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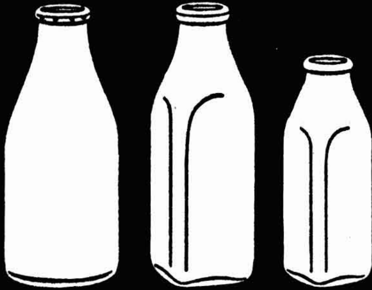

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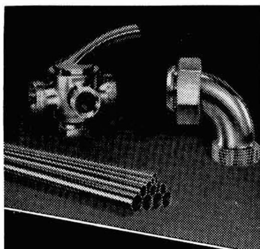

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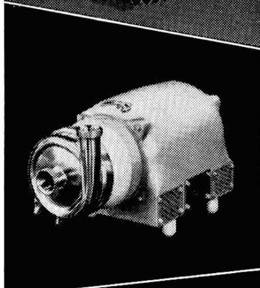

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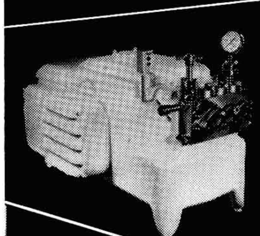

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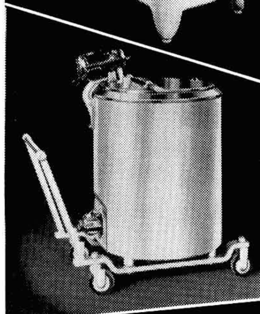

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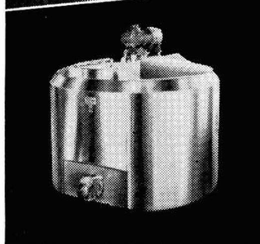

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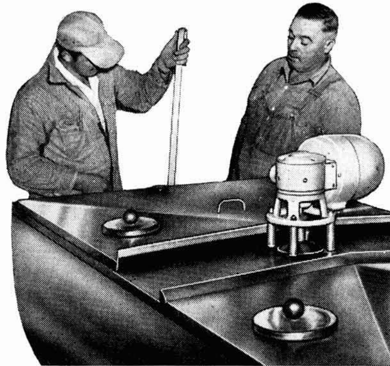

Left: W. D. Hahn, Ceresville, Md., in milk house of one of his two farms, watches driver of pick-up tanker "weigh" milk.

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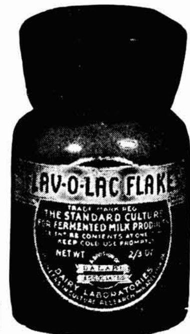

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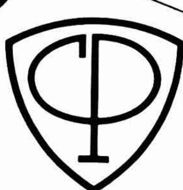

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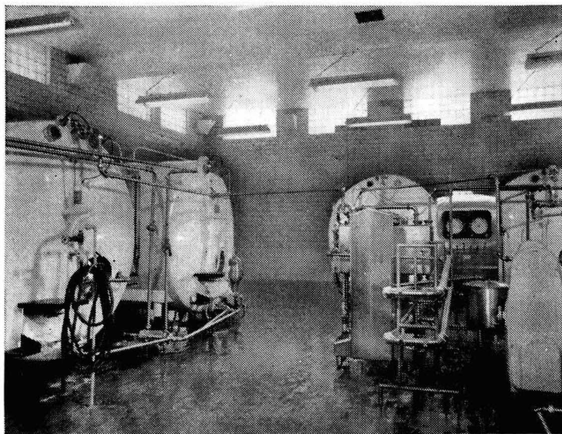

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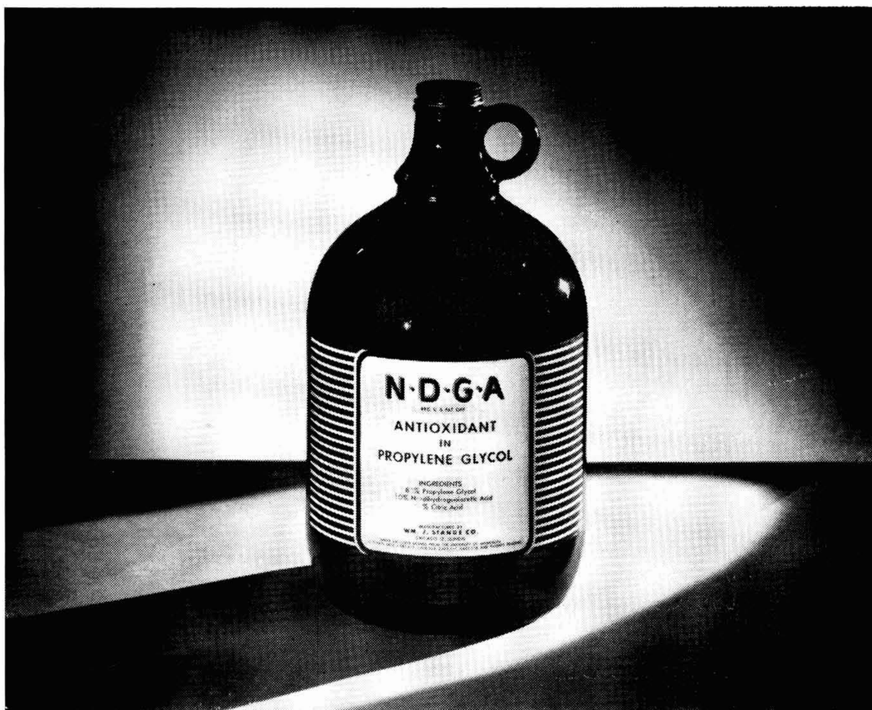

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THE ABSORPTION OF VITAMIN A NATURAL ESTERS AND OF CAROTENE BY YOUNG MALE HOLSTEIN CALVES[1]

MAGNAR RONNING[2] AND C. B. KNODT

Pennsylvania Agricultural Experiment Station, State College

The importance of vitamin A and its precursors in the rations of dairy calves has been well established. On the basis of the research published, however, information is needed relative to the site of absorption of these compounds in obtaining an understanding of the mechanisms involved in their utilization. Popper and Volk (10) determined that absorption of vitamin A in rats was most rapid in the upper part of the small intestine. Barrick *et al.* (3) indicated that the small intestine was most important in the absorption of vitamin A by sheep. Eden and Sellers (5) demonstrated that vitamin A was absorbed primarily in the duodenal area of the small intestine in sheep and in rats. The absorption of carotene and its subsequent conversion to vitamin A is not well understood and there appears to be considerable species variation in the mechanism of utilization of carotene (1, 3, 4, 6, 9). It was the purpose of this experiment to obtain information concerning the site of absorption of vitamin A natural esters and carotene by young dairy calves.

EXPERIMENTAL

Male Holstein calves were left with their dams for 24 hr. and then were placed on a vitamin A- and carotene-deficient diet for a period of 7 days. The purpose of this stabilization period was to deplete the digestive tract and its contents of carotene and vitamin A. The experimental ration consisted of a mixture of 0.2 lb. dry whole milk replacement, 1.8 lb. water and 3.0 lb. skimmilk fed twice daily. No hay, grain or additional fluids were made available. The calves were penned individually on wire screens to prevent eating of bedding.

After completion of this stabilization period, the calves were administered one of the following preparations dispersed in the experimental diet to insure more homogeneous distribution in the digestive tract: Group A, 5.0 g. commercial corn oil; group B, 2.0 g. concentrated fish liver oil containing 608,000 I. U. vitamin A per gram; group C, 5.0 g. cottonseed oil containing 255,000 I. U. vitamin

Received for publication Sept. 9, 1951.

[1] Data contained in this publication are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Authorized for publication as paper no. 1681 in the Journal Series of The Pennsylvania Agricultural Experiment Station.

[2] Present address: Department of Dairying, Oklahoma A. & M. College, Stillwater, Okla.

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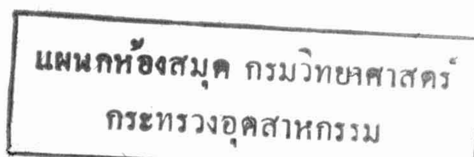

A equivalent in the form of β-carotene per gram; group D, 0.5 g. of the above vitamin A oil as used in group B; and treatment E, 1.25 g. of the above carotene oil used in group C. The total amount of oil was adjusted by adding corn oil as needed so that each calf received a total of 5.0 g. oil. The concentrate oil was added to 1 lb. of skimmilk of the ration, heated to 37° C. and dispersed by vigorous shaking. This mixture then was dispersed in the total test meal by thorough stirring and a sample for analysis was taken while the material was swirling in the bucket.

Eight hr. after feeding this meal the calves were stunned with a blow on the head and bled to death by severing the left carotid artery. The body cavity was exposed, the esophagus tied off and the digestive tract ligated as soon as possible in the following places to prevent as much as possible the movement of material from one portion to another: Between the rumen and abomasum; the pyloric and ileocecal valves; and at several places throughout the small intestine. The tract then was removed intact and taken to the laboratory and prepared for analysis.

The contents of the rumen, abomasum and the large intestine, including the cecum, were removed and identified as R, AB and LI, respectively. Because of a lack of sufficient material, the contents of the reticulum were included with those of the rumen for analysis. Similarly, the contents of the omasum were included with those of the abomasum. The small intestine was divided into three sections approximately equal in length and the contents removed and identified as SI-1, 2 and 3, beginning from the duodenal end.

Blood samples were taken for vitamin A and carotene analysis prior to administration and at time of slaughter. The livers were removed and prepared for analysis for vitamin A and carotene.

The mesenteric membranes, adhering fatty tissue and lymph nodules were removed from the outer surface of the digestive tract and the inner surfaces washed thoroughly with a 0.9 per cent saline solution. The mucous tissue of the abomasum and of the three sections of the small intestine were removed by carefully scraping the inner surfaces with a knife. The two layers of these sections and the tissues of the rumen and of the large intestine were prepared for analysis by grinding and macerating.

Analyses for carotene and vitamin A were made on the test meal, digestive tract contents, tissues of the digestive tract walls and livers after saponification and the extraction of the nonsaponifiable material with ethyl ether. The concentrations of vitamin A and carotene of the feed and digestive tract contents are reported in terms of I. U. per gram of dry matter. The concentrations of these compounds in the tissues are presented on the basis of I. U. per gram of fresh tissue. The Carr-Price reaction was used in the analyses for vitamin A, while the carotene was measured colorimetrically in petroleum ether, using the Evelyn colorimeter according to suggestions by the Association of Vitamin Chemists. Chromatographic purification (2) of the carotene extract was not employed since it had been predetermined that interfering pigments were not concentrated sufficiently to affect the final results. Blood analyses for vitamin A

and carotene were made according to a combination of methods proposed by Moore (8) and Kimble (7). The per cent dry matter of the feed and of the digestive tract contents were determined by using a Brabender semiautomatic moisture tester. The samples were dried at 100° C. with forced draft for 4 hr. which had been predetermined as the time required for constant weight. Three calves were subjected to each of the dietary treatments described.

RESULTS

The concentration of vitamin A and carotene in the digestive tract contents from the various sections are presented in figure 1. These levels may be com-

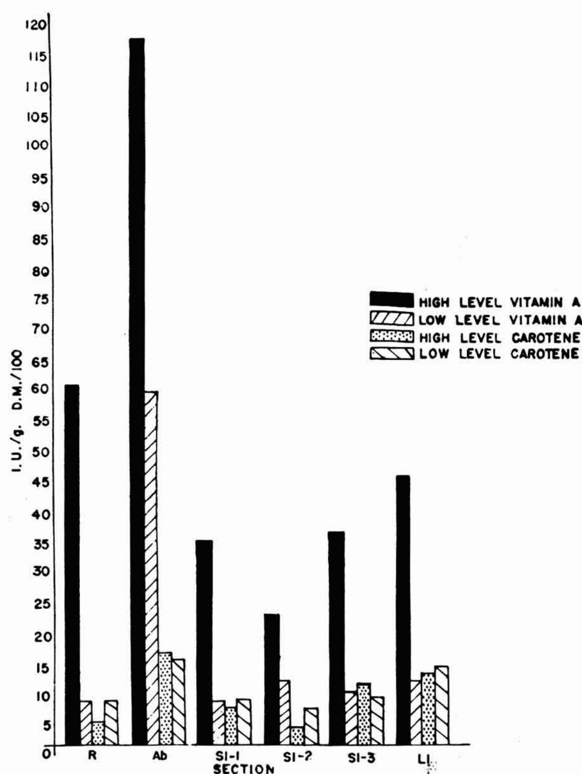

FIG. 1. Concentration of administered compound in digestive tract contents I.U./g. D.M.

pared with the average levels of the administred compound present in the feed, which were as follows as determined chemically: High level vitamin A, 5330 I. U. per gram dry matter; low level vitamin A, 938 I. U. per gram dry matter; high level carotene, 3079 I. U. vitamin A equivalent per gram dry matter; and low level carotene, 939 I. U. vitamin A equivalent per gram dry matter. The test meal and the digestive tract contents of the control group showed only traces of vitamin A and carotene values and these in all probability were due to the

presence of very small amounts of interfering substances. The chemically determined values of vitamin A and carotene of the feed were appreciably lower than the calculated values, which indicates that considerable loss in potency was suffered during the time of dispersing the oil with the feed. Some oxidative destruction may have taken place while shaking the heated mixture and further loss may have resulted from adherence of some oil to the utensils used.

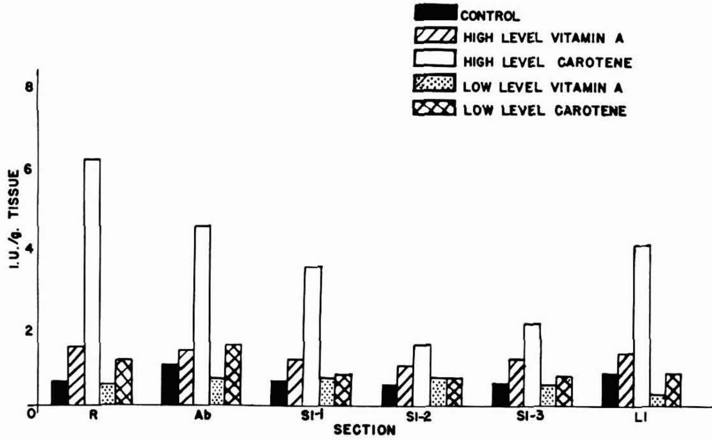

FIG. 2. Concentration of carotene G.I. tract tissue I.U./g.

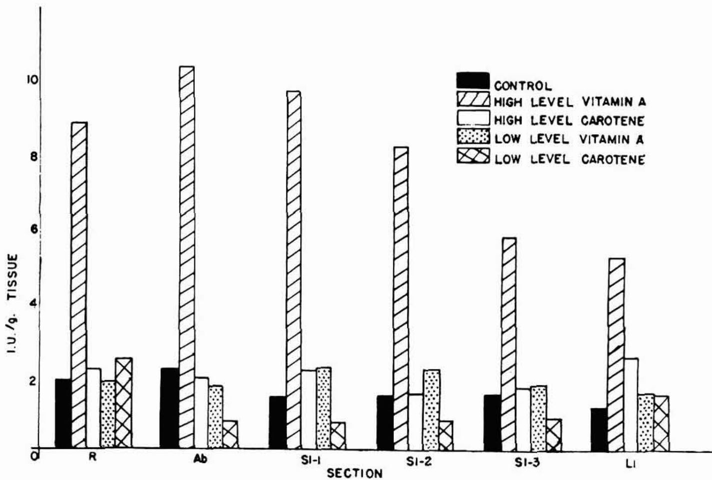

FIG. 3. Concentration of vitamin A G.I. tract tissue I.U./g.

A very high concentration of vitamin A in the abomasum resulted from the administration of this compound at both levels of dosage. The lowest level of vitamin A was observed in the upper one-third of the small intestine in the low-dosage group and in the middle one-third of the small intestine in the high-dosage group. There appeared to be a concentrating effect upon vitamin A in

the lower one-third of the small intestine and in the large intestine, which was particularly evident in the high-dosage group.

The carotene levels of the digestive tract contents of the two groups administered this compound were quite comparable, although the low-dosage group received only about one-third as much carotene as the high-dosage group. The concentration of carotene was lowest in the middle one-third of the small intestine

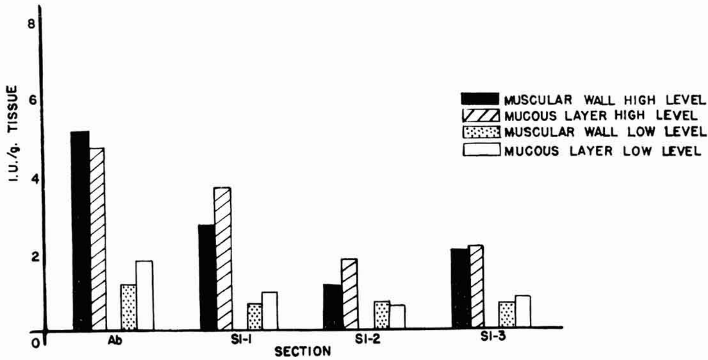

FIG. 4. The distribution of carotene between the mucous layer and muscular wall.

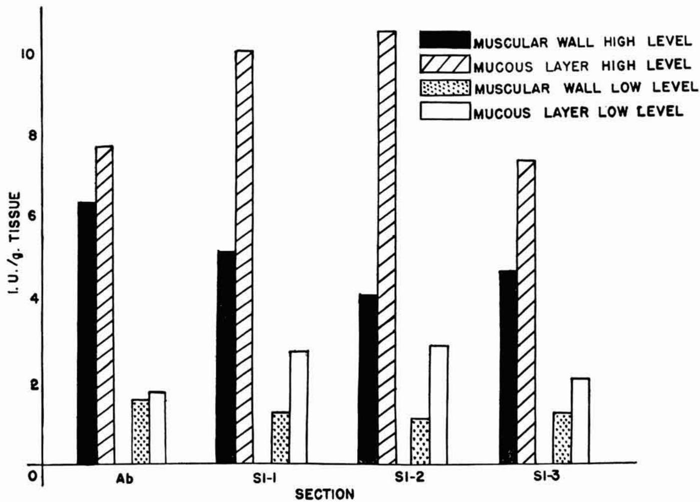

FIG. 5. The distribution of vitamin A between the mucous layer and muscular wall.

and highest in the abomasum. The concentration of carotene was quite high in the large intestine, approaching that observed in the abomasum. A great deal of individual variation was observed in the levels of carotene in the contents of the rumen, influenced probably by the passage of liquid material into this section.

The average dry matter content of the digestive tract contents was 61.5 per cent of that in the feed. This had the effect of increasing the concentration of

vitamin A and carotene per unit gram of dry matter. The total amount of these compounds, however, was somewhat less in the digestive tract contents than in the feed. The recovery of vitamin A was 83.7 and 65.4 per cent in the high- and low-administration groups, and the recovery of carotene was 21.6 and 64.3 per cent in the high- and low-intake groups, respectively.

