9, 7.2, 7.2 and 6.1 g. for the 0, 25, 50 and 75 ppm. DDT levels, respectively. This difference approaches significance (F value is 3.73 at $P = 0.05$ and it requires an F value of 3.86 to be significant at $P = 0.05$). The nitrogen stored for each protein level was not consistent for the different DDT levels. As the amount of nitrogen increased in the diet, the amount stored was greater. However, as the DDT in the diet was increased from 25 to 75 ppm. the amount of nitrogen stored decreased with each increase in DDT at the higher levels of protein equivalent. This protein DDT interaction is statistically significant ($P < 0.05$). These results agree with those of Harris *et al.* (6) in which they found that as the amount of DDT was increased in the diet, the nitrogen balance was decreased. Thus, it appears probable that DDT inhibits the synthesis of protein from non-protein nitrogenous materials in the paunch of ruminants. As urea was increased in the diet, the amount of nitrogen stored was significantly increased ($P < 0.05$). With protein equivalent levels of 10.2, 12.4, 14.5 and 16.5 per cent in the diet, the amounts of nitrogen stored daily per 100 lb. of body weight were 6.0, 6.7, 7.8 and 7.9 g., respectively. These results show that calves can utilize urea as a source of nitrogen.

The Ca and P balances were not affected by any of the treatments.

TABLE 2

Protein digestion coefficients for calves as affected by DDT and protein levels[a]

Protein levels (dry basis)	DDT levels, ppm. of diet (dry basis)				Grand av.
	0	25	50	75	
(%)	(%)	(%)	(%)	(%)	(%)
10.2	48	46	50	52	49
12.4	56	55	56	53	55
14.5	60	60	64	62	61
16.5	67	67	63	62	65
Grand av.	58	57	58	57	58

[a] Each value in the body of the table is the average for 4 calves.

Digestion coefficients. DDT had no measurable effect upon the digestion of any of the nutrients tested. As the amount of protein equivalent in the diet was increased, its digestibility was correspondingly increased (table 2). The average digestion coefficients for protein equivalent levels of 10.2, 12.4, 14.5 and 16.5 per cent were 49, 55, 61 and 65, respectively.

TABLE 3

Crude fiber digestion coefficients for calves as affected by DDT and protein levels[a]

Protein levels (dry basis)	DDT levels, ppm. of diet (dry basis)				Grand av.
	0	25	50	75	
(%)	(%)	(%)	(%)	(%)	(%)
10.2	46	49	45	49	47
12.4	56	50	53	52	53
14.5	55	56	52	60	56
16.5	59	60	58	54	58
Grand av.	54	54	52	54	53

[a] Each value in the body of the table is the average for 4 calves.

With each increase of urea in the diet there was an increase in the digestibility of crude fiber (table 3). The digestibility of crude fiber in the 10.2 per cent protein diet was 47 per cent, while that of the basal diet plus enough urea to make it up to 16.5 per cent protein equivalent was 58 per cent. Additions of urea

TABLE 4

Cellulose digestion coefficients for calves as affected by DDT and protein levels[a]

Protein levels (dry basis)	DDT levels, ppm. of diet (dry basis)				Grand av.
	0	25	50	75	
(%)	(%)	(%)	(%)	(%)	(%)
10.2	53	57	53	57	55
12.4	63	57	62	62	61
14.5	59	62	58	65	61
16.5	67	66	66	62	65
Grand av.	60	60	60	62	61

[a] Each value in the body of the table is the average for 4 calves.

to a low protein diet have given the same effects in the diets of sheep and cattle according to Harris and Mitchell (5) and Briggs *et al.* (2). The digestibility of cellulose shows the same trends as crude fiber, since each increase in urea in the diet caused an increase in cellulose digestibility (table 4). These results also agree with those of Harris and Mitchell (5) with sheep. The differences in digestibility of protein, crude fiber and cellulose between protein levels are statistically significant ($P < 0.05$).

SUMMARY

Digestion and balance trials were conducted with 16 Holstein bull calves. Each calf was fed one level of DDT and four levels of protein equivalent arranged in an orthogonal manner to take into account the time the protein level was fed in relation to the growth of the calves. The DDT levels were 0, 25, 50 and 100 ppm. of the diet and the protein equivalent levels were 10.2, 12.4, 14.5 and 16.5 per cent in the dry diet, respectively.

Symptoms consisting of skin wrinkling, thickening of the skin, profuse scaling, thinning of the hair, lacrimation from the eyes, watery discharge from the nose and tremors were noticed. These symptoms were more pronounced in the calves fed the highest levels of DDT.

As the DDT in the diet increased the amount of nitrogen stored decreased. The Ca and P balances were not affected by any treatment. DDT did not affect the digestibility of organic matter, crude protein, ether extract, cellulose, lignin, crude fiber and other carbohydrates. The addition of urea to the diet improved the digestibility of crude fiber and cellulose, but did not affect the digestibility of the other nutrients.

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THE EFFECT OF HEATING CREAM IN A SMALL-TUBE HEAT EXCHANGER ON SOME OF ITS PROPERTIES[1]

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The trend in the dairy and food industry is toward continuous operation with higher temperatures and shorter exposures for processing food products, the objectives being to secure greater destruction of microorganisms and enzymes, to improve keeping quality, to maintain and preserve nutritional properties and palatability and to save time and labor.

A small-tube heat exchanger, known as the Mallorizer, was made available to the Illinois Agricultural Experiment Station. Mallory (3), in 1942, obtained a patent for this heat exchanger in which liquid dairy products are forced through small tubes at a high velocity, resulting in turbulent flow and rapid heat transfer. Reynolds (5), in 1883, showed that the thickness of the laminar film was a function of velocity of the liquid across heated surfaces and as the velocity increased turbulent replaced laminar flow. This was confirmed by Prandtl (4) in 1904.

This paper deals with the effect of heating cream at different temperatures in the small-tube heat exchanger. Data also are presented to show some of the operating characteristics of this unit.

EXPERIMENTAL PROCEDURE

Before conducting experimental work with milk products, it was thought necessary to study some of the operating aspects of the small-tube heat exchanger. This unit consists of three independent heating sections and three cooling sections. Each section has eight lengths of stainless steel tubing, each length being 70.25 in. long and 0.25 in. in internal diameter. The entire length of the tubing in the three heating sections, including the header joints, was calculated to be 142.7 ft. The entire heating section is enclosed in an insulated jacket and is designed for use with high pressure steam. The cooling section is of similar construction and water is used as the coolant. In this study, however, one heating section was disconnected and an additional tube 1.0 in. in internal diameter was attached outside the heating jacket to serve as a holding tube. This reduced the length of the heating section to 95.1 ft.

Temperature was regulated with a compressed air-actuated regulator, known commercially as a "Fulscope", which controls the steam pressure. A Manton-Gaulin homogenizer was used as a positive pump to force the product through the exchanger and it operated at about 2,000 p.s.i. After the product was heated to the desired temperature, it flowed through an insulated holding tube of 1 in. internal diameter and which had a built-in thermocouple. The temperature was determined on a recording potentiometer.

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The heating rates in the small-tube heat exchanger at various temperatures were determined with water, milk and cream preheated to 140° F. as these products flowed through the unit. This was done with a header block with a built-in thermocouple which extended into the liquid and the temperature of each product was obtained at seven points with a potentiometer. Each temperature was determined at the end of two lengths of tubing or 11.7 ft. which is equivalent to about 0.5 sec. in flow time. The regulator was adjusted to give the desired temperature, which was determined with a thermocouple in the holding tube.

The small-tube heat exchanger was rated as having a capacity of 200 gal. per hr. Its actual capacity was determined by collecting the products into previously weighed cans and timing the flow with a stop watch. Knowing the capacity and also the dimensions of the heating section and of the holding tube, it was possible to calculate the velocity of the product through these parts of the exchanger and also to calculate holding times.

In this study, the cream was obtained from the mixed milk of the University herd. It was separated, placed in a vat, standardized and preheated to about 120° F. just before it was processed in the small-tube heat exchanger. The homogenizer, which served as a pump, was sterilized with water containing 200 ppm. of chlorine. The heating and the cooling sections of the exchanger were sterilized with water at 300° F. for approximately 5 min. The cream was pumped through the exchanger immediately behind the water and the product always was heated first at the highest temperature of 300° F. About 5 to 10 gal. of cream were pumped through the unit in order to obtain the desired temperature, and it was allowed to flow for 10 to 15 sec. before a sample was collected.

The size of fat globules was determined essentially by the method of Campbell (2). The cream samples were diluted 1 to 500 with water and the final dilution was made into a 50 per cent glycerol solution. The diluted sample was placed in a cell approximately 70 μ deep, covered with glass and allowed to stand for 20 min. before it was placed under the oil immersion lens. A Leitz XB projection microscope was used. It had a prism reflector which projected the field on a screen grid with lines $\frac{1}{16}$ in. apart, one space on the grid being made to equal 1μ. The fat globules were magnified 1,587 times and all those found in ten different fields were measured.

The viscosity determinations were made with a Hoeppler viscosimeter at 35.6 $\mp$ 0.5° F. in a room at 36° $\mp$ 2° F. The samples were cooled in iced water immediately after they were processed.

The pH determinations were made with a Beckman Model G instrument with the samples at 104° F. The acidity determinations were made on 10 g. of cream, using 0.1 *N* NaOH and phenolphthalein.

For flavor determination, the samples were cooled quickly in iced water and held in tightly stoppered bottles at 40° F. They were judged organoleptically at 70° F. by four experienced persons.

The stability of the cream was determined by adding 100 ml. of coffee at 195 to 200° F. to 9 ml. of cream. The coffee was brewed in a percolator for 10 min. after it had begun to boil. The water had a hardness of 250 ppm. (1).

EXPERIMENTAL RESULTS

Operating performance of the small tube heat exchanger. The actual capacity of the exchanger was found to be 218.7, 218.4, 216.0, 217.3, and 215.6 gal. per hr. for water, milk, ice cream mix, 12 per cent sucrose solution and cream containing 20 per cent of fat, respectively (table 1). This indicates practically the same capacity for all of these products.

The velocity of milk through the heating and cooling section was 23.78 ft. per sec. and through the holding tube was 1.49 ft. per sec., calculated as follows:

$$\text{Velocity (V)} = \frac{\text{(gal./hr.) (cu. in./gal.)}}{\text{(sec./hr.) (sq. in.)}} \tag{1}$$

The time required for the product to flow through the heating section was 4.0 sec., calculated as follows:

$$\text{Time (sec.)} = \frac{\text{Length}}{\text{Velocity}} \tag{2}$$

Determining the actual holding time presented a difficult problem as the dye method could not be used because glass would not withstand the high temperatures and the high pressures, and the salt method could not be used because it

TABLE 1

The operating characteristics of the small tube heat exchanger

Product	Sp. Gr.	Capacity		Holding time
		(lb./sec.)	*(gal./hr.)*	*(sec.)*
Water	0.999	0.5065	218.7	2.11
Milk	1.031	0.5217	218.4	2.14
Cream	1.015	0.5066	215.6	2.19
Ice Cream Mix	1.092	0.5409	216.0	2.24
Sucrose Solution	1.049	0.5280	217.3	2.15

would be very difficult to insert durable conducting wires into a tube of such small diameter. The holding time for different products was calculated as follows:

$$T = \frac{V\ D}{W} \tag{3}$$

Where: T = time in sec., V = volume or capacity of the holding tube in ft.^{3}, D = density of the product in lb. per ft.^{3}, and W = rate of flow in lb. per sec. The mass rate of flow (W) was determined by collecting the product for about 15 min. in previously weighed cans. The time was recorded with a stop watch and the weight of product was determined accurately. Precautions were taken to avoid spillage. Using milk, W was found to be 0.5217 lb. per sec. Next, the holding tube was removed and filled with each product from end to end, avoiding occlusion of air. The product then was poured into a graduate, and the volume (V) was found to be 0.0185754 ft.^{3} (526 ml.).

In calculating the density of water and other products in the holding tube, the pressure was estimated to be 1,200 p.s.i. Though the pressure at the pump was 2,000 p.s.i., the pressure on the product would decrease gradually from the

heating section to the holding tube and would decrease further in the cooling section. If the pressure had been calculated at 2,000 instead of 1,200 p.s.i., the holding time would have been affected by less than 0.3 per cent. The calculations for holding time were made as follows:

Density of water at 60° F. is 62.36 lb. per ft.[3] (4)

Density of water at 300° F. and 1200 p.s.i. would be 57.58 lb. per ft.[3] [2] (5)

Therefore, the difference in density of water at 60° F., and 300° F. is 62.36–57.58 = 4.78 lb. per ft.[3] (6)

The density of milk at 60° F. is 64.29 lb. per ft.[3], and it is assumed to contain 87 per cent water. It also is assumed that the difference in the density of milk at 68 and 300° F. would be 87 per cent of the difference of water between the two temperatures.

Therefore, the density of milk at 300° F. and 1200 p.s.i. would be

$$64.29 - (4.78 \times 0.87) = 60.13 \text{ lb. per ft.}^3 \tag{7}$$

$$T = \frac{V\ D}{W} = \frac{(\text{cu. ft.})\ (\text{lb. per ft.}^3)}{(\text{lb./sec.})} = \text{sec.} \tag{8}$$

$$= \frac{(0.0185754)\ (60.13)}{0.5217} = 2.14 \text{ sec.} \tag{9}$$

Thus, the holding time for milk was calculated to be 2.14 sec.

Since the capacity of the small tube heat exchanger is nearly the same for water, milk, ice cream mix, 12 per cent sucrose solution and cream containing 20 per cent of fat, then it is obvious that the holding time for each of these products also would be about the same. Furthermore, it is believed that the calculated holding times (table 1) for the various products represent closely the actual holding times. This belief is based on calculations of the Reynolds number (N) of the product passing through the heating section and through the holding tube. The equation for calculating the Reynolds number is:

$$N = \frac{2\ V\ R\ P}{n}, \tag{10}$$

in which N = Reynolds number, V = velocity in cm./sec., R = radius of the tube in cm., P = density in g./ml. and n = viscosity in poises.

To calculate the Reynolds number for milk at 68 and at 300° F., the following values were substituted in the equation: V (heating tube) = 23.78 ft. = 724.81 cm./sec., V (holding tube) = 1.49 ft. = 45.41 cm./sec., R (heating tube) = 0.125 in. = 0.3175 cm., R (holding tube) = 0.50 in. = 1.27 cm., P = 1.031 g./ml. at 68° F., P = 0.963 g./ml. at 300° F., n = 0.015 poise at 68° F., and n = 0.0079 poise at 300° F.[3]

[2] Table 4, Compressed Liquid of Steam Tables by Keenan and Keyes.

[3] Using n for milk = 1.5 cp. at 68° F., n for water = 1.0 cp. at 68° F., and n for water = 0.184 cp. at 300° F., n for milk was then calculated as follows: 1.0 – 0.184 = 0.816 cp. difference in viscosity of water at 68 and at 300° F. For milk containing 87 per cent water, the calculated viscosity at 300° F. = 1.5 – (0.816 × 0.87) = 0.79 cp. = 0.0079 poises. Information is lacking for calculating the effect of heat on the viscosity of the total solids at 300° F.

The Reynolds numbers for milk in the heating section and in the holding tube at 68° F. are 3.2×10^4 and 7.9×10^3, respectively, while the values at 300° F. are 5.6×10^4 and 1.4×10^4, respectively. In general, turbulent flow prevails when

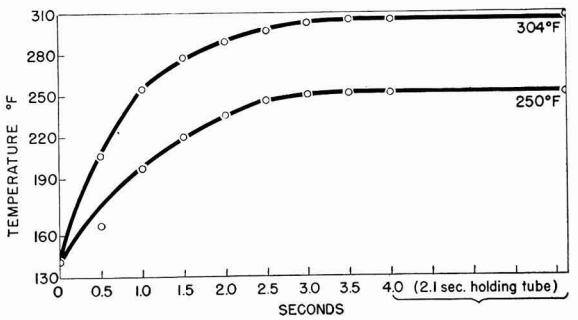

FIG. 1. Heating rates for water in the small tube heat exchanger.

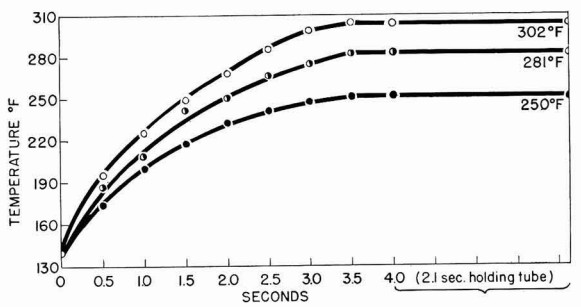

FIG. 2. Heating rates for milk in the small tube heat exchanger.

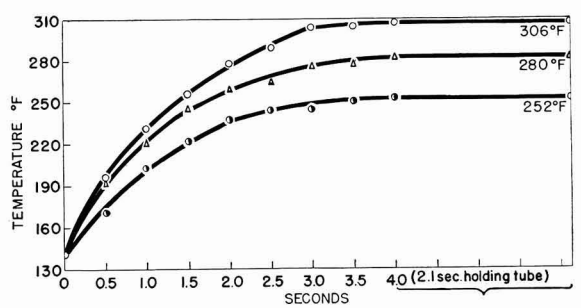

FIG. 3. Heating rates for cream in the small tube heat exchanger.

this number is greater than 2,000; however, the transition zone from laminar to turbulent flow may extend from 2,000 to 3,500. From the Reynolds numbers, it can be assumed that the flow is turbulent in the heating section and in the

holding tube of the heat exchanger. Therefore, the calculated holding time of 2.1 sec. should be close to that of the actual holding time.

The heating rates were essentially the same for milk (figure 2) and cream (figure 3), but slightly slower than those for water (figure 1). The time required to reach the desired temperature was 3.5 to 4.0 sec. The cooling rates for water are shown in figure 4.

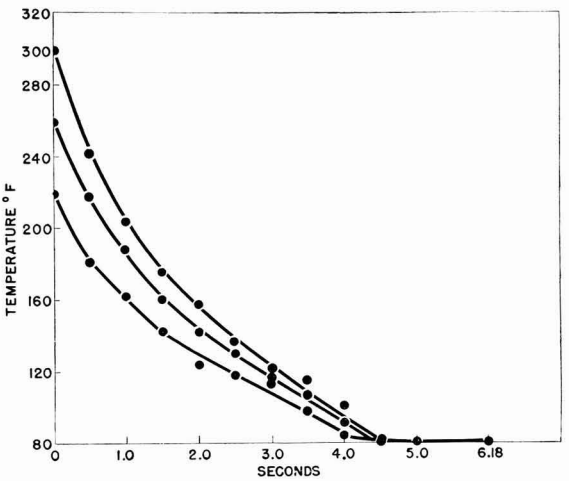

FIG. 4. Cooling rates for water in the small tube heat exchanger.

TABLE 2

The effect of heating cream in the small tube heat exchanger on fat globule size. (*Av. of 6 trials*)

Diameter	Unheated		180° F.		300° F.	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Percentage distribution					
1	6.7	2.0	15.6	3.9	27.4	5.7
2	29.2	2.2	41.8	5.1	48.9	2.3
3	31.3	2.5	26.6	2.1	18.9	3.9
4	18.6	0.7	11.0	1.6	3.1	1.9
5	8.9	0.6	4.0	2.3	0.9	0.2
6 or above	5.3		1.0		0.8	

Effect on fat globule size. The results in table 2 indicate that the fat globules were reduced in size by heating cream in a tubular heat exchanger at both 180 and 300° F. The effect was greater at higher temperature. In the raw cream, 67.2 per cent of the globules were 3 μ or less in diameter, while in the cream heated at 180° F., 84 per cent fell within this class and at 300° F., 95.2 per cent of the globules were 3 μ or less in diameter.

Effect on viscosity. Heating cream in the tubular heat exchanger at 170 to

300° F. reduced the viscosity by approximately 50 per cent, as compared to unheated cream, but the reduction was slightly less at 170 and 180° F. (table 3). With the exception of the creams heated to 190, 210, 220 and 240° F., there was a tendency for slightly higher viscosities in the heated sample after storage for 48 hr. However, it is questionable if these small variations are significant.

Effect on pH and titratable acidity. Heating cream in the tubular heat ex-

TABLE 3

The effect of heating cream in the small tube heat exchanger on viscosity. (Av. of 5 trials)

Temperature	4 hr.		24 hr.	
	Mean	Standard deviation	Mean	Standard deviation
(° *F.*)	(*cp.*)	(*cp.*)	(*cp.*)	(*cp.*)
Unheated	15.10	3.08	16.36	5.02
300	8.01	1.44	8.35	1.45
290	8.18	0.94	8.32	0.74
280	8.22	0.60	8.53	0.65
270	7.93	0.60	8.37	0.84
260	8.04	1.03	8.13	0.78
250	8.02	0.93	8.19	0.53
240	8.36	1.44	7.84	0.66
230	7.81	0.82	7.90	0.88
220	8.14	1.25	7.76	1.52
210	8.01	1.27	7.91	1.51
200	8.12	0.86	8.88	1.44
190	8.42	1.05	8.00	1.41
180	9.02	1.65	9.10	2.19
170	9.53	2.37	9.86	3.20

changer at various temperatures affects neither the hydrogen ion concentration nor the titratable acidity when these determinations were made before and after heating. Four trials were conducted with 35 per cent cream and five trials with 20 per cent cream.

Effect on flavor. The effect on flavor of heating ice cream at various temperatures in the small tube heat exchanger is shown in table 4. The cooked flavor

TABLE 4

The effect of high temperatures on the flavor of cream

Time	Relative intensity[a] of cooked flavor at:							
	300° F.	280° F.	260° F.	240° F.	230° F.	220° F.	210° F.	200° F.
(*hr.*)								
0	3.5	3.6	3.3	2.6	1.7	0.7	0	0
24	2.7	2.8	2.4	2.2	1.7	0.7	0	0
48	2.8	2.4	2.4	2.3	1.7	0.7	0	0

[a] 0 = no cooked flavor; 1 = doubtful; 2 = slight; 3 = pronounced; 4 = very pronounced.

became evident at 220° F. One of the judges scored the samples heated at 210° F. as doubtful, while the others scored these samples having no cooked flavor. For this reason the samples heated at 210° F. are recorded as having no cooked flavor.

Effect on stability of cream in coffee. Six trials were made to observe the

stability and the coloring ability in coffee of cream heated at various temperatures in the small-tube heat exchanger. There was no observable destabilization of either the fat emulsion or the proteins. The heated cream had more coloring ability in coffee than did the raw cream. This probably is due to the increased number of fat globules (6) and to precipitation of calcium phosphate and coagulation of the lactalbumin (7).

SUMMARY AND CONCLUSIONS

Some aspects of the operating performance of the small tube heat exchanger were studied. The capacity of this unit in gallons per hour is practically the same for water, milk, cream, ice cream mix and sucrose solution. Obviously then, the calculated holding periods for these products in the holding tube also would be essentially the same. Furthermore, the Reynolds numbers of these products in the heating, cooling and holding sections indicate turbulent flow. Therefore, it is believed that the calculated holding time for these products in the holding tube is the actual holding time.

The effects on fat globule size, viscosity, pH, titratable acidity, flavor and stability in coffee of cream heated at 170 to 300° F., in 10° intervals in the small tube heat exchanger were investigated. In the raw cream, 67.2 per cent of the fat globules were 3 μ or less in diameter, while in the cream heated at 180° F., 84 per cent were in this group and in that heated at 300° F., 95.2 per cent of the globules were 3 μ or less. The viscosity of the cream was reduced by approximately 50 per cent, whereas pH and titratable acidity were unchanged. A cooked flavor in the cream was detected at 210° to 220° F. Stability of the cream in coffee was not affected at any of the temperatures studied. Coloring ability in coffee was increased.