The concentrations of carotene and vitamin A in the tissues of the various portions of the digestive tract are summarized in figures 2 and 3. The total amount of these compounds in the tissues did not appear to be related to the concentrations of vitamin A or carotene in the corresponding digestive tract contents. At both levels of dosage the carotene concentrations were highest in the rumen tissue and lowest in the mid-section of the small intestine. The concentration of carotene in the tissues of the digestive tract wall was considerably higher in the high-dosage group than in the lower level intake group. However, at both levels of carotene dosage, the carotene concentrations in the digestive tract contents were nearly the same. The concentration of vitamin A in the tissues of the walls of the digestive tract was highest in the abomasum and became progressively lower throughout the remainder of the tract, in the high-dosage group. At the low-level intake, the digestive tract tissue levels of vitamin A were quite uniform, with somewhat higher levels in the upper two-thirds of the small intestine than elsewhere.

TABLE 1

Weights and carotene and vitamin A contents of calf livers

Calf	Treatment[a]	Wt.	Carotene[b]	Vitamin A
(no.)		*(g.)*	*(I. U.)*	*(I. U.)*
282	A	776	498	30,410
289		878	2,520	85,078
292		735	809	2,793
$\overline{X}$		796	1,276	39,427
278	B	825	2,368	61,215
285		790	869	6,565
291		763	1,099	10,758
$\overline{X}$		793	1,445	26,179
280	C	924	1,654	2,495
286		838	2,506	38,715
290		897	484	4,575
$\overline{X}$		886	1,548	15,262
287	D	1,199	2,290	54,914
294		958	1,494	40,332
296		1,024	2,202	77,414
$\overline{X}$		1,060	1,995	57,553
288	E	824	1,673	72,759
295		706	777	1,483
297		179	177	3,365
$\overline{X}$		570	876	25,869

[a] A, Control; B, high-level vitamin A; C, high-level carotene; D, low-level vitamin A; E, low-level carotene.

[b] Vitamin A equivalent.

The distribution of carotene and vitamin A between the mucous layer and the muscular wall is presented in figures 4 and 5. The concentration of carotene in these two tissue layers was nearly equal. Vitamin A, however, was predominantly higher in the mucous layer as compared to the muscular tissue. The concentration of vitamin A was highest in the mucous layer in the upper two-thirds of the small intestine in both dosage groups.

From the data summarized in table 1, liver storage of vitamin A apparently was not affected by any of these treatments during the 8-hr. period following administration. The highest concentration of vitamin A was found in the liver of a calf of the control group and only one of the four supplemented groups showed a higher average liver concentration of the vitamin than did the control group. It should be noted in table 1 that even after the period of stabilization there were large variations in the concentrations of liver carotene and vitamin A.

The changes in blood plasma vitamin A and carotene resulting from the treatments employed in this experiment are presented in table 2. A great deal

TABLE 2

Blood plasma carotene and vitamin A

Calf	Treatment[a]	Carotene (γ/*100 ml.*)			Vitamin A (γ/*100 ml.*)		
		Pre-adm.	Slaughter	Change	Pre-adm.	Slaughter	Change
(*no.*)							
282	A	11.66	10.69	− 0.97	14.16	13.74	− 0.42
289		15.55	14.57	− 0.98	23.32	23.32	0.00
292		7.77	7.77	0.00	3.91	2.91	− 1.00
$\overline{X}$		11.66	11.01	− 0.65	13.80	13.32	− 0.48
278	B	20.40	19.43	− 0.97	12.91	46.64	+ 33.73
285		11.66	9.72	− 1.94	7.77	13.32	+ 5.55
291		9.72	6.80	− 2.92	3.91	14.16	+ 10.25
$\overline{X}$		13.93	11.98	− 1.95	8.20	24.71	+ 16.51
280	C	6.80	7.77	+ 0.97	0.00	1.42	+ 1.42
286		13.60	15.55	+ 1.95	12.91	16.24	+ 3.33
290		4.86	3.89	− 0.97	8.61	11.24	+ 2.63
$\overline{X}$		8.42	9.07	+ 0.65	7.17	9.63	+ 2.46
287	D	7.77	6.80	− 0.97	5.25	4.58	− 0.67
294		1.94	4.86	+ 2.92	11.66	17.49	+ 5.83
296		6.80	5.83	− 0.97	17.07	19.57	+ 2.50
$\overline{X}$		5.50	5.83	+ 0.33	11.33	13.88	+ 2.55
288	E	14.57	17.49	+ 2.92	26.90	24.80	− 2.10
295		1.94	10.69	+ 8.75	3.91	5.58	+ 1.67
297		3.89	4.86	+ 0.97	5.25	6.25	+ 1.00
$\overline{X}$		6.80	11.01	+ 4.21	12.02	12.21	+ 0.19

[a] A, Control; B, high-level vitamin A; C, high-level carotene; D, low-level vitamin A; E, low-level carotene.

of individual variation was observed in the changes in blood plasma levels of both vitamin A- and carotene-fed calves. One calf of the vitamin A-administered groups did not exhibit an increase in blood plasma vitamin A. Only one calf

of the carotene groups showed an appreciable increase in blood plasma carotene. Changes in blood plasma vitamin A resulting from carotene administration were relatively small and quite inconsistent under the conditions of these experiments.

The increases in blood plasma vitamin A resulting from the administration of this compound at both levels of intake were significantly correlated to the concentrations of vitamin A in the mucous layers of the tissues of the walls of the small intestine. The calculated coefficient of correlation was $r = 0.97$, which was significant with $P > 0.01$. No other relationships could be shown between blood plasma changes and concentrations of carotene or vitamin A in the digestive tract tissues or contents.

SUMMARY AND CONCLUSIONS

The concentrations of vitamin A and carotene in the digestive tract contents and tissues after a single administration of either compound at a high or low level to young calves have been studied. Further observations were made with respect to the effect of such administrations upon liver storage and upon blood plasma carotene and vitamin A.

The resulting data indicate that the most active absorption of vitamin A takes place in the upper two-thirds of the small intestine. The concentration of vitamin A in the digestive tract contents after the administration of one single dose was lowest in this area. The mucous tissues of the upper two-thirds of the small intestine were more concentrated with vitamin A than those elsewhere. A highly significant correlation coefficient was calculated between the increase in blood plasma vitamin A and the concentration of vitamin A in the mucous tissue of the small intestine after the administration of a single dose of vitamin A. Absorption seemed to be limited in the lower one-third of the small intestine and in the large intestine, since there appeared to be a concentration effect upon the vitamin A that passed into these two sections.

The low concentration of carotene in the middle one-third of the small intestine after a single administration of this compound might indicate that absorption takes place most rapidly in this area. The low concentration of carotene in the tissues of the digestive tract wall may be evidence of carotene conversion, but concurrent increases in vitamin A could not be demonstrated under these conditions.

A large single dose of vitamin A or of carotene did not affect liver storage of vitamin A during the 8-hr. period following administration. The administration of vitamin A in a single dose increased blood plasma vitamin A in all instances but one. Carotene administrations resulted in inconsistent and relatively small changes in blood plasma carotene and vitamin A in these experiments.

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THE COPPER CONTENT OF BUTTER MADE BY A CONTINUOUS METHOD[1]

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The Cu content of freshly drawn milk has been determined by several investigators. The values reported vary from 0.06 to 0.80 ppm. Differences in technique possibly account for some of the differences in results reported. Some studies (12, 4) have been made of the Cu content of butter but none have been reported on the Cu content of butter made by one of the newer continuous processes developed in this country. It was for the purpose of obtaining such information that this study[3] was made. An attempt also was made to determine whether the Cu is associated with the fat or non-fatty portions of the butter.

METHOD OF DETERMINING COPPER

Ordinarily colorimetric methods are used for determining the amount of Cu in dairy products. Research workers, however, do not agree on the best method for preparing the sample for analysis (1, 3, 5, 6, 8, 9, 10, 11, 13).

Conn *et al.* (2) found sodium diethyldithiocarbamate most desirable as a colorimetric reagent in the determination of Cu. They state that the sensitivity for the carbamate method is 0.01 ppm. Moir and Andrews (10) also found it desirable. Hetrick and Tracy (7) state that few metals interfere with the carbamate determination and, with the exception of cobalt, nickel and bismuth, these interferences can be eliminated by extraction of the Cu carbamate with carbon tetrachloride from an ammoniacal citrate solution at pH 8.5 to 9.0. These workers also found that if the nickel concentration is equal to the Cu concentration, a high degree of accuracy cannot be had with the carbamate method.

The dry ashing method was selected for this study because of its greater simplicity and because of the satisfactory results obtained in a previous investigation made in this laboratory (7).

METHODS OF ANALYSIS

The apparatus used consisted of 100-ml. pyrex beakers, platinum-tipped tongs, Coleman Universal spectrophotometer, matched pyrex cuvettes, muffle furnace equipped with rheostat and pyrometer, heat lamps, pyrex glassware, 125-ml. pyrex separatory funnels and pyrex still to prepare all distilled water.

Distilled water was used for all dilutions. All glassware was cleaned and rinsed with nitric acid, followed with distilled water. All reagents were stored in pyrex containers. The reagents were prepared as follows:

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[1] The Cherry-Burrell continuous method of butter making.

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[3] This report is a portion of a thesis submitted by the senior author in partial fulfillment of the requirements for a M.S. degree in Dairy Technology in the Graduate School of the University of Illinois.

(a) HCl—50 per cent solution of concentrated HCl in water.

(b) citric acid (C.P.)—15 per cent solution.

(c) ammonium hydroxide (C.P.).

(d) cresol red—0.02 g. in 100 ml. redistilled water.

(e) sodium diethyldithiocarbamate solution made by dissolving 1 g. in 1 l. of redistilled water and stored in brown bottle.

(f) carbon tetrachloride (C.P.).

(g) standard Cu solution made by dissolving 0.5000 g. of electrolytic Cu foil in 15 ml. 6 *N* HNO_3, warmed gently to dissolve metal and expel fumes. This solution then was cooled and diluted to 500 ml. with water. One ml. of this solution was diluted to 1 l. and contained 1 γ of Cu per milliliter.

Ashing procedures. In the course of the study, it was necessary to make a Cu analysis of butter, fat concentrates, cream, skimmilk, salt and water used for standardizing the butter. The methods used for ashing were:

(a) Butter, fat concentrates and cream. Weigh 35 g. of butter or fat concentrate, or 20 g. of cream into a clean 100-ml. pyrex beaker. Remove water from the product by placing beaker under an infra-red lamp and regulate heat by varying the distance of the lamp from the beaker. Removing water prior to ashing prevents spattering and loss of product in the muffle furnace. About 2 hr. are required to remove all traces of moisture from the product, as indicated by the browning reaction. Place the beaker in a cold muffle furnace and raise temperature slowly to 550° C. The sample is left in the muffle furnace for approximately 9 hr. Remove beaker from the furnace, cool and wash down the ash adhering to the side of the beaker with approximately 10 ml. redistilled water. Again dry the sample under the lamp, then place it in a cold muffle furnace and raise temperature slowly to 550° C. and leave it in the muffle furnace overnight. This double ashing technique removes all carbonaceous material. Add 5 ml. of the HCl solution to the ash and carefully heat to boiling, holding at this temperature for 5 min. to ensure complete solution. Transfer this solution to a separatory funnel. Rinse the beaker with six successive 5-ml. portions of redistilled water and transfer washings to the separatory funnel.

(b) Skimmilks. The skimmilk samples are ashed in the same manner as the butter, fat concentrates and cream samples except that 20 g. of skimmilk are dried in the 100-ml. pyrex beakers over a boiling water bath, rather than with the heat lamp.

(c) Salt. Weigh 5 g. of dried salt directly into the separatory funnel and dissolve with redistilled water and 5 ml. HCl. Add 10 ml. of citric acid and determine Cu.

(d) Standardizing water. Pipette 60 ml. of water into a clean 100-ml. pyrex beaker and place it under the heat lamp and evaporate to a volume of approximately 20 ml. Wash contents directly into the separatory funnel and add 5 ml. of HCl and determine Cu, beginning with the addition of 10 ml. citric acid.

Transmittance determination. Transfer sample solution of ash, prepared as previously described, to a 125-ml. pyrex separatory funnel. Add 10 ml. of the citric acid and 5 drops of cresol red indicator. Adjust to pH 8.5 to 9.0 by adding

about 0.5 ml. of C.P. NH_4OH after solution reaches the violet color of cresol red. Make volume up to 55 ml. with redistilled water, and then add 10 ml. carbamate solution. Allow it to stand for 5 min. for Cu carbamate color formation and add exactly 10 ml. carbon tetrachloride. Shake vigorously for 5 min. and let mixture stand for 2 min. to allow the carbon tetrachloride layer to settle to the bottom of the funnel. Draw off carbon tetrachloride containing the colored Cu carbamate into a clean dry test tube. Stopper tube and allow it to stand for 20 min. Transfer colored carbon tetrachloride to the cuvette and determine transmittance in the spectrophotometer at a wave length of 440 mμ with a blank of carbon tetrachloride in the other cuvette set at 100 per cent transmission. Cu was estimated from a standard reference curve.

Precautionary measures. The 100-ml. beakers used for the Cu determinations should be handled with platinum-tipped tongs to avoid possible metal contamination of the sample. Only beakers free from excessive etching should be used, as badly etched glassware leads to inconsistent results. The distilled water used should be redistilled from pyrex to avoid Cu contaminations. All glassware should be pyrex. The carbon tetrachloride and the sodium diethyldithiocarbamate solutions should be protected against light .

Samples to be ashed should be moisture-free to prevent spattering in the muffle furnace.

RESULTS

To determine whether or not Cu was lost during the ashing procedure, known amounts of Cu were added to weighed portions of butter. From the results in table 1, it is evident that satisfactory recoveries were made.

TABLE 1

Recovery of added amounts of Cu to butter

Sample no.	Cu added	Cu calculated	Cu found
	(ppm.)	*(ppm.)*	*(ppm.)*
A	....	....	0.26
A	....	....	0.26
A	....	....	0.26
A	0.06	0.32	0.31
A	0.06	0.32	0.32
A	0.06	0.32	0.33
A	0.11	0.37	0.39
A	0.11	0.37	0.35
A	0.11	0.37	0.38
B	....	....	0.13
B	....	....	0.13
B	....	....	0.13
B	0.15	0.28	0.29
B	0.21	0.34	0.34
B	0.21	0.34	0.31

A series of tests were made of the Cu content of butter made in the University dairy plant by the batch system. Twelve samples were analyzed. The lowest value found was 0.13 ppm. and the highest was 0.38 ppm. of Cu. The average was 0.27 ppm.

Eight samples of butter made by the continuous method also were analyzed for Cu. The lowest value found was 0.12 ppm. and the highest was 0.27 ppm. of Cu. The average was 0.19 ppm.

To study the relation of each step in the process of making butter by the continuous methods, samples were obtained from four different plants. Table 2 gives the results. The Cu values are expressed on the basis of ether-soluble material, as well as total weight. Since it is commonly thought that the Cu-fat relationship is important from the standpoint of keeping quality of butter, the discussion is based upon Cu as expressed in ppm. of ether-soluble material.

TABLE 2

Cu content of samples taken after each step in the continuous process of buttermaking

Product	Cu content	
	Ether-soluble extract basis	Total weight basis
	(ppm.)	*(ppm.)*
Plant A		
Raw cream	0.03	0.01
First cream entering the separator	0.10	0.05
First cream from the separator	0.18	0.14
First heavy skimmilk	1.65	0.11
First light skimmilk	1.20	0.09
Fat concentrate	0.21	0.19
After standardizing concentrate	0.21	0.17
Coloring material	......	0
Salt	......	0.71
Standardizing water	......	0.09
First butter through printing machine	0.35	0.29
Last butter through printing machine	0.21	0.16
Plant B		
Raw cream	0.62	0.21
Heavy skimmilk	8.45	0.66
Light skimmilk	1.70	0.15
Concentrate from vacreator	0.13	0.11
Concentrate in composition control vat	0.20	0.19
Butter from printing machines	0.17	0.14
Plant C		
Raw cream	0.40	0.14
Cream entering the separator	0.37	0.13
First fat concentrate	0.11	0.10
Light skimmilk	3.19	0.29
Heavy skimmilk	3.29	0.25
Concentrate in composition control vat	0.13	0.12
Concentrate after standardization	0.12	0.10
Butter from the texturator	0.12	0.10
Wrapped printed butter	0.19	0.15
Plant D		
Raw cream	0.40	0.14
Cream entering cream vat	0.51	0.18
Cream entering the separator	1.57	0.55
Fat concentrate	0.47	0.42
Light skimmilk	2.33	0.21
Heavy skimmilk	22.37	1.70
Second separation of concentrate—99% fat	0.04	0.04
Second separation of concentrate—99.5% fat	0.02	0.02

Plant A. Data on the various products in plant A indicate that considerable Cu contamination occurred in the creamery. The cream had a low original Cu content but as it entered the separator the Cu content was three times greater than the original amount. After separation, the Cu in the concentrate was six times greater than the original amount, indicating significant contamination between the cream receiving vat and the composition control vat.

Standardization with water did not alter the Cu content enough to be significant. However, the first butter through the printing machine had a much higher Cu content than did the last, indicating that the first product absorbed most of the free Cu. The last butter through the printing machine had the same Cu content as the standardized concentrate.

Plant B. Data from plant B indicate that most of the Cu in the raw cream passed into the skimmilk in the separation process, because the Cu content of the concentrate was less than one-fifth of the original. No Cu contamination of the butter took place in the process.

Plant C. Data from plant C indicate again that most of the Cu present in cream was eliminated in the separation process, because the Cu content of the concentrate was only one-fourth as much as that of the cream. These data also indicate that no contamination of the product occurred until after printing and packaging. Since the butter was wrapped in parchment, some contamination could have occurred in the printing and wrapping processes.

Plant D. Data for plant D indicate that much Cu contamination took place in the creamery itself, as the cream entering the separator had a Cu content almost four times greater than the content of the raw cream. However, the important fact is that almost all of the Cu present was eliminated in the first separation of the cream. The fat concentrate was reseparated into butter oils containing 99.0 and 99.5 per cent fat. In both of these products the Cu content was extremely low, being 0.04 and 0.02 ppm., respectively. This indicates that separation removed most of the Cu and that it was not dissolved in or combined with the fat.

SUMMARY

A method for measuring the Cu content of butter has been presented that has been found satisfactory.

The Cu in milk products has been found to be associated with the non-fat portion rather than the fat phase.

In two cases out of three, butters made by the continuous process contained less Cu than the original cream when the Cu content was expressed on the fat basis.

Butter made by the continuous process in four commercial creameries contained less Cu than butter made by the batch system in the University dairy plant.