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THE ORIGIN OF OXIDIZED FLAVORS AND FACTORS RESPONSIBLE FOR THEIR DEVELOPMENT IN MILK AND MILK PRODUCTS

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The objectionable flavors which develop in milk and milk products such as chalky, tallowy, metallic and fishy, often are referred to as oxidized flavors. Few attempts have been made, however, to trace their origin. It has been known for some time that ascorbic acid plays an important part in reactions which produce the oxidized flavors (2, 4, 5, 6), and that in fresh milk oxidized flavors are not associated with the deterioration of fat, but with unstable lipids which are mostly a part of the fat-globule membrane (5, 12, 13). Studies of the stability of fat in cream and butter, as determined by the re-emulsification test, also have indicated that with passage of time the fat itself may undergo deterioration in the presence of ascorbic acid, resulting in the development of oxidized flavors and losses in the fat-soluble vitamins (5, 8). It has been pointed out, that the sensitization of fat to this type of deterioration may take place within the limits of time during which neither organoleptic nor chemical changes can be accurately determined in the fat-containing food products (5).

Furthermore, a relationship was shown to exist between the tocopherol levels of the fat, as influenced by type and quality of the roughages fed to the cow, and the resistance of fresh milk to oxidized flavors (7, 9), and also between the ability of milk fat to resist deterioration and the stability of tocopherols (5, 8).

The re-emulsification test revealed, however, that oxidized flavors associated with deterioration of milk fat were of a somewhat different nature from those associated with deterioration of the fat-globule membrane, and that occasionally, in the presence of ascorbic acid and a small amount of copper, a flavor difficult to describe developed in the skimmilk below the gravity cream layer of reconstituted milk. Neither this skimmilk flavor nor any other type of oxidized flavor was detected in the gravity cream layer of reconstituted milk made of stable fat, and its development apparently was prevented or appreciably retarded by the redistribution of fat throughout the body of milk during storage. Later, it was observed that when the reconstituted milk made of unstable fat remained undisturbed during storage, each layer of milk developed its characteristic flavor, and the development of these flavors was neither prevented nor retarded by mixing of milk during storage.

These observations suggested the possibility that the antioxidant activity centered in the fat phase of the milk might extend its protective influence, not only to vitamin A and the fat, but also to the whole body of milk when the tocopherol content of the fat is sufficiently high and the fat remains scattered.

In view of these observations, the following experiments were performed, not only to trace and identify types of oxidized flavors which develop in milk and factors responsible for their promotion, but also to learn whether some of the

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causative reactions could be induced, in the absence of vitamin C, by Cu alone, especially after the exposure of milk to light.

Evidence also is presented to show that the iodine and thiocyanogen values can be used successfully to determine initial chemical changes in fat other than the destruction of fat-soluble vitamins.

EXPERIMENTAL

It already has been remarked that the organoleptic character of oxidized flavors which develop in milk apparently is determined by the nature of the substances undergoing deterioration. Consequently, to trace and identify the oxidized flavors and the factors responsible for their development, 72 samples of milk varying in different factors were prepared as follows. Mixed, morning, 4 per cent fat, whole milk of low tocopherol content, obtained from the Cornell University herd on successive days, was divided into three lots. Two lots of milk were depleted of their total vitamin C content by rapid oxidative methods, such as adding 0.03 ml. of 30 per cent H_2O_2 per liter of milk, or, photochemically by exposing milk to sunlight for 30 min., then pasteurizing at 61.6° C. for 30 min. (4). The third lot of milk, containing 20 mg. ascorbic acid per liter, was pasteurized at the same temperature and retained as a control. Parts of the vitamin C-containing and depleted lots of whole milk were separated to obtain the corresponding three lots of 0.03 per cent fat skimmilk.

Subsequently, a part of each of the three skimmilk lots was used to prepare 4 per cent fat reconstituted milks, all of which were made with the same fat. The fat was prepared by oiling and centrifuging the butter churned from cream separated from milk treated with H_2O_2. This was done to remove the fat-globule membrane and to prevent the development of oxidized flavors during the preparation of fat for tests (6).

Each of the resulting nine lots of milk (three of whole, three of skim and three of reconstituted) then was subdivided into eight aliquot portions. One portion was retained as control, and, to the others, 0.1, 0.2, 0.4, 0.5, 1, 5 and 10 mg. of Cu per liter were added in the form of $CuSO_4$ solution. These portions of milk then were held at 0 to 5° C. up to 10 days in glass bottles protected from the light. During this time, the milk samples were tasted each day.

At the end of 10-day storage, gravity cream and skimmilk of duplicate samples of reconstituted milk, which remained undisturbed during storage, were carefully separated by siphoning. Cream was churned and fat oiled, centrifuged and analyzed for its tocopherol content. This experiment was repeated using fat of high tocopherol content to ascertain whether susceptibility of fat to deterioration is affected by tocopherol level. Furthermore, in order to determine the chemical changes in fat other than tocopherol destruction, the stable and oxidized samples of both low and high tocopherol fats were analyzed for their acid degrees, iodine, thiocyanogen and saponification values.

Finally, a collaborative experiment was performed to identify definitely the oxidized flavors associated with deterioration of the fat-globule membrane and to show again that the prevention of oxidized flavors by H_2O_2-heat destruction of

vitamin C is not caused by a complete oxidation to tasteless compounds of the substances involved, as some investigators are inclined to believe. For this reason, 20 mg. of ascorbic acid and 0.5 mg. of Cu per liter were added, alone or together, to buttermilk and skimmilk totally depleted of their vitamin C content by H_2O_2 and heat.

The tocopherol content of the fat was determined using a four-unit molecular semi-micro still, as described by Quaife and Harris (10). Distillates of fat of low tocopherol content were dissolved in 25 ml. of solvent for colorimetric assay for total tocopherols. The vitamin C content of the milk and the fat constants were determined using Gunsalus and Hand (3), Sharp (11) and A.O.A.C. methods (1). The flavors described in the manuscript were identified as such by the author, graduate students and the staff members of the Department of Food and Nutrition, College of Home Economics.

RESULTS AND DISCUSSION

The rates of development of oxidized flavors in 72 previously described samples of milk are shown in figure 1. In the absence of vitamin C, Cu catalyses

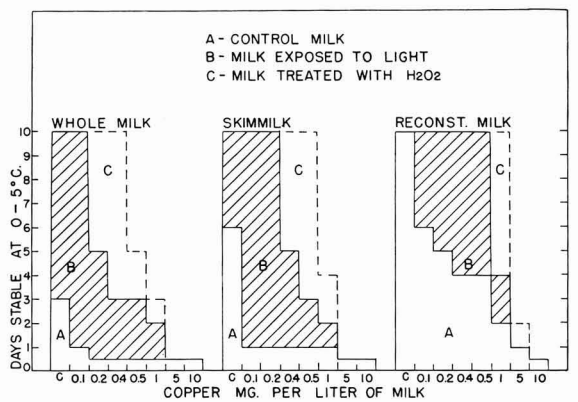

FIG. 1. The rates (in days) of development of oxidized flavors in vitamin C-containing and depleted samples of whole milk, skimmilk and reconstituted milk in the presence of varying amounts of Cu.

of the reactions which produce the oxidized flavors were either prevented or appreciably retarded in whole milk, and increasingly so in skimmilk and reconstituted milk. It also was evident that, although the depletion of milk of the total vitamin C content by either one of the methods used increased the resistance of milk to oxidized flavors, nevertheless the H_2O_2-heat treatment proved more effective.

Prevention or postponement of onset of reactions which produced oxidized flavors in samples of reconstituted milk containing ascorbic acid alone and with increasing amounts of Cu could be attributed to removal of the fat-globule

membrane on one hand, and on the other, to extension of the antioxidant activity of the fat to constituents of the milk plasma, when reconsituted milk is mixed during storage. A comparison of the stability of this milk with that of the corresponding samples of whole milk and skimmilk, which developed oxidized flavors very rapidly, indicates that the antioxidant activity of fat of low tocopherol content was not sufficient to stabilize the whole body of the milk and that development of oxidized flavors associated with deterioration of constituents of milk plasma is more readily prevented or retarded by the fat antioxidant.

The types of oxidized flavors identified in whole, skim and reconstituted milks are described in table 1. Flavors associated with deterioration of skimmilk con-

TABLE 1

The types of oxidized flavors developed in vitamin C-containing and depleted samples of whole, skim and reconstituted milks in the presence of varying amounts of Cu

Product held	Cu added	Criticisms		
		Control milk containing ascorbic acid[a]	Milk depleted of the total vitamin C content by:	
			Photochemically and heat	H_2O_2 and heat
	(*mg./l.*)			
Whole milk	.....	Chalky to tal.[c]	.....	.....
4% fat[b]	0.1	Met., then	.....	.....
	0.2– 0.4	Soapy-to-tal.	Chalky-met.	.....
	0.5– 1	and again	Chalky-met.	Chalky
	5 –10	Met.-to-fi.	Met.-to-fi.	Met.-to-fi.
Skimmilk	.....	Chalky-(Tal.)?	.....	.....
0.03%	0.1– 0.2	Chalky-to-	.....	.....
	0.4– 0.5	Soapy-	Chalky	.....
	1 –10	Tal.	Chalky	Chalky
Reconstituted	.....	.....	.....	.....
milk	0.1– 0.5	Chalky then chalky-	.....	.....
4% fat[b]	1	to-soapy-tal.	Chalky	.....
	5 –10	and again	Met.-to-fi.	Met.-to-fi.
		Met.-to-fi.		

[a] Ascorbic acid content was adjusted to 20 mg./l.
[b] Low-tocopherol winter fat (2,053 γ/100g. fat).
[c] Tal. = tallowy; Met. = metallic; Fi. = fishy.

taining ascorbic acid were dominantly chalky with a trace of tallowiness; in the presence of both ascorbic acid and Cu they were identified as chalky-to-soapy-tallowy. Intensity of the soapy flavors increased with Cu content of skimmilk. In the absence of vitamin C only chalky flavor was promoted by Cu. This flavor was detected in both light-exposed and H_2O_2-treated skimmilks, but only in those samples which contained at least 0.4 and 1 mg. of Cu, respectively.

The flavors associated with the deterioration of reconstituted milk were essentially the same as those developed in skimmilk samples containing ascorbic acid and Cu, except that, toward the end of the experimental trial, samples with 1 mg. or more Cu developed metallic-to-fishy flavors. The fishy flavor was dominant and exceedingly repulsive. In the absence of vitamin C, 5 to 10 mg. of Cu were needed to promote development of fishy flavor after initial development of a

purely chalky flavor in both kinds of reconstituted milk. The chalky flavor also was detected in reconstituted milk made of light-exposed skimmilk, and containing 1 mg. of Cu.

These flavors also were detected in the whole milk. However, in contrast to reconstituted milk, a dominantly metallic flavor developed during the first day of storage in all samples of milk containing ascorbic acid and Cu. Although this metallic flavor was persistent throughout storage, in milk with 0.2 to 0.4 mg. of Cu it definitely was dominated later by soapy-to-tallowy flavors, and with a further increase in Cu content from 0.5 to 10 mg., a fishy flavor became progressively dominant. The sample of whole milk containing ascorbic acid and no added Cu developed a chalky-to-tallowy flavor. However, some random samples of the natural whole milk containing no added Cu developed metallic flavor as well. Development of metallic flavor during the first day of storage was due to deterioration of the fat-globule membrane, as indicated by additional observations reported in table 2. The intensification of this metallic flavor and its supercedure

TABLE 2

The effects of readdition of ascorbic acid and Cu to skimmilk and buttermilk totally depleted of their vitamin C content by H_2O_2 and heat, upon the development of oxidized flavors

Held at 0 to 5° C.	Product held	No vitamin C or added Cu	Flavor[a] criticisms of milk containing:		
			Cu 0.5 mg./l.	Ascorbic acid 20.0 mg./l.	Ascorbic acid and Cu
(*d.*)					
1–3	buttermilk	.....	.....	Tal.-to-met.	Met.-to-fi.
5–7		.....	.....	then chalky	then chalky-to-soapy-tal.
1–3	skimmilk	.....	.....	Sl. chalky	Sl. chalky,
5–7		.....	.....	then chalky-to-tal.	then chalky-to-soapy-tal.

[a] Sl. = slightly; Tal. = tallowy; Met. = metallic; Fi. = fishy.

by fishy flavor toward the end of storage in samples of milk with 0.5 to 10 mg. of Cu, was caused primarily by deterioration of the fat. This is clearly evident from a comparison of the data in table 3. In the absence of vitamin C, a dominantly fishy flavor developed finally in the whole milk as in the reconstituted milk when at least 5 mg. of Cu were added to the samples. Only chalky-metallic on one hand and chalky on the other were detected in light-exposed and H_2O_2-treated whole milk samples containing 0.2 and 0.5 mg. of Cu, respectively.

The differences in the amounts of Cu required to catalyze the reactions which produce chalky-metallic flavors in whole milk and purely chalky flavors in skimmilk and reconstituted milk, when depleted of the total vitamin C content by the two previously described methods, could be explained by assuming that the unstable lipids of the fat-globule membrane and constitutuents of milk plasma were made more sensitive to Cu catalysis of oxidized flavors by exposure of milk to light. The sensitizing effect of light on milk to Cu catalysis of oxidized flavors diminished when the fat-globule membrane was removed by the separation of

whole milk, followed by addition of freshly prepared fat to the skimmilk thus obtained.

The flavors associated with deterioration of the fat-globule membrane, concentrated in the buttermilk, are described in table 2. It is important to note that the reactions which produce the oxidized flavors were induced again by the addition of ascorbic acid to skimmilk and buttermilk which first were depleted of the total vitamin C content by H_2O_2 and heat. Neither of these two samples developed oxidized flavors when Cu alone acted as a catalyst. In the buttermilk containing ascorbic acid and Cu, as in the whole milk, the metallic flavor developed first. This flavor was initially so intense that it created a sensation of

TABLE 3

Effects of the readdition of Cu and ascorbic acid to reconstituted milk made of fat of low and high tocopherol content and skimmilk depleted of total vitamin C content upon the development of oxidized flavors and the stability of tocopherols at the end of 10 d. storage at 0–5° C.

Reconstituted milk—4% fat				
Additions		Flavor[a] criticisms of gravity		Tocopherols
Ascorbic acid	Cu	Skimmilk	Buttermilk	
(mg./l.)	*(mg./l.)*			*(γ/100 g. fat)*
Control fat		.....	.....	2053
.....	0.1– 1	.....	.....	2005[b]
.....	5	Chalky	Metallic	1408
.....	10	Ex. chalky	Ex. met.-to-fi.	960
20.0	.....	.....	.....	2024
20.0	0.1– 0.4	Chalky, then	.....	1979[b]
20.0	0.5	Chalky-to-	Sl. tal.-met.	1875
20.0	1	soapy-	Met.-to-fi.	1659
20.0	5 –10	Tal.	Ex. met.-to-fi.	930[b]
Control fat		.....	.....	4650
.....	0.1– 1	.....	.....	4632[b]
.....	5	Chalky	.....	4598
20.0	.....	.....	.....	4611
20.0	0.1– 1	Chalky-to-	.....	4577[b]
20.0	5	soapy-tal.	Met.-to-fi.	3002

[a] Sl. = slightly; Tal. = tallowy; Met. = metallic; Fi. = fishy; Ex. = extremely.
[b] Average values.

fishiness. However, the metallic flavor was superseded by chalky-to-soapy-tallowy flavors, the soapy flavor being dominant. The corresponding sample of skimmilk developed chalky flavor first, which later was superseded by chalky-to-soapy-tallowy flavors. The soapy flavor again was dominant. These observations indicate that oxidation of the substances involved might be carried on preferentially in the milk.

A comparison of data in table 3 also reveals that the ability of fat to resist deterioration involving ascorbic acid oxidation is governed by the antioxidant activity of the fat as determined by its tocopherol content. Fat containing 2,053 γ of tocopherols per 100 g. of fat underwent deterioration in the presence of 0.5 mg. of Cu, while 5 mg. of Cu were needed to produce the same effect in fat containing 4,650 γ of tocopherols. Likewise, in the absence of vitamin C, 5 mg.

of Cu, which were sufficient to promote the oxidative deterioration of fat of low tocopherol content, failed to affect high-tocopherol fat. Oxidative deterioration of fat was manifested by development of metallic and dominatly fishy flavors in the cream layer of reconstituted milk and the destruction of tocopherols. Flavors developed in the skimmilk below the gravity cream layer of reconstituted milk were identified as chalky when Cu alone acted as a catalyst, and as chalky-to-soapy-tallowy when both ascorbic acid and Cu were present.

Under certain environmental conditions the fat might become extremely unstable during storage without affecting its palatability (5). In such a case, the fat would undergo deterioration immediately upon addition of ascorbic acid and as little as 0.1 mg. of Cu. This would explain the development of objectionable flavors when unstable storage fat is used in the preparation of foods containing ascorbic acid.

Data concerning acid degrees, iodine, thiocyanogen and saponification values of stable and oxidized samples of fat obtained in the preceding experiment are shown in table 4. While iodine values of oxidized fat decreased, thiocyanogen

TABLE 4

Comparison of iodine,[a] thiocyanogen and saponification values, tocopherol content, and acid degrees of stable and oxidized high- and low-tocopherol fats

Type of fat	I.V.	T.V.	S.V.	Tocopherol	A.D.
				(γ/100 g. fat)	
		Summer fat			
Stable	37.60	30.63	228.1	4650	0.10
Oxidized	35.01	30.72	228.3	3002	0.10
		Winter fat			
Stable	31.24	26.53	.....	2053	0.15
Oxidized	30.11	26.58	.....	880	0.16

[a] Hanus method.

values remained practically the same. This suggests that one double bond of the linoleic acid in the triglyceride molecule was oxidized, since the thiocyanogen radical attaches itself to all double bonds of oleic acid and only to one double bond of linoleic acid. Consequently, it is logical to assume that oxidation of one double bond of the two-double-bond fatty acids would produce no changes in thiocyanogen values. On the other hand, the iodine number, which indicates total degree of saturation, would decrease. Apparently, this type of deterioration does not result in break-down of long-chain fatty acids, because saponification values and acid degrees of fat at the end of the experimental trial were found to be the same.

Apparently iodine and thiocyanogen values can be used successfully to determine the initial chemical changes in the structure of unsaturated fatty acids in the triglyceride molecules.

SUMMARY

In fresh milk the oxidized flavors such as chalky and chalky-to-soapy-tallowy have been demonstrated to be associated with the deterioration of milk plasma

(skimmilk), and metallic and metallic-to-fishy flavors with the deterioration of the fat-globule membrane and oxidation-sensitive fat, respectively.

Evidence is presented that oxidative deterioration of the substances involved, which is stimulated in the presence of ascorbic acid and enhanced by Cu, is carried on preferentially. With passage of time, flavors developed in whole milk containing ascorbic acid and 0.1 to 0.4 mg. Cu per liter, changed from metallic to soapy-tallowy, the soapy flavor being dominant; with further increase in the Cu content from 0.5 to 10 mg. the soapy-tallowy flavors were superseded by metallic and metallic-to-fishy flavors associated with fat deterioration. Corresponding samples of reconstituted milk, from which the fat-globule membrane was removed, developed chalky-to-soapy-tallowy flavors first, which then were superseded by metallic and metallic-to-fishy flavors when the fat underwent deterioration as well. Only chalky and chalky-to-soapy-tallowy flavors developed in the skimmilk samples.

In contrast to this, in whole, skim and reconstituted milks totally depleted of vitamin C content by H_2O_2 and heat, 0.5, 1 and 5 mg. of Cu, respectively, were needed to promote the development of chalky flavor, and 5 mg. of catalyst to cause oxidative deterioration of fat.

The photochemical-heat destruction of vitamin C resulted in sensitization of milk to Cu catalysis of oxidized flavors. This effect was diminished when the fat-globule membrane was removed by separation of whole milk, followed by addition of pure stable fat to skimmilk thus obtained.

Oxidative deterioration of fat in presence of ascorbic acid and Cu was shown to be accompanied by oxidation of one double bond of the two double-bond fatty acids in the fat molecule.

The data indicate that the antioxidant activity of fat as determined by tocopherol content and extension of this activity to the body of milk by redistribution of fat, play an important part in the prevention of retardation of reactions which produce oxidized flavors.

The observations reported in this paper also explain the relative immunity of homogenized milk to oxidized flavors, since in such milk the fat remains scattered and the unstable lipid component of the fat-globule membrane is split and withdrawn from the surface into the interior of the fat-globules, where it is efficiently protected by the antioxidant.

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UNIDENTIFIED DIETARY FACTORS IN DAIRY CATTLE NUTRITION. I. DIGESTIBILITY OF PEANUT HULLS AND THEIR USE IN "BALLAST" STUDIES WITH MILKING COWS DEPLETED ON HAY ALONE[1]

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Ever since the work of Kellner and Köhler (20), it has been universally recognized that a pound of total digestible nutrients (T.D.N.) in roughages, other than young pasture and hay grown in certain areas in the Rocky Mountain region, was inferior for production purposes in the ration of livestock when compared with rations of roughages plus concentrates. Kellner and Köhler concluded from the results of their studies with steers that the productive value of feeds varied more closely with the crude fiber content than with any other ingredient in the feeding stuff. Consequently, a method of feed evaluation was worked out which was based on the crude fiber content and expressed as starch equivalent. Armsby (2) calculated net-energy values for many feeding stuffs by discounting the digestible nutrients on the basis of the amount of crude fiber. Woodman and Evans (29) determined starch equivalent values by deducting 0.58 times the undigestible crude fiber from the T.D.N. value. Lehmann (21) used undigested organic matter as an index of "ballast" as the basis for a discount system for calculating net-energy values. In 1932, Forbes (6) stated the modern concept of a balanced ration as follows: "—there is no present, or possible scientifically significant and defensible single measure of nutritive value of individual feeding stuffs; and that in order to place our interest in such a measure on a rational basis it is necessary to revise the terms of the interest itself, and to base it on *rations as wholes*[2] rather than upon individual feeding stuffs."

In earlier reports (13, 14, 15, 16, 17, 18, 19) it was shown that cows placed on alfalfa hay alone, following calving, made efficient use of the T.D.N. for several months (usually 2 to 3) and then declined in milk production. This was referred to as a depletion technique, since it appeared that some unidentified factor(s) stored in the body was being withdrawn to balance the T.D.N. in the hay. After the factor(s) had become exhausted milk production declined to a level capable of being supported by the nutriments in the all-hay ration. The efficiency of milk production prior to depletion was not always accompanied by a loss in body weight. When part of the T.D.N. in the hay was replaced by an equal amount of T.D.N. in concentrates, milk production increased against the natural tendency for cows to decline with advance in lactation. Milk production did not increase, however, when either corn starch or corn sugar was added to the

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[2] Italics by authors.

ration of depleted cows. When the starch or sugar was replaced by either corn or wheat, on an equal T.D.N. basis, milk production increased without a change in the crude fiber content of the ration. It appears that hays vary in the amount of the unidentified lactation factor(s) that they contain as indicated by the results obtained with Montana hay (10). The cows used in the Montana study made efficient use of the T.D.N. for milk production, but the chemical analysis of the hay gave no clue as to why this hay was superior to Michigan-grown hays. In line with the modern concept of a balanced ration, these results were explained on the hypothesis that certain hays were deficient in the unidentified lactation factor(s) which could be supplied by grain. This assumption has been questioned on two counts: first, that the increase in milk production resulting from the replacement of part of the T.D.N. in hay with an equivalent amount of T.D.N. in concentrates was due to an increase in calculated net-energy (22, 28); and secondly, that the replacement of part of the T.D.N. with concentrates resulted in a reduction in crude fiber intake (26). Both of these criticisms overlook the results with sugar and starch (15) and the conclusions reported by Burroughs *et al.* (4) that corn starch does not depress digestibility when fed with alfalfa hay. Axelsson (3) concluded that the optimum amount of crude fiber in the ration of cattle was between 18 and 23 per cent on the dry basis. Recently, Nordfeldt *et al.* (26), using Napier grass as the crude fiber variable, concluded that the level of crude fiber should not exceed 16 per cent of the dry matter in the ration of liberally milking cows

This experiment reports the results of a study on the digestibility of peanut hulls, the efficiency of milk production of cows depleted of their milk-stimulating factor(s) by maintenance on an all-hay ration and when part of the hay had been replaced with corn and peanut hulls. The peanut hulls were used to increase the crude fiber and "ballast" contents of the ration and to decrease the T.D.N., calculated net-energy and starch equivalent.

EXPERIMENTAL

Eight milking Holstein cows which had been properly depleted were used in this investigation (13). Four of the cows (A27, A73, A80 and 499) received the basal hay alone and were used as controls. The other four cows (A61, A53, A46 and A42) were used to determine the coefficients of digestibility of the basal hay alone and again after 15 lb. of the hay had been replaced with 6 lb. of corn and 9 lb. of peanut hulls. The basal hay consisted of an alfalfa-timothy-brome mixture, U. S. grade no. 2 on leaf and color. This hay contained about 50 per cent alfalfa and had been cured without being rained on. Six-day collection periods were used in each trial. The collection period for the hay-corn-peanut hulls ration was started on the seventh day following the switch to this ration. The cows were fed twice daily. The feces were weighed and mixed and aliquot samples preserved with HCl were taken daily for chemical analysis. Samples of hay, corn and peanut hulls were analyzed for the various constituents by A.O.A.C. methods (1). The chemical analysis of feeds, feces and average daily weights of the feces are presented in table 1.

TABLE 1

The chemical analysis of feeds and feces used in digestion trials with peanut hulls (P.N.H.) and the average daily weights of feces

	Dry matter	Org. matter	CHO	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	T.D.N.	Feces	
										Wet	Dry
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(*lb./day*)	
					Feeds						
Corn	89.6	87.5	73.6	2.09	9.06	4.77	2.4	71.2	84.3		
Hay	90.3	84.8	71.3	5.43	11.75	1.79	31.2	40.1	51.0		
P.N.H.	89.8	87.0	79.2	2.84	6.44	1.31	62.4	16.9	18.9		
					Feces from hay alone						
Cow											
A61	15.6	14.2	12.0	1.38	1.63	0.54	6.0	6.0		72.5	11.3
A53	14.1	12.8	10.9	1.24	1.45	0.46	5.6	5.3		102.4	14.4
A46	14.8	13.4	11.4	1.37	1.53	0.49	5.8	5.6		98.0	14.5
A42	14.2	13.0	11.0	1.28	1.54	0.49	5.5	5.5		101.6	14.4
					Feces from hay-corn-peanut hulls ration						
A61	21.0	19.8	17.3	1.23	1.96	0.55	10.8	6.4		61.1	12.8
A53	18.4	17.2	15.0	1.11	1.71	0.50	9.1	5.9		85.5	15.7
A46	18.8	17.5	15.2	1.27	1.82	0.52	9.3	5.9		81.7	15.4
A42	18.6	17.4	15.2	1.23	1.75	0.49	9.2	6.0		86.2	16.0

The coefficients of digestibility for the peanut hulls were calculated by using the following coefficients for corn as given in Henry and Morrison (12) and Morrison (24): protein, 77; ether extract, 90; crude fiber, 57; nitrogen-free extract (N.F.E.), 93; and dry matter, 90. The percentage of undigested dry matter (D.M.), organic matter (O.M.), carbohydrate or any feed constituent can be obtained by subtracting the coefficient of digestibility of the given constituent from 100.0. This value is termed the coefficient of undigestibility. The amount of the undigested constituent then can be calculated by multiplying the pounds of the constituent in the ration by its per cent of undigestibility. The method for calculating undigested carbohydrates is illustrated by using the data for cow A61 on hay alone (table 1) as follows:

Coefficient of digestibility for crude fiber	= 54.6%	
Coefficient of undigestibility for crude fiber	= 100.0 − 54.6	= 45.4%
Coefficient of undigestibility for N.F.E.	= 100.0 − 64.8	= 35.2%
Undigested crude fiber in hay	= 31.2 × 0.454	= 14.2%
Undigested N.F.E. in hay	= 40.1 × 0.352	= 14.1%
Total and undigested CHO or "Ballast"	= 71.3	28.3%

Per cent undigested CHO = $28.3/71.3 \times 100 = 39.7$

Calculated net-energy (C.N.E.) was determined by the formula suggested by Moore (23), *i.e.*, E.N.E. = −324.7 + 213.2 log of T.D.N. The starch equivalent (S.E.) values were determined by the standard method. The number of pounds of T.D.N. in 100 lb. of feed minus the product of the number of pounds of crude fiber times 0.58 equals the starch equivalent, *i.e.*, 51.0 − (31.2 × 0.58) = 32.9 (table 1). In calculating the coefficients for peanut hulls, the individual coefficients for each cow on the basal hay were used.

The milk was weighed at each milking and 3-day composite samples were taken for butterfat determinations. The equivalent number of pounds of 4 per cent fat-corrected milk (F.C.M.) was calculated from the formula proposed by Gaines (9). In the milk production study, the periods on hay alone were 12 days and the hay-corn-peanut hulls periods were 15 days in length. During each of these periods, feces were collected for 6 consecutive days. The animals were weighed at the same hour every third day.

RESULTS

The data concerning the various coefficients of digestibility of the peanut hulls and the total ration for each cow, together with their mean values and the mean values obtained from the four cows on the basal hay ration are compiled in table 2. The coefficients for the various constituents are in good agreement among all of the cows. The coefficient obtained for dry matter, organic matter and carbohydrates for the peanut hulls are of about the same order for each cow.

The coefficients of digestibility for the total ration of hay, corn and peanut hulls also show good agreement. The coefficient of the crude fiber for cow A46 however, was 41.1 compared with 38.3, 39.5 and 38.8 per cent for the other three cows. This slight increase affected the T.D.N. because of the larger amount of crude fiber in the ration. The T.D.N. value of the total ration of A46 was 50.5

TABLE 2

Summary of the coefficients of digestibility of the peanut hulls (P.N.H.), the total ration (T.R.) and the basal hay alone (B.H.A.)

Coefficients	A61		A53		A46		A42		Mean		
	P.N.H.	T.R.	P.N.H.	T.R.	P.N.H.	T.R.	P.N.H.	T.R.	P.N.H.	T R.	B.H.A.
Dry matter	20.7	54.0	20.7	54.2	25.7	55.4	17.4	53.3	21.1	54.2	58.3
Org. matter	20.7	54.7	20.1	54.9	25.8	56.1	17.8	54.0	21.1	54.9	59.6
CHO	21.0	53.7	18.7	54.1	26.4	55.6	17.3	53.1	20.9	54.1	59.1
Protein	21.3	60.0	25.0	61.8	22.4	61.0	26.2	60.5	23.7	60.8	66.5
Ether ext.	11.3	51.4	5.6	47.6	11.3	48.8	23.9	48.8	13.0	49.2	29.3
Crude fiber	23.6	38.3	24.0	39.5	28.0	41.1	20.2	38.8	24.0	39.4	52.6
N.F.E.	10.5	67.2	5.4	66.0	20.4	68.0	5.7	66.0	10.5	66.8	64.1
T.D.N.	18.2	48.4	17.7	48.1	22.7	50.5	16.0	48.5	18.7	48.9	51.0
C.N.E. (therms)	−56.1	21.6	−58.6	23.4	−35.6	28.9	−68.0	20.8	−54.6	23.7	39.3
S.E. (%)	−18.0	28.4	−18.5	28.4	−13.5	29.7	−20.2	28.8	−17.6	28.8	32.9
Ballast as percentage of:											
Undig. D.M.	79.3	46.0	79.3	45.8	74.3	44.8	82.6	46.9	78.9	45.9	41.9
Undig. O.M.	79.3	45.3	79.9	45.2	74.2	43.9	82.2	46.0	78.9	45.1	40.6
Undig. CHO	80.0	46.0	81.3	46.0	73.6	44.3	82.7	46.8	79.4	45.8	40.8

compared with mean of 48.9 per cent. The undigested dry matter, organic matter and carbohydrates for the total ration showed good agreement among all of the animals. Since starch equivalent values are calculated by discounting the T.D.N. on the basis of the amount of crude fiber, the variations obtained among the cows followed the T.D.N. content of the ration. Calculated net-energy values are based on the summation of the amount of C.N.E. in each ingredient that is included in the ration; however, Moore's equation can not be used to determine the C.N.E. of the total ration when a negative C.N.E. value is assigned to one of the feed ingredients. The high T.D.N. value obtained for A46 was responsible for the high calculated net-energy value.

A comparison of the mean coefficients of digestibility obtained for the all-hay ration and the hay-corn-peanut hulls ration shows consistently lower values for all components of the peanut hulls ration except for ether extract and nitrogen-free extract (table 2). The per cent of T.D.N. on hay alone averaged 51.0 compared with 48.9 for the peanut hulls ration. The average hay ration contained 39.3 therms per cent of calculated net-energy compared to 23.7 for the hay-corn-peanut hulls ration. Starch equivalent values for the hay alone and hay-corn-peanut hulls ration averaged 32.9 and 28.8 per cent, respectively.

Table 3 shows the data relative to milk production. Three of four cows increased in milk production against the tide of declining lactation when 15 lb. of hay were replaced with 6 lb. of corn and 9 lb. of peanut hulls. Cows A61, A46 and A42 were 157, 148 and 167 days in gestation at the beginning of the experiment. Cow A42 declined 0.1 lb. in F.C.M., which was a smaller decline than any of the four control cows fed hay alone during the same time. It should be pointed out that there was a decrease in the T.D.N. intake during the peanut hulls period of about 1 lb. per day per cow, but milk production increased. The intakes of crude fiber and undigested carbohydrates also increased significantly during hay-corn-peanut hulls periods, but the amounts of calculated net-energy and starch equivalents received decreased markedly. As indicated by the increased number of pounds of F.C.M. per 100 lb. of T.D.N., the over-all efficiency of the cows on the hay-corn-peanut hulls ration increased when compared to the production on the all-hay ration. All of the control cows declined in milk production when maintained on the all-hay ration.

The weight losses of the experimental cows were not excessive after they were changed to the hay-corn-peanut hulls ration, while only one control cow (A73) showed a slight loss in body weight.

DISCUSSION

Fifteen lb. of hay were replaced with 6 lb. of corn and 9 lb. of peanut hulls in order to determine whether the unidentified lactation factor(s) supplied by grain could be accounted for on the basis of an increase in calculated net-energy (Moore), starch equivalent or a decrease in crude fiber. The value of peanut hulls as a supplement to replace part of the hay is indicated by the negative calculated net-energy, starch equivalent and high crude fiber contents (−54.6 therms, −17.6 and 62.4 per cent, respectively), but their merit as a source of

TABLE 3

The effect of replacing 15 lb. of the basal hay with 6 lb. of corn and 9 lb. of peanut hulls as a source of crude fiber and "ballast" in an all-hay ration on average daily yield of 4 per cent F.C.M. as compared with the control cows

Cow	Expt. period	Body wt.	F.C.M. yield	Hay intake	T.D.N. rec.	C.N.E. rec.	S.E. rec.	Total undigested		Crude fiber in D.M.	F.C.M./ 100 lb. T.D.N.
								CHO	CHO		
(*no.*)	(*d.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*therms*)	(*lb.*)	(*lb.*)	(%)	(%)	(*lb.*)
					Cows used in digestion trials						
A61	12	1089	7.1	31.2	16.1	12.7	10.5	8.9	39.9	34.6	44
	15	1079	8.3	16.0[a]	15.0	6.7	8.8	10.6	46.3	38.3	55
A53	12	1248	17.2	37.9	19.3	14.9	12.4	11.2	41.5	34.6	89
	15	1221	18.4	23.0[a]	18.4	8.9	10.8	12.8	45.9	37.9	100
A46	12	1233	16.4	37.9	19.3	14.9	12.4	11.2	41.5	34.6	85
	15	1207	16.8	23.0[a]	18.8	11.0	11.3	12.4	44.4	37.6	89
A42	12	1228	11.0	37.9	19.1	14.5	11.2	11.2	41.6	34.6	58
	15	1220	10.9	23.0[a]	18.1	7.9	10.6	13.1	46.9	37.6	60
					Controls						
A27	12	1168	12.5	35.8	18.3	14.1	11.8	10.5	41.1	34.6	68
	15	1187	8.9	37.4	19.1	14.7	12.3	11.0	41.1	34.6	47
A73	12	849	18.9	29.3	14.9	11.5	9.6	8.6	41.1	34.6	126
	15	838	18.2	28.4	14.5	11.2	9.4	8.3	41.1	34.6	126
A80	12	931	16.2	29.8	15.2	11.8	9.8	8.7	41.1	34.6	107
	15	944	15.0	29.4	15.0	11.6	9.7	8.6	41.1	34.6	100
499	12	1110	10.6	34.0	17.4	13.4	11.2	9.9	41.1	34.6	61
	15	1123	10.3	33.9	17.3	13.4	11.2	9.8	41.1	34.6	60

[a] Replaced 15 lb. of hay with 6 lb. of corn and 9 lb. of peanut hulls.

"ballast" is manifested by the large amounts of undigested dry matter, organic matter and carbohydrates that they contain (78.9, 78.9 and 79.4 per cent, respectively).

Fraps (7, 8) determined coefficients of digestibility of peanut hulls by using four sheep. Peanut hulls also have been used in other digestion trials reported in the literature, but they appear to have contained some peanuts, as was indicated by their high ether extract content. It appeared desirable, therefore, to secure coefficients of digestibility of peanut hulls for milking cows. The peanut hulls used in this study contained 1.31 per cent ether extract, which indicated that they were low in peanuts. The two samples of peanut hulls used by Fraps contained 1.26 and 1.66 per cent ether extract. The coefficients of digestibility of the crude fiber for the four cows used in this study varied from 20.2 to 28.0, compared to 7.7 to 38.2 secured for sheep. The crude fiber content of the peanut hulls was 62.4, as compared to 56.0 and 57.2 per cent in the trials with sheep.

The increases obtained in F.C.M. from three of four cows when 15 lb. of hay were replaced with 6 lb. of corn and 9 lb. of peanut hulls were associated with a marked decline in calculated net-energy and starch equivalent intakes and indicate the unreliability of these two methods as scientific measures of feed evaluation. Even when the peanut hulls were given a zero calculated net-energy value, as suggested by Morrison (24), the calculated net-energy value failed to express the production value of the hay-corn-peanut hulls ration. It should be emphasized that true net-energy of a mixed ration can not be expressed by a fixed value, because higher or lower values can be obtained depending on the balance of the ration and also on the genetic characteristics of the individual cow. The concept of true net-energy must take into consideration the ration as a whole and not just the summation of the amount of calculated net-energy in each individual feed included in the ration. The results obtained from this work support the modern concept of a balanced ration as first expressed by Forbes (6).

The increase in F.C.M. which was obtained when 15 lb. of hay were replaced with 6 lb. of corn and 9 lb. of peanut hulls was associated with an increase in the per cent of crude fiber in the dry matter from 34.6 to 38.0. These results do not confirm the conclusions of Axelsson (3) that the optimum crude fiber content of the dry matter is between 18 and 23 per cent. The results also fail to confirm the conclusions of Nordfeldt *et al.* (26), who reported that the crude fiber level should not exceed 16 per cent of the dry matter in the ration of liberally milking cows. The level of milk production of the cows used by Nordfeldt *et al.* was higher than in the cows used in this study; nevertheless, Graves *et al.* (10) reported high levels of milk production by cows fed Montana hay which contained 34 per cent crude fiber on the dry basis. It is likely that the Napier grass which Nordfeldt *et al.* fed as the crude fiber variable had some factor present which tended to depress milk production.

The role of "ballast" in dairy cattle nutrition has received scant attention in the past, except that crude fiber has been used as a criterion for feed evaluation. The amount of crude fiber in a ration is not a reliable index of "ballast" because of the wide variation in the coefficients of digestibility (5). Lehmann (21) de-

fined "ballast" as the undigested organic matter and used it as a basis for calculating the net-energy value of feeds for livestock. The undigested organic matter includes metabolic nitrogen and ether extract. These constituents only make up a small part of the undigested organic matter, but it does account for the waxes and chlorophyll that may be present in roughages. Guillemet and Jacquot (11) defined "ballast" as undigested carbohydrates (crude fiber + N.F.E.). This fraction accounts for all the lignin which is found in crude fiber and N.F.E. (25, 27). On theoretical grounds, the undigested crude fiber plus the undigested N.F.E. appear to be the most logical basis of "ballast". When milking cows were changed from hay alone to the hay-corn-peanut hulls ration, the change in the different forms of "ballast" (undigested dry matter, organic matter and carbohydrates, calculated as per cent of the dry matter) was greater in the case of undigested carbohydrates. It appears, therefore, that of the three forms of "ballast" used in this investigation, undigested carbohydrates is a superior index for measuring "ballast". These results failed to show that by increasing the "ballast" (undigested carbohydrate) from about 41 on the hay alone to about 46 per cent on the hay-corn-peanut hulls ration, milk production was affected adversely.

When 15 lb. of hay were replaced by 6 lb. of corn and 9 lb. of peanut hulls, there was a decline of about 0.9 lb. of T.D.N. per day. It is universally recognized that the T.D.N. concept is not a completely reliable criterion of the balance of a ration. The increase in the number of pounds of F.C.M. per 100 lb. of T.D.N. appears to be a more acceptable measure of the balance of the ration. There was an increase in the efficiency of T.D.N. conversion to F.C.M. in all four cows during the hay-corn-peanut hulls periods in comparison to a consistent decrease in the control cows fed the basal hay. These results confirm previous reports that grains and root crops supply some unidentified factor(s) needed to balance the T.D.N. of hay (13, 14, 15, 16, 17, 18, 19).

The loss in weight of all of the cows during the hay-corn-peanut hulls periods appears to be associated with the decrease in the net weight of the feces, because of a lack of fill in the digestive tract. There was, however, an increase in the weight of the dry matter of the feces due to the increase in "ballast" intake (table 1). The difference in the water-holding capacity of the feces after the addition of corn and peanut hulls may have been due to a decline in hydrophilic colloids in the ration.

SUMMARY

Four depleted milking Holstein cows were used to determine the digestibility of hay when fed alone and after 15 lb. of the hay had been replaced with 6 lb. of corn and 9 lb. of peanut hulls. Four Holstein cows were maintained on the basal hay ration throughout the experiment as controls.

The coefficients of digestibility of the peanut hulls were found to be: dry matter, 21.1; organic matter, 21.1; carbohydrates, 20.9; protein, 23.7; ether extract, 13.0; crude fiber, 24.0; and nitrogen-free extract, 10.5. The "ballast"

values were calculated to be: undigested dry matter, 78.9; undigested organic matter, 78.9; and undigested carbohydrates, 79.4 per cent.

Three of four cows increased in the production of F.C.M. when changed from hay alone to a hay-corn-peanut hulls ration, whereas all of the control cows declined in F.C.M. All of the cows increased in the number of pounds of F.C.M. per 100 lb. of T.D.N. on the hay-corn-peanut hulls ration, while all of the control cows showed a decline.

The amount of F.C.M. increased appreciably in spite of the fact that the calculated net-energy and starch equivalent values declined markedly and the crude fiber and undigested carbohydrates increased.

The results of this investigation support the modern concept of a balanced ration and illustrate some of the imperfections in both the calculated net-energy and the starch equivalent concepts as scientific methods of feed evaluation.

The data presented in this paper give further support to the contention that grain supplies the unidentified factor(s) needed by some hays for more efficient milk production.

Undigested carbohydrates appear to be a better index for "ballast" evaluations than either undigested dry matter or undigested organic matter.

The losses in body weight of the cows on the hay-corn-peanut hulls ration appeared to be due to a decrease in wet fecal weights due to a lack of fill, although the daily amount of fecal dry matter increased.

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UNIDENTIFIED DIETARY FACTORS IN DAIRY CATTLE NUTRITION. II. FURTHER EVIDENCE OF AN UNIDENTIFIED FACTOR(S) IN GRAIN NEEDED TO BALANCE ROUGHAGE FOR MILK PRODUCTION[1]

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In the preceding paper (12), digestion trial data were presented on four milking cows receiving a ration of hay alone and again after 15 lb. of the hay had been replaced with 6 lb. of corn and 9 lb. of peanut hulls. Three of four cows increased in milk production against the tide of declining lactation on the hay-corn-peanut hulls ration, in spite of the fact that there was a marked decrease in the consumption of T.D.N., calculated net-energy and starch equivalent and a significant increase in the amount of crude fiber in the dry matter and in ballast.

The work presented in this paper is a continuation of the study on the effect of replacing part of the hay in an all-hay ration with grain and ballast in the form of wheat straw, wood meal or peanut hulls on milk production, with emphasis on the possibility of explaining the unidentified factor(s) in grain on the basis of the calculated net-energy and starch equivalent concepts.

EXPERIMENTAL

Seventeen Holstein cows were used in 25 trials to determine the effect on milk production when part of the hay was replaced with grain plus either wheat straw, oakwood meal or peanut hulls. They had been depleted of their milk-stimulating factor(s) by the technique described previously (9, 10, 11). Six cows were used in seven trials with grain and wheat straw, three cows were used in three trials with wood meal and 11 cows were used in 15 trials with various amounts of corn and peanut hulls. Some of the cows were used in two different trials at later stages of lactation. The cows varied from 42 to 318 days in lactation at the start of the experimental period and from 0 to 146 days in gestation. Detailed data are presented for 10 representative cows, but complete data are available upon request. The milk was handled as reported previously (12). The cows were weighed at the same hour every third day. The length of time that the cows were on the basal ration varied from 9 to 18 days and the supplemented periods ranged from 9 to 27 days.

The description of the feeds, their chemical composition, coefficients of digestibility, digestible protein, T.D.N., calculated net-energy, starch equivalent and ballast values are shown in table 1. When the cows were depleted on a mixture of alfalfa and brome hays, part or all of the brome hay was replaced with grain and ballast. The coefficients recommended by Morrison (20) were used for some of

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TABLE 1

The description, chemical composition, coefficients of digestibility, digestible protein, T.D.N., calculated net-energy (C.N.E.), starch equivalent (S.E.) and undigested carbohydrates of feeds used in the various trials

Feed	Used in trials	D.M.	CHO	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	Dig. prot.	T.D.N.	C.N.E.	S.E.	Ballast
(*no.*)	(*no.*)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(*therms*)	(%)	(%)
						Hay							
1[a, b]	1	88.8	67.2	6.59	12.6 72	2.47 31	32.5 43	34.6 70	9.1	49.0	35.7	30.1	28.9
2	2, 3, 6, 8	90.0	77.3	4.72	6.8 51	2.15 39	36.9 59	39.4 64	3.5	52.4	41.9	31.0	29.3
3	2, 3, 6, 8	89.4	67.0	5.91	14.2 72	2.32 34	28.7 43	38.3 71	10.2	51.5	40.3	34.9	27.5
4	4	90.0	75.8	4.53	8.6 51	1.09 39	32.8 59	43.0 64	4.4	52.2	41.7	33.2	28.9
5	5, 7	87.8	66.4	5.93	14.0 70	1.55 40	27.6 46	38.7 69	9.8	50.6	38.8	34.6	26.9
6	9–10	89.3	69.9	5.66	12.1 66	1.60 29	30.3 53	39.6 64	8.0	50.4	38.3	32.8	28.5
						Wheat straw							
7	1–3	90.9	77.9	5.56	5.1 8	2.35 41	37.9 52	40.9 47	0.4	41.0	19.1	19.5	39.5
						Wood meal (oak)							
8	4	91.5	88.7	0.61	1.6	0.60	59.2	29.5	0.0	10.0	− 111.5	− 24.3	87.4

TABLE 1—Concluded

Feed	Used in trials	D.M.	CHO	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	Dig. prot.	T.D.N.	C.N.E.	S.E.	Ballast
(*no.*)	(*no.*)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(*therms*)	(%)	(%)
						Peanut hulls							
9	5–10	89.8	79.3	2.84	6.4	1.31	62.4	16.9	1.5	18.9	−52.6	−16.8	62.2
					24	13	24	12					
						Soybean oil meal							
10	1	87.5	32.0	5.24	46.4	3.85	5.0	27.0	39.0	74.6	74.7	74.6	3.8
					84[c]	85	73	91					
						Corn gluten meal							
11	2, 3	91.7	42.7	2.99	42.4	3.59	4.5	38.2	36.1	81.7	83.0	81.7	4.6
					85[c]	93	58	93					
						Corn (U. S. grade 2)							
12	1, 4	88.0	71.1	2.16	9.1	4.66	2.6	69.5	7.0	82.6	83.9	82.6	6.0
					77[c]	90	57	93					
13	5–10	87.1	72.4	2.31	8.6	3.78	2.4	70.0	6.6	80.7	82.0	80.7	5.9
					77[c]	90	57	93					

[a] The first line in each trial represents the chemical composition of the feed, whereas, the second line represents the coefficients of digestibility of the various feed fractions. All values were obtained experimentally, except those lines marked with a footnote c which represent calculated values.