Eleven samples of butter made by the continuous process were found to contain from 0.12 to 0.27 ppm. of Cu on a total weight basis with an average of 0.19 ppm.

The centrifugal treatment given cream in the process of making butter by the continuous method results in concentration of the Cu in the non-fat portion.

Butter oils made by the centrifugal process in the continuous method of making butter contain only minute traces of Cu.

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THE LIBERATION OF WATER-INSOLUBLE ACIDS IN CREAM BY *GEOTRICHUM CANDIDUM*

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Recently attention has been directed to the lipolytic reaction in cream because the extent of lipolysis, as measured by the accumulation of water-insoluble acids (WIA), has been proposed as a means of evaluating the quality of cream and butter (6).

Numerous studies have demonstrated that lipolysis of butterfat in raw cream results from the action of lipoclastic microorganisms and/or natural milk lipases. Lipolytic microorganisms, including *Geotrichum candidum*, have been considered by some authors (3) to be of greater significance than milk lipase as a cause of fat hydrolysis in raw cream. Other investigators (8) have revealed that the natural milk lipases may cause significant increases in the WIA values of cream. Peters *et al.* (9) reported that the types of microorganisms, the amount of lipase present and the time and temperature of cream storage determine the degree to which WIA values change in a given sample of raw cream.

Hillig *et al.* (6) attributed the inferior quality of some cream to improper production practices and reported that the rate of cream decomposition depends chiefly on the storage temperature. Water separation was considered also by these authors to be a contributing factor to early decomposition of cream.

Babel (2) was unable to find a significant difference in the WIA content of butters made from raw cream held at 55 or 75° F. for periods up to 10 days. The same author indicated that when cream sours rapidly, the lipases are inhibited because of the unfavorable pH. In addition, cream having a low acidity and containing putrefactive types of microorganisms usually produced butter with a high WIA content.

Many investigations dealing with factors influencing the growth of mold in cream and its significance in cream quality have been reported and summarized (4). However, data demonstrating the release of WIA in cream by the action of specific organisms are very meager. The purpose of this report is to present data which indicate that certain environmental factors have a profound influence upon the rate at which WIA are released in sterilized cream by the action of *G. candidum*. The data obtained serve to emphasize the lipolytic potential of this commonly encountered dairy contaminant and demonstrate that *G. candidum* is capable of releasing unusually large quantities of WIA from the butterfat in cream.

EXPERIMENTAL PROCEDURE

The rate at which *G. candidum* produces WIA from butterfat was studied by growing the test organism in sterilized cream incubated at 20° C. or other selected storage temperatures for a period of 9 days. WIA values of the samples were determined at 3-day intervals during the incubation period.

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The lots of pasteurized cream used in these experiments were obtained from the University of Illinois creamery and contained approximately 35 per cent butterfat. The WIA content was measured gravimetrically in accordance with the method described by Hillig (5). The mean molecular weight of the WIA was frequently determined as an analytical control procedure. The butterfat content of each sample was determined by the Babcock method (1).

The cream was dispensed into 1-l. Erlenmeyer flasks in 150-ml. amounts and sterilized at 121° C. for 15 min. The samples were cooled to incubation temperature and inoculated with 1-ml. portions of a standardized inoculum prepared from a 24-hr. agar slant culture of *G. candidum*. All inocula were standardized by diluting the suspension with sterile water to 97 per cent light transmission using a Lumetron colorimeter, model 400-A, equipped with a no. 530 filter.

The cultures employed in this investigation were from the culture collection of the Dairy Science Department, University of Illinois. *G. candidum* no. 13 was originally isolated from commercial cream and identified in this laboratory, while those cultures designated by alphabetical symbols were originally obtained from the Northern Regional Research Laboratory, USDA, Peoria, Illinois.

Duplicate flasks of cream were used for all analyses. The WIA content was determined on sterile control samples at the beginning and completion of each experiment. All results are recorded as milligrams of WIA per 100 g. of butterfat.

RESULTS

Storage conditions. The influence of the time and temperature of storage on the rate of WIA liberation in cream supporting the growth of *G. candidum* strain no. 13 is shown in table 1. The WIA values increased only slightly when cream samples were held at 4° C. for 9 days. Higher storage temperatures

TABLE 1

The effect of storage temperature on rate of WIA production in cream by G. candidum 13

Experiment no.	Storage temp.	Mg. WIA/100 g. fat after:				
		0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
	(° C.)					
I	4	175	230[a]	242	357	222
		....	227[a]	262	332	......
	10	257	453	4,370	4,913	264
		....	575	4,197	4,902	......
	16	253	2,687	10,387	13,550	259
		....	2,328	9,690	12,913	......
II	21	162	6,245	12,784	18,969	293
		....	5,407	12,722	18,147	......
	29	162	8,064	14,675	23,652	228
		....	7,188	14,533	22,132	......
	37	218	12,027	16,635	30,793	356
		....	10,086	15,627	28,742	......

[a] Values of duplicate flasks of cream shown for 3, 6 and 9 d. of storage.

up to 37° C. increased the rate of WIA formation in a manner directly proportional to the increase in incubation temperature. The WIA values also were proportional to the length of storage time. The volatile acidity of the cream samples did not increase during the 9-day incubation period. Therefore, this analysis was not repeated in other experiments.

In actual practice cream may be subjected to varying storage temperatures during production and shipping operations. Thus, an experiment was designed to test the influence of secondary storage temperatures on the rate of WIA formation in samples of cream where growth and lipolysis previously had been established by primary incubation. For this purpose, cream cultures of *G. candidum* were prepared in the usual manner. Mycelial growth was established by incubation at 27° C. for 2 days, after which the cultures were stored at 4, 10, 16, and 27° C. for 2 additional days. The WIA values increased from 244 to 4,978 mg. per 100 g. fat during the first 2 days storage at 27° C. Two additional days' storage at 27° C. resulted in an additional increase of 4,091 mg. WIA, whereas the WIA values of samples stored at 4, 10 and 16° C. for 2 additional days increased 520, 1,515, and 2,645 mg., respectively, per 100 g. fat. These data show that storage at lower temperatures, even after appreciable mold growth and fat hydrolysis had occurred, retarded the subsequent rate of WIA production. Secondary storage at 4° C. resulted in almost complete inhibition of lipolysis, whereas only a slight depression of rate occurred at 16° C.

Cream acidity. The influence of cream acidity on WIA production was tested by comparing the rate of WIA formation in low-acidity (0.13 per cent) cream with that in cream acidified with lactic acid prior to inoculation. As table 2 shows lower WIA values were obtained in the samples adjusted to 0.50

TABLE 2

The effect of cream acidity on rate of WIA production by G. candidum 13

Initial cream acidity	Mg. WIA/100 g. fat after:				
	0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
(%)					
0.13	155	11,777	15,357	19,318	160
0.50	155	8,381	12,873	15,665	156
0.88	155	2,064	8,120	10,475	160

and 0.88 per cent lactic acidity than in the unacidified flasks. The decrease in WIA levels was directly proportional to the initial lactic acid content of the cream. However, after 3 days of incubation, the acidified samples contained sufficient WIA to indicate extensive fat hydrolysis. Apparently *G. candidum* is capable of causing lipolysis in acidified cream. This conclusion is supported further by the fact that essentially the same results were obtained when *G. candidum* was cultivated at 20° C. in association with *Streptococcus lactis* and *Lactobacillus casei.* The 3-day WIA values for cream supporting *G. candidum* in association with either *L. casei, S. lactis* or *S. lactis* and *L. casei* together

were 6,608, 5,366 and 5,996 mg. per 100 g. of fat, respectively. The titratable acidities of these samples were 0.80, 0.84 and 0.91 per cent. After 9 days these values had increased to 11,194, 8,861 and 9,886 mg. WIA per 100 g. of fat, and 1.56, 1.29 and 1.50 per cent titratable acidity. The WIA production by *S. lactis* and *L. casei* in the absence of *G. candidum* was nil. These results show that considerable quantities of WIA were produced by *G. candidum* in acid cream.

Surface area. The growth of *G. candidum* is largely confined to the surface of an undisturbed medium. Consequently, development of this organism can be limited by restricting the cream surface area. This in turn should cause a decrease in the rate of WIA production. To test this possibility, constant volumes of cream were added to various flasks so as to present widely different surface : volume ratios. The samples then were inoculated equally and incubated at 20° C. The results presented in table 3 demonstrate that the lipolysis rate

TABLE 3

The influence of cream surface area on the rate of WIA production by G. candidum 13

Surface area of cream	Mg. WIA/100 g. fat after:				
	0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
(in.²)					
7	216	2,380	4,882	7,540	240
12.6	222	6,854	10,886	19,204	247
25.8	224	10,406	14,613	25,857	240

in a constant volume of cream varied directly with the surface area, and lipolysis was greatly retarded by restricted surface area.

Culture variation. Great variations are encountered in cultures classified as *G. candidum*. Distinct differences in morphology and biological activity are to be expected. The data shown in table 4 illustrate the differences in the

TABLE 4

Comparison of WIA values produced by 9 strains of G. candidum grown in 35% cream

Culture	Mg. WIA/100 g. fat after:			
	0 d. (Sterile)	3 d.	6 d.	9 d.
Sterile control	220	....	....	240
G. candidum 13	....	8,770	18,550	25,360
" A	....	7,770	14,500	23,060
" B	....	7,980	17,260	23,750
" C	....	8,630	17,370	24,940
" D	....	260	510	1,670
" E	....	4,090	12,180	16,410
" F	....	6,960	13,360	19,750
" G	....	4,610	13,140	17,610
" H	....	5,680	13,390	18,240

lipolytic activity of nine cultures of *G. candidum*. Eight of the nine cultures caused a rapid and extensive hydrolysis of the butterfat in cream. Four

cultures yielded WIA values ranging from 23,000 to 25,000 mg. per 100 g. of fat. The values obtained from four other cultures ranged from 16,000 to 19,000 mg. per 100 g. of fat. Although strain D was lipolytic, it hydrolyzed the fat at a comparatively slow rate. This strain also grew poorly on laboratory media.

A comparison of WIA values produced in cream by *G. candidum*, *Candida lipolytica* and three unidentified lipolytic bacterial species isolated from commercial cream is shown in table 5. These data emphasize the rapid accumulation of WIA in cream contaminated with *G. candidum* or *C. lipolytica*, as compared with several other natural contaminants of cream.

TABLE 5

WIA values produced by lipolytic microorganisms

Organism	Mg. WIA/100 g. fat after:				
	0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
G. candidum	160	4,175	12,049	21,010	160
C. lipolytica	....	2,750	10,120	16,670	....
Species A (gram-rod)	....	1,023	2,910	7,410	....
Species B (gram-rod)	....	887	1,785	3,192	....
Species C (gram-rod)	....	706	1,244	2,642	....

Extent of contamination. Production sanitation and storage conditions are among the many factors influencing the mold content of cream. The original contamination of cream by *G. candidum* may be slight or extensive, depending upon the level of production sanitation. Accordingly, an experiment was designed to test the influence of the extent of contamination on the rate of WIA production. Samples of cream were artificially contaminated at different levels by inoculation with 1.0 ml. of a *G. candidum* suspension containing either 48,000, 4,800 or 48 organisms per milliliter. The calculated final concentrations of *G. candidum* in the three series of samples were 320, 32 and less than 1 per milliliter of cream, respectively. The WIA levels in cream having a calculated original plate count of less than one colony of *G. candidum* per milliliter did not change appreciably during the first 3 days of incubation at 20° C. (table 6). Only a moderate increase in WIA liberation occurred between the third and sixth day of storage. Maximum lipolysis occurred between the sixth and ninth day. Other samples having calculated original *G. candidum* plate counts of 32

TABLE 6

The influence of the extent of contamination on the rate of WIA production by G. candidum 13

Plate count of inoculum /ml.	Mg. WIA/100 g. fat after:				
	0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
48	250	299	1,853	11,537	253
4,800	....	2,468	11,251	19,671	....
48,000	....	5,656	15,722	22,392	....

and 320 underwent rapid and extensive fat hydrolysis, although a short lag in WIA formation was encountered in the intermediate samples. In all instances the rate of WIA production was essentially proportional to the extent of contamination.

Utilizable carbohydrates. Usually the growth of pure cultures of *G. candidum* in milk and other lactose-containing media is not accompanied by an acid reaction resulting from carbohydrate degradation. Under such conditions, maximum levels of lipase are encountered. The addition of utilizable compounds, such as glucose or glycerol, causes increased growth but decreased levels of lipase (7). An experiment was conducted to test the effect of utilizable carbohydrates, such as glucose, upon the rate of WIA production in cream. The decrease in the rate of WIA production in cream containing glucose is illustrated in table 7.

TABLE 7

The effect of glucose on the rate of WIA production by G. candidum 13

Glucose added	Mg. WIA/100 g. fat after:				
	0 d. (Sterile)	3 d.	6 d.	9 d.	9 d. (Sterile)
(%)					
0	244	8,514	11,431	16,800	285
3.3	233	2,158	3,685	5,751	257
6.6	229	1,850	3,128	4,564	260

DISCUSSION

The foregoing experiments demonstrate that those conditions which are known to influence the growth of *G. candidum* (4) also influence the rate of WIA formation by this organism. Under most conditions *G. candidum* produced an extremely rapid and progressive decomposition of the fat in cream. In some instances WIA values as high as 20,000 to 25,000 mg. per 100 g. of fat were encountered. This represents approximately 22 to 28 per cent hydrolysis of the fat.

The observed WIA values produced by *G. candidum* were of such a magnitude as to be of importance in cream quality, especially if one assumes that the WIA values of butter correspond approximately to those of the cream used in its manufacture and that values in excess of 400 mg. of WIA per 100 g. of butterfat are indicative of decomposition of cream resulting from improper production and handling practices.

SUMMARY

Data which emphasize the lipolytic potential of pure cultures of *G. candidum* have been presented. These data show that eight of nine test cultures produced a rapid and extensive hydrolysis of butterfat in cream.

The rate and extent of WIA liberation in sterilized cream increased with incubation time, storage temperature, inoculum size and cream surface area. This rate decreased in direct proportion to the added lactic acid content of the

cream. The addition of 3.3 per cent glucose to cream markedly decreased the rate and extent of WIA formation.

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ADAPTATION OF THE BARKER–SUMMERSON LACTIC ACID METHOD TO ICE CREAM AND INGREDIENTS FOR ICE CREAM

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There has been a need for a reasonably fast, dependable method for determining lactic ion in ice cream, ice milk and related dairy products. Such a method is particularly needed in the case of ice cream containing alkaline stabilizing salts, where it is difficult to obtain a quantitative measure of developed acidity by direct titration. The Hillig (7, 9) method, now the official method of the A.O.A.C., while quite reliable, is time-consuming and can be used for relatively few samples a day. Troy and Sharp (11) stated that their method was not applicable to sweetened products. The simplified $FeCl_3$ procedure of Venekamp and Kruisheer (12) is not sufficiently sensitive in the intermediate and lower ranges of developed acidity. The work of Heinemann (6) and of Tiersma and Venekamp (10) with the veratrole method indicated that the color development is hard to control. Barker and Summerson (1) presented a very sensitive and specific method for lactic acid in biological fluids, based on the color reaction between p-hydroxydiphenyl and the acetaldehyde formed by the oxidative decarboxylation of lactic acid. Cupric ions were used to intensify the color obtained and a high degree of specificity was obtained by careful treatment of the sample to remove interfering substances. Davidson (2) applied the Barker-Summerson method to milk and milk powders, obtaining very good results, but they did not present any data on sweetened products. The modification used by Davidson—simultaneous deproteinization and removal of lactose by lime and $CuSO_4$—cannot be used for the removal of interfering substances from a sweetened or flavored product. The original Barker and Summerson method has been applied to ice cream, and to its ingredients with satisfactory results. A technician, using comparatively simple and inexpensive equipment, can make 30 analyses in an 8-hr. day.

METHOD

The Barker and Summerson method was followed in all essential details, which are discussed thoroughly in their original publication (1). Particular attention must be paid to the precautions, some of which are included in the following discussion. For example, contact of the sample with skin surfaces, directly or indirectly, can add as much lactic acid as was in the sample originally.

The basic steps in the procedure are: (a) Removal of protein by precipitation with Na_2WO_4 and H_2SO_4. (b) Removal of interfering substances from the protein-free filtrate with $CuSO_4$ and $Ca(OH)_2$. (c) Conversion of the lactic acid

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to acetaldehyde by heating in concentrated H_2SO_4. (d) Development of color through the reaction of the acetaldehyde and p-hydroxydiphenyl in the presence of Cu ions. (e) Measurement of the color in a photoelectric colorimeter equipped with a filter giving maximum transmission at 565 mμ.

The deproteinization was carried out by the Folin and Wu Na_2WO_4–H_2SO_4 procedure, as outlined in Hawk *et al.* (5). A Cenco photelometer was used for color measurement. A Rubicon #565 filter was easily installed after removing the regular filter carrier. The effective diameter of the cell used in the photelometer was 1.5 cm., instead of 1.0 cm. as in the instruments used by Barker and Summerson. This necessitated using 9 ml. H_2SO_4 in step *b*, instead of 6 ml.

Preparation of standard curve. Lithium lactate is made by the following procedure: USP lactic acid (85 per cent) is diluted with an equal volume of distilled water and a few drops of phenol red indicator are added. A saturated solution (approximately 20 per cent) of C.P. LiOH is added to slight excess, as indicated by the phenol red. The solution is heated to boiling and the alkali again added to slight alkalinity before cooling. Four volumes of 95 per cent alcohol are added and, after cooling for some time, the mass of crystals is floated on a Buchner funnel and washed thoroughly with 95 per cent alcohol. The lithium lactate is recrystallized from water and dried at 100° C.

The stock standard solution of lithium lactate for the standard curve is prepared by dissolving 0.213 g. of pure dry lithium lactate in about 100 ml. of distilled water in a 1-l. volumetric flask, adding about 1 ml. of concentrated H_2SO_4 (C. P.), then diluting to the mark and mixing. This is stable if kept in a refrigerator. It contains 1.0 mg. of lactic acid in 5 ml. of solution. The dilute standard is made by measuring 10 ml. of the stock standard into a 100-ml. flask and bringing to volume with distilled water and mixing. This preparation contains 0.020 mg. per milliliter and must be prepared fresh before use.

Several portions of the dilute standard are measured into separate centrifuge tubes covering the range of 0.01 to 0.10 mg. of lactic acid. The procedure is started at the Cu-lime stage after bringing all volumes to 9.0 ml. with distilled water. The final readings are plotted on semilog paper against the micrograms of lactic acid present in the 1 ml. of the supernatant from the Cu-lime treatment. The series is repeated to insure accuracy.

A straight line is obtained if micrograms lactic acid are plotted against per cent transmission on semi-logarithmic paper. Barker and Summerson prefer running known levels of lactic acid with each set of unknowns in order to obtain a proportionality factor from which the lactic acid content may be calculated. However, if care is taken in performing the test in exactly the same way from day to day, a calibration curve is quite satisfactory. The curve and the technique are checked occasionally by running a sample of known lactic acid content.