[b] Description of feeds used: 1. Alfalfa, 1st cut. 2. Brome-timothy, 1st cut. 3. Alfalfa, 2nd cut. 4. Brome-timothy-alfalfa, 1st cut. 5. Alfalfa, 2nd cut. 6. Alfalfa-timothy-brome, 1st cut.

the feeds, but actual coefficients of digestibility were used for the majority of the feed stuffs. The requirements suggested by the National Research Council (15) were used for T.D.N. and the upper limits suggested by Morrison (20) were used for calculating net-energy. Moore's method (19) also was used to determine the calculated net-energy values.

The wood meal was made by grinding oakwood shavings. The T.D.N. content of the meal was assumed to be 10 per cent on the basis of the work of Kirsch and Jantzon (13), who determined the T.D.N. content of birch and pine sawdust silage.

RESULTS

The pertinent data showing the representative results obtained by replacing part of the hay in an all-hay ration with an equivalent amount of grain and either wheat straw, oakwood meal or peanut hulls are presented in table 2. The data given for the three cows are representative of the seven trials in that they include the minimum and maximum increases in milk yield when straw and grain were fed. The cow used in trial 1 produced 2.2 lb. more of 4 per cent F.C.M. per day on the hay-straw-grain ration with the same amount of T.D.N., calculated net-energy and ballast, than that produced on hay alone. When corn gluten meal and straw replaced part of the hay in trials two and three, the amounts of F.C.M. increased slightly, although there was decline in T.D.N. and calculated net-energy and an increase in the ballast content. The number of pounds of F.C.M. per 100 lb. of T.D.N. increased in all seven trials following the change from hay alone to the hay-straw-grain ration.

When 10 lb. of hay were replaced with 5 lb. of a grain mixture and 5.5 lb. of wood meal, in trial 4, the amount of milk increased on the same amount of T.D.N., less calculated net-energy, more ballast and the same amount of starch equivalent and crude fiber in the dry matter. Milk production increased still further, however, when the wood meal was removed from the ration. This increase was associated with an increase in calculated net-energy and starch equivalent intake and a decrease in the per cent of crude fiber on the dry basis and in ballast. The results presented for this cow are typical of those obtained from the other two cows. The number of pounds of F.C.M. increased in all three trials on the hay-grain-wood meal ration, but an additional increase was obtained when the wood meal was withdrawn from the ration.

Peanut hulls are an excellent source of ballast because of their high crude fiber and low T.D.N. content, but wheat straw is not a satisfactory source of ballast, because the spread between some of the hays and wheat straw is not wide enough to accentuate the effect of ballast. Also, the per cent of crude fiber in some of the hays and wheat straws does not vary enough to properly evaluate the role of the level of crude fiber in the ration.

Corn and peanut hulls replaced part of the basal hay in 15 trials. In trials 5 to 8, inclusive, 15 lb. of hay were replaced by 6.5 lb. of corn and 8 lb. of peanut hulls. All of the cows showed an increase in the production of F.C.M. on this ration compared to the periods on hay alone. The increases in milk production occurred even though the amounts of calculated net-energy and starch equivalent

TABLE 2

The effect on milk yield when part of the T.D.N. in hay was replaced with an equivalent amount of grain and ballast

Trial no.	Exptl. period	Body wt.	Fat test	F.C.M. yield	Feed[1]			T.D.N. rec.	C.N.E. received[2]		S.E.	Crude fiber in D.M.	Ballast	F.C.M./ 100 lb. T.D.N.
					Hay	Corn	Ballast		(a)	(b)				
	(*d.*)	(*lb.*)	(%)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*therms*)		(*lb.*)	(%)	(%)	(*lb.*)
							Wheat straw							
1	18	1175	4.0	6.9	35.8	.....	.....	17.5	12.8	.....	10.8	36.6	43.0	39
	15	1203	4.0	9.1	10.0	6.0[a]	17.3	17.6	12.6	.....	12.0	32.0	44.0	52
2	15	942	3.4	8.3	29.5[b]	.....	.....	15.5	12.4	.....	9.5	37.7	39.2	54
	12	934	3.4	9.5	10.0[c]	4.0[d]	15.7	14.9	10.4	.....	9.7	33.0	44.0	64
3	9	1002	3.3	25.0	39.7[e]	.....	.....	20.7	16.8	.....	12.7	38.8	39.0	121
	12	1045	3.3	25.2	10.0[c]	4.0[d]	16.0	15.0	10.4	.....	9.8	33.0	44.2	168
							Wood meal							
4	15	937	2.9	17.1	35.0	.....	.....	18.3	14.6	.....	11.7	36.4	38.2	93
	15	937	3.1	19.3	25.0	5.0[f]	5.5	18.5	9.3	.....	11.9	35.4	42.1	104
	15	913	3.0	20.1	25.0	5.0[f]	.....	18.0	15.4	.....	13.2	30.1	31.9	112
							Peanut hulls							
5	12	1319	3.5	17.6	40.0	.....	.....	20.3	15.5	15.5	13.9	31.4	40.6	85
	12	1305	3.3	20.1	25.0	6.5	8.0	19.4	10.8	15.0	12.5	34.6	43.8	104
	15	1257	3.2	20.4	25.0	6.5	.....	17.6	15.0	15.0	13.8	25.6	33.5	116
6	12	1130	3.6	19.6	34.0[g]	.....	.....	17.1	12.9	12.9	11.5	33.0	41.4	115
	12	1112	3.3	19.7	19.0	6.5	8.0	16.3	7.7	12.4	10.0	38.3	46.8	121
	18	1082	3.1	19.7	19.0	6.5	.....	14.7	12.4	12.4	11.5	26.8	33.4	134
7	12	1097	3.4	15.0	35.8	.....	.....	18.2	13.9	13.9	12.4	31.4	40.6	93
	12	1100	3.1	18.3	20.3	6.5	8.0	17.1	9.0	13.2	10.9	35.1	44.2	107
8	12	1092	3.1	22.9	34.0[g]	.....	.....	17.0	13.0	13.0	11.7	31.4	40.6	135
	9	1093	3.1	28.3	19.0	6.5	8.0	16.4	8.5	12.7	10.5	35.2	44.3	173
9	12	1036	3.6	11.7	34.0	.....	.....	17.1	13.0	13.0	11.2	34.0	41.0	68
	15	1048	3.6	12.2	26.5	3.0	4.5	16.6	10.3	12.6	10.3	36.0	43.6	73
10	12	1096	3.3	17.6	38.0	.....	.....	19.2	14.6	14.6	12.5	33.9	41.0	92
	12	1134	3.5	19.8	30.5	3.0	4.5	18.6	11.8	14.1	11.6	35.8	43.3	106
	27	1146	3.3	20.1	23.0	6.0	9.0	18.1	9.0	13.7	10.8	37.6	45.6	111

[1] Components of the ration: (a). 1 lb. of soybean oil meal and 5 lb. of corn. (b). 19.5 lb. hay no. 2 and 10 lb. of hay no. 3. (c). Hay no. 3. (d). Corn gluten meal. (e). 29.7 lb. hay no. 2 and 10 lb. hay no. 3. (f). 4 lb. of corn and 1 lb. of corn gluten meal. (g). 10 lb. of hay no. 2 and 23 lb. of hay no. 3.

[2] (a) C.N.E. values determined by Moore's method, (b) C.N.E. values when peanut hulls equal zero.

were reduced and the per cent of crude fiber in the dry matter and ballast were increased. The cow used in trial 5 increased 2.5 lb. of F.C.M. per day on the hay-grain-peanut hulls ration over that produced on the all-hay ration and milk production increased an additional 0.3 lb. when the peanut hulls were removed, whereas, the F.C.M. produced by the cow used in trial 6 was unchanged on the same ration. There was no significant difference between the calculated net-energy and starch values after the 8 lb. of peanut hulls had been discontinued and the values on the all-hay ration, but an increase was obtained in the number of pounds of F.C.M. per 100 lb. of T.D.N. consumed. In trials 9 and 10, 3 lb. of corn and 4.5 lb. of peanut hulls replaced about 7.5 lb. of hay. This ration produced an increase in F.C.M. in all of the trials, although the T.D.N., calculated net-energy and starch equivalent values were reduced and the amount of ballast and crude fiber in the dry matter were increased. The supplements were doubled in the last part of trial 10 and the basal hay was decreased by 7.5 lb. per day for a 27-day period. This change resulted in a further increase of 0.3 lb. of F.C.M. per day and an additional drop in the T.D.N., calculated net-energy and starch equivalent intakes and a marked increase in the crude fiber and ballast intakes. The results presented for the six cows are typical of the results obtained from the other nine trials.

In all trials, the amount of 4 per cent F.C.M. increased against the tide of advancing lactation when part of the basal hay was replaced with corn and wheat straw, wood meal or peanut hulls, even when a zero value was used for the net-energy content of the peanut hulls. The efficiency of caloric conversion of T.D.N. to milk is strikingly illustrated in table 2 when the number of pounds of F.C.M. per 100 lb. of T.D.N. consumed is examined.

The per cent of fat in the milk remained unchanged after the ration was changed from hay alone to the hay-grain-wheat straw ration and to the hay-grain-wood meal ration, but when corn and peanut hulls replaced part of the hay, there was a decline in the fat content of the milk in six trials, no change in seven trials and an increase in two trials.

The differences in body weights recorded in table 2 for the cows on the various rations do not appear to be significant.

DISCUSSION

The seven trials with wheat straw indicate that the unidentified factor(s) present in grain improves the balance of the ration. These findings are in agreement with the results reported by Burroughs *et al.* (2), who found that corncobs fed with good hays and grain were almost as productive per pound of T.D.N. for body gains in steers as that in corn grain. The increased ballast intake when oakwood meal was fed failed to suppress the milk producing factor(s) in the grain, as was shown by an increase in the amount of F.C.M. in each trial. The wood meal exerted some inhibitory effect, however, because milk production increased in all trials following withdrawal of the meal from the ration. Although the amount of ballast decreased from 42 to 32 per cent from the periods on the hay, grain and wood meal to the hay-grain ration, it appears unlikely that ballast

was a factor in view of the higher ballast values in all of the trials with peanut hulls. The increased production of F.C.M. can not be explained on the basis of calculated net-energy, even when a zero value is used for peanut hulls, since the amount of F.C.M. increased in all trials which contained the same or less calculated net-energy. These results are not in agreement with Moore (18), Saarinen *et al.* (22) or Shaw *et al.* (23), who have postulated that the unidentified lactation factor(s) in grain is due to an increase in calculated net-energy, but they do confirm previous findings (9, 10, 11, 12), which indicated that concentrates supply an unidentified factor(s) needed to balance the nutrients in some hays for efficient milk production. These results also are in agreement with those of Smith *et al.* (24), who showed that the replacement of 13 to 25 per cent of the T.D.N. in an all-alfalfa hay ration with various concentrates, on an equal T.D.N. basis, resulted in an increase in milk production. Davis and Kemmerer (5) found that milk production was increased when dried grapefruit peel was added to an alfalfa ration. Loosli (14) reported that an increase in F.C.M. occurred when hay was replaced with an equal amount of T.D.N. in either corn distillers grains or corn distillers solubles.

The increases obtained in F.C.M. when the cows were changed from an all-hay ration to a hay-corn-peanut hulls ration were associated with an increase in the per cent crude fiber in the dry matter and in the per cent of ballast. These results are in agreement with those of Graves *et al.* (8), who showed that high levels of crude fiber do not depress milk production when the total ration is reasonably well-balanced. The work of Graves *et al.* also indicated that some hays may contain a sufficient amount of the unidentified lactation factor(s) for efficient milk production when fed as the only feed. The results, however, are not in accord with Nordfeldt *et al.* (21) who reported an optimum crude fiber content of 16 per cent on the dry basis for liberally milking cows, or with Axelsson (1) who stated that the optimum crude fiber content of the dry matter of the ration for cattle was 18 to 23 per cent. The highest values (about 38 per cent on the dry basis) observed in this work may have been close to the maximum under the conditions of this experiment, since one cow went off-feed when 22.5 lb. of hay were replaced by 9 lb. of corn and 13.5 lb. of peanut hulls. The T.D.N. supplied by peanut hulls may be as poorly balanced as that in the hay which was replaced. The efficiency of the conversion of T.D.N. to milk is a better index of the balance of a ration than either calculated net-energy, starch equivalent or any system of feed evaluation based on the summation of empirical values assigned to individual feed stuffs when they are fed in combination.

The increase in milk production which resulted when part of the hay was replaced with grain also may apply to gains in body weight by beef cattle. Forbes *et al.* (7) studied the metabolism of steers fed alfalfa hay alone, corn alone and a mixture of hay and corn and concluded that the net-energy (actual, not calculated) of corn in the mixed ration was greater than when the components were fed alone. The results of Burroughs *et al.* (2) also showed that the T.D.N. of corncobs exerted about the same production in steers as the T.D.N. in corn.

The unidentified lactation factor(s) present in concentrates may be required

by the rumen bacteria to improve the balance of the ration. Burroughs *et al.* (3) found that the digestibility of the dry matter of corncobs was depressed by the addition of starch, but when the same amount of starch was added to alfalfa hay, the depression in the digestibility of dry matter did not occur. These workers (4) showed later that alfalfa hay ash contained essential nutrients for the growth of rumen microorganisms. It is difficult to determine whether the factor(s) is needed by the rumen microflora or by the host or by both.

The results reported herein support the modern concept of a balanced ration which was first expressed by Forbes (6) and officially defined by Mitchell (16) in 1935. They also are in agreement with Maynard (17) who stated: "Since individual feeds are seldom if ever balanced rations, it is evident that the summation of the net-energy values of the feeds making up a ration is not an accurate measure of the energy value of the ration as a whole and that the values for certain individual feeds may be highly misleading as to their effects as constituents of completely balanced rations. Herein lies another practical limitation of the net-energy system."

These results suggest that an objective in forage crop research should be to produce hays high in milk-stimulating factors in order to reduce the amount of grain-feeding.

SUMMARY AND CONCLUSIONS

Seventeen depleted cows were used in 25 trials to determine the effect of replacing part of the hay in an all-hay ration with grain and either wheat straw, oakwood meal or peanut hulls.

In all but one trial, the replacement of part of the hay with these feed stuffs resulted in an increase in 4 per cent F.C.M., even though the intakes of T.D.N., calculated net-energy and starch equivalent decreased and the crude fiber in the dry matter and ballast increased.

Crude fiber values up to 38 per cent of the dry matter did not depress milk production when the cows were changed from hay alone to a ration of hay, corn and peanut hulls.

The results of this investigation indicate that calculated net-energy and starch equivalent values of individual feeds are not additive, but that they support the modern concept of a balanced ration.

Further evidence is presented to show that the unidentified lactation factor(s) in grain is needed to improve the balance of roughages.

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THE PRESENCE OF AUREOMYCIN IN MILK AND ITS EFFECT ON CHEESE MAKING AND STARTER ACTIVITY

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Numerous reports have appeared during the last 3 yr. concerning the treatment of bovine mastitis with antibiotics and the effect of using milk from treated cows for products requiring acid development. Penicillin was introduced first as a treatment for mastitis caused by streptococci. Numerous reports have appeared describing the inhibition of starter activity in milk from cows treated with penicillin (3, 4, 5, 6, 8, 9, 10, 11, 14, 15, 16).

McCulloch and Kiser (13) reported the use of aureomycin as a treatment for staphylococcic mastitis in 1949. Katznelson and Hood (8) reported the inhibitory action on starter activity with aureomycin added to milk. Krienke (12) reported practically no acid production at 7- and 18-hr. incubation when the milk contained 0.0005 mg. aureomycin hydrochloride per milliliter.

The first known report on the making of cheese from milk produced by cows treated with aureomycin was by Bradfield (1, 2). Further studies were reported by Hanson *et al.* (4).

EXPERIMENTAL

Trouble was experienced in making cheddar cheese at the University of Vermont creamery with pasteurized milk from the College herd. Some of the cows had been treated with aureomycin HCl for staphylococcic mastitis. The trouble consisted of failure to develop lactic acid during the making of the cheese.

Trials were set up to coincide with monthly treatment of the herd. Treatment consisted of injection into each infected quarter of one tube (7.1 g.) of aureomycin hydrochloride ointment. Each gram contained 30 mg. aureomycin HCl. Therefore, each treatment contained 213 mg. of the antibiotic. The cows were treated soon after the morning milking. In the herd of 67 milking cows, 7.5 per cent of the quarters were treated. Three lots of cheddar cheese were made on successive days after treatment, from the mixed milk of the entire herd. The cheese was aged for 14 mo. and scored each month.

Another set of trials was conducted each month as follows: Lots of milk in 200-ml. quantities from each treated quarter were placed in a water bath, brought to 98° F. and held. As soon as the milk reached 98° F., 2 per cent active cheese starter was added. Acidity increase was measured each hour for 6 hr. by titrating with 0.1 *N* NaOH, the bottles being inverted each half hour. Each trial was continued on successive milkings until no starter inhibition was evident. The control was conducted with milk procured from cows that had no history of

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mastitis and had never been treated with an antibiotic. Samples were procured from each milking and tested for the presence of aureomycin by microbiological assay. This was an agar cup assay, employing *Bacillus subtilis* as previously reported (7).

At a later date a third set of trials was conducted to ascertain the smallest quantity of aureomycin necessary to produce inhibition of starter. These trials were conducted in the same manner as the previous set, except that aureomycin was added to normal milk instead of using the milk from treated cows.

The last trials were set up to ascertain the degree of treatment in a herd that would cause inhibition of starter activity under practical conditions. Several herds near the College were selected. A certain proportion of the quarters of

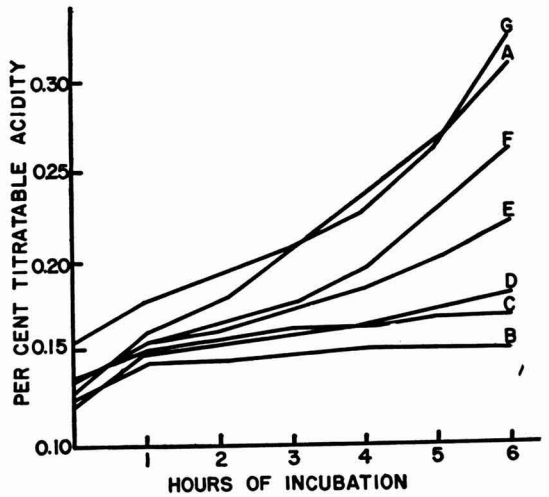

FIG. 1. Inhibition of starter activity in milk from cows treated with aureomycin HCl.
A—Acidity before treatment.
B—Milkings 1 to 4 after treatment (averaged).
C—Fifth milking.
D—Sixth milking.
E—Seventh Milking.
F—Eighth milking.
G—Control (average of all milkings).

each herd was treated after the morning milking. The next day the combined supply of the night and morning milkings was brought to the College. A sample of the mixed milk was used for the starter activity test.

Chr. Hansen's culture of mixed organisms was used for starter in all of the trials.

RESULTS

The three lots of milk used for cheese had an acidity of 0.16 per cent. Starter from a different source was used with each batch. At the time of adding rennet the acidity was 0.18 per cent. The acidity at dipping was 0.13 per cent, which is normal at the College creamery. During cheddaring the acidity rose

very slowly, the three lots reaching 0.25, 0.26 and 0.31 per cent, respectively, 7 hr. after setting. It was realized at this stage that no further increase in acidity could be expected and the curd was milled and placed in the press.

The cheese was stored at 45 to 50° F. for the first 9 mo. During this period it had little or no cheese flavor and the body was weak, pasty and distinctly wet. The cheese then was transferred to storage at a temperature of 55 to 60° F. Two mo. later it had developed a strong bitter flavor with no change in body. There was still no cheese flavor. The cheese was left at this temperature until a total of 14 mo. had elapsed. By this time the bitterness had disappeared and the cheese had a sharp cheese flavor scoring 38. The body, however, remained weak and pasty. It appears from these results that the presence of aureomycin in the milk merely delays the development of cheese flavor. However, the body never was good. The cheese did not become gassy as reported by Schaaf (15) in connection with the use of penicillin in Holland.

TABLE 1

Aureomycin content of milk after udder treatment (av. of triplicate microbiological determinations)

Cow	Quarter	Hours after treatment								
		12	24	36	48	60	72	84	96	108
					(γ/*ml.*)					
444	C	3.5	3.5	2.0	2.0	0	0	0	0	0
431	A	20.0	20.0	3.5	0	0	0	0	0	0
218	C	5.5	4 5	2.0	0	0	0	0	0	0
309	D	4.5	3.5	3.5	1.5	1.0	0	0	0	0
462	B	5.5	5.5	4.5	1.5	0	0	0	0	0
257	B	25.0	5.5	2.0	1.5	1.0	1.0	0	0	0

The second set of trials showed definite inhibition of starter activity. Acidity increase was negligible in milk from the first four milkings. There was so little increase that the acidity readings for the first four milkings were averaged (fig. 1). At the fifth milking some increase was evident. By the time the eighth milking was reached acid increase followed approximately the same curve as the control, although the final acidity was not as high. The first set of these trials was examined by microbiological assays to ascertain the quantity of aureomycin remaining in the milk at each milking (table 1). Comparing these amounts with figure 1, it was evident that there was some inhibition of starter even after the assays showed no evidence of aureomycin. Amounts of the antibiotic less than 1 γ per milliliter could not be measured by the bioassay.

Table 2 shows the amount of milk produced by various quarters at each milking and the quantity of aureomycin found in each lot of milk. After the first milking there was no correlation between the amount of milk produced by treated quarters and the quantity of aureomycin in the milk.

Hanson *et al.* (4) and some other workers have reported that the use of aureomycin gave a slight orange color to the milk. This was not observed in any of these experiments. In one case only the milk exhibited a slight green color.

TABLE 2

Concentration of aureomycin present in varying amounts of milk produced by cows that had all received the same treatment for mastitis

Cow	Quarter	Milkings									
		1		2		3		4		5	
		lb.	*γ/ml.*	*lb.*	*γ/ml.*	*lb.*	*γ/ml.*	*lb.*	*γ/ml.*	*lb.*	*γ/ml.*
479	C	2.9	5.0	1.7	5.0	2.5	5.0	1.9	2.0	2.2	0.5
462	B	3.0	7.0	2.3	15.0	2.5	7.0	3.0	1.5	2.5	1.0
382	C	3.6	6.0	2.5	7.0	2.6	2.0	3.0	1.0	3.2	1.0
479	A	3.7	7.0	3.5	5.0	3.0	3.0	3.5	1.0	3.4	1.5
393	C	5.2	7.0	4.0	5.0	3.5	7.0	5.7	1.5	3.9	0.0
392	B	5.3	10.0	5.9	1.0	5.4	3.0	7.4	0.0	6.0	0.0

However, this was an experimental lot of aureomycin in a different type of vehicle. This particular mixture was not put on the market.

The third set of trials consisted of selecting milk from untreated cows. This was divided into 15 lots and a different amount of aureomycin added to each lot. This was accomplished by making a 100 : 1 solution of aureomycin with distilled water (pH 7.2), and adding it to the milk in the desired quantity 30 min. before adding the starter. Active starter at the rate of 1 per cent was used in this trial. The rest of the trial was conducted in the same manner as the previous one. The results (table 3) indicate that when the concentration of the drug is 0.10 γ or less per milliliter the inhibitory effect is greatly reduced.

Another series was conducted in this trial, consisting of ten lots of milk from the same supply and using three different quantities of aureomycin. The results (table 4) show inhibition at the 0.25 γ per milliliter level but not at the 0.10 γ per milliliter level. This varies somewhat from the report by Krienke (12) that 0.0005 γ per milliliter of aureomycin HCl greatly retarded the production of lactic acid by starters.

TABLE 3

Effect on acid production of adding aureomycin to milk

Quantity (γ/ml.)	Acidity[a] at:						
	Original	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
0	0.14	0.15	0.15	0.16	0.17	0.18	0.21
10.0	0.14	0.14	0.14	0.15	0.14	0.14	0.14
5.0	0.14	0.15	0.15	0.15	0.15	0.15	0.14
2.5	0.14	0.14	0.14	0.15	0.14	0.14	0.14
1.0	0.14	0.14	0.14	0.14	0.14	0.14	0.15
0.5	0.14	0.15	0.15	0.14	0.15	0.15	0.15
0.25	0.14	0.15	0.15	0.15	0.15	0.15	0.16
0.10	0.14	0.15	0.15	0.15	0.16	0.17	0.18
0.05	0.14	0.14	0.15	0.16	0.16	0.18	0.20
0.03	0.14	0.14	0.14	0.15	0.16	0.18	0.19
0.01	0.14	0.14	0.15	0.16	0.16	0.19	0.21
0.005	0.14	0.14	0.15	0.16	0.17	0.19	0.21
0 0025	0.14	0.14	0.15	0.16	0.17	0.19	0.22
0.0010	0.14	0.15	0.15	0.16	0.17	0.19	0.21
0.0005	0.14	0.15	0.15	0.16	0.18	0.19	0.22

[a] Per cent acidity calculated as lactic acid.

TABLE 4

Effect on acid production of adding aureomycin to milk (Averages of 10 lots of milk)

Quantity added (γ/ml.)	Acidity[a] at:						
	Original	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
0.00	0.17	0.18	0.21	0.26	0.30	0.38	0.47
0.25	0.15	0.17	0.19	0.20	0.22	0.24	0.26
0.10	0.15	0.18	0.20	0.22	0.26	0.31	0.41
0.05	0.15	0.18	0.22	0.24	0.31	0.35	0.42

[a] Per cent acidity calculated as lactic acid.

Hanson (4) and Krienke (12) have reported that if 1 per cent of the milk supply came from treated quarters, serious inhibition of starter activity could be expected. Whitehead (16) and others reported that there is some inhibition for 6 and even 12 milkings after treatment. The results reported here are in agreement with these observations. It was felt that this might not be entirely true under ordinary farm conditions. Therefore, three local herds were selected for study. One per cent of the quarters in each herd were treated with 213 mg. of aureomycin HCl in ointment per quarter. The milk was tested for starter activity in the same manner as the previous trials. Tests were carried on until there was no apparent inhibition of starter growth. The data presented in table 5 show that there was very little restriction of starter activity the day after treatment when 1 per cent of the quarters were treated. On the second day there was no evidence of any effect from the antibiotic with two of the herds and very slight inhibition with herd H. A week later when 2 per cent of the quarters were treated (table 6) there was some effect on the first day after treatment and none at all on the second, except again a slight inhibition with the milk from herd H.

After another interval of a week, 3 per cent of the quarters were treated.

TABLE 5

Effect on acid development after treating 1% of the quarters in a herd

Herd	Before adding starter	Acidity[a] at: Hours after adding starter					
		1	2	3	4	5	6
		1st day after treatment					
C	0.14	0.16	0.17	0.18	0.21	0.23	0.25
W	0.14	0.17	0.18	0.19	0.20	0.20	0.21
H	0.13	0.15	0.15	0.16	0.17	0.17	0.18
Control	0.14	0.17	0.18	0.20	0.22	0.25	0.28
		2nd day after treatment					
C	0.14	0.17	0.18	0.19	0.21	0.22	0.25
W	0.14	0.17	0.18	0.19	0.21	0.22	0.25
H	0.14	0.16	0.17	0.17	0.18	0.19	0.21
Control	0.15	0.17	0.18	0.19	0.20	0.22	0.24

[a] Per cent acidity calculated as lactic acid.

TABLE 6

Effect on acid development after treating 2% of the quarters in a herd

Herd	Before adding starter	Acidity[a] at: Hours after adding starter					
		1	2	3	4	5	6
		1st day after treatment					
C	0.14	0.17	0.17	0.18	0.18	0.18	0.18
W	0.14	0.16	0.17	0.18	0.18	0.18	0.19
H	0.14	0.16	0.16	0.16	0.16	0.16	0.17
Control	0.15	0.17	0.18	0.19	0.20	0.21	0.22
		2nd day after treatment					
C	0.14	0.17	0.17	0.18	0.20	0.20	0.21
W	0.14	0.16	0.17	0.18	0.19	0.21	0.21
H	0.13	0.15	0.16	0.17	0.18	0.18	0.19
Control	0.15	0.17	0.17	0.18	0.19	0.20	0.21

[a] Per cent acidity calculated as lactic acid.

In table 7 it is evident that there was inhibition on the first day after treatment but none on the second day with any of the three herds. According to these results, it appears necessary to have at least 2 per cent or probably 3 per cent of the quarters treated before any serious restriction of starter activity is evident. Even with 3 per cent treatment, the effect does not last more than 2 days after treatment.

Several attempts were made to manufacture cottage cheese from the skim-milk from these lots of milk. The milk was set at 72° F. with 2 per cent active starter. Instead of setting up in the usual 16 to 18 hr., it was 36 hr. before there was any sign of curd. The curd, when it finally developed, was of soft gelatinous nature. It was impossible to cut or handle this curd in any way that would produce a normal product.

TABLE 7

Effect on acid development after treating 3% of the quarters in a herd

Herd	Before adding starter	Acidity[a] at: Hours after adding starter					
		1	2	3	4	5	6
		1st day after treatment					
C	0.14	0.16	0.17	0.17	0.17	0.18	0.18
W	0.14	0.16	0.17	0.17	0.18	0.19	0.20
H	0.13	0.16	0.16	0.17	0.18	0.19	0.19
Control	0.15	0.17	0.18	0.19	0.20	0.21	0.22
		2nd day after treatment					
C	0.15	0.17	0.18	0.19	0.20	0.21	0.24
W	0.15	0.17	0.18	0.19	0.20	0.22	0.24
H	0.14	0.16	0.17	0.18	0.19	0.20	0.22
Control	0.15	0.17	0.18	0.19	0.20	0.22	0.24

[a] Per cent acidity calculated as lactic acid.

Tests for starter activity were made on the cream from the above mentioned milk. These tests showed the same general trend as the milk from which the cream was separated.

Since these trials were completed the supplier of aureomycin tubes for mastitis treatment has doubled the strength of the drug in each tube. A single treatment now consists of 426 mg. of aureomycin instead of the 213 mg. used in these experiments. No work was done to ascertain the results of this increased degree of treatment with regard to its effect on starter activity.

It has been reported by some milk plant operators that treatment of cows with an antibiotic resulted in the presence of an oily substance floating on the surface of the milk. Veterinarians claim that this is caused by improper treatment or treatment just prior to milking. This effect was not evident in any of the trials run in this work. It is not likely that the absence of this substance can be taken as an indication that the cows have not been treated.

The only way that a plant operator can avoid trouble from this source is to keep milk out of the supply for 2 or 3 days after treatment. In the case of penicillin treatment, Whitehead (16) in New Zealand recommends that the producer keep the milk from treated cows out of the supply on the day of treatment and for 2 days thereafter. In Holland, according to Schaaf (15), an arrangement has been made that permits only veterinarians to make treatment. They in turn notify the plant receiving the milk as to the date of treatment and the number of quarters treated. The plant operator then can divert the milk from treated herds to uses that do not require bacterial activity.

SUMMARY

Varying amounts of aureomycin were found in the milk from treated cows for as long as 72 hr. after treatment.

After the first milking following treatment there was no correlation between the amount of milk produced and the concentration of aureomycin remaining in the milk.

There was very little evidence of the inhibition of starter activity when the amount of aureomycin was less than 0.25 γ per milliliter.

Inhibition of starter activity was definitely evident for six milkings (3 days) after treatment.

Treatment of commercial herds indicated that when using the mixed milk from such herds little or no trouble is experienced unless 3 per cent or more of the quarters in the milking herd are treated. If inhibition of starter activity is evident under such conditions, it does not continue in any harmful degree beyond the fourth milking (2 days).

Cheddar cheese-making operations are definitely disrupted by the presence of appreciable amounts of aureomycin in the milk. Acid development is retarded, and during curing, flavor develops slowly and may be bitter. Body is weak and pasty. Cottage cheese making was rendered impossible.

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THE ALPHA–NAPTHOLPHTHALEIN (ANP) METHOD FOR MEASURING FAT HYDROLYSIS. I. APPLICATION TO BUTTER[1]

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Within the past few years, the proposal has been made to relate the degree of fat hydrolysis in butter to the quality of the cream used in its manufacture. Hillig's WIA method for the measurement of water-insoluble acids (4) has generally been accepted as the standard procedure to use to determine the extent of fat hydrolysis in butter. This method is time-consuming, requires highly trained personnel, and under ideal conditions a trained technician is limited to about eight determinations a day (4).

If the free water-insoluble-acids content of butterfat is to continue to serve as an index of cream quality, the development of a rapid method that could be used generally by the dairy industry would be a valuable aid in quality improvement programs. A method which appears to offer possibilities in this connection is the α-naphtholphthalein colorimetric method suggested by Roberts *et al.* (6, 7). Armstrong *et al.* (1) recently compared this method to the Hillig procedure for determining water-insoluble acids and found a close correlation for 17 samples of butter.

The α-naphtholphthalein method described by Roberts *et al.* (6, 7) has certain weaknesses which limit its accuracy and its use. Therefore, further work was thought necessary to modify, perfect and adapt the test to routine dairy plant use.

EXPERIMENTAL PROCEDURE

Basically, the method of Roberts *et al.* (6) consists of preparing a solution of α-naphtholphthalein in 57 per cent alcohol and neutralizing it with 0.1*N* NaOH to a blue-green color just before use. To 5 ml. of the neutralized solution in a test tube is added (with a medicine dropper) 12 drops of purified butter oil and the tube shaken vigorously. After standing for 5 min., and no longer than 20 min., the color of the alcohol layer is compared with color standards. Several inadequacies of this procedure became apparent when it was being applied to commercial butter. These inadequacies are: (a) Neutralizing dye to a blue-green color before beginning analyses introduces a possible error, since different technicians do not always reproduce the same starting alkalinity. (b) Instability of the neutralized dye makes it necessary to use neutralized dye within 1 hr. after neutralization. (c) Inaccuracy in quantity of fat used due to possible human error arising from the counting of 12 drops and to using medicine droppers of markedly different sizes. In order to overcome these disadvantages, the original

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method of Roberts *et al.* (6) was modified, and the following procedure was established:

(a) *Preparation of dye solution.* Exactly 0.1 g. of the α-naphtholphthalein dye (Coleman-Bell, pH 7.3 – 8.7) is made to a volume of 150 ml. with 95 per cent ethyl alcohol. This alcoholic dye solution remains stable if kept tightly stoppered in a cool place.

(b) *Preparation of neutralizing solution.* A neutralizing solution containing a borate buffer is prepared according to the method of Clark (3). The strength of the buffer solution is varied with different lots of dye, since each dye lot differs in its buffer capacity. The buffer is prepared so that 2 ml. of the neutralizing solution mixed with 3 ml. of the alcoholic dye solution gives a density of 0.495 when measured against distilled water with a Beckman DU spectrophotometer at a wave length of 750 μ and a slit width of 0.035 mm. This establishes a balance permitting the use of 0.5 ml. of fat in the test.

(c) *Preparation of neutralized dye solution.* Three ml. of the alcoholic dye solution are pipetted into a test tube 12 × 120 mm. Two ml. of the neutralizing solution are added to give 5 ml. of neutralized dye. If desired, enough neutralized dye may be prepared, using the above proportions, for use during 1 day, since the weak buffer prevents fading for at least 12 hr.

TABLE 1

Color standards for colorimetric fat hydrolysis test[a]

Color of test	Name of color[b]	Plate no.[b]	Degree of hydrolysis
Dark green	Dusky dull green	42	None
Medium green	Dusky olive green	41	Very slight
Light green	Buffy olive	30	Slight
Orange	Ochraceous buff	15	Definite
Yellow	Chamois	30	Pronounced
White	Cartridge buff	30	Very pronounced

[a] Standards selected by Roberts (7).

[b] Name of color and plate number refer to Ridgway, R. *Color Standards and Nomenclature.* Ridgway, Washington, D. C. 1912.

(d) *Determining the degree of fat hydrolysis.* Melt butter in a water bath at 140° F. until a clear separation of fat and serum is obtained. If necessary to remove traces of serum from the fat, centrifuge in a heated Babcock centrifuge and return the clarified butter oil to the water bath maintained at 140° F. Measure 0.5 ml. of the butter oil into the test tube containing 5 ml. of the neutralized dye solution. A heated pipette is desirable in order to deliver the proper amount of fat. Stopper the test tube with a rubber stopper or finger. Shake it vigorously with a vertical motion five times through a 1-ft. arc and read the test. The color is stable for 20 min. Color standards were prepared as suggested by Roberts *et al.* (7). They are given in table 1.[2]

A single analysis may be completed within 10 to 15 min. Attempts to adapt the method to a colorimeter were unsuccessful because of the high density of the

[2] Color standards have been prepared and may be obtained from the Department of Dairy Technology at a nominal cost.

beginning dye solution and the extreme change in density with small increases in acidity. Spectrophotometric analyses may be possible over a narrow range of the visual color range.

Early observations revealed that lots of α-napholphthalein manufactured by the Coleman-Bell Co. were variable and did not always yield the same color when treated with a uniform quantity of a standard buffer. This variation results from the formation of various isomers during the synthesis of α-naphtholphthalein from phthalyl chloride, all of which are not removed during purification (2). Because of the variations in different lots of dye, each lot of dye must be tested to determine the proper molarity and concentration of buffer to use in the neutralizing solution. Also, extremely old dye should not be used, since it may yield unsatisfactory results regardless of strength or type of buffer.

For purposes of simplicity and to differentiate from the test of Roberts *et al.* (6), the modified method has been designated as the ANP method, after the indicator, and this nomenclature will be used henceforth.

EXPERIMENTAL RESULTS

Determining concentrations of pure fatty acids. In order to determine the specificity and quantitative nature of the ANP method, the test was made on purified butterfat containing varying concentrations of added pure acids. For these trials, purified fat was prepared as follows: Butter was melted, the fat collected, the acids extracted in the manner described in Hillig's WIA method (4) and the fat then washed with alcohol and water, and dried.

Varying concentrations of lactic, butyric, caproic, capric, palmitic and oleic acid were added to the purified butter fat and thoroughly incorporated prior to the analysis. Enough distilled water was added to simulate the volume of serum present in the butter.

The results presented in table 2 reveal three important points. First, as shown by Roberts *et al.* (6), increases in the concentration of free fatty acids cause the indicator to undergo a series of definite color changes from dark green to medium green, light green, orange, yellow and white. Second, for caproic, capric, palmitic and oleic acids, the method is roughly quantitative within a range of acid concentrations not exceeding 400 mg. Within this range of concentration, a major color change occurs with each 100 mg. change in acid. When close color comparisons are made, changes in color may be observed with increments of 25 mg. acid per 100 g. of fat; when the water-insoluble acid concentration is above 400 mg. per 100 g. fat, the increase of acid necessary for a definite color change is much higher than at the lower concentrations. However, the lack of sensitivity with acid concentrations above 400 mg. per 100 g. of fat is not a serious limitation, since the critical concentration of water-insoluble acids for acceptable butter is less than 400 mg. per 100 g. of fat. The yellow color, covering a WIA range of approximately 400 to 500 mg. of acid per 100 g. of fat, would always indicate to the manufacturer that his product was not satisfactory because of excessive fat hydrolysis.

Third, lactic and butyric acids do not affect the application of the test for

TABLE 2

Effect of adding acids to purified butter fat on the accuracy and reliability of the ANP test

Mg. acid added per 100 g. fat	Color obtained for following acids[a]				
	Lactic	Butyric	Caproic	Palmitic	Oleic
0	d. green	d. green	d. green	d. green	d. green
25	d. green	d. green	d. green	d. green	d. green
50	d. green	d. green	d. green	d. green	d. green
100	d. green	d. green	d. green	d. green	d. green
125	d. green	d. green	d. green	d. green	d. green
150			d. green	m. green	m. green
200	d. green	d. green	d. green	m. green	m. green
225				l. green	l. green
250	d. green	d. green	m. green	l. green	l. green
300	d. green	m. green	m. green	l. green	l. green
325				orange	orange
350	d. green		l. green	orange	orange
400	d. green	m. green	l. green	orange	orange
425				yellow	yellow
450	d. green	l. green	orange	yellow	yellow
500	d. green		orange	yellow	yellow
550			yellow		yellow
600	d. green			yellow	yellow
700	d. green			yellow	yellow
750					yellow
800	d. green		yellow	white	white

[a] d. = dark, m. = medium, l. = light.

water-insoluble acids. Lactic acid is not detected by the method, even when present in high concentrations. Thus, the method may be applied accurately to soured dairy products, such as cream used for buttermaking, and large concentrations of butyric acid cause only insignificant changes in the color.

Butter from cream containing added acids. Samples of cream with known concentrations of butyric, caproic, capric, and lactic acids were churned in the

TABLE 3

Effect of acids added to cream on ANP test of the resulting butter

Mg. acid added per 100 g. fat	Color[a] obtained for following acids:				
	Lactic	Butyric	Caproic	Capric	Oleic
0	d. green	d. green	d. green	d. green	d. green
30	d. green	d. green	d. green		d. green
75	d. green	d. green	d. green		d. green
100	d. green	d. green	d. green	d. green	d. green
150	d. green	d. green			m. green
200	d. green	d. green	m. green	m. green	m. green
225	d. green	d. green			l. green
300	d. green	d. green	l. green	l. green	l. green
325	d. green	m. green			orange
400	d. green	m. green	l. green	orange	orange
475	d. green	m. green			yellow
500	d. green	m. green	l. green	yellow	yellow
650	d. green	m. green	orange		yellow

[a] See foot note table 2.

laboratory. The resulting butters were washed twice with a volume of distilled water equal to the original cream, then the ANP test made on the washed butter. The results in table 3 corraborate those in table 2 in that butyric and lactic acid again were found not to be measured by the method. Also, caproic acid in high concentrations in the cream has only a slight effect on the method, but capric acid is quantitatively detected within the limits of the method. It is likely that butyric and caproic acids are removed to a large extent by the washing of the butter.

TABLE 4

A comparison of WIA and ANP methods for determining water-insoluble acids in butter

ANP test		Mg. WIA per 100 g. of fat		
Color[a]	Equivalent WIA range (mg. WIA/ 100 g. fat)	Predicted[b]	Experimental	Difference from predicted value
m. green	100 to 225	162	137	− 25
m. green	100 to 225	162	141	− 21
m. green	100 to 225	162	155	− 7
m. green	100 to 225	162	175	+ 13
m. green	100 to 225	162	183	+ 21
l. green	200 to 325	262	200	− 62
l. green	200 to 325	262	201	− 61
l. green	200 to 325	262	222	− 40
l. green	200 to 325	262	230	− 32
m. green	100 to 225	162	232	+ 70
l. green	200 to 325	262	251	− 11
l. green	200 to 325	262	252	− 10
l. green	200 to 325	262	283	+ 21
l. green	200 to 325	262	285	+ 23
l. green	200 to 325	262	285	+ 23
l. green	200 to 325	262	281	+ 19
l. green	200 to 325	262	290	+ 28
orange	300 to 425	362	372	+ 10
orange	300 to 425	362	376	+ 14
yellow	400 to 900	650	384	−278
orange	300 to 425	362	409	+ 47
yellow	400 to 900	650	416	−234
yellow	400 to 900	650	492	−158
yellow	400 to 900	650	498	−152
yellow	400 to 900	650	868	+218

[a] See footnote table 2.
[b] Predicted value taken as mid-point of equivalent WIA range.

Comparison to WIA. The data in tables 2 and 3 suggest that water-insoluble acid values could be predicted from the results of the ANP test in butter. In order to determine the validity of such predictions, a comparison of the ANP test and Hillig's WIA procedure (4) was made on 25 samples of butter.

The predicted WIA value was selected arbitrarily as the mid-point of the equivalent WIA range established experimentally for each color of the ANP test. The results of the ANP test, predicted WIA and experimental WIA are presented in table 4. The last column of the table shows the differences between the experimental and predicted WIA values. For WIA values of less than 400 mg., the differences between the results exceeded 62 mg. for only two of 21 samples.

In one exception a butter sample gave an ANP color of yellow and a WIA value of 384.

DISCUSSION

The results reveal that the method gives a fairly reliable measure of fat hydrolysis in butter. The rapidity and comparative accuracy of the test makes it useful for routine analyses and as a quality control test. A single analysis may be conducted in 10 to 15 min. Thus, at least 100 determinations may be made in the time required for eight WIA determinations by the Hillig procedure (4).

One disadvantage of the method is the necessity to standardize each lot of dye to obtain the proper neutralizing solution. However, this may be handled conveniently in a central laboratory and information as to the proper ratio of buffer to dye be distributed from that point. The ANP method should be helpful as a screening method for the detection of butter which has been made from cream containing a high water-insoluble acid content. Further investigation is being made to apply the test to cream, an application which would permit the manufacturer to control the water-insoluble acid content of cream being manufactured into butter.

SUMMARY

A rapid colorimetric method using α-naphtholphthalein originally developed by Roberts *et al.* (6) has been modified to eliminate certain inherent weaknesses. For convenience, the modified procedure is designated as the ANP test.

The method may be applied to butter to give a roughly quantitative measure within an accuracy of 100 mg. per 100 g. fat of water-insoluble fatty acids below a concentration of 400 mg. acid per 100 g. of fat. With extra precautions greater accuracy may be achieved, since color changes may be detected with variations of 25 mg. water-insoluble acids at concentrations of less than 400 mg. per 100 g. fat.

The ANP method is not affected by the presence of butyric and lactic acids, but does measure caproic and higher fatty acids. The results indicate a close agreement between the ANP method and the WIA method of Hillig (4).

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THE EFFECT OF PROGESTERONE ON OVULATION TIME IN DAIRY HEIFERS

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Evidence for the existence of a nervous mechanism in the release of luteinizing hormone and ovulation in the cow recently has been presented (7). In these experiments the parasympathetic blocking drug, atropine, administered to heifers at the beginning of estrus, was found to block ovulation. However, when atropine and chorionic gonadotrophin (as a source of luteinizing hormone) were administered concurrently to heifers at the beginning of estrus, ovulation occurred at the normal time or earlier. These results were interpreted to indicate the existence of a neural mechanism having a parasympathetic component involved in luteinizing hormone release and ovulation in the cow.

As a result of these experiments, it was decided to study the effects of the ovarian hormones on ovulation time. The first of the ovarian hormones studied in this respect was progesterone.

EXPERIMENTAL PROCEDURE

The length of estrus and the time of ovulation were determined for 11 heifers in a control estrous period and in an estrous period in which progesterone was administered as soon as possible after the beginning of estrus. The beginning and end of estrus usually were determined by checking the heifers with a teaser bull at 2-hr. intervals. In some cases, more frequent checks were made to determine the beginning of estrus. The time of ovulation was determined by rectal palpation. In one case a double ovariectomy was performed in order to verify the findings of the rectal palpations. In some cases the control data were obtained prior to the treatment period, and in other cases they were obtained subsequently. In the latter event, one complete estrous cycle was allowed to elapse before the control data were taken. Some heifers were treated several times and data on several control periods also were taken in some instances. The progesterone was dissolved in cottonseed oil at the rate of 5 mg. per milliliter and injected subcutaneously. The dosages ranged from 5 to 50 mg. but were 10 mg. or less in all but three cases. Heifers were considered to be in estrus when they would stand when mounted and out of estrus when they no longer stood. The heifers were grade Holsteins and Guernseys between 1 and 3 yr. of age. Data were collected during the first 5 mo. in 1951. Additional data also have been collected in the same way on the effects of progesterone given prior to the beginning of estrus and later than the second hour of estrus. Sham injections were not made during the control estrous periods.