Calculation of results. From the standard curve read the micrograms of lactic acid present in the 1-ml. aliquot taken from the Cu-lime supernatant. A convenient general formula for calculating the results is:

$$\text{(a)} \quad \frac{100}{\text{grams of sample contained in aliquot to be deproteinized}} \times \frac{100}{\text{ml. protein-free filtrate used}} \times \frac{\text{micrograms read from curve}}{1{,}000} = \text{mg./100 g. ice cream or other product}$$

This formula may be used to calculate the lactic acid level in the sample of original dairy product either when the amount of sample deproteinized is different from that suggested, or when the milliliters of protein filtrate used are varied from that suggested. If the exact quantities are used, as suggested for the ice cream analysis, the formula simplifies to the following:

(b) $5 \times$ micrograms read from standard curves = mg./100 g. original material and

(c) $0.001 \times$ mg./100 g. original material = percent lactic acid in original material

Procedure for ice cream. Weigh out 20.0 g. of ice cream into a 100-ml. beaker on an accurate torsion balance. Add some distilled water and allow to soften. Transfer with washing to the 100-ml. volumetric flask and make up to volume with distilled water. After thorough mixing transfer a portion to a dry beaker for pipetting. With an Ostwald pipette transfer 5 ml. of the ice cream solution (representing 1 g. of ice cream) to a 22×145 mm. test tube. Add exactly 3.0 ml. of distilled water from the burette. Add 1.0 ml. of 10 per cent solution of C. P. $Na_2WO_4 \cdot 2H_2O$ solution and swirl to mix. Add 1.0 ml. of 0.66 N H_2SO_4 solution, 2 to 3 drops at a time, swirling between additions. (Precipitation should begin when about 0.8 ml. have been added.) Let stand 10 to 15 min.

Filter through Whatman #5 filter paper, being careful to wear rubber gloves when preparing the paper (see Precautions). Transfer 2.0 ml. of the filtrate to a 15-ml. centrifuge tube. Add 1.0 ml. of a 20 per cent solution of $CuSO_4 \cdot 5H_2O$. Add 7 ml. of distilled water from the burette. Add approximately 1 g. of C. P. $Ca(OH)_2$ powder, using a small piece of parchment paper to aid in the transfer. Shake vigorously after closing the tube by means of a square of parafilm, fresh surface down, held in place by the finger. Let stand at room temperature for 0.5 hr. with occasional shaking. Centrifuge at 1,200 to 1,500 r.p.m. for about 7 min. to pack the solids.

Pipette a 1.0-ml. aliquot of the supernatant solution (the sample should be pipetted from beneath the surface film which usually is present, and the tip of the pipette wiped free of any adhering solids before transferring) to a 22×145 mm. test tube. Add 1 drop (0.05 ml.) of 4 per cent $CuSO_4$ solution. Finally, add 9.0 ml. of concentrated H_2SO_4 from a burette, swirling the tube to mix while the acid is being added. Place upright in boiling water bath for 5 min. Remove and cool immediately in ice water to 20° C. or below. When cold, add 2 drops (0.1 ml.) of the p-hydroxydiphenyl reagent, so that the drops fall directly upon the acid surface. (The p-hydroxydiphenyl is a 1.5 per cent solution in 0.5 per cent NaOH. It is prepared by dissolving, by means of warming and stirring, 1.5 g. dry p-hydroxydiphenyl[2] in 10 ml. of 5.0 per cent NaOH to which has been

[2] Eastman Kodak.

added from 10 to 20 ml. distilled water. The solution is cooled and made up to 100-ml. volume. Stored in a brown bottle, the solution is stable for months. High blanks indicate deterioration.) Swirl the tube carefully but thoroughly to disperse the precipitated p-hydroxydiphenyl uniformly throughout the solution. Place the tube in a 30° C. Water bath for 30 min., swirling to mix at least once during this period. After 30 min. at 30° C. transfer to a boiling water bath for 90 sec. Remove and cool immediately to room temperature with ice water. Fill the photelometer absorption cell to the required level with the resulting liquid and take reading in the Cenco photelometer equipped with a Rubicon 565 mμ filter. A reagent blank should be run through the procedure with each set of samples. Start reagent blank using 9.0 ml. water, 1.0 ml. 20 per cent $CuSO_4 \cdot 5H_2O$, 1 g. $Ca(OH)_2$ powder and continue through remaining steps of the procedure. This blank, which should be colorless, is used to set the photelometer at 100 per cent transmission.

The standard curve is calibrated in terms of the micrograms of lactic acid present in the 1-ml. aliquot taken from the Cu-lime supernatant. This aliquot should contain between 1 and 10 γ of lactic acid. If a sample of ice cream is known to be made of high quality ingredients, a 1:50 dilution of a 1-g. sample is most suitable, as outlined in the procedure. If the quality of the ice cream is unknown, a 1:100 dilution, or 1 ml. of the filtrate from the protein precipitation, should be used in the Cu-lime treatment. This dilution will cover all samples containing between 10 and 100 mg. lactic acid per 100 g. ice cream. Occasionally, further dilution may be necessary; in this case the filtrate from the protein precipitation should be diluted before transferring an aliquot to the Cu-lime.

Procedure for ice cream ingredients.

(a) *Dairy products.* The procedure which has been outlined in detail for ice cream, or the simplified method of Davidson (2) may be applied to any of the unsweetened dairy ingredients. The level of milk solids not fat must be known in order to determine the sample size of dairy products. Thus, while a 20-g. sample of ice cream was used, convenient samples for other products were: Cream (36 per cent fat), 40 g.; condensed skim (32 per cent T.S.), 7 g.; nonfat dry milk solids, 2.2 g.; fluid whole or nonfat milk, 25 g.

(b) *Sugar and flavoring.* The solutions are made up to have the same concentration that they would have in ice cream. Start the analysis with the Cu-lime step, using a 2-ml. aliquot.

RESULTS

Reliability of method.

(a) *Recovery of added lactate.* A standard lithium lactate solution equivalent to 1 mg. of lactic acid per milliliter was prepared. The desired volume of lactate solution was added to 20-g. samples of ice cream, the volume made up to 100 ml. with distilled water and the analysis carried out in the usual way. In typical recovery tests, 10, 20, 30, 40, 50 and 75 mg. lactate were added to

100-g. samples of ice cream. The recoveries were 10.5, 19.5, 29, 41.5, 51.5 and 75.5 mg., respectively. The results obtained by the use of a standard curve compare favorably with those reported for the Hillig method (4).

(b) *Values on milk compared with published values.* Values obtained by Gould (3) on milk from individual cows of different breeds were compared with values obtained on eight samples of manufacturing grade milk received at a commercial condensing plant. Gould found an average of 1.95 mg. lactic acid per 100 g., whereas the average of the condensing plant samples was 2.5 mg. per 100 g. Troy and Sharp (11) found an average of 2.0 mg. per 100 g. fluid milk by their method.

TABLE 1

Lactic acid values for ingredients and final product in two similar unflavored mixes

Ingredients	Level in mix		Lactic acid[a] contributed to mix		Lactic acid[a] of mix by analysis of complete mix	
	Mix A	Mix B	Mix A	Mix B	Mix A	Mix B
	(% by weight)		(mg./100 g.)		(mg./100 g.)	
Nonfat dry milk solids	5.0	5.0	1.4	1.4		
Nutrimix (Nonfat dry milk solids containing stabilizing salts)	5.5	5.5	2.4	2.4		
Cream (36% butterfat)	30.50	30.50	0.4	0.4		
Gelatin	0.20	0.20	0	0		
Dariloid	0.15	0	0	–		
CMC	0	0.15	–	0		
Liquid sugar (66.5% syrup)	24.0	24.0	7.6	7.6		
Water	34.65	34.65	0	0		
Total	100.00	100.00	11.8	11.8	12.5	11.7

[a] Lactic acid values for individual ingredients and for complete mixes, as given in last column, were determined by the Barker-Summerson Method.

(c) *Effect of sugar and flavorings.* Table 1 shows the results of the application of the method to complete mixes and to a number of major ingredients separately. The composition of the mix is shown, with the lactic acid values obtained by analysis and those predicted from the results of the separate analyses of individual ingredients. Actual and predicted analyses agree closely.

As a result of the apparent lactic acid value of 7.6 mg. per 100 g. for the liquid sugar at the level used in the mixes, a series of tests was carried out on different sugar sources. The range of values on five sugars, including cane syrup, cane sugar and beet sugar, was 7.6 to 9.0, with an average value of 8.4 mg. per 100 g. Since the effect of the sugar appears to be consistent and additive, an allowance of 8.5 mg. per 100 g. "lactic acid" will correct for the sugar in ice cream of standard composition (14–16% sugar).

Table 2 shows the lactic acid value of ice creams prepared from a single mix and flavored with different vanillas, chocolates, coffee and strawberry. The only increase of any magnitude is in the case of vanillas 2 and 3; analysis of solutions of these flavorings gave values almost identical with the increase. These two vanillas were found to contain 15 per cent propylene glycol in addi-

tion to alcohol. Propylene glycol is one of the seven compounds listed by Barker and Summerson as interfering with the lactic acid test, 1 γ giving a color equivalent to 0.16 γ of lactic acid. The apparent lactic acid due to the vanilla may be calculated if its composition is known, or it may be tested if a sample is available.

The lactic acid value was increased measureably by the two chocolates, but the increase was less than that reported by Gould and Potter (4) using the Hillig procedure. The coffee and strawberry flavors contributed very little to the lactic acid value, whereas Gould and Potter found that chocolate and coffee

TABLE 2

Lactic acid values for ice creams of various flavors made from the same basic mix

Flavor		Lactic acid value of mix	Increase in lactic acid value caused by flavoring ingredients	
	Level in mix		By difference	By analysis
Description		(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
None	....	12.5	.....	....
Vanilla #1. Pure Bourbon alcohol extract	3.25 fluid oz. in 5 gal. mix	13.5	1.0	....
Vanilla #2. Single strength extract containing 15% propylene glycol	"	22.5	10.0	10.5
Vanilla #3. Double strength extract containing 15% propylene glycol	"	27.5	15.0	16.0
Chocolate #1. American processed cocoa	3.0%			
Chocolate liquor (50% cocoa fat)	1.5%	16.5	4.0	....
Chocolate #2. Dutch processed cocoa	3.0%			
Chocolate liquor (50% cocoa fat)	1.5%	17.2	4.7	....
Coffee. Water extract containing 8% coffee solids and 7% dextrose	40 fluid oz. in 5 gal. mix	13.5	1.0	....
Strawberry. Fruit sugared 1 : 3	1 gal. fruit mixture to 5 gal. unflavored mix	12.0	−0.5	....

extract caused increases in the lactic acid values amounting to 7.1 and 5.6 mg. per 100 g., respectively, using the Hillig method. Gould (3) reports interference with the Hillig method from sodium benzoate, which commonly is used in coffee extracts.

Table 3 gives both the titratable acidity and lactic acid for a group of unflavored and flavored test mixes.

(d) *Effect of neutralization.* Davidson (2) showed that his simplified Barker and Summerson procedure recovered lactic acid quite dependably from neutralized milk. Gould (3) found that the Hillig method gave slightly increased values on neutralized milk, and Hillig (8) found that the Troy and Sharp distillation

TABLE 3

Comparison of apparent titratable acidity with actual lactic acid in various ice cream mixes

Mix no.	Composition of mix				Comments	Apparent titratable acidity	Lactic acid found	Lactic acid corrected[a]
	Fat	MSNF	Sugar	Flavor				
	(%)	(%)	(%)			(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
1	11.0	10.5	16.0	None	5.5% serum solids from Nutrimix.	125	12.5	....
2	11.0	10.5	16.0	None	"	120	11.7	....
3	11.0	10.5	16.0	Vanilla	Same s.s. Pure Bourbon vanilla 3.25 fl. oz./ 5 gal. mix.	120	13.5	....
4	11.0	10.5	16.0	Vanilla	Same s.s. Vanilla containing 15% propylene glycol. Same level as *3*.	155	22	12
5	11.0	14.0	16.0	Vanilla	Same s.s. Same vanilla as *4*.	120	22	12
6	11.0	14.0	16.0	Vanilla	Serum solids all from condensed skim. Same vanilla as *4*.	195	24	14

[a] 1 mg. propylene glycol gives color equivalent to 0.16 mg. lactic acid.

TABLE 4

Barker and Summerson method applied to ice cream made from a soured, neutralized mix

	Apparent titratable acidity		Lactic acid	Increase in acidity over control
	(%)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
Control mix	0.22	220	13.5	....
Soured mix	0.35	350	....	130[a]
Soured mix neutralized with $MgCO_3$	0.23	230	140.0	126.5[b]

[a] Difference in titratable acidity values.
[b] Difference in B&S lactic acid values.

method gave somewhat lowered values. Table 4 shows that neutralization has no effect on the accuracy of the determination.

(e) *Preservation of samples.* Formaldehyde cannot be used as a preservative, as Barker and Summerson found that it interfered with their method. However, it is shown in table 5 that neither $HgCl_2$ nor sodium benzoate affected

TABLE 5

Comparison of lactic acid values on an ice cream with and without preservatives

Preservative	Amount of preservative /200 g. ice cream	Lactic acid value
		(*mg./100 g.*)
None	....	12.5
Commercial $HgCl_2$ (with dye)	1 tablet	12.0
C. P. $HgCl_2$	0.2 g.	12.0
Sodium benzoate	15 g.	12.5

the test significantly when added at the levels usually used for the preservation of dairy products.

(f) *Effect of lipolysis.* Hydrolytic rancidity of fat was found by Troy and Sharp (9) to cause high results with the distillation method. Gould (3) found no interference from rancid fat with the Hillig method. To test the effect on the Barker and Summerson method, 0.4 g. very rancid butterfat was added to 100 ml. skimmilk. The lactic acid value of the control skim was 2.7 mg. per 100 g. and that of the skim plus rancid fat was 2.8 mg. per 100 g., a difference within experimental error.

DISCUSSION

The Barker and Summerson method is practical for routine determinations of lactic acid in ice cream. The equipment necessary is not expensive, the method is fast, and the results are at least as good as those obtained by the Hillig method.

It is essential that the procedure be followed carefully. The standard curve should be checked from time to time, using the standard lactic acid solution. The analytical precautions outlined by Barker and Summerson are extremely important. Reagent grade chemicals should be used. The p-hydroxydiphenyl was obtained from Eastman Kodak and needs no purification. It is essential that the directions to cool the H_2SO_4 solution to 20° C. or below before the addition of the p-hydroxydiphenyl be insured by cooling until the tube is chilly to the touch after swirling.

Stopcocks in contact with the H_2SO_4 must be free from grease. Either a "greaseless" stopcock or a regular stopcock lubricated with graphite or with the acid itself should be used. The concentrated H_2SO_4 should be protected from organic matter and moisture. Since it must be measured exactly, a burette with provision for filling from the acid bottle with vacuum or pressure is best.

The pipettes and burettes used must be accurate and the analyst should be precise in all measurements. In addition, care should be taken that the skin does not come in contact with any glassware, filter papers or solutions which are used either in or in contact with the sample. The tips of pipettes are best wiped with fresh pieces of cleansing tissue which are discarded after one use. As mentioned before, parafilm is used, fresh surface down, when shaking in the Cu-lime step.

In cleaning the glassware, chromic acid cleaning mixtures should not be used unless followed by an alkaline rinse. It is preferred to use a detergent wash followed by rinsing in hot and then distilled water, taking care that the glassware is kept free from dust and perspiration. The centrifuge tubes need an extra wash, before soaping, with dilute HCl to remove the very tenacious lime.

SUMMARY

When applied to a wide variety of dairy products, the Barker and Summerson method for lactic acid has been found to be accurate and reasonably rapid.

It is accurate in unflavored ice cream when a constant allowance is made for the lactic acid value contributed by the sugar.

It is accurate in ice cream of various flavors, when the flavoring agent does not contain propylene glycol.

The lactic acid value of ice cream containing propylene glycol may be corrected by use of an accurate constant allowance, when the amount present is known.

Preservatives such as $HgCl_2$, pure or commercial grade with indicator, and sodium benzoate were found not to influence the lactic acid value by this method.

The presence of neutralizers in an ice cream mix will not interfere with the determination of developed lactic acid.

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A CLASSIFICATION FOR THE IDENTIFICATION OF BOVINE NOSEPRINTS[1]

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The problem of positively identifying cattle always has been difficult. Some breed associations have resorted to sketches and some to photographs, but sketches always have the element of error resulting from the poor artistic ability of the one sketching the animal. A more positive means of identification is by noseprints. Petersen (1) has suggested the ease of identifying noseprints of the same animal. However, a rapid means of classifying them would be essential for their rapid identification. Thus, this study concerns a workable classification for bovine noseprints.

MATERIAL AND PROCEDURE

The noseprints for this experiment were obtained from the college herd and from cattle on neighboring farms. The procedure and equipment used were similar to those described by Petersen (1). That is, ink from an ordinary ink pad was applied to the dry nose. Then a 4×7 inch no. 120 Blue Bird blotting paper was rolled against the nose to obtain a clear print.

METHOD OF CLASSIFICATION AND DISCUSSION

After careful consideration, a system similar to that of the fingerprint classification (2) was attempted. A glass disc having equi-distant concentric circles cut into the glass and marked from the center A, B, C, D, E and F was used. The disc was placed over a print which was read by determining the number of ridges in each area marked by the above designated letters. A study of the noseprints was made and it was found that no central point or core could be established for all noseprints; also, as the animal grew the nose enlarged. This made the use of the disc of no value, since the ridges on the nose became larger and fell into different areas on the disc.

Another attempt was made to establish a core or center point. The narrowest area was obtained on each print and a core established. This was accomplished by circumscribing a circle just bordering the outer edges of the narrowest area of the noseprint. In this manner the print was divided into four sections with the ridges counted in each section of the circle. As the print enlarged, the compass would compensate by drawing a larger circle from the narrowest part of the print which enlarged in proportion to the growth of the nose. How-

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[2] These data are taken from a thesis presented to the graduate school of South Dakota State College by Morris Hirsch in partial fulfillment of the requirement for the Master of Science degree.

ever, this system was unsuccessful too, even though it seemed to work well on different prints. It failed when duplicated on the same noseprint because the same center point was not consistently obtained.