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RESULTS

Table 1 shows that administration of progesterone at the beginning of estrus reduced average length of estrus by 3.6 hr., average time from the end of estrus to ovulation by 5.4 hr. and average time from the beginning of estrus to ovulation by 9 hr. The reduction in the length of the time elapsing from the beginning of estrus to ovulation (5.4 hr.) and in the length of time elapsing from the end of estrus to ovulation (9 hr.) are significant at the 1 per cent level when the t test is applied. The reduction of the length of the estrous period is significant at the 5 per cent level. Particularly noteworthy is the performance of heifer no. 80,

TABLE 1

The effect of progesterone given at the beginning of estrus on ovulation time in dairy heifers

Heifer no.	Control period			Progesterone period				
	Length of estrus	Time from end of estrus to ovulation	Time from beginning of estrus to ovulation	Progesterone injected[a]	Time of injection from beginning of estrus	Length of estrus	Time from end of estrus to ovulation	Time from beginning of estrus to ovulation
	(*hr.*)	(*hr.*)	(*hr.*)	(*mg.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)
68	16.0	14.0	30.0	9.14	0	11.0	7.0	18.0
79	12.0	14.0	26 0	10.00	1.7	11.5	2.5	14.0
	14.0	10.0	24.0					
76	19.0	8.0	27.0	7.29	1.0	21.0	11.0	32.0
				10.00	0.5	21.0	2.0	23.0
75	23.0	8.5	31.5	8.15	1.5	20.0	4.0	24.0
	19.0	10.0	29.0					
74	22.0	17.0	39.0	8.13	2.0	14.0	11.5	25.5
81[b]	.....	.....	.....	10.00	1.5	17.0	3.0	20.0
77[c]	12.5	14.0	26.5	.....	.....	.....	.....	.....
73	16.0	17.0	33.0	50.00	1.0	6.0	12.5	18.5
67	21.0	9.0	30.0	5.00	2.0	17.0	6.0	23.0
80	22.0	12.0	34.0	5.00	1.0	17.0	−2.0	15.0
62	20.5	11.5	32.0	20.00	2.0	8.5	29.0	37.5
				5.00	0.5	19.0	20.0	39.0
				10.00	2.0	23.0	7.0[d]	30.0
72	22.0	12.0	34.0	10.00	0.5	10.0	5.5	15.5
				30.00	2.0	18.0	8.0	26.0
Av.	18.6	12.3	31.0	13.18	1.3	15.0	6.9	22.0

[a] Subcutaneously in cottonseed oil.
[b] Ovariectomized after progesterone treatment.
[c] Serves as control for heifer no. 81.
[d] A second follicle ruptured 16 hr. from the end of estrus.

in which ovulation occurred 2 hr. before the end of estrus. The ovulatory process could not be hastened in heifer no. 62 despite three attempts. This is the only heifer in which ovulation could not be induced at the normal time or earlier by the simultaneous administration of atropine and luteinizing hormone in earlier experiments, so that her general response to treatment is abnormal.

The data in table 2 indicate that the injection of progesterone later than 2 hr. after the beginning of estrus is without effect. Progesterone injected 4 to 5 hr. before the onset of estrus seems to have hastened the ovulatory process to a small degree.

A corpus luteum was formed after the progesterone treatment in all cases except one. A corpus luteum could not be felt on rectal palpation on the 7th and 16th days after ovulation in heifer no. 76 following treatment, with 7.29 mg. of progesterone. The subsequent estrous cycle was normal, however. Heifer no. 72 returned to estrus twice at 8-day intervals after treatment with 30 mg. of progesterone. Subsequent cycles were normal. Heifer no. 73 had a normal estrous cycle after treatment with 50 mg. of progesterone.

DISCUSSION

These results are not in keeping with the present concepts of the mechanisms for the reciprocal control of the ovary and pituitary. However, a search of the literature reveals that they do not stand alone. Everett and co-workers (3, 4, 5) have stimulated ovulation by appropriately timed injections of progesterone in

TABLE 2

The effect of progesterone given before and after the beginning of estrus on ovulation time in dairy heifers

Heifer no.	Control period			Progesterone period				
	Length of estrus	Time from end of estrus to ovulation	Time from beginning of estrus to ovulation	Progesterone injected	Time of injection from beginning of estrus	Length of estrus	Time from end of estrus to ovulation	Time from beginning of estrus to ovulation
	(*hr.*)	(*hr.*)	(*hr.*)	(*mg.*)		(*hr.*)	(*hr.*)	(*hr.*)
72	22.0	12.0	34.0	10[a]	5 hr. before	20.0	6.0	26.0
80	22.0	12.0	34.0	5[a]	4 hr "	13.0	10.0	23.0
75	23.0	8.5	31.5	10[b]	5 hr. after	20.0	13.0	33.0
	19.0	10.0	29.0					
73	16.0	17.0	33.0	15[b]	6.5 hr. "	17.5	14.0	31.5

[a] Subcutaneously in cottonseed oil.
[b] Subcutaneously in propylene glycol.

the rat. Neher and Fraps (9) have increased the number of eggs in the hen's clutch by progesterone injections. Progesterone given at the proper time also appears to stimulate ovulation in the monkey during anovulatory summer cycles (10). Recently, Sawyer *et al.* (11) have found that progesterone injections following estrogen treatment cause spontaneous ovulation in the rabbit without the stimulus of coitus. Neither estrogen nor progesterone alone causes this effect. Pregnanediol excretion in women appears to rise before ovulation occurs. The follicular fluid of sow's ovaries has been found to contain some progesterone (8). Thus, it may be inferred that some progesterone is produced before a corpus luteum is formed, although the possibility that this follicular progesterone was actually produced by adjacent corpora lutea cannot be ignored.

Ulberg *et al.* (12) recently have reported that daily doses of 50 mg. of progesterone inhibit estrus and ovulation in dairy heifers if the injections are started before the beginning of estrus. Apparently, the response of the ovary to exogenous progesterone is conditioned by the stage of the estrous cycle and the

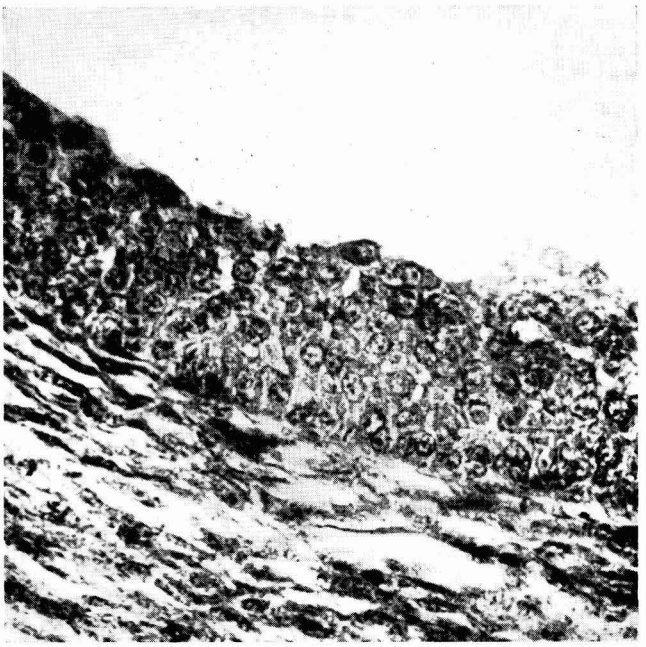

FIG. 1. Granulosa cell layer in the ovary of a grade Holstein heifer no. 37 at 19 days postestrum. × 450.

FIG. 2. Granulosa cell layer in the ovary of a grade Holstein heifer no. 48 during estrus and prior to ovulation. The nuclei are densely chromatic and the cytoplasm in many cells appears vacuolated. × 450.

size of the dose administered. Increased progesterone secretion prior to ovulation may occur normally in the cow and this may be an important factor in the release of luteinizing hormone and ovulation in the normal animal. Marked changes normally occur in the granulosa cell layer prior to the rupture of the follicle. Figures 1 and 2 show the granulosa cells in the ovaries of two heifers at 19 days postestrus and during estrus. The nuclei become densely chromatic and many cells appear vacuolated, giving the granulosa a "foamy" appearance just prior to ovulation. Corner (2) described similar changes in sow's ovaries some years ago.

Whether or not progesterone operates through the nervous mechanism for the release of luteinizing hormone is not yet known.

The possibilities of certain practical applications of these results may be mentioned. The most obvious of these is in connection with the nymphomaniac cow. All investigators are agreed that the granulosa cell layer is absent or very poorly developed in most nymphomaniac cows and the question arises whether some of these cows fail to ovulate and develop cystic ovaries because of the presence of too little progesterone at the normal time of ovulation. Experimental treatment of cows with cystic ovaries with small doses of progesterone shows some promise. Further study may reveal practical uses of this information in other types of infertility as well. Properly timed injections of small doses of progesterone may prove useful in attempts to induce ovulation and estrus in anestrus ewes.

The decreased length of the estrous period, especially in those cases where the large doses of progesterone were administered, is in keeping with the results of Cole *et al.* (1) and Fraps *et al.* (6) in sheep and goats. Sheep, goats and cattle appear to differ from rodents in this respect, since there is ample evidence that progesterone plays an important role in determining sexual receptivity in the latter species.

SUMMARY

The subcutaneous injection of small doses (5 to 10 mg.) of progesterone at the beginning of estrus hastens the ovulatory process in dairy heifers. Both the length of estrus and the time from end of estrus to ovulation are significantly reduced. These results and histological changes occurring in the ovary at estrus suggest that progesterone produced by the ovary before ovulation normally plays a role in luteinizing hormone release and ovulation in the cow. These facts may be significant in an understanding of the deranged physiology of the nymphomaniac cow and in other types of infertility in farm animals.

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EFFECT OF THE ADMINISTRATION OF UNFRACTIONATED GONADOTROPHIC PITUITARY EXTRACTS DURING ESTRUS ON TIME OF OVULATION IN THE BOVINE[1]

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The interaction of pituitary and ovarian hormones on the processes regulating estrus and ovulation has been extensively studied in laboratory animals. The hormonal interrelations of these processes are not too well understood in the bovine. Furthermore, the relationship of estrus to ovulation in the bovine differs markedly from that of most species in that ovulation does not generally occur until after the end of estrus.

The range in the time between the end of estrus and ovulation in the bovine is 10 to 15 hr. (1, 3, 15, 17). The interval from estrus to ovulation for heifers is somewhat shorter (3, 11, 17).

The follicle-stimulating hormone (FSH) content of the anterior pituitary of cattle is the least of any species studied (18, 19). Hammond (8) postulates that the relatively low FSH in comparison to the levels of luteinizing hormone (LH) is responsible for the short estrus and "silent" estrus in the bovine. Asdell (1) is of the opinion that the nervous system of the cow is refractive to low levels of estrogen. Thus, ovulation occurs after estrus in the cow because the cow becomes refractive in the early stages of follicular development and estrus is terminated.

Release of gonadotrophic hormones from the pituitary is not only dependent upon ovarian hormones, but apparently is intimately associated with the nervous system. Rabbits will copulate but will not ovulate after surgical section of the infundibular stalk (4). Sawyer *et al.* (16) found that the injection of estrogen into rats 4 days pregnant was followed by ovulation and cholesterol storage in the corpus luteum. Dibenamine and atropine prevented the action of estrogen by blocking nervous passages to the brain. However, the action of the luteinizing hormone was not blocked by atropine or dibenamine. Everett *et al.* (7) injected dibenamine into rats during proestrus and were able to delay or prevent ovulation. Atropine uniformly prevented preovulatory swelling and ovulation. Hansel and Trimberger (9) delayed ovulation 20 to 66 hr. in the cow by administration of atropine at the beginning of the estrous period. The average interval between the end of heat and ovulation in dairy heifers was found to be shortened by the copulatory stimulus (11).

The literature concerning the activities of gonadotrophic hormones when administered to females of various species is quite voluminous. However, it is

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virtually impossible to compare results of the many reports, since they are easily altered by such factors as the origin of the gonadotrophic substances administered, age of the experimental animal, route of administration, stage of the estrous cycle at the time of administration, the formation of pro- and anti-gonadotrophic substances, as well as the individuality of the animal.

Gonadotrophic substances have been used with varying degrees of success, as a treatment for most of the infertile conditions known to exist in livestock (2). Mirskaja and Petropovlovskii (13), Mirskaja *et al.* (14), Day (6), McKenzie (12) and Davidson (5) found that treating mares which were in heat with a pregnancy urine extract or pregnancy mare serum usually would induce ovulation within 48 hr. after the injection, if a mature follicle was present in the ovary at the time of injection. In the bovine, except when dealing with nymphomania, the gonadotrophic hormones have previously been administered during some phase of the sexual cycle other than estrus.

This study was undertaken to determine the effect of administration of an unfractionated gonadotrophic pituitary extract during estrus on the time of ovulation in dairy heifers.

EXPERIMENTAL METHODS

Fourteen heifers, consisting of three groups from the University herd, representing three dairy breeds, were used for this study. Group A included five heifers that varied in age from 15 to 18 mo. Group A was observed through four normal estrous cycles. Heifers in group A were confined to pasture lots during the experimental period (July 12, to Oct. 28, 1950). Group B, consisting of four heifers ranging in age from 11 to 15 mo., was observed through two normal sexual cycles. This group was confined to a barnyard during the experimental period (Sept. 1, to Oct. 10, 1950). Group C included five Holstein heifers that ranged in age from 12 to 15 mo. Four of the heifers were observed through six normal sexual cycles, and a fifth heifer was observed through four normal cycles. The sixth consecutive sexual cycle of this heifer was abnormal, and since time did not permit the continuation of the study, the corresponding control cycle also was omitted. These heifers were confined to a pen barn throughout the experimental period (Nov. 30, 1950, to Apr. 2, 1951). The estrual periods were alternately designated as control and experimental. Heifers in groups A and B were injected intramuscularly with 20 rat units, and those of group C with 10 rat units, of an unfractionated gonadotrophic pituitary extract.[3] These injections were made as early as possible after an estrus check when the heifers were noted in heat during an experimental period.

The animals were observed for the onset of estrus twice daily at 6 a.m. and 6 p.m. Willingness of the heifer to stand while being mounted by a bull or another heifer was the only criterion accepted as indicating estrus. As soon as a heifer was noted in estrus, she was confined to the barn. Beginning of estrus was dated back 6 hr., midpoint between checks, from the check when the heifer was first noticed in estrus.

[3] Vetrophin, a commercial product prepared by Abbott Laboratories, was the extract used in these studies.

TABLE 1

Effects of injections of unfractionated gonadotrophic pituitary extracts during estrus in the bovine

Animal no.	Control		Experimental			Control		Experimental			Control		Experimental		
	End of estrus to ovulation	Length of estrus	End of estrus to ovulation	Length of estrus	Injection to ovulation	End of estrus to ovulation	Length of estrus	End of estrus to ovulation	Length of estrus	Injection to ovulation	End of estrus to ovulation	Length of estrus	End of estrus to ovulation	Length of estrus	Injection to ovulation
	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)
							Group A—20 Rat units								
42	10.25	36.75	−1.50	29.25	21.75	13.00	28.75	− 6.50	43.50	19.00					
15	12.00	29.00	−3.50	37.00	27.00	8.75	26 50	0.00	27.50	21.75					
28	10.50	22 00	−2.00	29.50	21.50	6.50	32.00	6.00	19.50	18.75					
31	7.00	25.50	2.00	16.50	12.00	19.75	16.75	7.50	18.50	16.00					
39	13.00	21.25	7.50	21.50	22.00	5.50	26.50	2.50	20.25	16.50					
Mean	10.55	26.90	0.50	26.75	20.85	10.70	26.10	1.90	25.85	23.00					
							Group B—20 Rat units								
13			4.25	13.75	10.50	14.75	25 50								
32			−4.50	28.50	17.50	8.75	16.75								
38			−0.25	28.50	22.75	15.00	20.00								
33			6.50	18.75	19.75	4.50	21.00								
Mean			1.63	22.38	17.63	10.75	20.81								
							Group C—10 Rat units								
38	16.00	20.25	−5.00	18.75	15.75	5.25	26.75	1.00	22.25	19.25	7.00	25.00	−1.75	20.00	12.00
25	7.75	15.00	4.25	15.00	21.00	5.00	25.00	6.25	19.00	17.75	22.00	25.00	−2.25	22.00	13.00
232	15.50	21.00	−0.75	25.00	15.75	19.25	24.50	7.25	22.50	22.00	14.75	33.00	−4.50	29.25	18.25
13	13.50	19.50	3 25	22.00	17.75	19.00	20 00	2.00	9.00	10.00	13.00	13.00	7.00	24.00	24.75
141	9.25	20.75	6.75	7.25	16.75	12.25	20.00	−14.00	37.00	16.50					
Mean	12.40	19.30	1.70	17.60	17.40	12.15	23.45	0 50	21.95	17.11	14.19	24.00	0.38	23.81	17.00

	End of estrus to ovulation	Length of estrus
Over-all experimental mean	1.01 hr.	23.06 hr.
Over-all control mean	11.74 hr.	23.50 hr.

The time of ovulation was determined by the rectal palpation method. The heifers were examined per rectum shortly after being noticed in heat. The size, position and tone of any follicle in either ovary were determined and recorded. If the follicle was turgid, the next examination was made after the animal went out of estrus, from which time palpations were made at 2-hr. intervals until ovulation was detected. If the follicle was soft, rectal palpations at 2-hr. intervals were begun immediately. Time of ovulation was established as the midpoint between the last examination when the follicle was intact and the subsequent examination 2 hr. later when the follicle had collapsed.

The end of estrus was determined by checking the heifers every 2 hr. with other females, with a bull or with both. The heifers were aproned to prevent copulation when a bull was used. End of estrus was dated from the midpoint between the last check when the heifer stood quietly for mounting and the subsequent check when mounting was not permitted.

RESULTS

The results of the experiment are presented in table 1. The average time interval from the end of heat to ovulation during all experimental periods for groups A, B and C was 1.01 hr. compared with 11.74 hr. for all control periods. The individual ovulation intervals varied greatly from one cycle to the next for all groups of heifers. These heifers were not consistent with respect to time of ovulation from end of heat during control periods, as judged from the limited number of observations in this study. The interval between hormone injection and rupture of the follicle varied from 10.00 to 27.0 hr., with no apparent consistency in the length of time required for the hormone to show an effect.

As shown in table 1, average length of estrus of all three groups of heifers was 23.06 hr. during experimental periods and 23.50 hr. during control periods. These data indicate the treatment had no particular effect on the length of time that a heifer remained in heat.

During the course of the experiment, one of the heifers was unintentionally serviced by the bull used for checking heat and therefore was excluded from the analysis. The heifer was 14 mo. of age and it was hoped that she would not settle to the service. It was decided that conception might be prevented if the follicle was ruptured prematurely. The development of the follicle was followed by periodic rectal palpations until it was found to protrude from the surface of the ovary. At this time the follicle was turgid and was approximately 15 mm. in diameter. Judging from the usual length of time required for a follicle to reach a flabby condition when ovulation is imminent, it was estimated that ovulation would not occur for at least 6 hr. The follicle then was clasped between the thumb and index finger and sufficient pressure exerted to rupture the follicle. The heifer conceived to this service and gave birth to a living calf. Krüsa (10) reported conception in cows after manual rupture of a ripe follicle.

DISCUSSION

These data show that the follicle can be caused to rupture earlier than occurs naturally if the proper stimulus is applied. Previous work (11) showed that

sterile copulation hastened ovulation. The stimulus of copulation may contribute to earlier ovulation by inducing a release of factors essential to ovulation. Whether copulation hastens ovulation by causing a release of pituitary gonadotrophins remains to be proved.

Since the heifers were checked for estrus only twice daily, some of them may have been in estrus as much as 11 hr. before an estrous check. The effect of the hormonal treatment depended upon the degree of follicular development at the time of treatment. Heifers that had only a small amount of follicular development in the ovary at time of treatment ovulated sooner with the respect to end of estrus than heifers in which the follicles were well developed at time of treatment.

The hormonal treatment did not produce superovulation. No more than one follicle was ever palpated during mid-cycle examinations following experimental periods.

Considerable variation in the average length of estrus was noted among the three groups of heifers. The heifers in groups B and C were younger, on the average, than heifers of group A. Whether age or season affect length of estrus is not known.

SUMMARY

The effect of the administration of an unfractionated gonadotrophic pituitary extract during estrus on time of ovulation of dairy heifers was observed. The mean time from the end of estrus to ovulation for periods when the heifers were treated was 1.01 hr. and for the untreated periods 11.74 hr. Length of estrus was not changed by the treatment as measured under conditions of this experiment.

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SOME PROPERTIES OF FREEZE-DRIED MILK[1]

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Spray-dried whole milk powder has some limitations for beverage purposes (1). The powder is not readily miscible with water and many reconstituted samples leave greasy films on the containers. Furthermore, normal whole milk powder, even when fresh, has a typical cooked and somewhat astringent flavor. During storage there is a rather rapid initial decrease in flavor quality, even under the best conditions.

During World War II, the preparation of dry blood plasma illustrated the applicability of freeze-drying techniques for the preservation of heat-labile materials. Drying from the frozen state takes place at low temperatures and low oxygen levels so that little chemical change would be expected during drying. In addition, freeze-dried products generally are readily soluble in water (9).

No published data are available on the physical characteristics and keeping qualities of freeze-dried milk. This study was undertaken to determine whether a superior product could be produced by freeze-drying techniques.

FREEZE-DRYING EQUIPMENT

The drier used to prepare all of the freeze-dried powders consisted essentially of a box of $\frac{3}{16}$-in. sheet iron, in one end of which was installed a refrigeration coil ($12 \times 18 \times 8$ in.) operating on freon. The other end of the box consisted of a chamber ($9 \times 12 \times 10$ in.) into which a rack of five shelves (8.25×11.5 in.) could be placed. The front of the box could be closed by a heavy metal cover which was clamped to flanges. The entire box could be evacuated by means of a Cenco Megavac pump to a pressure under 1 mm. of mercury. The vacuum outlet was so placed that air and water vapor had to pass through the refrigeration coil, thus freezing out the water and consequently maintaining a low pressure of water vapor in the system.

Sublimation of the water was hastened by supplying heat during the process by means of nichrome heating coils strung above each shelf of the rack. The amount of current flowing through this circuit could be controlled by a rheostat (Variac) outside of the chamber.

EXPERIMENTAL

Preliminary. Lots of freeze-dried milk were prepared from frozen pasteurized fluid milk and from frozen condensed milk of various concentrations.

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[2] The data in this paper are taken from a thesis presented by T. A. Nickerson in partial fulfillment of the requirements for the Ph.D. degree, University of Minnesota, 1950.

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The frozen milk was pulverized in a Dazey Triple Ice Crusher before being placed in the drier.

The size and shape of the frozen milk particles do not change during freeze-drying and, as a result the density of the powder varies directly with the solids content of the milk being dried. The product obtained by freeze-drying whole milk is soft and fluffy, while that obtained from condensed milk (40 per cent total solids) is hard and brittle. Microscopic examination showed that freeze-dried milk consists of irregularly shaped porous particles in which the fat globules are dispersed throughout the mass.

The color of both spray- and freeze-dried whole milk powder is the same, namely a light cream. In the case of skimmilk, however, the spray-dried product is a chalky white, while the freeze-dried product is a pale yellow.

State of fat. It was observed early in the investigation that reconstituted freeze-dried milk always produced an oily film on glassware. Evidently the fat emulsion was partially destabilized during processing. Trials were instituted to determine the effect of various processing methods on stability of the fat emulsion. Freeze-dried powders were prepared using various processing techniques and the amount of free fat determined by extracting the powders with petroleum ether for 2 hr. in a Soxhlet extractor.

Data presented in table 1 show the effect of the various methods used in processing on the amount of free fat extracted from the powder. The only procedure that greatly reduced destabilization of the fat emulsion was homogenization of the milk before freezing. Freezing the milk at a very slow rate (−25° F. in still air) appeared to decrease slightly the amount of free fat. Such factors as total solids content and temperature at which the milk was frozen had no apparent effect on the emulsion. Other processing methods, such as spray freezing or passing the frozen milk through a milling machine to increase the surface for faster drying, increased the amount of fat extracted from the powder.

Standard method of manufacture. As a result of this investigation, the following procedure was adopted as standard to prepare the freeze-dried powders: Milk from the University of Minnesota herd was pasteurized, condensed to approximately 40 per cent total solids and homogenized at 2,500 and 500 p.s.i. The condensed milk was frozen rapidly in ice-cube trays set in the air stream of a large circulating fan at $-18 \pm 2°$ F. The milk froze solid in approximately 30 min. and there was no visible evidence of diffusion of milk solids during freezing. The frozen cubes were ground in a Dazey Triple Ice Crusher to increase the drying surface before placing them in the freeze-drier. Sufficient heat was supplied by means of the electric heating element to dry the milk within 24 hr. when the chamber was maintained at less than 1 mm. mercury absolute pressure.