Since there was no possibility in sight of developing a classification system

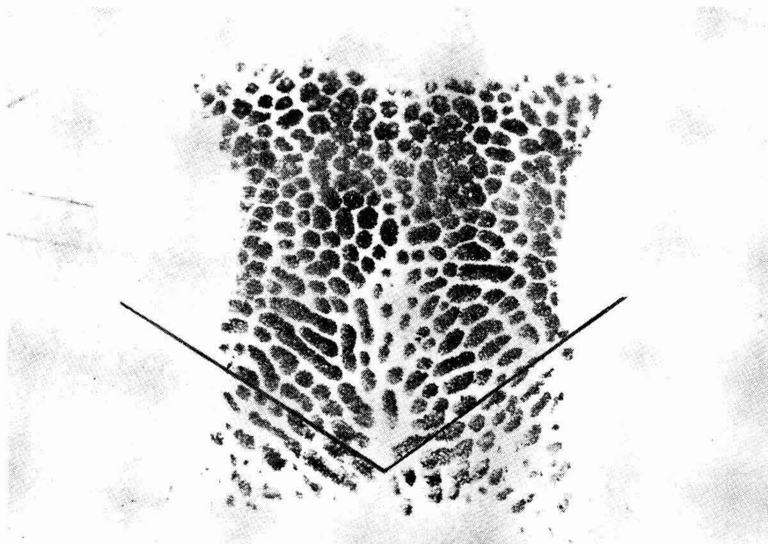

FIG. 1. Plain inverted arch is shown by angle of more than 90°.

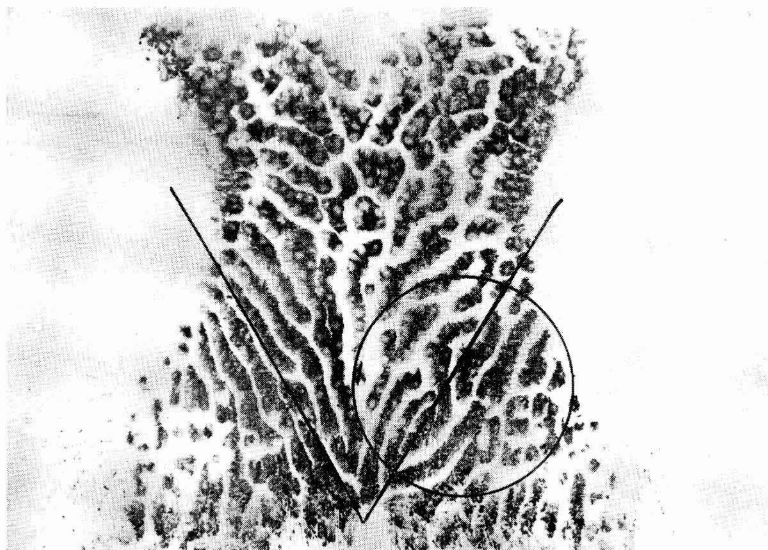

FIG. 2. Tented inverted arch is shown by angle of less than 90°. Area within circle indicates broken irregular ridges.

by means of a core, the system of using patterns was formulated. After an intensive study of the noseprints was made, four distinct general patterns were found in which the prints could be grouped. The patterns were classified according to three different types of inverted arches and no arch at all. The inverted arches were broken down to the plain inverted arch (fig. 1), the tented

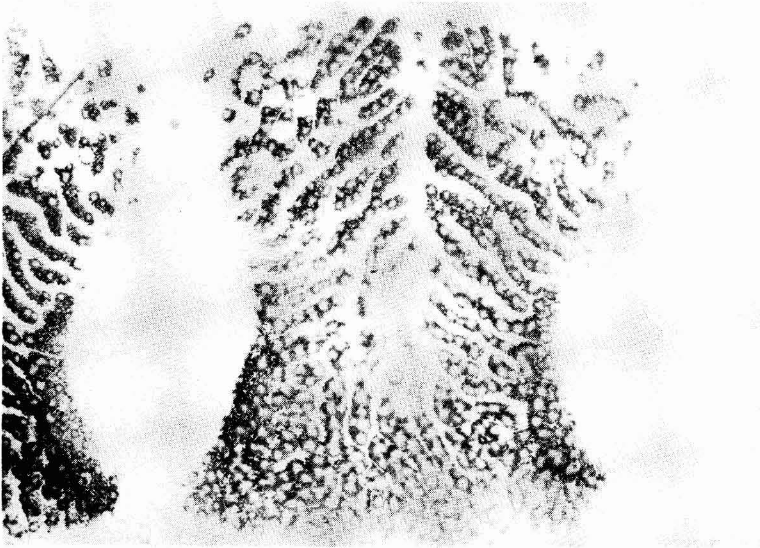

FIG. 3. Open inverted arch.

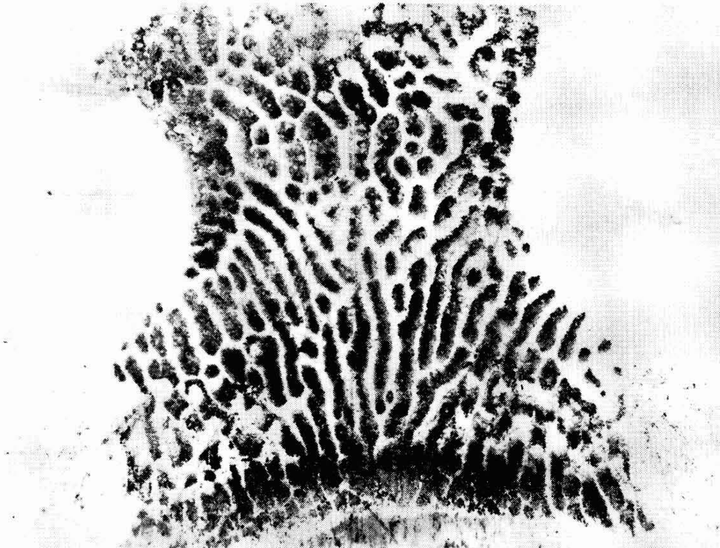

FIG. 4. No arch.

inverted arch (fig. 2) and the open inverted arch (fig. 3). The angle of the arch was determined by the ridges as they radiated to the left and right from the mid-line of the noseprint. The pattern with no arch is shown in figure 4. The plain inverted arch was characteristically recognized as being over 90 degrees. The tented inverted arch is one containing a rather sharp acute angle, being less than 90 degrees. The open inverted arch is a characteristic pattern that contains an inverted arch of varying magnitude with no definite apex, since the print has an open area down the mid-line from about the middle of the print to the base. In the pattern which contains no arch, there is no definite mid-line.

With these four groups established, there was need for further breakdown. Each print was divided in half down the center line, separating it into a left and a right side. All further characteristics then were broken down according to the two sides. The left side of the print was designated as numerator and the right side as denominator in the formula. Note that the left side of the print is from the right side of the nose.

Further breakdown was concerned with the types of ridges in the noseprint pattern. The ridges considered were only those from the lower half of the print. The first breakdown deals with the length of ridges. If the majority of ridges extended from the outer edge to the apex in one or two sections, they were considered long ridges designated by no. 1. If the ridges were broken into more than two sections they were considered broken ridges designated by no. 2. Then there were those prints where the ridges had a combination of broken ridges and long ridges in equal amounts; these were designated no. 3. Hence, figure 2 was classified: tented inverted arch 1/3 since it had a tented inverted arch and a no. 1 means that it contained long ridges on the left side and the no. 3 denominator refers to a combination of long and broken ridges on the right.

Continuing the breakdown of the ridges there were characteristic straight ridges, irregular ridges and combinations of both. These were broken down as were the previous traits by the nos. 1, 2 and 3, respectively, as shown in figure 2. The ridges on the left side were straight while those on the right were irregular. Thus, this print would now be classified: tented inverted arch 1/3 1/2.

The ridges were further classified as to whether they were all dots, all lines or a combination of both; these were designated by 1, 2 and 3, respectively. Since figure 2 has no dots it would be classified: tented inverted arch 1/3 1/2 2/2 with the last set of numbers referring to all lines on the left and right side of the print. Figure 5 is a representative example of a print with dots.

In addition to the classification of the animals by nose patterns there were two more groupings. One separated the prints by breed and the other separated them still further by sex. For example: figure 2 is a noseprint of a Guernsey cow. The entire classification would be as follows: Guernsey-female-tented inverted arch 1/3 1/2 2/2.

Since bovine noseprints have been used to establish positive identification (1) any means to facilitate identification would be highly desirable. Thus a simple

classification has been formulated. Of the 200 different noseprints inspected, only four appeared under one classification. Again, enough individual variations were evident so that no difficulty was encountered in distinguishing between the four prints. Actually the individual variations in the angle of the ridges are so exact and different as to be clearly differentiated. In addition, the relative lengths and arrangements of the ridges differ on each animal leaving unquestionably different nose prints.

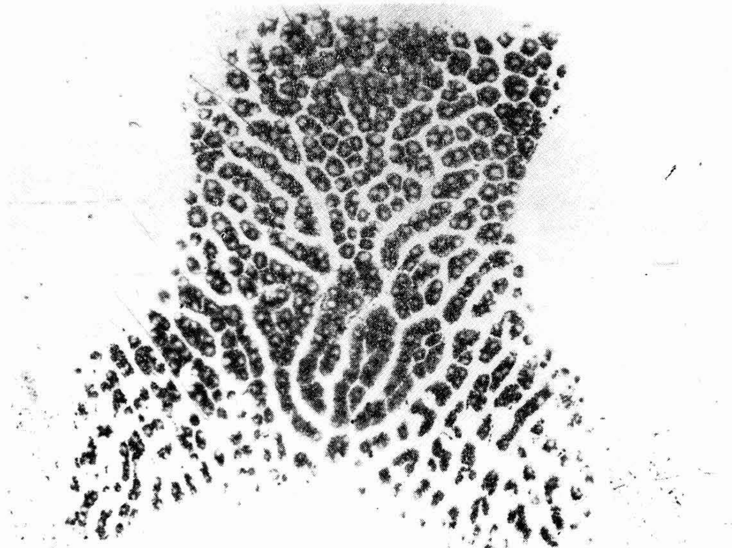

FIG. 5. A print with dots.

To further expedite the location of any print on file, a code has been completed so that the desired information may be obtained on any standard tabulating machine. Thus if noseprints should be filed with necessary registration certificates and at a later date a positive substitution should occur, the time required to determine the exactness or difference would be totally a matter of minutes. Therefore, large numbers of noseprints could be kept on file at all times.

Outline of the Classification System

A brief outline of the classifying system is as follows:

I. Breed

II. Sex

III. Patterns

- A. Plain inverted arch
- B. Tented inverted arch
- C. Open inverted arch
- D. No arch

IV. Types of ridges
(Note: The left side is designated by a numerator and the right side by a denominator.)

Group a: 1. Long ridges
2. Broken ridges
3. Combination of both

Group b: 1. Straight ridges
2. Irregular ridges
3. Combination of both

Group c: 1. All dots
2. All lines
3. Combination of both

SUMMARY

A method of classifying bovine noseprints by patterns has been described. According to this classification, it is possible to segregate the noseprints of any breed into 5,832 different groups. Thus, this allows only a few prints in each category, simplifying absolute identification. Even if several specimens have the same general classification, enough minute individual differences are present for an accurate decision.

ACKNOWLEDGMENTS

We are grateful for the assistance offered by C. O. Vickmark and L. Miller, finger print experts for the F.B.I. from Pierre, S. D., for their helpful suggestions in classifying the noseprints.

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THE RATE OF ABSORPTION OF VITAMIN A NATURAL ESTERS AND OF CAROTENE BY MALE HOLSTEIN CALVES AS MEASURED BY CHANGES IN BLOOD PLASMA LEVELS[1]

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Blood plasma levels have been used widely as criteria for the study of the effects of various sources and methods of administration of vitamin A and carotene in the nutrition of dairy calves. Baumann *et al.* (2) have shown that the peak of absorption of vitamin A is reached in 6 hr. in rats. Popper and Volk (8) found that absorption of vitamin A begins in rats 25 min. after administration and continues for a long time due to storage in the mesenchymal cells of the intestinal villi. Less information is available with respect to the rate of absorption of carotene, since some species of experimental animals do not absorb carotene into the blood stream. Barrick *et al.* (1) showed that massive doses of carotene resulted in only a trace of carotene in the blood plasma of sheep. Goodwin *et al.* (4, 5) have shown that while certain herbivorous animals do not have carotene in their blood stream, the bovine may have large amounts. Calves have considerable amounts of carotene in the blood plasma. It was the purpose of this experiment to study the rate of absorption of vitamin A and carotene and to ascertain the time interval at which a maximum concentration of each of these nutrients appeared in blood plasma following the administration of a single dose of either compound to young Holstein calves.

EXPERIMENTAL METHODS

Male Holstein calves were left with their dams for 24 hr. after which they were placed in individual pens in an artificially lighted and heated experimental calf barn. They are placed on a vitamin A- and carotene-deficient diet for a period of 7 days for stabilization. The ration used consisted of a mixture of 0.2 lb. dry whole milk replacement (9) (with the vitamin A constituent omitted), 1.8 lb. warm water and 3.0 lb. skimmilk fed twice daily. No hay, grain or additional fluids were made available to the calves.

After the completion of the stabilization period, the calves were assigned arbitrarily to one of four experimental groups. Group I received 1,216,000 I. U. vitamin A in the natural ester form; group II, 304,000 I. U. vitamin A in the natural ester form; group III, 1,275,000 I. U. vitamin A equivalent as carotene; and group IV, 319,000 I. U. vitamin A equivalent as carotene. Single doses of the vitamin A and carotene preparations were dispersed in the experimental diet. The total amount of oil was adjusted by adding commercial corn oil as needed

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so that each calf received a total of 5 g. of oil. Blood samples were drawn 1, 2, 4, 8, 12, 24, 48 and 72 hr. after administration of the test material and were analyzed for vitamin A and carotene. Two calves were observed in each of the high-dosage groups and three calves were subjected to each low-dosage treatment.

All calves were subjected then to a second stabilization period which was followed by daily oral administrations by capsule of 12,750 I. U. vitamin A equivalent as carotene to the one group of calves and 12,160 I. U. vitamin A in the natural ester form to the other. Blood samples were collected daily for 7 days, followed by weekly samples through the fourth week. The blood plasma was analyzed for vitamin A and carotene. The purpose of this latter investigation was to ascertain again the response of the same calves to the vitamin A and carotene preparations used in these experiments.

Blood plasma analyses for carotene were made according to the methods of Moore (7) and plasma vitamin A was determined according to Kimble (6). Measurements of light transmission were made with an Evelyn photoelectric colorimeter (3).

RESULTS

The blood plasma vitamin A and carotene levels after the administration of single doses of carotene are presented in figure 1. The administration of

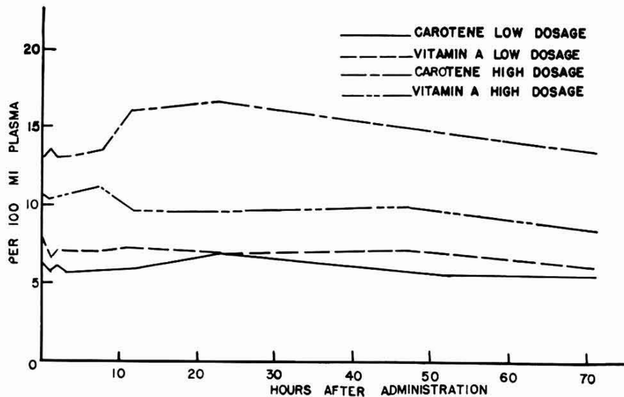

FIG. 1. Blood vitamin A and carotene levels after a single dose of carotene.

carotene at the low level had no effect upon blood levels of vitamin A or carotene during the 72-hr. observation period. The higher dosage of carotene appeared to increase blood plasma carotene slightly from 10 to 24 hr. after administration. However, the concentration of plasma carotene had receded to pre-administration levels by the end of 72 hr. and no apparent effect upon blood plasma vitamin A concentration was observed during this period.

The administration of vitamin A in single doses resulted in marked increases in blood plasma vitamin A, as shown in figure 2. At the highest level of dosage the peak of concentration appeared to be reached sooner, at about 4 to 8 hr. as compared to 8 to 12 hr. in the low-dosage group. Although both levels of dosage

of vitamin A resulted in about the same maximum blood plasma vitamin A concentrations, the calves receiving the lower dosage exhibited a less persistent increase in plasma vitamin A.

The blood plasma vitamin A and carotene concentrations resulting from

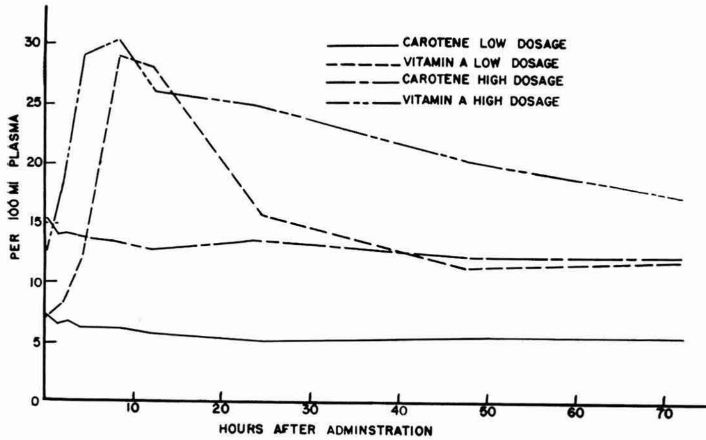

FIG. 2. Blood vitamin A and carotene levels after administration of single dose of vitamin A.

the daily administration of these compounds for a period of 4 wk. at levels approximating the daily recommended allowance are presented in figure 3. The administration of carotene resulted in a rapid increase of this compound in the

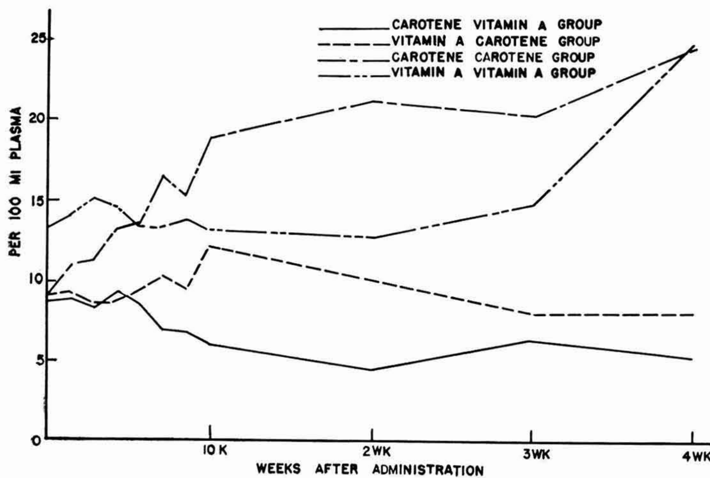

FIG. 3. Blood levels of vitamin A and carotene daily administration 10,000 I. U.

blood plasma during the first week, after which only a slower increase was exhibited. A slight increase in blood plasma vitamin A appeared by the first week as a result of the carotene administrations. The blood plasma concentra-

tion of vitamin A, however, had receded to the initial level by the end of the 4-wk. period of observation.

The administration of vitamin A resulted in an irregular increase in blood plasma vitamin A during the first week of observation. This was followed by a stable level for 2 wk. and finally a large increase during the last week.

SUMMARY AND CONCLUSIONS

The administration of carotene in single doses of 1,275,000 and 319,000 I. U. vitamin A equivalent had little or no effect upon blood plasma carotene, and did not appear to affect blood levels of vitamin A within 72 hr. after administration.

The administration of vitamin A in single doses of 1,216,000 and 304,000 I. U. resulted in about the same maximum blood plasma vitamin A levels, but the blood from the calves constituting the low-dosage group exhibited a less persistent increase.

The daily administration of vitamin A and carotene at levels approximating the daily recommended allowance for a period of 4 wk. resulted in increases of both of these compounds in the blood plasma. The initial response appeared to be somewhat faster in the carotene group. Only a small and temporary increase in blood plasma vitamin A resulted from the daily administration of carotene.

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PREPARATION OF MILK FAT.[1] I. A STUDY OF SOME ORGANIC COMPOUNDS AS DE-EMULSIFYING AGENTS[2]

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In dairy products research the need for preparing pure milk fat for such things as oxidation studies, determinations of fat constants, assays of fat-soluble vitamins and other trace materials frequently arises. Available methods for recovering fat from milk or cream are rather tedious. Destruction of the natural fat emulsion of dairy products and the separation or extraction of pure fat are made difficult by proteinaceous material, some of which is strongly adsorbed on the surfaces of the fat globules. One commonly employed procedure involves churning the cream to butter, melting and washing the butter until it is free of serum constituents and then drying the resulting oil. Other procedures utilize various fat solvents, either singly or in combination, to extract the fat. These, in order to be effective, must be made exhaustive either by several batch extractions or protracted continuous extraction. There is always the possibility that such procedures may be complicated by emulsion formation.

In an effort to develop more rapid and convenient methods of preparing pure milk fat, a rather large number of simple organic compounds was studied with regard to their capacity for destroying the natural emulsion in cream. This research led to the development of an aqueous reagent, containing *n*-butylamine and *n*-butanol, which has singular properties for de-emulsifying milk, cream and a number of other fluid dairy products.

EXPERIMENTAL

To 9 ml. of fresh cream (40 per cent fat) in a 15-ml. graduated centrifuge tube was added 1 ml. of the compound to be tested. The tube and contents were shaken vigorously for a few seconds to insure thorough mixing, then placed in an 82° C. water bath for 15 min., after which they were centrifuged for 5 min. at 1,800 r.p.m. The extent of de-emulsification then was observed.

The solubility in milk fat of the compounds tested varied widely. In addition, a precise line of demarcation between oil and aqueous phases could not always be obtained. For these reasons, effectiveness of the compounds has been expressed in terms of ranges in milliliters of oil layer as follows: effective, 3.5 to 4.5 ml.; partially effective, 1.0 to 3.5 ml.; ineffective, less than 1 ml. A tabulation relative to the performance of the various compounds studied is as follows:

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[1] The research reported in this series of papers relates to U. S. patent application no. 221, 723 by The Pennsylvania State College.

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Effective: *acids*—propionic, butyric, valeric, iso-valeric, caproic, heptylic; *alcohols*—*n*-propyl, iso-propyl, *n*-butyl, sec-butyl, tert-butyl, iso-amyl, furfuryl, benzyl, diethyleneglycol monobutyl ether; *aldehydes*—crotonic; *amines*—*n*-butyl, iso-butyl, *n*-amyl; *ketones*—methyl ethyl.

Partially effective: caprylic acid, heptyl alcohol (2-heptanol), furfural, *n*-butyric aldehyde, *n*-caproic aldehyde, methyl *n*-propyl ketone.

Ineffective: *acids*—acetic, capric, lauric, myristic, palmitic, stearic, oleic, levulinic; *alcohols*—*n*-octyl, diacetone, trimethylene glycol, glycerol, ethylene glycol; *aldehydes*—heptylic, salicyl; *amines*—ethanol, methyl (25 per cent aqueous), ethyl (70 per cent aqueous), diethyl, triethyl, *n*-propyl, β-methoxy *n*-propyl, aniline, pyridine; *esters*—(ethyl) of acetic, butyric, caproic, caprylic and levulinic acids, butyl butyrate; *ketones*—acetone, diacetyl, methyl *n*-amyl, methyl *n*-hexyl, methyl *n*-heptyl, acetonyl acetone, acetyl acetone; *miscellaneous*—*n*-butyl bromide, γ-butyrolactone, 1,4-dioxane, benzene, toluene.

Control samples of cream were tested with each group of compounds. In no instance did these samples show any measurable degree of ''oiling off''. Certain lower members of the various homologous series could not be included for reason of their low boiling point. However, in the particular classes of compounds that were found effective a trend was evident. In general, optimum results were obtained with the C_3 to C_6 members, whereas higher or lower homologues, with some exceptions, were ineffective. Of the classes of compounds, the acids, alcohols and amines appeared to have superior de-emulsifying power to the aldehydes, ketones and esters. The effective amines were observed to have certain singular properties. Their de-emulsifying action was rapid and accompanied by complete peptization of the milk proteins. These characteristics suggested that further work should be conducted on use of the amines to prepare milk fat from milk, cream and other fluid dairy products.

With this objective in mind, various C_3 to C_6 amines and alcohols were tried both singly and in combination as de-emulsifying agents for milk. The C_5 and C_6 members, although effective in certain instances, were too soluble in milk fat and consequently difficult to eliminate therefrom. The C_3 and certain isomeric C_4 compounds were found unsatisfactory because of their inability to disperse the material adsorbed at the surfaces of the fat globules. Although it was not possible to conduct an exhaustive study of all appropriate compounds as well as their qualitative and quantitative combinations, the data from more than 300 tests resulted in development of a suitable reagent which may be prepared as follows: To 310 ml. of water in a 1-l. reagent bottle are added 420 ml. of *n*-butylamine and 132 ml. of *n*-butyl alcohol. The contents of the bottle are shaken to yield the reagent which should be single-phased. This reagent is stable and easily prepared and its constituents are inexpensive and readily available.

For use of the reagent to prepare milk fat, a 50 g. sample of milk or cream in a 125-ml. Erlenmeyer flask is tempered to 20° C. Where evaporated, con-

densed or dry milks are employed, it is first necessary to reconstitute the product to normal fluid basis. Ten ml. of reagent are added, this amount being satisfactory for all products. The mixture is agitated with a rotary motion, either mechanically or by hand. Effective action of the reagent may be observed at the time the serum has acquired a watery translucent appearance and small light-yellow particles of fat have begun to rise toward the surface. One minute of agitation should be sufficient. The flask and contents are immersed in a gently boiling water bath for 1 min. to allow the liberated fat to melt and coalesce. With cream samples it is necessary to rotate the flask contents occasionally. The de-emulsified sample is transferred to a small separatory funnel and, as soon as a distinct and complete oil layer has formed, the lower phase is withdrawn. In order to remove residual reagent, the fat is washed twice with two 50-ml. portions of hot (60° C.) water and then dried under vacuum. Fat yields from the various dairy products studied were determined by taking up the fat in the separatory funnel, after draining off the serum, in two 25-ml. portions of ethyl ether. The ether solution was transferred to an evaporating dish, the solvent removed on a hot plate, the last traces of the solvent, water and reagent removed by drying under vacuum at 135° C. for 5 min. and the weight of the purified fat determined.

Where it is necessary to prepare more or less fat, the amounts of sample and reagent are varied proportionately. In preparing a comparatively large quantity of fat, it is advisable to use cream of reasonably high (40 per cent) fat content. If subjecting the de-emulsified mixture to heating in a boiling water bath is considered too rigorous a treatment, the procedure can be carried out at room temperature. The sample after agitation with the reagent, is centrifuged, preferably in a small separatory funnel adapted for such use; the serum layer is withdrawn and the fat washed several times and dried as previously described. When properly washed and dried, milk fat prepared with the aid of the butylamine-butanol reagent has no off-flavor or odor.

TABLE 1

Yields of fat obtained by de-emulsification of milk with various amounts of n-Butylamine-n-Butanol reagent

	Ml. of reagent					
	6	8	10	12	14	16
Grams of fat recovered[a]	0.33	1.73	1.75	1.70	0.44	0.31
% recovered	17	89	90	87	23	16

[a] From 50-g. samples of a milk testing 3.9% fat.

The optimum quantity of reagent for use with milk was investigated. Data relating to this variable (table 1) revealed that amounts of reagent ranging between 8 and 12 ml. give satisfactory results, but that above or below this range decreased yields of fat may result. Below 8 ml. peptization of the milk proteins is incomplete; above 12 ml. the liberated fat appears to undergo partial re-emulsification into the serum.

Representative fat yields obtained from a number of fluid dairy products by subjecting them to de-emulsification with the reagent are presented in table 2. Dry whole milk reconstituted to the fluid basis of normal milk also was subjected to treatment with the reagent. Although satisfactory de-emulsification of the product was noted, data relative to recovery of fat were not secured for this product.

TABLE 2

Yields of fat obtained by de-emulsification of certain dairy products with a n-Butylamine-n-Butanol reagent

Product	Grams of fat[a]		
	By analysis[b]	Recovered	Yield (%)
Pasteurized milk	1.95	1.80	92
Homogenized milk	1.95	1.76	90
Evaporated milk	1.99	1.82	92
Light cream	9.0	8.7	97
Heavy cream	19.0	18.6	98
Ice cream mix	5.04	4.90	97

[a] Per 50-g. sample.
[b] Babcock or Roese-Gottlieb methods (1).

As shown in table 1, the butylamine-butanol reagent gave satisfactory de-emulsification of the dairy products investigated. The yields of fat were found to average 90 per cent or more in all instances, with somewhat better results being obtained in the case of the higher fat products.

DISCUSSION

This investigation has shown that a considerable number of simple organic compounds can promote more or less quantitative de-emulsification of cream. Only a single set of conditions, arbitrarily selected, was employed in the study of these compounds. However, the results are no less significant since, from a practical standpoint, they suggest more convenient methods for preparing and analyzing milk fat; and from a fundamental standpoint, they reveal interesting surface active properties of the compounds with regard to the emulsions existing in certain dairy products. The effectiveness of the compounds appears to depend upon their ability to penetrate and disperse the proteinaceous materials of the fat globule membrane. In the case of the amines, coalescence of liberated fat may be further facilitated through peptization of the proteins. Although certain compounds containing as few as three and as many as eight carbon atoms were observed to de-emulsify satisfactorily, a trend toward optimum effectiveness in the various classes of compounds was evident in the normal C_4 members. Their solubility in both the lipid and aqueous phases, with consequent lowering of interfacial tension, seems the most logical explanation as to how they effect removal of adsorbed materials from the globule surface.

A comparison of physical and chemical constants of milk fat prepared by several methods, including that employing the butylamine-butanol reagent, will be the subject of a subsequent paper. In addition, this reagent is being investigated with regard to its use for quantitative fat testing of dairy products.

SUMMARY

A group of 69 organic liquids was studied concerning their capacity to destroy the normal emulsion existing in fresh cream (40 per cent fat) during heating. Of these, 20 were found effective, 6 partially effective and 43 ineffective. As groups, the acids, alcohols and amines were found most effective, the aldehydes, ketones and esters least effective. Maximum de-emulsifying activity within a given group appeared to occur in the vicinity of the normal C_4 member. However, effective action was noted in the case of certain C_3 compounds and in one other containing eight carbons. Based on these findings, an aqueous reagent containing *n*-butylamine and *n*-butanol was developed and found capable of de-emulsifying a number of fluid dairy products. Products from which milk fat was readily recovered with the reagent included pasteurized and homogenized milks, light and heavy creams, ice cream mix, evaporated and dry whole milks, the latter two after reconstitution to normal fluid basis. Advantages of the method employing this reagent are that it is rapid, reasonably quantitative, involves no tedious extraction procedure and does not require a rigorous digestion of the sample. Preparation of milk fat for vitamin analyses, for the determination of fat constants, as well as for studies concerning its composition, should be facilitated considerably through use of this reagent.

ACKNOWLEDGMENT

The technical assistance of Mrs. Jean Baker is gratefully acknowledged.

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SULFUR DIOXIDE PRESERVATION OF FORAGE CROPS[1]

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One of the most difficult problems in grassland farming is that of storing the crop for later feeding. Most forage crops are produced and should be harvested for their maximum feeding value at a time of year when it is very difficult to field-dry and make them into hay. In efforts to find a better method of preserving forage crops, Knodt (4) treated successfully the contents of a silo with SO_2 at the rate of 5 lb. per ton of green material. Alternate loads of timothy-red clover were placed in two silos which were filled using 200 lb. of hominy and 5 lb. of SO_2, respectively, per ton of green material. In a feeding trial with lactating Holstein dairy cows fed 60 lb. of silage per day, the two silages were equal in feeding value, on a pound-for-pound basis. Twice as much carotene was found in the SO_2 silage as in the hominy-treated silage when fed.

As a result of this previous work, the experiments described in this report were conducted in 1950, to study the use and value of SO_2 for preserving various forage crops. This report presents the chemical studies of SO_2 silages compared to two control silages made from four different forage crops and corn.

EXPERIMENTAL

For these chemical studies the silages were made and stored at atmospheric temperature in 50-gal. steel barrels with removable tops. For each combination of treatment and material a set of eight barrels of silage was prepared or a total of 40 barrels for each crop studied.

Materials tested included timothy and red clover cut at the pre-bloom stage of the timothy, brome grass cut when in bloom, second cutting orchard grass and ladino clover, second cutting alfalfa just at the beginning of the bloom stage, and corn cut in September. Each of these materials was treated with liquid SO_2 at rates of 3, 5 and 7 lb. per ton of green material. Two control silages were made from each material, the first without treatment and the second with the addition of 200 lb. of finely ground corn-and-cob meal per ton of chopped material. The corn-and-cob meal treatment was omitted in the corn silage series. Thus, the SO_2 silages could be compared, chemically, with the two sets of control silages which depended on fermentation for their preservation.

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The data presented in this report are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE 1

Chemical studies on hay-crop and corn silages[a]

Treatment	pH values				% Reducing sugar			% Lactic acid			Carotene (micrograms/g.)	% Ammonia	% Volatile acids
	2 hr.	Low	Days to reach low	120 d.	2 hr.	7 d.	120 d.	2 hr.	7 d.	120 d.	120 d.	120 d.	120 d.
	Timothy and clover silages												
Untreated	5.9	4.3	7	4.5	6.72	0.70	0.54	0.02	3.22	0.80	44.1	0.20	0.85
C & C meal	5.9	4.1	30	4.2	6.80	0.52	0.80	0.02	2.64	1.40	29.1	0.16	0.81
7 lb. SO_2	5.7	4.3	120	4.3	7.58	3.23	3.38	0.03	0.40	0.30	120.9	0.05	1.58
5 lb. SO_2	5.9	4.3	14	4.5	7.01	1.55	3.63	0.02	1.44	0.23	84.6	0.06	1.98
3 lb. SO_2	5.7	4.2	14	4.6	7.35	3.37	3.11	0.03	0.39	0.82	90.4	0.07	1.40
	Brome grass silages												
Untreated	4.6	3.9	60	5.0	0.37	1.31	0.37	1.88	3.06	1.42	94.9	0.05	1.37
C & C meal	4.5	3.9	60	5.1	0.41	0.57	0.44	2.02	2.52	1.29	75.3	0.08	1.16
7 lb. SO_2	4.8	4.2	1	5.3	3.32	1.45	2.17	0.01	0.09	0.03	104.5	0.01	1.00
5 lb. SO_2	4.8	4.4	3	5.3	3.18	4.25	2.37	0.02	0.43	0.13	104.9	0.01	1.29
3 lb. SO_2	5.1	4.2	7	5.3	3.77	1.70	3.00	0.22	0.10	0.24	95.5	0.02	1.20
	Orchard grass-ladino clover silages												
Untreated	5.3	4.8	3	5.4	0.09	0.12	0.06	1.29	3.09	0.05	135.0	0.13	8.30
C & C meal	5.1	4.4	7	4.9	0.06	0.19	0.07	1.01	3.37	1.06	158.2	0.17	4.80
7 lb. SO_2	4.7	4.0	3	4.6	1.85	3.59	1.87	0.08	0.62	0.42	409.8	0.04	3.21
5 lb. SO_2	5.3	4.0	7	4.5	2.60	1.81	1.48	0.13	0.46	1.27	368.0	0.06	3.04
3 lb. SO_2	5.0	4.0	7	4.5	2.44	1.61	2.75	0.12	1.22	0.78	364.8	0.03	4.58
	Alfalfa silages												
Untreated	5.7	4.8	14	5.9	0.32	0.06	0.05	1.68	0.90	0.03	155.1	0.13	9.49
C & C meal	5.5	4.3	30	5.1	0.22	0.09	0.10	1.10	1.88	1.06	152.9	0.12	8.97
7 lb. SO_2	5.3	3.9	1	5.3	3.37	1.90	0.55	0.07	0.07	0.42	208.2	0.11	6.70
5 lb. SO_2	5.7	3.7	1	5.0	2.46	0.59	0.93	0.20	0.85	1.27	208.2	0.08	6.82
3 lb. SO_2	5.3	3.8	1	5.1	3.31	1.51	0.76	0.11	0.51	0.78	203.1	0.08	6.96
	Corn silages												
Untreated	5.9	3.7	14	4.4	11.40	0.35	0.61	none	2.00	1.64	89.3	0.03	2.65
7 lb. So₂	3.3	3.3	2 hr.	4.5	7.14	8.98	7.71	none	0.61	0.61	141.6	0.01	2.20
5 lb. SO_2	4.4	3.8	1	4.5	12.50	5.77	11.24	none	1.09	0.03	99.8	0.01	1.94
3 lb. SO_2	4.3	3.9	3	4.6	15.60	13.29	15.60	none	0.28	0.11	131.8	0.01	1.90

[a] Dry matter basis.

Silages were sampled 2 hr. after preparation and at intervals of 1, 3, 7, 14, 30, 60 and 120 days after preparation. Samples which could not be analyzed immediately were frozen and stored at –10° F. until they could be analyzed. The hay crops were mowed and windrowed in one operation, chopped and loaded on wagons with a field harvester and placed in the barrels as quickly as possible, without any wilting.

Barrels were tramped carefully while being filled, then weighed and treated with liquid SO_2 by means of a hollow pipe applicator which was inserted down the center of the barrel and withdrawn as the SO_2 flowed into the silage. The corn-and-cob meal was mixed with the green material by running the proper amounts of the two materials through a screw-type silo blower simultaneously before packing into the barrels.

Fresh, finely ground sub-samples of the silages were used for the following determinations: Dry matter was determined in the Brabender Forced Draft Moisture Tester at 100° C. to constant weight. Carotene, by an adaptation of the chromatographic separation method of Wall and Kelley (8). Total sulfur was determined by the official magnesium nitrate method described in A.O.A.C. Official and Tentative Methods of Analysis (1).