This standard procedure was the most satisfactory found in reducing the amount of free fat, but even it did not produce as stable a produce as spray drying. As shown in table 2, 35 to 75 per cent of the total fat in the freeze-dried milk was extracted with petroleum ether in 2 hr., while only 12 to 19 per cent was extracted from spray-dried powder under the same conditions.

State of lactose. It is generally accepted that the lactose in spray- and roller-

TABLE 1

The effect of various processing methods on the extractability of fat from freeze-dried whole milk powder

Treatment	Solids content of sample frozen[b]	Freezing time	Per cent of total fat extracted[a]			
			Lot A	Lot B	Lot C	Lot D
	(%)	(*min.*)				
I. Effect of method of freezing:						
Frozen on trays:						
In contact with dry ice	20	ca 5	72.2[c]	73.6	.....	.....
In air stream @ −25° F.	20	ca 30	71.0	71.2	.....	.....
As above but milled to pass 0.062 mesh screen	20	ca 30	85.2	74.2	.....	.....
In still air @ −25° F.	20	180–480	72.3	57.8	.....	.....
On refrigerator coil @ 20° F.	20	45	.....	.....	70.9	68.0
In air stream @ −4°F.	20	30	.....	.....	75.2	75.2
In air stream @ −35° F.	20	30	.....	.....	73.3	.....
In still air @ −4° F.	20	360	.....	.....	72.0	.....
In still air @ −35° F.	20	360	.....	.....	66.8	.....
Sprayed on cold plates @ −20° F.	20	Instantaneous	.....	.....	.....	83.1
Sprayed in room @ −20° F.	20	"	.....	.....	.....	92.3
II. Effect of solids content:						
Frozen in trays in air stream @ −25° F.	10	30	73.3	69.0	.....	.....
	20	30	74.5	70.6	.....	.....
	30	30	75.3	71.2	.....	.....
III. Effect of homogenization:						
Condensed milk (40%) homogenized @ 2000 and 500 p.s.i. diluted to 20% and frozen on trays in air stream @ −25° F.	20	30	48.3	35.5	.....	.....

[a] Extracted for 2 hr. with petroleum ether in Soxhlet apparatus.
[b] Diluted in every case from 40% solids condensed milk.
[c] Each figure represents average of duplicate determinations.

TABLE 2

he extractability of fat from spray- and freeze-dried whole milk powders by petroleum ether

Lot	Temperature of preheating	Per cent of total fat extracted[a]	
		Spray-dried	Freeze-dried
	(° *F.*)		
III	143	12.3	43.0
	160	11.6	51.4
	180	14.9	51.4
IV	143	13.3	57.7
	160	17.6	72.0
	180	18.0	61.9
		Freeze-dried, reconstituted and spray-dried	Spray-dried, reconstituted and freeze-dried
V	143	18.6	64.5
	160	18.6	75.5
	180	17.5	60.2

[a] Figures are averages of duplicate determinations.

dried powders exists as a concentrated syrup or glass and that α and β lactose are present in the equilibrium ratio (3, 13, 19, 20, 21). It seemed possible that some crystallization of the lactose could have taken place in the freeze-dried powder, because the drying process was relatively slow (24 hr.) and the granular appearance of the powder suggested that crystals might be present. Alpha and β lactose were determined quantitatively in a number of lots of powder by the polarimetric method of Sharp and Doob (20).

The data presented in table 3 are good evidence that normally no crystallization of lactose takes place in either type of powder. Crystallization takes place in both powders when the moisture content is high. Crystallization also occurs when a portion of the frozen milk melts during drying. The data show further that the ratio of β to α lactose in the powder is dependent upon the temperature of drying. In the spray-dried powders 41.75 to 43.47 per cent of the total lactose

TABLE 3

Alpha and beta lactose contents of spray- and freeze-dried milk

Sample Lot III	Temp. of preheating	Total anhydrous lactose	Fraction of total lactose present as		Ratio of Beta/Alpha
			Alpha	Beta	
	(° F.)	(%)	(%)	(%)	
(1) Spray	143	34.3	42.87	57.12	1.33
		34.6	43.47	56.51	1.30
(2) Spray	160	34.8	41.75	58.24	1.39
		35.0	43.17	56.82	1.32
(3) Spray	180	34.5	42.16	57.83	1.37
		34.8	42.26	57.73	1.37
(4) F.D.	143	34.7	37.41	62.58	1.67
		34.5	37.91	62.08	1.63
(5) F.D.	160	34.8	37.71	62.28	1.65
		.....	37.91	62.08	1.63
(6) F.D.	180	34.8	37.20	62.78	1.69
		34.8	38.32	61.67	1.61
(7) F.D. (7.8% H_2O)	143	34.8	49.54	50.45	1.02
		34.9	48.53	51.46	1.06
(8) F.D. Skim (melted)	143	51.8	45.19	54.89	1.21
		50.7	46.20	53.79	1.16

was present in the alpha form, whereas in the freeze-dried powders 37.20 to 38.32 per cent of the total lactose was present as α lactose. Therefore, the ratio of β to α in the spray-dried powders varied between 1.30 and 1.37 and closely approached the 1.33 quoted in the literature as the equilibrium ratio in a lactose solution at 212° F. (10). The ratio in the freeze-dried powders varied between 1.61 and 1.69 and approached the equilibrium ratio at 32° F., which has been reported to be 1.65 (10). Troy and Sharp (21) suggested in 1930 that "theoretically, at least, an indication of the temperature at which the powders are dried can be gained from the ratio of β to α lactose in the non-crystalline dried material." It is interesting to note that the data obtained in this study confirm their statement.

Ease of reconstitution. The ease with which a milk powder may be reconstituted depends in part on the rate the powder is wetted by water and in part on the rate and extent to which the individual particles will disperse after wetting.

Wettability of the powders was determined by the time required for the particles to sink below the surface of water on which they were dusted. In general, the freeze-dried whole milk powders wetted more slowly than the spray-dried powders made from the same milk. Wettability and dispersibility characteristics of freeze-dried skimmilk were excellent. The presence of free fat in the freeze-dried whole milk powder decreased the rate of wetting and, as a result, the powder did not reconstitute as easily and readily in cold water as did the spray-dried powder. Freeze-dried powder disperses more readily in warm than in cold water. Dispersibility of the powders was determined by noting the time required for particles to lose their identity when immersed in water at room temperature. Fine particles of freeze-dried powder characteristically dispersed as they wetted, but spray-dried powder rarely exhibited such rapid disintegration.

Flavor and keeping quality of freeze-dried and spray-dried milks. A total of four series of powders was prepared for keeping quality tests. In the first two series the milk was preheated at 160 and 180° F. for 30 min. An additional preheating temperature of 143° F. for 30 min. was included in the last two series. The condensed milk in each case was split into two lots, one of which was spray-dried in the drier described by Coulter (4) and the other freeze-dried.

The dry milk powders were packed in cans (200 × 210) at the rate of 35 g. per can. The cans were hermetically sealed; half were air-packed and half nitrogen-packed by means of the equipment described by Coulter and Jenness (5). All cans were stored in an insulated oven thermostatically controlled at 100 ± 4° F.

At intervals during storage, the milk powders were reconstituted and judged for flavor by at least two experienced milk judges. Initially distilled water at room temperature was used for reconstitution, but at this temperature excessive churning occurred in the freeze-dried milks. For this reason the powders were reconstituted at 110 to 120° F. and passed through a hand homogenizer to disperse the fat. When the samples were prepared in this manner, it was not possible to distinguish physically between reconstituted spray-dried and freeze-dried milks.

Thiamine disulfide- and acid ferricyanide-reducing capacities and fat peroxide determinations were used to measure quantitatively some of the changes in these powders during storage. Acid ferricyanide-reducing substances were estimated by the Chapman and McFarlane procedure (2) as modified by Crowe *et al.* (6), thiamine disulfide-reducing substances by the method of Harland and Ashworth (11) and fat peroxides by the method of Hills and Thiel (14).

It was not possible to produce a freeze-dried powder that had a better initial flavor than spray-dried powder made from the same milk. The freeze-dried powders usually had a slightly more pronounced heated flavor than their spray-dried counterparts, but only powders produced from milk heated at 180° F. were criticized regularly as being "cooked." Both types of powder were criticized as having an astringent flavor. On one occasion, the condensed milk, used in preparing the powders, was diluted to the solids content of fluid milk and scored along with the reconstituted powders. It was not possible to distinguish

among spray-dried, freeze-dried and condensed milks when they were reconstituted to the same total solids content. The astringent flavor was detected in all of the samples. It is apparent, therefore, that drying *per se* was not responsible for the production of this flavor. There was no correlation between the incidence of astringent flavor and the temperature at which the fluid milk was preheated.

The flavor score data obtained from one series of powders stored at 100° F. are presented in table 4 and are typical of the results obtained. These powders were subject to the same types of flavor deterioration and were criticized for the same flavor defects, such as tallowy, stale, "burnt feathers," gluey, astringent and cooked. The data show that except for oxidation of the fat, the flavor of these powders deteriorated at about the same rate.

TABLE 4

Flavor score of spray- and freeze-dried whole milk powders

Sample Lot III	Temp. of preheating	Flavor score after storage at 100° F.					
		Initial	In air for:				In nitrogen for:
			2 wk.	4 wk.	7 wk.	9 wk.	12 wk.
	(° F.)						
Spray	143	39	33	32	N.S.[a]	N.S.	28
"	160	39	32	32	30	N.S.	28
"	180	39	34	36	31	28	30
Freeze-dried	143	39	34	32	28	N.S.	N.S.
"	160	39	34.5	36	31.5	29	30
"	180	39	30	36	34	32	31

[a] N.S. = No Score.

The mechanism of oxidation appears to be the same in these powders, but the rates of oxidation are different. The freeze-dried powders neither became tallowy nor developed peroxides as rapidly as did the spray-dried powders. Both types of powder became tallowy and were influenced similarly by the oxygen level at which they were stored and by the temperature at which the fluid milk was preheated. Increasing preheating temperatures increased the keeping quality of the powders with respect to tallowy flavor, which is in agreement with the findings of others (7, 8, 15, 16, 17, 18). The peroxide values presented in table 5 are typical and show that, the fat of spray-dried powders had higher initial peroxide values.

Several factors apparently are responsible for the slower rate at which tallowiness developed in the freeze-dried powders. The higher initial peroxide values of the spray-dried milk indicate that the induction period of the fat was reduced by oxidation during spray-drying, and, therefore, subsequent oxidative changes during storage would be accelerated. The greater capacity of freeze-dried powder (160 and 180° F.—30 min.) to reduce thiamine disulfide also may be a factor, since Harland *et al.* (12) have reported that thiamine disulfide-reducing substances appear to be associated with increased resistance to oxidation

when present in dry whole milk. The data presented in table 6 show the initial capacities of the dry milks to reduce thiamine disulfide. No significant change was observed in these reducing substances during storage.

Off-flavors associated with the Maillard reaction were found in both types of powder during storage. This type of deterioration manifests itself in high moisture powders as "gluey," "burnt feathers" flavors and results in the development of large quantities of acid ferricyanide-reducing substances. Data not

TABLE 5

Effect of preheating, method of drying and oxygen level on peroxide values of dry whole milk during storage at 100° F.

Sample Lot III	Temp. of preheating	Peroxide values as O_2 per kg. powder after storage at 100° F.					
		Fresh	In air for:				In nitrogen for:
			2 wk.	4 wk.	7 wk.	9 wk.	12 wk.
	(° *F.*)	(*m.eq.*)	(*m.eq.*)	(*m.eq.*)	(*m.eq.*)	(*m.eq.*)	(*m.eq.*)
Spray	143	0.04	0.11	0.18	0.43	0.38	0.06
Spray	160	0.09	0.09	0.26	0.37	0.29	0.02
Spray	180	0.15	0.06	0.21	0.10	0.14	0.06
F. D.	143	0.10	0.09	0.16	0.35	0.43	0.02
F. D.	160	0.08	0.03	0.08	0.08	0.09	0.02
F. D.	180	0.06	0.04	0.04	0.09	0.05	0.03

presented in detail show that the acid ferricyanide-reducing capacity increased in both types of powder at rates dependent upon the moisture content.

Although deterioration of these two types of powder was similar in most respects, development of a fruity flavor during storage was characteristic of a number of freeze-dried samples, but was not noted in the spray-dried samples. Fruitiness usually was evident only after several months of storage, but one lot

TABLE 6

The effect of preheating and method of drying on thiamine disulfide-reducing substances in dry whole milk

Lot III Temp. of preheating	Thiamine disulfide-reducing substances as cysteine-HCl per g. powder	
	Spray-dried	Freeze-dried
(° *F.*)	(*mg.*)	(*mg.*)
143	0	0
160	0	4.2
180	11.0	12.7

became fruity within 3 wk. This defect was not related to the preheating temperature used in processing.

SUMMARY

The effect of freeze-drying milk on the physical state of certain milk constituents and on the keeping qualities of the resulting powder has been studied. The fat emulsion of freeze-dried powder is partially destabilized. The presence of free fat in the powder makes reconstitution difficult.

No crystallization of the lactose is evident in the spray- and freeze-dried powders. The data show that the ratio of β to α lactose in these powders is dependent upon the temperature of drying. The ratio in spray-dried powder closely approaches the equilibrium ratio in a lactose solution at 212° F., while the ratio in freeze-dried powder closely approaches that at 32° F.

Flavor and keeping quality characteristics of freeze-dried milk are essentially the same as for spray-dried whole milk powder. Both dry milks have essentially the same flavor characteristics when fresh and both become tallowy in storage. They also may exhibit deterioration of the type associated with the Maillard reaction. The freeze-dried powder, however, frequently acquires a fruity flavor which is not apparent in spray-dried milk.

Freeze-drying by the methods used in this study does not produce a more satisfactory product than spray-dried milk.

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ASSOCIATION ANNOUNCEMENT

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The University of California is honored, after twenty-one years, to welcome you again to its Davis campus. We are looking forward to June 24–26, with much pleasure and are happy that you have chosen to hold your 1952 meeting in California, one of the leading states in dairying as in many other fields of agriculture.

The Annual Meeting of the American Dairy Science Association provides an opportunity for exchange of the latest scientific information in the many fields of dairying. This is the time when new discoveries are reported, when theories are debated, and when scientific bases are laid for the advancement of the dairy industry. It is our hope that your visit to the Pacific Coast will be productive, stimulating, and pleasant.

We hope, too, that you will have opportunity to visit other campuses of the far-flung University of California, for all which it does for the advancement of dairy science is by no means confined to the Davis campus. And we would be amiss if we did not attempt to persuade you to save some time in your busy summer schedule, now that many of you have travelled so far, to enjoy the unsurpassed beauties of California's mountains, forests, lakes, and seashore—and above all the redwoods, the oldest living organisms of the world.

We extend to you a welcome—physically as well as emotionally, warm.

Very sincerely yours,

C. B. HUTCHISON,
Dean of the College of Agriculture

PAPERS FOR THE 1952 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The 47th annual meeting of the American Dairy Science Association will be held June 24 to 26, 1952, as guests of the University of California on the Davis campus. All members planning to present papers should submit the titles of their papers accompanied by two copies of an abstract of not more than 200 words not later than March 15 to the Chairman of the Program Committee of their Section. Terminology used in abstracts should be understandable by the general reader as well as the specialist, according to a resolution adopted in 1951. Titles and abstracts must be received promptly since abstracts of the final program must be made available in printed form for the annual meeting. The respective committee chairmen are as follows:

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The general program committee believes that no person should present more than two papers, thereby making it possible for active participation by more members. No title will be considered that is unaccompanied by a suitable abstract. It is desired that more contributions be submitted by senior staff members and from the laboratories of industry.

Because of problems in slide projection, it is desirable for speakers to distribute mimeographed copies of pertinent data, together with a brief summary of the paper.

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

MILK PRODUCTION

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JOURNAL OF DAIRY SCIENCE

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Prepared in cooperation with the
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and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

1. A comparison of the Brucella ring test and the blood serum agglutination test in 955 cows. H. S. BRYAN, G. T. WOODS and M. E. MANSFIELD, Univ. of Ill., Urbana. N. Am. Vet., **32,** 9: 618–620. Sept., 1951.

Results compare simultaneous ring and blood tests of 955 lactating cows in 14 different Holstein herds. Calfhood vaccination with B.A.I. strain 19 was practiced in each herd and all cows used in the study except a few of the older animals had been calfhood vaccinated. Approximately 85% were negative to the ring test and 75% were negative to the blood test. The over-all agreement between the ring and blood tests was 78.7%, indicating considerable discrepancy. Results of a comparison of the antigen used for the ring test with an experimental ring test antigen furnished by the U. S. Bureau of Animal Industry also are given. R. P. Niedermeier

2. A clinical pathological study of experimental leptospirosis of calves. K. R. REINHARD, U.S.P.H.S., Hamilton, Mont. Am. J. Vet. Research, **12,** 45: 282–291. Oct., 1951.

Six calves were inoculated with 2 strains of leptospira which had been isolated from field cases. Rectal temperature and blood and urine samples were taken daily. Fever appeared beginning the 4th–9th d. and lasted 2–4 d. Leptospira were found in the blood during the febrile period. In 2 cases leptospiruria appeared following the leptospiremia and persisted until autopsy, when leptospira were found in the kidneys. A transitory anemia appeared with the fever and some cases showed hemoglobinuria. At this time erythrocyte fragility was high and lymphocytes and neutrophiles were low. The blood changes are particularly significant in diagnosis since gross physical signs may be indicative of a number of diseases. In all cases the kidneys showed marked damage. Small white foci on the surface of the kidneys was the most significant gross lesion.
E. W. Swanson

3. A comparative study of the intradermal johnin test on cattle artificially and naturally sensitized with Mycobacterium paratuberculosis. D. SIKES, H. W. JOHNSON and W. T. OGLESBY, La. State Univ., Baton Rouge. Am. J. Vet. Research, **12,** 45: 302–305. Oct., 1951.

Cattle artificially sensitized with *M. paratuberculosis* were more sensitive than naturally sensitized ones in all respects. When injections of johnin were repeated at the same site at 1-, 2-, 3- and 4-wk. intervals, the respective percentage positive response was 56, 75, 82 and 88. Normal reaction occurred when previously unused sites were injected. A lapse of time exceeding 4 wk. is required for repeatable results if the same injection site is used. E. W. Swanson

4. Attempts to produce bovine hyperkeratosis. C. OLSON and R. H. COOK, Nebr. Agr. Expt. Sta., Lincoln. Am. J. Vet. Research, **12,** 45: 261–272. Oct., 1951.

This report is of an extended investigation with pelleted feeds previously proved to produce susceptibility to hyperkeratosis (X disease). A new group of susceptible calves was mixed with calves already affected by hyperkeratosis and 1 pen of new calves was maintained separately. Half of the calves were fed prairie hay and soybean meal and half were fed prairie hay plus the pelleted feed supplement for 74 d. In addition, the suspected pellets were fed to another group of calves at a distant experiment station. Of the calves receiving no pellets, none developed hyperkeratosis even though they were with calves recovering from previous illness. Hyperkeratosis appeared in some of the new calves in all lots receiving the suspected pellets. Papillomas of the mouth appeared at 40–80 d., dermatosis at 60 d. and definite hyperkeratosis at 133–164 d. Some of the calves only mildly affected, apparently recovered. Following resumption of the pellet supplement feeding to 5 calves, 100% mortality occurred. Detailed necropsy findings are reported. Significant items were anemia, edematous gall bladder and heart changes. The development of the disease was definitely attributed to the feed, but required a longer feeding period with 15-mo.-old than with younger calves.
E. W. Swanson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

5. Alfa-Smörets Struktur (The structure of Alfa butter). N. KING, Swedish Dairy Expt. Sta., Meddelande, **30,** 1951.

During the ripening of cream over a period of several hours the fat globules have time to undergo certain changes. Phase inversion and the chemical composition of the globular and free fat are affected. The composition of the 2 phases influences the consistency of the butter. In the Alfa

process there is insufficient time for the complicated changes involved during the ripening of cream. The crystallization process takes place in the Alfa butter mass, where the fat has formed a globular and a free phase, with the fat globules dispersed in the free fat.

At a temperature of 11–15° C., phase inversion can take place in 80% cream by cooling alone.

In the Alfa method phase inversion takes place throughout the entire cream mass, whereas in usual churning butter passes through the stage of granule formation. This results in a very close consistency in the structure of Alfa butter, but a less close consistency by the normal method.

At the beginning of working of ordinary butter, the percentage of globular fat is considerably higher than in Alfa butter but only a small difference exists in the finished butter of both types.

The fat globules in both types of butter appear identical after short periods of storage. At low temperatures Alfa butter can show larger quantities of birefringent fat (crystal-mosaic) in the fat globules, indicating a difference in composition of the globular fat. In Alfa butter the crystallization processes continue after leaving the transmutator; in ordinary butter small water droplets are enclosed during churning and larger droplets during working. There are no fat globules in the water droplets of normal butter.

In Alfa butter there are 2 different types of water-phase droplets. Since phase inversion takes place throughout the whole cream mass during manufacture, the water phase is finely dispersed in small interstices between the fat globules. The diameter of the droplets are 1–3 μ, none larger than 5–7 μ. These droplets increase somewhat in size during storage. The other type of water-phase droplet in Alfa butter is larger and packed with fairly large fat globules (cream droplets). This gives the Alfa butter the character of a dual emulsion and indicates incomplete inversion of phases. The cream droplets disappear to some extent during storage. A birefringent sheath encloses the cream droplets in Alfa butter. The sheath is part of the free fat and its birefringency indicates that it consists of several layers of molecules of fat fractions of higher melting point, orientated perpendicular to the surface of the droplets.

The short period of treatment in the transmutator may account for the presence of the half-moon-shaped droplets occasionally encountered in Alfa butter which is an indication of differences in the hydrophilic properties of the fat globule surfaces. Under certain conditions it is possible for air bubbles to be present in Alfa butter, but this is not usually the case. G. H. Wilster

6. Metallic surface for butter churns. F. J. J. J. Henrard (assignor to Ecremeuses Melotte). U. S. Patent 2,571,573. 4 claims. Oct. 16, 1951. Official Gaz. U. S. Pat. Office, **651,** 3: 823. 1951.

The surface of butter workers and other parts of churns is so designed that it remains wet with water, preventing the butter from sticking to it. The surface is kept wet by water from capillary cells, the walls of which converge and intersect in lines, presenting a minimum of exposed area.

R. Whitaker

7. Butter-forming device. R. R. Steans. U. S. Patent 2,572,960. 2 claims. Oct. 30, 1951. Official Gaz. U. S. Pat. Office, **651,** 5: 1368. 1951.

A chamber containing ice telescopes into a cylinder containing butter and forces the chilled butter through apertures in the top of the butter holding vessel to form servings of various shapes. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

8. En Aktuell Orsak till Röda Prickar i Ost (A current cause of rusty spots in cheese). K. E. Thomé and B. Lindgren, Swedish Dairy Expt. Sta., Meddelande, **31,** 1951.

Reddish or rusty-brown spots, chiefly in whole-milk Herrgård cheese, were not more than 1 mm. and generally less, but cheese thus affected was not approved for the "Rune Brand." The defect was caused by a rod-shaped lactic acid bacterium, most closely resembling *Lactobacillus plantarum var. rudensis.* The pigment was produced when the bacterium was permitted to form colonies on a surface or in an agar stab. Favorable bacterial growth temperatures were 13–40° C., with an optimum of 25° C. Growth and pigment production were dependent on the presence of certain sugars, but fairly independent of the pH and salt content of the medium.

A method was devised for the detection of the bacterium, which was found to be present in the milk of certain suppliers. The bacterium was present in the feces of certain cows.

With HTST pasteurization, the lethal temperature of the organism was slightly under 72° C., and the bacterium thus is destroyed with normal pasteurization.

Growth of the bacterium in cheese depends on the rapidity of the reduction in the redox potential. The defect can be related to low activity of the starter bacteria in the milk, caused by penicillin or bacteriophage.

Cheesemaking experiments showed that the pigment was not produced when the lactose concentration was reduced, for example by dilution with lactose-free whey. In large scale cheesemaking, such dilution would not be practical.

The defect occurred before the cheese was 1 mo. old. It remains to be determined how infection of milk with the organism responsible for the defect may be controlled . G. H. Wilster

9. Molkenlöcher am Käserand (Whey pockets in the rind of cheese). J. Ludwig. Milchwissenschaft, **5,** 11: 376. Nov., 1950.