A 1 : 4 silage-water extract was used (9) for determining pH values on the silages by a glass electrode using a Beckman instrument. Titratable acidity was determined on this water extract by the official A.O.A.C. method (1). Reducing sugar values were determined by a combination of the methods of Nelson (5) and Somogyi (7). Lactic acid was measured by the method of Barker and Summerson (2). Aliquots of this water extract were used for determining the sulfurous acid values by the official A.O.A.C. method (1), volatile acids by the steam distillation method of Osborn *et al.* (6) and ammonia by the method described by Hawk *et al.* (3).

RESULTS

The silages made in these experiments ranged from excellent to poor in quality, when judged by odor and appearance 120 days after preparation. Analyses of the various silages are presented in table 1. Invariably, the SO_2-treated silages were superior in appearance and odor to those made from the same materials by either of the control methods. When SO_2 was used at levels of 5 and 7 lb. per ton, the resulting silages seemed nearly as fresh and green as the materials when they were prepared. SO_2 at the 3 lb. per ton level appeared to preserve much of the original color, but did not prevent an appreciable amount of fermentation, especially in the wetter materials and after 2 mo. in storage. The control silages, especially those made from the high-moisture, high-protein materials, darkened in color and developed offensive odors long before 120 days of storage. The SO_2 silages all were free of the strong odors suggestive of putrefaction.

Many reports indicate that the development and maintenance of pH values of approximately 4.0 are necessary for production and preservation of high-quality silage. Among the experimental silages prepared in this work, very few

were found in which the lowest pH values attained were maintained for any appreciable length of time. Most of these silages reached their lowest pH values within 2 wk. after preparation. From the time the lowest values were reached until the end of the 120 days of storage, the pH values gradually rose, until, in some of the silages, they were almost at the same point as recorded for the fresh material at the time of ensiling.

In general, pH values close to 4.0 were recorded for the SO_2 silages within 1 to 3 days after treatment, while it required from 1 to 2 wk. for the control silages to develop comparable pH values. Some of the control silages failed to approach the pH value of 4.2 at any time during the experimental period. These silages were definitely inferior in quality to the silages which reached a pH value of 4.2 or lower. In these studies, it appeared that any silage which reached a pH value of 4.2 or lower within 2 wk. after preparation suffered comparatively little fermentation loss after developing this acidity, even though the pH values often rose to 5.0 or higher at 120 days. Apparently, the quicker an effective pH value was developed after ensiling, the lower were the fermentation losses of the silages. The SO_2 silages developed a low pH almost immediately. The control silages developed the necessary acidity through the fermentation processes and as a result there were losses, particularly in the carbohydrate fraction of the forage ensiled.

The reducing sugar content of the materials, as ensiled, ranged from about 12 per cent in the corn to about 0.1 per cent in the orchard grass-ladino clover mixture. Corn, brome grass and a mixture of timothy and red clover made acceptable quality silage with no treatment, but the orchard grass-ladino clover and the second cutting alfalfa failed to make satisfactory silage, either without treatment or with the addition of 200 lb. of corn-and-cob meal per ton. Neither of the two materials contained sufficient sugar to support proper silage fermentation. The addition of corn-and-cob meal did not add appreciably to the amounts of sugar, according to chemical analysis, although fermentation in these corn-and-cob meal silages was more satisfactory when judged by the pH values and the levels of lactic acid found in the silages.

In all of the control silages, except those made from brome grass, the sugar values declined rapidly, until most of the sugar had disappeared in 3 to 7 days after ensiling. This indicates that the major part of the lactic acid fermentation was completed during the first week of storage. The brome grass silage showed little change in sugar content, despite the fact that the lactic acid concentration rose markedly, the pH dropped rapidly and preservation of the nutrients was satisfactory.

In contrast to the control silages, the SO_2 silages at the two higher levels of treatment maintained sugar levels even higher than found in the original material throughout the storage period. This may have been the result of the breakdown of some of the more complex carbohydrates in the plant material to simple sugars, through the action of the sulfurous acid in the silages. Although the sugar levels in these SO_2 silages declined toward the end of the

storage period, they never reached the low levels found in the control silages after the first 7 days of storage.

The control silages on this experiment developed lactic acid levels ranging from 2.5 to 4.4 per cent on a dry matter basis on or before the seventh day of storage. After this time there was a gradual decrease in the lactic acid content of these control silages. At 120 days the lactic acid values found in the control silages remained at about one third of the peak values, found earlier.

In striking contrast, the SO_2 silages, at the two high levels of treatment, never developed lactic acid values as high as 1 per cent, except in a few cases where sulfurous acid determinations indicated that the SO_2 treatment had been lighter than planned. In silages treated with only 3 lb. of SO_2 per ton, the lactic acid values reached somewhat higher levels than with larger applications of SO_2, especially during the latter part of the experimental period. From this, it appears that some fermentation does occur after a considerable storage period in SO_2 silages treated at this low level.

The levels of carotene were another point of sharp contrast between the SO_2 silages and the control silages. At all three levels of application, the SO_2 treatment resulted in the preservation of most of the carotene present in the original materials. In the control silages, it was found that the greatest loss of carotene occurred during the first day after the materials had been ensiled. Prompt treatment of the chopped material with SO_2 prevented any considerable loss during this time or at any later time during the experimental period of 120 days. Carotene values in the SO_2 silages were almost twice as high after storage for 120 days as those in the control silages on this experiment.

Volatile acids, largely made up of acetic acid which comes from the bacterial decomposition of lactic acid or from protein breakdown, may be considered one of the identification marks of destructive decomposition in hay-crop silages. In these experimental silages, volatile acid levels were not found excessively high except in some of the alfalfa and the orchard grass-ladino clover silages. Untreated silages made from both of these materials developed very high levels of volatile acids, over 8 per cent on a dry matter basis. These silages were very dark in color and had a strong odor suggestive of putrefactive changes in the materials.

The corn-and-cob meal-treated alfalfa silage also developed a high concentration of volatile acids, almost 9 per cent. This particular silage was very high in its losses of sugar and protein, which amounted to close to 50 per cent by the end of the experimental period.

Ammonia nitrogen, another indication of destructive change in silages, comes from decomposition of the protein in the material. In the control experimental silages, after 120 days the ammonia nitrogen values had increased to approximately twice the values found in the fresh materials. On the other hand, SO_2-treated silages failed to show this increase and remained close to the original ammonia levels. Some of the SO_2 silages treated at the 3 lb.-per-ton levels did show some increase in ammonia nitrogen but these changes were much less than found in the control silages.

Sulfurous acid analyses were conducted to check on the precision of application of the SO_2 in the experimental barrels of silage. Total sulfur analyses were conducted to determine how much of the sulfur remained in the silage during the storage period. Data indicated that, at the two lower levels of SO_2 application, the sulfur values remained fairly constant throughout the experimental period. At the 7 lb.-per-ton level, there was considerable decline in total sulfur toward the end of the storage period, indicating that some of the SO_2 may have evaporated from the ensiled material at this higher level of application.

SUMMARY

Data on the chemical analysis of 24 experimental silages, made and stored in 50-gal. steel barrels, are presented for the purpose of comparing the preservative effect of SO_2 with that of corn-and-cob meal. Four different types of hay-crop silages and corn silage were tested with three levels of sulfur dioxide treatment, no treatment and with 200 lb. of finely ground corn-and-cob meal per ton.

Liquid SO_2, at levels of 5 and 7 lb. per ton of green material, proved to be very effective in developing the desired acidity for proper silage preservation in a very short time, as compared to the time required for the development of such acidity by fermentation.

Reducing sugar levels in the SO_2 silages increased over the levels found in the original fresh grass materials. These high levels of sugar were maintained throughout the 120 days storage period.

Lactic acid values in the SO_2 silages did not increase markedly during the storage period, as did those in the control silages. The high sugar values and the low lactic acid values in the SO_2 silages indicate that bacterial fermentation had been decreased by this treatment.

Carotene destruction, which was most evident during the first day of storage, was kept to a minimum by treating the silages with SO_2. The SO_2 silages maintained much higher levels of carotene than the control silages during the entire storage period.

Ammonia nitrogen levels in the SO_2 silages were much lower than in the control silages. Evidently, the use of SO_2 in these silages prevented considerable protein decomposition.

Volatile acid values were lower in the SO_2 silages, compared to those in the control silages.

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MILK REPLACEMENTS FOR DAIRY CALVES[1]

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There have been numerous reports (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 17, 18, 19) that it is possible to raise dairy calves on limited amounts of whole milk through the use of skimmilk, remade skimmilk, dry calf starters and, more recently, milk replacement formulas.

Presented in this report are studies relative to the further development and improvement of milk replacement formulas developed at this station. It was desired to evaluate blood meal, fish solubles, dried corn fermentation solubles, red dog flour, corn gluten meal, dried ethyl and butyl molasses solubles and a simplified formula that was high in dried skimmilk.

EXPERIMENTAL PROCEDURE

The male Holstein calves used in the two trials presented in this report were secured from Pennsylvania state institution herds. Housing was in a steam heated, ventilated and artificially lighted stable. The temperature was maintained at 65° F. by thermostatic control. Each calf was assigned at random to a solid-wall pen bedded with straw and equipped with a water bowl, concentrate box, hay rack and iodized salt block. In determining growth rate, measurements of body weight, withers height and chest circumference were taken once each week by the same person, at the same time of day and in the same order. The general appearance, vigor and the feces condition of each calf also were noted daily. In cases of scours that persisted longer than 24 hr., 8 g. of sulfathalidine in bolus forms were given, followed by a 4-g. dose at each of the next two successive feedings.

Trial 1. The 36 calves were divided into six groups of six calves each on the basis of body weight, withers height and chest circumference and were placed on experiment not later than 4 days after birth. They were fed the milk replacement formula for their respective group, as shown in table 1. Group I was designated as the control group. Each replacement was fed at 100° F., according to the following schedule: First through fourth day—dam's milk; fifth through seventh day—2 lb. whole milk, 0.2 lb. milk replacement and 2 lb. water (twice daily); eighth through 10th day—1 lb. whole milk and 0.4 lb. milk replacement, 3 lb. water (twice daily); 11th through 21st day—0.5 lb.

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milk replacement and 5 lb. water (twice daily); 22nd through 35th day—0.6 lb. milk replacement and 6 lb. water (twice daily); 36th through 49th day—0.7 lb. milk replacement and 6 lb. water (twice daily); 50th through 56th day—0.7 lb. milk replacement and 7 lb. water (once daily).

Each calf was fed a fair grade of alfalfa hay *ad libitum* during the entire 12-wk. trial. Calf starter was fed *ad libitum* up to a maximum consumption of 6 lb. daily, the upper limit for the duration of the trial. The calf starter was composed of 416.5 lb. ground yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal, 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skimmilk, 100 lb. dis-

TABLE 1
Milk replacement formulas—Trial 1

Ingredient	Group					
	I	II	III	IV	V	VI
			(*lb.*)			
Dried skimmilk	50	50	48	45	65	35
Dried whey	10	10	10	10	10	......
Dist. dr. sol. (Corn)	15	15	15	15	20	20
Blood flour	10	......	......	......	......	10
Blood meal	......	10	10	10	......	......
Oat flour	5	5	5	5	......	5
Dextrose	7	7	7	7	......	7
Red Dog flour	......	......	......	......	......	10
Corn gluten meal	......	......	......	......	......	10
Fish solubles[a]	......	......	2	......	2	......
B-Y 500[b]	......	......	......	5	......	......
Stabilized vitamin A and D feed[c]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mixture[d]	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0

[a] Furnished by Amburgo Co. Inc., Philadelphia, Pa.
[b] Dried corn fermentation solubles. (Furnished by Commercial Solvents Corp., New York, N. Y.)
[c] Contained 500 D_2 and 4000 A USP units/gram. (Furnished by Nopco Chemical Co., Harrison, N. J.)
[d] Mineral mixture contained 32% calcium, 2.6% magnesium, 1% manganese, 0.175% iron, 0.225% iodine, 0.125% copper, 0.009% zinc and 0.010% cobalt. (Furnished by Limestone Products Corp. of America, Newton, N. J.)

tillers' dried corn solubles, 0.5 lb. irradiated yeast (9F), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt and 3 lb. vitamin A feed (2,270,000 USP units of A per pound in dry meal form).

Trial 2. Thirty calves were divided into five comparable groups of six calves each and were placed on experiment at not later than 4 days of age. The formulas in table 2 were fed at 100° F. according to the same schedule as that for the first trial, with the exception that in this trial 7 lb. of water were used in preparing the replacement during the 36th- through 49th-day period. Group I again constituted the control group.

Good quality, fine-bladed grass hay was fed *ad libitum* throughout this trial. The same calf starter that was used in trial 1 was fed in this trial and its consumption regulated in the same manner.

TABLE 2

Milk replacement formulas—Trial 2

Ingredient	Group				
	I	II	III	IV	V
			(*lb.*)		
Dried skimmilk	50	50	50	40	40
Dried whey	10	5	......	10	......
Blood meal	10	10	10	......	10
Ethyl solubles[a]	......	5	10	10	10
Butyl solubles[a]	......	......	......	5	5
Dist. dr. sol. (corn)	15	15	15	15	15
Soybean oil meal	......	......	......	5	5
Oat flour	5	5	5	5	5
Dextrose	7	7	7	7	7
Stabilized vitamin A and D feed[b]	0.5	0.5	0.5	0.5	0.5
Mineral mixture[c]	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0

[a] Furnished by Publicker Industries Inc., Philadelphia, Pa.
[b] Vitamins A and D feed same as table 1.
[c] Mineral mixture same as table 1.

EXPERIMENTAL RESULTS

Trial 1. All of the replacements studied were palatable and the calves were easily taught to drink from open pails. Ration VI, however, had a tendency to settle out more rapidly than was desirable.

The summary of growth data in table 3 shows that groups I and V made comparable weight gains that were superior to all other groups. This also was true for gains in withers height and chest circumference. Although group V exceeded all others in mean gains, analysis by the method of Snedecor (16) indicated that differences in daily gains were not statistically significant.

Scours were not a serious problem and those cases that did occur seemed not to be restricted to any particular group. During the first 3 wk., the feces of most of the calves were looser than was thought to be desirable. This looseness appeared to differ from typical scours, as in most cases the condition was not accompanied by other symptoms of scours, such as characteristic odor of feces or extreme weakness and dehydration of the calf.

In general appearance and thriftiness calves in group IV seemed slower in getting started and had rougher hair for a longer proportion of the trial.

TABLE 3

Mean daily gains in body weight, withers height and chest circumference—Trial 1

Group	Body weights			Withers height			Chest circumference		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
		(*lb.*)			(*in.*)			(*in.*)	
I	0.44	0.86	1.13	0.06	0.05	0.05	0.03	0.06	0.08
II	0.33	0.69	1.04	0.05	0.06	0.05	0.04	0.07	0.09
III	0.33	0.66	1.03	0.05	0.05	0.05	0.03	0.06	0.08
IV	0.35	0.69	1.02	0.04	0.05	0.04	0.02	0.06	0.08
V	0.44	0.78	1.21	0.06	0.05	0.06	0.03	0.06	0.09
VI	0.22	0.51	0.87	0.05	0.04	0.05	0.01	0.05	0.07

TABLE 4

Mean daily gains in body weight, withers height and chest circumference—Trial 2

Group	Body weights			Withers height			Chest circumference		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
		(lb.)			*(in.)*			*(in.)*	
I	0.21	0.79	1.16	0.02	0.04	0.05	0.03	0.07	0.09
II	0.19	0.92	1.17	0.03	0.05	0.06	0.03	0.08	0.09
III[a]	0.19	0.87	1.28	0.03	0.04	0.05	0.02	0.07	0.10
IV	0.15	0.67	1.06	0.02	0.04	0.04	0.03	0.06	0.09
V[a]	0.24	0.61	1.00	0.03	0.04	0.05	0.03	0.06	0.08

[a] Means of 5 calves in each group.

The average calf starter consumption at 8 and 12 wk. is presented in table 5. Group I calves consumed the greatest amount of starter to 8 wk. and group V was highest for the 12 wk. of the trial. Groups III, IV and VI consumed considerably lower amounts of calf starter than the other groups.

Trial 2. Palatability was not a problem in this trial but the replacements fed to calves of groups IV and V, containing soybean oil meal, tended to settle out somewhat. One calf in group III died from a navel infection and one in group V from persistent loss of weight, diarrhea and finally pneumonia. Sulfathalidine treatment failed to alleviate the condition. These calves died after being on experiment from 6 to 7 wk. Due to these losses, a statistical analysis described by Love (10), based on total gains and calculated values for the missing calves, was applied. It indicated no significant differences between gains made by the various groups.

From the growth data summarized in table 4, it can be seen that slow growth during the first 4 wk. was characteristic of most of the groups. The data for 12 wk. shows that by that time most of the group mean daily gains approached normal standards.

Table 5 gives the average consumption of calf starter. Groups I, II and III consumed considerably more than groups IV and V at both 8 and 12 wk.

Looseness of the feces was noted, especially in the calves of groups IV and V, in which this condition persisted for the longest period of time. The soybean oil meal in these two rations may have contributed somewhat to the condition observed.

TABLE 5

Average consumption of calf starter to 8 and 12 wk.

Group	Trial 1		Trial 2	
	8 wk.	12 wk.	8 wk.	12 wk.
	(lb.)		*(lb.)*	
I	65	208	68	211
II	54	205	70	209
III	47	182	73	227
IV	51	192	54	196
V	58	218	59	194
VI	51	181	.....	....

From the standpoint of general appearance and thriftiness the calves in groups I, II and III were superior to those in groups IV and V. The calves in the latter groups seemed to develop more slowly and to have a rough hair coat.

DISCUSSION

A large and rapidly increasing number of dairy calves is now being raised successfully on milk replacement formulas. The objective of the experimental work presented in this report was to develop new milk replacement formulas which would decrease cost and make use of more readily available sources of ingredients. While replacement formulas high in dried skimmilk have been shown to give normal growth, because of the frequent shortage of this product and large demand for its use in milk replacements which have developed, other ingredients must be found which will replace it. Similarly, blood flour is often unobtainable and, therefore, it was desirable to compare growth obtained on this product with that on a high grade of blood meal.

Data presented in this report indicate that a good quality blood meal may be used to replace blood flour which frequently is difficult to obtain and much higher in price. It also was found that a replacement formula containing 50 per cent dried skimmilk gave growth comparable to that on mixtures containing 65 per cent of this product. The reduction of dried skimmilk to 35 per cent of the total formula did result in decreased growth rates. It also was found that ethyl molasses dried solubles effectively replaced dried whey at the 5 and 10 per cent levels. Combinations of ethyl and butyl molasses at the 5 and 10 per cent levels, respectively, resulted in decreased growth. The addition of fish solubles at the expense of dried skimmilk did not increase rates of growth. Because of the demand for milk and milk products as a result of an increasing human population, the saving of milk for human use as a result of the use of milk replacements in calf raising is essential. Reports from many dairymen indicate that milk replacements which are properly formulated are raising better calves than were raised previously on whole milk on many farms.