Among the most important factors for controlling proper rind formation in cheese are control of milk acidity at renneting time and rate of acid development thereafter. A high milk acidity at renneting time and/or rapid acid formation will tend to bring about a rough rind containing whey pockets in camembert cheese. I. Peters

10. Über Aromastoffe des Tilsiter Käses (Aromatic substances in tilsit cheese). English summary. G. SCHWARZ and J. TOMASOW. Milchwissenschaft, **5,** 11: 376–379; 12: 412–416. Nov., Dec., 1950.

By means of appropriate analytical methods, the presence of the following substances was established in 4–4.5-mo.-old, part-skim tilsit cheese: (a) volatile acids: valeric, butyric and propionic; (b) nonvolatile acids: pyruvic, lactic and oxyphenylcarbonic acids are most probable; (c) volatile basic compounds: predominantly ammonia, also methylamine and dimethylamine, together with traces of trimethylamine; (d) nonvolatile basic compounds: histidine, arginine, lysine, ornithine, guanidine, tyramine, cadaverine and putrescine. The presence of histamine is assumed; (e) volatile carbonyl compounds: methylketones and traces of aldehydes.

Valeric and butyric acids, ammonia, methylamines and methylketones are considered most important in aroma formation of the cheese investigated. I. Peters

11. Die Beziehungen zwischen der Tätigkeit der Milchsäurebakterien und dem Kochsalz bei der Käsebereitung (The relationship between the activity of lactic bacteria and common salt in cheese making). English summary. G. GODBERSEN. Milchwissenschaft, **5,** 11: 379–383. Nov., 1950.

Comparisons were made using single- and multiple-strain lactic cultures as to their suitability as starter cultures in the manufacture of cheddar cheese. Cultures were examined for rapid acid production, growth rate at different temperatures and growth in the presence of NaCl.

A considerable difference in activity was found in the 8 cultures examined when grown at 37 and 30° C. in the absence of added NaCl and at 30° C. in the presence of 5% added NaCl.

I. Peters

12. Cheese handling apparatus. R. MIOLLIS (assignor to The Borden Co.). U. S. Patent 2,567,957. 8 claims. Sept. 18, 1951. Official Gaz. U. S. Pat. Office, **650,** 3: 698. 1951.

A device for handling cheese in curing rooms consists of racks each suspended from a wheel on a monorail attached to the ceiling. The racks, holding a series of cheeses one over the other, are so arranged that each cheese rests on doubly-hinged supports. All cheeses on 1 rack can be inverted by lifting or lowering 1 side of each support by means of a cable and pulley attached to the top of each rack and extending down to the bottom support in each tier. R. Whitaker

13. Roasted cheese products and process for making the same. J. J. RUYS (assignor to Eru-Kaasfabrick N. V.). U. S. Patent 2,571,765. 8 claims. Oct. 16, 1951. Official Gaz. U. S. Pat. Office, **651,** 3: 875. 1951.

Cheese, containing less than 15% moisture, is roasted to form porous small thin brittle crisp pieces. R. Whitaker

14. Prüfung von schimmelverhütenden Wandanstrichen für Mauerwerk (Examination of fungistatic wall paints). W. KUNDRAT. Milchwissenschaft, **5,** 12: 410–412. Dec., 1950.

Commercial paints, A, B and C were examined for fungistatic properties in the laboratory and later by application to the walls of curing rooms for camembert and tilset cheese. These paints varied in their fungistatic properties when tested against various strains of molds in the laboratory. Of the 2 water-miscible paints, A was much superior to B in fungistatic properties when applied to walls. Paint C was oily in nature, having an undesirable odor and therefore was unsuitable for plant use, although it exhibited strong fungistatic properties in laboratory tests. I. Peters

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

15. Problems created for the dairy industry by antibiotic mastitis treatments. C. S. BRYAN, Mich. State College, East Lansing. J. Milk & Food Technol., **14:** 161–162. Sept.–Oct., 1951.

Mastitic cows treated with antibiotics should have the milk withheld from the market for at least 3 d. Antibiotics present in the milk are not appreciably affected by pasteurization. Humans may become sensitive to penicillin injections when milk is consumed containing this antibiotic.

H. H. Weiser

16. A buffered boric acid lactose medium for enrichment and presumptive identification of Escherichia coli. R. H. VAUGHN, M. LEVINE and H. A. SMITH, Univ. of Calif., Berkeley; and Dept. of Health, Honolulu, T. H. Food Research, **16,** 1: 10–19. Jan.–Feb., 1951.

A study of a medium containing buffered boric acid as a means of distinguishing between *E. coli* and members of the *Aerobacter* and *E. freundii* groups is presented, together with the factors such as incubation temperature, concentration of medium constituents, pH, etc. which influence the selectivity. F. J. Doan

17. The numbers and types of bacteria found on the hands of food handlers. M. P. HARWOOD and V. A. MINCH, M. I. T., Cambridge. Food Research, **16,** 2: 133–136. Mar.–April, 1951.

Considerable numbers of bacteria, including coliforms, hemolytic streptococci, hemolytic staphylococci and aerobic spore-forming bacilli, were found on the hands of food handlers in public eating places. Hemolytic streps and staphs were found in 29 out of 30 examinations. This study emphasizes the need for better personal hygiene among those employed in handling foods.

F. J. Doan

18. Bacteriological studies on margarine. V. D. FOLTZ and T. H. LORD, Kansas State College, Manhattan. Food Research, **16,** 3: 216–221. May–June, 1951.

The bacterial quality of 50 samples of margarine was judged to be on a par with other processed foods. Plate counts were 100 or less/ml. on 42% of the samples. The general practice of adding 0.1% sodium benzoate to the margarine doubtless is partly responsible for the low

populations. Fifty four % of the samples were negative for yeasts and molds, 58% negative for mold mycelia and only 8% gave positive results for coliform bacteria. F. J. Doan

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

19. Separation of alpha-casein from whole casein. N. J. Hipp, M. L. Groves and T. L. McMeekin (assignors to the United States of America). U. S. Patent 2,572,026. 3 claims. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1020. 1951.

An aqueous alkaline 0.5–15% solution of casein at a pH of about 8 at room temp. is treated with a neutral soluble inorganic salt at a concentration of 0.05–0.2 and a saturated lower alkanol at a concentration of 25–75% by volume. The α-casein is precipitated and removed at a pH of 6–7. R. Whitaker

20. Studien über die Säure-und Salzverhältnisse in Milch und Molke (Studies concerning the relationship of acid and salt in milk and whey). English summary. G. Roeder. Milchwissenschaft, **5,** 11: 391–400; 12: 426–428; **6,** 1: 91–92. Nov., Dec., 1950; 2: 54–57; 3: 91–92; 5: 165–169. Nov., Dec., 1950; Feb., Mar., May, 1951.

This study is concerned with the relationship between titratable acidity and pH in milk and whey. This relationship can best be expressed by the formula ($p_s = -\log [-SH]$).

Graphical demonstration of the titration curve results at first in a flat curve followed by a sudden rise in the slope and a final flattening. In the case of whey it seemed that the speed with which the curve changed direction depended upon the point at which the Ca, originally present as Ca caseinate, combined with lactic acid. Thus, whey of different acidities behaved differently. However, the formula used for milk also may be used for whey above pH 4.5, employing the appropriate constant.

Due to variation in composition of salts and other constituents of milk it seems advisable to report values for metals and acids separately. I. Peters

21. The isolation from beef serum of a survival factor for Treponema pallidum. F. A. H. Rice and R. A. Nelson, Jr., Johns Hopkins Univ., Baltimore, Md. J. Biol. Chem., **191,** 1: 35–41. July, 1951.

From beef serum ultrafiltrate (whole serum filtered through cellophane) a crystalline compound ($C_{12}H_{22}O_{10}$) was isolated which was shown to be a survival-promoting factor of virulent *Treponema pallidum*. The material was designated the TPS factor. After 5 d. at 30° C., 15 γ TPS factor/ml. medium were sufficient to permit 8% of *T. pallidum* to remain motile. H. J. Peppler

22. Spectrophotometric analysis of amino acids and peptides with their copper salts. J. R. Spies and D. C. Chambers, U. S. Dept. Agr., Washington, D. C. J. Biol. Chem., **191,** 2: 787–797. Aug., 1951.

Conversion of Cu complexes of amino acids to the Cu salt of alanine is the basis for a rapid, accurate and simple spectrophotometric method of determining amino acids and peptides. The method was used to study the rate of hydrolysis of casein. Chromogenic nitrogen found for complete acid hydrolysates of casein and β-lactoglobulin compared favorably with values calculated from data determined by others. H. J. Peppler

23. Competition in the binding of long chain fatty acids and methyl orange to bovine serum albumin. G. E. Cogin and B. D. Davis, U. S. Public Health Service, Cornell Univ. Medical College, Ithaca, N. Y. J. Am. Chem. Soc., **73,** 7: 3135–3138. July, 1951.

The interaction between albumin and oleic, elaidic and stearic acids was studied at pH 6.6 in 0.1 *M* phosphate buffer at 6° C. by observing the competitive binding by the protein of each fatty acid and methyl orange. Little competition in the region of small molar ratios of added acid to albumin indicates the existence of more than 1 binding site. The addition of more fatty acid increases competition until 1 fatty acid displaces more than 1 dye anion, probably caused by steric hindrance at other sites. H. J. Peppler

24. The binding of organic ions by proteins. Optical evidence of cooperative interactions with hydrogen ions. I. M. Klotz and J. M. Urquhart, Northwestern Univ., Evanston, Ill. J. Am. Chem. Soc., **73,** 7: 3182–3186. July, 1951.

Changes in the optical properties of buffered solns. of iodinated bovine serum albumin were used to demonstrate the uptake of hydrogen ions by the protein molecule concurrently with the binding on anions. H. J. Peppler

25. Study of protein-ion interaction by the moving boundary method. The combination of bovine serum albumin with chloride ion. R. A. Alberty and H. H. Marvin, Jr., Univ. of Wis., Madison. J. Am. Chem. Soc., **73,** 7: 3220–3222. July, 1951.

Crystallized bovine serum albumin in 0.15 *M* NaCl at 0° C. was found by the moving boundary method to bind 8, 9 and 29 chloride ions per molecule of albumin at pH 7, 5.4 and 3.2. The values are comparable to those obtained by the membrane equilibrium and electromotive force methods. H. J. Peppler

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

26. Die Verteilung der Milch im Tellerraum der Separatoren (The distribution of milk between the discs of the separator bowl). English summary. F. J. Schmitz. Milchwissenschaft, **5,** 12: 418–425. Dec., 1950.

The author was able to verify the theoretical assumption that during separation milk is distributed uniformly between the conical plates in the separator bowl. This verification was made possible by photographing the milk currents

with the aid of a stroboscope and glass windows in the bowl. Movement of milk in the bowl was at a speed similar to that of the discs.

I. Peters

27. Method and apparatus for treating mixtures to make or break emulsions. G. J. STREZYNSKI (assignor to The DeLaval Separator Co.). U. S. Patent 2,572,287. 10 claims. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1092. 1951.

A mechanical device is described for reconstituting milk or cream from melted butterfat, milk powder and hot water, consisting of a centrifuge, so designed that the blend is intermittently discharged by centrifugal force through a constricted opening. The same device, suitably operated, may be employed to break the fat emulsion and produce free milkfat. R. Whitaker

28. Conveying apparatus for milk cans. W. L. H. E. WURDEMANN (assignor to Vennootschap onder firma Postma & Feenstra). U. S. Patent 2,572,233. 1 claim. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1077. 1951.

A device is described for moving milk cans on a rail through a straight line can washer.

R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

29. Automatic merchandising. R. Z. GREENE, The Rowe Corp. Milk Dealer, **40,** 12: 44–45, 64–66. Sept., 1951.

Automatic merchandising has definitely established itself as a modern marketing medium. Equipment specifically designed to meet dairy industry needs now is available. These machines not only refrigerate milk to keep it fresh and palatable but are adaptable to vari-sized containers and are equipped with a flexible price range running from 5–25¢; a coin changer can be incorporated to facilitate sales. Milk vending offers the dairy these principal considerations: (a) Vendors can be used to open up new outlets where milk is not now sold, building extra gallonage and revenue for the dairy; (b) Automatic merchandisers can supplement manual facilities; (c) They can do much to build the daily milk "habit" in a variety of locales, popularizing the product as a between-meals pick-up and thirst quencher; (d) Vendors can be utilized to obtain what has been termed "paid sampling" for a dairy product; and (e) Automatic merchandisers are valuable as advertising medium that pays for itself. Milk vending equipment either is operated by the dairy or an independent vending concern.

C. J. Babcock

30. Absenteeism can be reduced . . . here are some suggestions. P. LOCKWOOD. Sou. Dairy Prod. J., **50,** 5: 62–63. Nov., 1951.

The following suggestions are made to reduce absenteeism in milk plants: (a) Identify offenders by a system of colored time cards and remove time cards for third offense. (b) Enlarge parking area for employees' automobiles. (c) Thoroughly indoctrinate new workers on company policies and procedures by the use of booklets and personal instruction. (d) Place the right man on the right job. (e) Curtail overtime. (f) Apply the rules of good human relations through the full cooperation of supervisors. (g) Hold contests for the lowest rate of absenteeism. (h) Penalize absences. (i) Require a complicated return procedure such as physical examinations for those absent for more than 1 d. F. W. Bennett

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

31. The influence of nutrition on reproductive efficiency in cattle. 1. The effect of calcium and phosphorus intake on the fertility of cows and heifers. S. L. HIGNETT and P. G. HIGNETT, Wellcome Vet. Research Sta., Frant, Sussex. Vet. Record, **63,** 38: 603–609. Sept., 1951.

Authors studied influence of Ca and P intake on breeding efficiency in over 800 cows in 39 herds. Herds under varied management conditions were included and feed analyses were made. Data support the authors' tentative conclusions that there is a relationship between P intake and fertility and between Ca intake and fertility when the P intake is high or low. R. P. Niedermeier

32. The effect of dried citrus products on the flavor of milk. N. P. TARASSUK and C. L. ROADHOUSE, Univ. Calif., Davis. Milk Plant Monthly, **40,** 9: 38–39. Sept., 1951.

Feeding up to 4 lb. of dried citrus pulp (lemon and grapefruit pulp) or dried orange pulp/cow 1–1.5 hr. before milking did not impart an objectionable flavor to the milk. Five or more lb. of dried orange pulp imparted a distinct feed flavor which was considered undesirable.

E. B. Collins

33. Lead poisoning in cattle and sheep. RUTH ALLCROFT. Vet. Record, **63,** 37: 583–590. Sept., 1951.

A review presents published and unpublished investigations by Blaxter and Allcroft of lead absorption, excretion, retention and general metabolic effects in cattle and sheep. Data show blood and tissue levels of animals following both single dose and cumulative lead poisoning cases, using various lead compounds. Lethal dose for calves up to 4 mo. of age is reported as 0.2–0.4 g./kg. body weight of lead ingested in 24 hr. as the acetate, the basic carbonate or oxide. Older cattle required larger doses. In making a diagnosis, the symptoms, plus the lead content of blood and feces, or in case of death the kidney cortex, offer best evidence. Authors regard kidney cortex values of 40 ppm. or more (wet tissue) and liver values of 20 ppm. or more as evidence that lead poisoning caused death. R. P. Niedermeier

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

34. Über die Beziehunger zwischen den Jahresleistungen und dem Lebensgewicht des schwarzbunten Niederungsrindes (Concerning the rela-

tionship between the annual production and the live weight of the Hostein-Friesian cattle). English summary. E. LAUPRECHT and H. DÖRING. Milchwissenschaft, **5,** 11: 383–389; 12: 416–418. Nov., Dec., 1950.

About 10,000 Holstein-Friesian cows from 4 different regions in northern Germany were used in determining the correlation between body weight and annual milk and milk fat production. Cows weighing from 700–800 kg. yielded from 900–1,500 kg. milk and from 30–60 kg. milk fat more than cows weighing from 400–500 kg. The correlation coefficient for yield ranged for milk between +0.185 and +0.248, and for milk fat between +0.183 and +0.289.

By means of the regression coefficient, it was found that a 100-kg. increase in body weight resulted in an annual increased yield of milk by from 300–500 kg. and of milk fat by from 10–20 kg. I. Peters

35. Rolling cart. F. NEAL. U. S. Patent 2,571,601. 10 claims. Oct. 16, 1951. Official Gaz. U. S. Pat. Office, **651,** 3: 830. 1951.

A 2-wheel underslung hand-operated cart for transporting cans of milk is described. R. Whitaker

36. Test-milking apparatus. E. REDIN and H. RYDE. U. S. Patent 2,572,518. 6 claims. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1156. 1951.

A vacuum milker having a means for taking a small representative sample of milk as the cow is milked is described. R. Whitaker

37. Teat cup liner for milking machines. F. E. RICHWINE. U. S. Patent 2,574,063. 1 claim. Nov. 6, 1951. Official Gaz. U. S. Pat. Office, **652,** 1: 179. 1951.

Structural features are given for a teat cup liner. R. Whitaker

38. Portable miking machine. J. R. ORELIND (assignor to International Harvester Co.). U. S. Patent 2,573,927. 5 claims. Nov. 6, 1951. Official Gaz. U. S. Pat. Office, **652,** 1: 143. 1951.

A mechanism is described for causing pulsations in vacuum-operated teat cups. R. Whitaker

39. Automatic teat cup release device for milking machines. A. G. PERKINS. U. S. Patent 2,572,658. 25 claims. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1194. 1951.

A description is given of a device, located in the vacuum line, which automatically releases the teat cup from the teat when milking is complete. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

40. Method of making ice cream. R. HORTON (assignor to National Dairy Prod. Corp.). U. S. Patent 2,571,136. 7 claims. Oct. 16, 1951. Official Gaz. U. S. Pat. Office, **651,** 3: 701. 1951.

Ice cream mix is frozen in the conventional manner to at least 125% overrun, then chilled until it is sufficiently hard to hold its shape after being compressed to an overrun of not over 100%. R. Whitaker

41. Ice cream disher. B. F. LAWRENCE and E. E. LAWRENCE. U. S. Patent 2,571,729. 3 claims. Oct. 16, 1951. Official Gaz. U. S. Pat Office, **651,** 3: 866. 1951.

An ice cream dipper of the conventional bowl shape, has a scraper blade for ejecting the ball of ice cream, pivoted on the center of the bowl and rotated by a shaft parallel to the handle. R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

42. Bottle pouring cap and closure. C. P. ZURLINDEN. U. S. Patent 2,573,378. 2 claims. Oct. 30, 1951. Official Gaz. U. S. Pat. Office, **651,** 5: 1485. 1951.

A pivoted cap attaches to glass milk bottles, closing when the bottle is upright and swinging to the open position when the bottle is tilted for pouring. R. Whitaker

43. Milk can dispenser closure. T. LEWIS. U. S. Patent 2,574,338. 3 claims. Nov. 6, 1951. Official Gaz. U. S. Pat. Office, **652,** 1: 254. 1951.

A closure attaches to the top of evaporated milk cans, permitting easy pouring of the contents and a sealing mechanism to close the pouring and vent holes. R. Whitaker

44. Cream remover. G. SEVERUD. U. S. Patent 2,573,500. 8 claims. Oct. 30, 1951. Official Gaz. U. S. Pat. Office, **651,** 5: 1517. 1951.

A small cylinder-shaped scoop for removing cream from the top of a bottle of milk which has been creamed is described. R. Whitaker

45. Strainer. E. J. GALLOWAY (assignor to Neenah Milk Prod. Co.). U. S. Patent 2,572,131. 4 claims. Oct. 23, 1951. Official Gaz. U. S. Pat. Office, **651,** 4: 1048. 1951.

A strainer for milk consists of a nest of perforated tubes clamped in a step-wise position between 2 partitions. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

46. Some effects of processing on the nutritive properties of proteins. R. R. BALDWIN, J. R. LOWRY and R. THIESSEN, JR., General Foods Corp., Hoboken, N. J. Food Research, **16,** 2: 107–117. Mar.–April, 1951.

Loss of nutritive value of casein heated sufficiently in the presence of dextrose to produce a "browning reaction" is due to a rapid destruction of some of the essential amino acids, especially lysine and arginine, but apparently also methionine, histidine and threonine. F. J. Doan

47. Consumer preference for bread containing different levels of non-fat dry milk solids. E. L. JACK and VESTA M. HAYNES, Univ. of Calif., Davis. Food Research, **16,** 1: 57–61. Jan.–Feb., 1951.

A study of consumption of bread by 320 boys aged 8–16 yr., when different levels of non-fat

milk solids were contained therein, revealed that consumption averaged 104.4, 107.5 and 112.6%, respectively, when the bread contained 6, 10 and 14% of the milk product and where 100% was used as the consumption figure for bread containing no added milk solids. The general diets of the subjects were varied and nutritionally adequate during the study. F. J. Doan

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

48. Studies of electrolytes in body fluids of dairy cattle. II. Effects of estrogen on electrolyte levels in body fluids in late pregnancy. A. F. SELLERS and M. H. ROEPKE. Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet. Research, **12,** 45: 292–295. Oct., 1951.

A 75–100-mg. dose of diethylstilbestrol was given intramuscularly to 5 cows in late pregnancy. Following the injection, a slight fall in serum Ca was noted. Following parturition a much larger decline in serum Ca accompanied by a fall in inorganic P was observed. Ratios of plasma Mg to K did not show significant changes due to estrogen or parturition. Plasma Na and Cl also were not altered. E. W. Swanson

49. Studies of electrolytes in body fluids of dairy cattle. III. Effects of potassium on electrolyte levels in body fluids in midlactation. A. F. SELLERS, T. L. GITIS and M. H. ROEPKE. Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet Research, **12,** 45: 296–301. Oct., 1951.

Diuresis was induced in 5 lactating cows by giving 0.5 g. KCl/lb. body wt. in 1 gal. water, 0.5 g. NaCl/lb. in 10 gal. water, 0.5 g. KCl/lb. in water preceded by 3 mg. biotin/lb., and water alone. Changes in blood K, Na, Cl, Mg and inorganic P were determined. Ca and Mg showed no regular change. Na and P increased in all the salt diuresis. Urine excretion of Na increased and Ca was slightly increased. Urine excretion of Mg was not changed. K excretion following NaCl was not increased. Biotin did not alter the K pattern in these studies. E. W. Swanson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

50. A time and motion analysis of the cleaning of dairy equipment. W. E. SHIFFERMILLER, The Borden Co., Chicago, Ill. Milk Dealer, **40,** 12: 116–122. Sept., 1951.

Time and motion analyses of the cleaning of dairy equipment in 3 plants receiving 20,000, 40,000 and 100,000 lb. of milk daily are reported. Data show the percentage of the total labor cost represented by cleaning labor in the 3 dairies and percentages of total cleaning labor represented by: the assembly and disassembly operation; rinsing equipment before and after washing; washing equipment exterior; washing milk contact surfaces; preparing cleaning solutions and getting and returning hose. Conclusions drawn are: (a) Use of time and motion study as a means of analyzing cleaning operations brings out some hidden information that would be difficult to observe by any other method. (b) Considerable time is involved in assembly and disassembly operations. (c) Simplified design would be necessary to reduce this assembly and disassembly time much. (d) Good plant layout of equipment, sanitary lines and cleaning equipment aid in reducing cleaning time. (e) The cleaning operation should be planned in detail for each piece of equipment. (f) A considerable number of cleaning aids now available show some promise in motion economy. C. J. Babcock

51. Lye solution for milking machine rubber parts. C. K. JOHNS, Canada Dept. of Agr., Ottawa. J. Milk & Food Technol., **14:** 153–154, 160. Sept.–Oct., 1951.

Lye solution has valuable detergent properties in addition to its germicidal activity. Rubber parts immersed in lye soak solution accompanied by weekly brush washing will maintain the equipment in good sanitary condition. The author lists 12 advantages of wet storage in lye solution between milkings as an acceptable method to remove milk residue. H. H. Weiser

52. Machine for successively cleaning both sides of centrifugal separator disks. C. H. ABBOTT. U. S. Patent 2,573,173. 7 claims. Oct. 30, 1951. Official Gaz. U. S. Pat. Office, **651,** 5: 1427. 1951.

A motor-driven, cone-shaped brush for cleaning separator disks in a wash tank is described. R. Whitaker

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