SUMMARY

Blood meal and blood flour appeared to be of comparable value for growth in dairy calves in these milk replacements. Adding fish solubles or dried corn fermentation solubles resulted in no increase in growth rate over the control ration. A ration containing 65 per cent dried skimmilk produced more rapid growth rates than other complex mixtures. Red dog flour and corn gluten meal did not appear to effectively replace dried whey and dried skimmilk in these rations. A combination of 10 per cent ethyl solubles and 5 per cent butyl molasses solubles with soybean oil meal caused excessive scours and retarded growth. Ethyl solubles at levels of 5 or 10 per cent replaced dried whey effectively in these trials.

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THE ALPHA-NAPHTHOLPHTHALEIN (ANP) METHOD FOR MEASURING FAT HYDROLYSIS. II. APPLICATION TO CREAM[1]

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In recent years there has been increasing emphasis on the relationship of water-insoluble fatty acids to cream quality. The method of Hillig (3) which has been used for the measurement of water-insoluble acids (WIA) is highly technical and is time consuming; consequently it is not entirely suitable for quality control work. In the first paper of this series (1), a rapid colorimetric method for measuring water-insoluble acids in butter is described. The method, named the ANP test, utilizes the color changes produced by the fatty acids upon an alcoholic solution of alpha-naphtholphthalein. In view of the fact that this ANP test appeared to show considerable promise as a screening test for determining the WIA content of butter, it was deemed desirable to ascertain if the method could be adapted to cream. If so, then it could be used as a rather rapid test for grading cream for its WIA content.

This paper presents results obtained in adapting and applying the ANP test to cream. Since the completion of this work several workers have presented rapid methods for the determination of water-insoluble acids. One is a colorimetric procedure developed by Greenberg *et al.* (2), and the other is a measure of the acid degree presented by Parmalee and Babel (5). Roberts *et al.* (8) found that their method generally revealed the acid degree of the fat.

EXPERIMENTAL

The colorimetric test of Roberts *et al.* (6) upon which the ANP test is based, was applied to cream by first churning the cream into butter, and then determining the acids in the fat obtained by melting the butter. Roberts *et al.* (7) were able to conduct a single test within about 20 to 25 min. by use of an automatic shaker, but without such an automatic shaker about 60 min. were required to make one test.

In order to reduce the time required to complete an analysis, a solvent extraction procedure was developed for obtaining the fat. A mixture of alcohol and petroleum ether was selected for making the extraction. Although petroleum ether is recognized as a less efficient fat solvent than ethyl ether, it was used to exclude the extraction of the water-soluble acids, such as lactic, acetic and butyric, which are present in sour cream. The extraction procedure finally adopted was: (a) A 10-g. sample of cream was weighed into a large test tube

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(32 × 175 mm.); 5 ml. of 95 per cent ethyl alcohol were added and the mixture shaken vigorously for 30 sec. (b) Thirty ml. of petroleum ether (benzene, B.P. 30 to 60° C.) were added to the test tube and the mixture shaken vigorously for 1 min. (c) The mixture was allowed to stand until the solvent layer separated completely, or the separation was hastened by centrifuging the tubes in a Babcock centrifuge for 1 or 2 min.

The efficiency of the extraction procedure for recovery of fat is shown in table 1 for sweet and sour creams of various fat contents. The sour cream was from the same original cream as the sweet sample, but was inoculated with a *Streptococus lactis* starter and incubated until it contained 0.6 per cent acidity. The extractions were conducted in the same manner for both sweet and sour cream. The results revealed that 80 to 91 per cent of the fat in the sweet cream and 96 to 104 per cent of the fat in the sour cream were extracted; not only was the fat extracted more efficiently from the sour cream than from the sweet cream, but also the recoveries were more consistent. By adding pure lactic acid

TABLE 1
Recovery of fat from cream by alcohol-petroleum ether extraction[a]

Fat test of cream	Recovery of fat	
	From sweet cream	From sour cream (about 0.6% acidity)
(%)	(%)	(%)
20	82.1	103.7
25	84.0	97.0
30	83.5	99.8
36	91.0	96.8
39	80.1	99.2
42	89.0	98.5

[a] 5 ml. ethyl alcohol and 30 ml. petroleum ether/10-g. sample.

to sweet cream to produce a 0.5 per cent titratable acidity, the recovery of the fat could be increased 10 to 13 per cent without influencing the ANP test. In addition, it was found that more consistent recoveries of fat could be obtained when lactic acid was added to the sweet cream. The value of acidification in increasing the extraction of milk fat was demonstrated by Johnson and Gould (4).

Method used for the solvent extract for ANP test. The ANP test was performed in essentially the same manner as previously described by Armstrong and Harper (1). This consisted of preparing a 0.04 per cent solution of alphanaphtholphthalein in 0.57 per cent alcohol and buffering with the proper amount of borate buffer to produce a blue-green color. The molarity of the buffer depended on the lot of dye, as previously reported (1). The fat from the cream was added to 5 ml. of the dye solution.

Two methods of preparing the fat for the test were studied. These are designated as the "solvent extract" and as the "solvent-free fat". In the solvent extract procedure, 5 ml. of the petroleum extract, *i.e.*, the top layer in the test tube, were added directly to 5 ml. of the prepared dye solution. The tubes

were shaken vigorously and the layers permitted to separate. The color of the lower layer was compared to the prepared color standards.[2] To obtain solvent-free fat, about one-half of the ether extract from the cream was decanted into a Babcock cream test bottle. The bottle was placed in a hot water bath (160 to 170° F.) for 5 min. Hot water was added to the test bottle, shaken and enough hot water added to bring the fat well up in the neck of the bottle. The bottle was centrifuged for 1 min., and then 0.5 ml. of clear fat was removed. This was added to the dye solution in the same manner as described previously (1).

Effect of added acids on the ANP test in cream. Various water-soluble and water-insoluble acids were added to fresh 35 per cent cream to detect their influence on the ANP test. The results of this experiment are summarized in table 2. The data reveal that lactic acid and butyric acid are not removed from the cream by the alcohol-petroleum ether extraction and will not influence the results of the ANP test. In contrast, oleic and palmitic acids are extracted from the cream and are measured by the ANP test to the same degree of accuracy as when these acids were added to purified butter oil. The results again show that for these two acids, the range of WIA values for a given color of the ANP test is approximately 100 mg. per 100 g. of fat in the range of 0 to 400 mg. WIA.

Caproic and capric acids also are removed from the cream by the extraction procedure, but the completeness of removal is less than for oleic and palmitic. For example, the concentration of acid necessary to produce the orange color was found to be nearly 200 mg. of WIA per 100 g. of fat higher than that required by either oleic or palmitic acids. This naturally results since these acids possess more affinity for the water than do oleic and palmitic acids; consequently, a more exhaustive extraction procedure would need to be applied to achieve their removal. Table 2 also reveals that 5 ml. of solvent extract give essentially the same results as 0.5 ml. of fat; therefore either method could be used with equally satisfactory results.

Comparison of ANP test on churned and solvent-extracted fat. In connection with the application of the ANP extraction procedure involving the alcohol and petroleum ether, it was compared to the standard churning method as a means of removing the fat from the cream. A sample of sour cream was divided into two parts. One portion was extracted as previously described and the other portion was churned by means of a mechanical shaker. The churned fat was washed twice with water, and the ANP test was completed as previously reported for butter (1). The results in table 3 reveal that the results using solvent extraction procedure often indicated slightly more fat hydrolysis in the cream than was obtained when using the churned fat. This would be expected from the work of Johnson and Gould (4), who found that higher acid degrees were obtained by solvent extraction. This may be explained by the fact that the lower fatty acids, such as caproic and capric, are removed from the churned fat during the washing process, but are included to some extent in the petroleum ether extract.

[2] Color standards available at nominal cost from the Dept. of Dairy Technology, The Ohio State University, Columbus 10, O.

TABLE 2

Effect of the addition of various acids on the ANP test applied to cream

Cream sample no.	Acid added	Mg. acid/ 100 g. fat	Color obtained with the ANP test[a]	
			5 ml. solvent extract	0.5 ml. solvent-free fat
1	none	....	dark green	dark green
1	butyric	135	dark green	dark green
1	butyric	270	dark green	dark green –
2	none	....	dark green	dark green
2	lactic	165	dark green	dark green
2	lactic	330	dark green	dark green
2	lactic	660	dark green	dark green
3	none	....	dark green	dark green
3	caproic	100	medium green	medium green
3	caproic	200	light green	medium green
3	caproic	300	orange	light green
3	caproic	400	yellow	orange
4	none	....	dark green	dark green
4	capric	100	dark green	dark green
4	capric	200	medium green	light green
4	capric	300	light green –	orange
4	capric	400	orange	yellow
5	none	....	dark green	dark green
5	oleic	80	dark green	dark green
5	oleic	164	medium green	medium green
5	oleic	246	light green +	light green
5	oleic	330	orange	orange –
5	oleic	490	yellow	yellow
5	oleic	660	yellow	yellow
6	none	....	dark green	dark green
6	palmitic	130	medium green	medium green
6	palmitic	260	light green	light green
6	palmitic	390	orange	yellow
6	palmitic	520	yellow	yellow

[a] Color of alpha-naphtholphthalein changes in the following manner with increases in water-insoluble acids: dark green, medium green, light green, orange, yellow and white.

TABLE 3

Comparison of solvent extraction and churning as a means of obtaining fat for the ANP test

Sample	Color obtained with the ANP test[a]		
	0.5 ml. churned fat	0.5 ml. solvent-extracted fat (Solvent free)	5 ml. of solvent extract
28% cream	medium green	medium green	medium green
36% cream	orange	yellow	yellow
35% cream	yellow	yellow	yellow
35% cream	dark green	dark green	dark green
35% cream	light green	light green	orange
30% cream	dark green	dark green	dark green
32% cream	medium green	medium green	medium green
20% cream	light green	orange	orange
25% cream	medium green	light green	medium green
42% cream	medium green	medium green	medium green
35% cream 1[a]	dark green	dark green	dark green
35% cream 2	dark green	medium green	medium green
35% cream 3	medium green	medium green	medium green

[a] 1, 2, 3 = same sample of cream: 1—normal sample; 2—capric acid added at rate of 100 mg./100 g. fat; 3—oleic acid added at rate of 100 mg./100 g. fat.

TABLE 4

Reliability of the solvent extraction procedure in obtaining fat and fatty acids from creams of varying fat contents containing a fixed amount of added oleic acid

Fat in cream	Oleic acid added		Color obtained with the ANP test	
	Mg./10 g. cream[a]	Equiv. WIA (mg./100 g. fat)	5 ml. solvent extract	0.5 ml. solvent-free fat
(%)				
10	10	1000	white	white
20	10	500	yellow	yellow
30	10	333	orange	yellow
40	10	250	light green	light green
50	10	200	light green	medium green

[a] WIA of original cream = 25 mg./100 g. fat.

However, the results obtained in these trials reveal that the quantity of lower fatty acids recovered by solvent extraction would be insufficient to invalidate the application of the extraction procedure to cream to obtain fat for the ANP test.

The effect of the fat test of the cream on the ANP test. The use of a solvent-extraction procedure for removing the fat and fatty acids for analysis immediately raises the question as to the validity of the results when the method is applied to creams of widely varying fat contents. The established practice is to express the water-insoluble acids as milligrams per 100 g. of fat. Obviously, if the solvent extraction procedure is to be used directly in making the determination by the ANP method, it must extract both the fat and the water-insoluble acids from creams of different fat contents in a manner to reveal the true ratio of fat to acids. To ascertain if the correct ratio could be maintained, the solvent extraction procedure was applied to fresh cream varying in fat content and to which was added 10 mg. oleic acid per 10 g. cream. Results are presented in table 4. In addition, a second experiment was conducted in which the acids were added at the same concentration per 100 g. of fat. These results are presented in table 5.

The results in table 4 show clearly that when the extraction procedure is used on creams of varying fat contents containing the same concentration of acid per weight of cream, the ANP test varies directly with the concentration of acid

TABLE 5

Reliability of the solvent extraction procedure in obtaining fat and fatty acids from creams of varying fat contents and containing varying concentrations of added oleic acid

Sample no.	Mg. of oleic acid added/100 g. fat	21% cream		42% cream	
		5 ml. solvent	0.5 g. fat	5 ml. solvent	0.5 ml. fat
1	none	med. green	med. green	med. green	med. green
1	200	l. green	l. green	l. green	l. green
1	400	orange	orange	orange	orange
1	600	yellow	orange	yellow	yellow
2	none	med. green +	med. green +	med. green	med. green +
2	250	l. green	orange +	l. green	l. green −
2	500	orange	yellow	orange +	orange
2	750	yellow	yellow −	yellow −	yellow −

per weight of fat. In such a case the cream with the lower fat content will possess the highest WIA per 100 g. of fat, a fact revealed by the ANP test.

Table 5 presents additional information, demonstrating that the ANP test when conducted on the solvent extract yields the same results for creams of the same WIA concentration per gram of cream, regardless of the fat content. Although unexpected, this may be explained possibly by the fact that the alcohol-petroleum ether solvent extracts the fatty acids to the same extent as it does

TABLE 6

A comparison of the ANP and the Hillig methods for the determination of water-insoluble acids in cream

Hillig WIA method (3)	ANP method			
	Color description	Estimated WIA range	Mid-point of range	Difference ANP from WIA[a]
78	d. green	0–125	62	+ 18
89	d. green	0–125	62	− 27
100	d. green	0–125	62	+ 38
100	d. green	0–125	62	+ 38
102	d. green	0–125	62	+ 40
113	m. green	100–225	162	− 49
113	m. green	100–225	162	− 49
118	d. green	0–125	62	− 44
155	m. green	100–225	162	− 7
163	m. green	100–225	162	+ 1
164	m. green	100–225	162	+ 2
165	l. green	200–325	262	− 97
200	l. green	200–325	262	− 62
209	l. green	200–325	262	− 53
214	l. green	200–325	262	− 48
215	l. green	200–325	262	− 47
228	l. green	200–325	262	− 34
245	m. green	100–225	162	+ 83
249	l. green	200–325	262	13
265	l. green	200–325	262	− 3
279	l. green	200–325	262	+ 17
300	orange	300–425	362	− 62
337	orange	300–425	362	− 25
356	orange	300–425	362	− 6
462	yellow	400–1000	700	+238
510	orange	300–425	362	148
540	yellow	400–1000	700	160
688	yellow	400–1000	700	18
948	yellow	400–1000	750	248
994	yellow	400–1000	700	294

[a] ANP arbitrarily taken as midpoint of range.

the fat. Therefore, the relationship of the fatty acids to fat appears to remain constant when this extraction is conducted.

Comparison of ANP and WIA procedures. As a final measure of the reliability of the solvent extraction ANP test on cream, this method was compared to the WIA procedure of Hillig (3). Thirty samples of commercial sour cream were selected and analyses completed. A comparison of results of the ANP test and Hillig's (3) procedure is given in table 6. As previously reported for butter (1), the method has its greatest sensitivity in the WIA range of 0 to 400 mg. per

100 g. In every case, an ANP test result of orange or yellow was associated with a WIA value of over 300 mg. per 100 g. of fat. In three of the 30 comparisons, the WIA value was outside of the estimated WIA range indicated by the ANP test.

In the WIA range of 0 to 400 mg. per 100 g. of fat for 22 samples, and when the acid value representing the mid-point of each color range was used for the basis of comparison, there was an average difference of 36 mg. between the ANP and Hillig's method. This is 8 mg. greater difference than the average difference of 28 mg. between the two methods previously reported for butter (1). However, the results indicate a reasonably good agreement between the two methods.

The greatest value of the method appears to be in its use as a field test to differentiate high and low water-insoluble acid content. Based on the results in table 6, WIA values of less than 300 would give a green ANP test, whereas values of over 300 mg. WIA per 100 g. of fat would give an orange or yellow ANP test.

SUMMARY AND CONCLUSIONS

The rapid alpha-naphtholphthalein colorimetric (ANP) test for water-insoluble acids, previously used for butter, has been applied to cream. The method involves the use of a solvent extraction method utilizing a mixture of ethyl alcohol and petroleum ether for obtaining the fat for analysis. The method yielded satisfactory results when applied to cream containing added water-insoluble and soluble fatty acids.

The solvent extract from cream may be used directly in the ANP test or the solvent may be evaporated and the fat used. The method may be used as a rapid screening method for field work by using 5 ml. of solvent extract directly, or as a laboratory method by evaporating the solvent and using 0.5 ml. of the fat for each 5 ml. of dye solution. Both of these methods give comparable results, with the method utilizing the solvent-free fat being somewhat more reliable for laboratory work. The difference is traced to differences in the turbidity of the test when using fat and when using the solvent containing fat and fatty acids.

The solvent extraction ANP method for cream gives comparable results with cream of markedly different fat contents containing the same concentration of WIA per 100 g. of fat.

The solvent extraction ANP method was found to compare to Hillig's WIA method (3) for 30 samples of cream.

WIA values of less than 300 mg. acid per 100 g. of fat give a green ANP test, whereas WIA concentrations of 300 or more result in an orange or yellow color. By using the mid-points of each color range, in the range of 0 to 400 mg. water-insoluble acids per 100 g. of fat, there was an average difference of 36 mg.

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A COMPARISON OF BIMONTHLY AND QUARTERLY TESTING WITH MONTHLY TESTING FOR ESTIMATING DAIRY CATTLE PRODUCTION[1]

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One method of effecting economies in production testing of dairy cattle involves increasing the interval between tests. Several studies have been published concerning the amount and importance of the accuracy lost when this interval is lengthened. At least seven investigators have reported the accuracy of lactation records computed from monthly butterfat tests and milk weights as compared with an accuracy from daily measures (2, 5, 6, 7, 8, 11, 12). Their results indicate an average deviation of the monthly records from the daily records of about ± 2 per cent for milk yield and ± 3 per cent for fat yield. McCarthy and Boyle (7) reported that 95 out of 100 of the errors they observed in monthly milk records were between + 6.16 and – 5.35 per cent. Campbell (2) found that 86 out of 100 errors regarding milk yield were less than ± 5 per cent. Seventy-six out of 100 errors in fat records were within ± 5 per cent in his data.

Comparisons concerned with the accuracy of computing lactation records from weights taken every 2 mo. have indicated that bimonthly milk yield records vary from records computed from daily weights on an average of about ± 4 per cent (5, 9). McCarthy and Boyle (7) reported that 95 out of 100 observed errors were within ± 10.11 per cent. McDowell (9) found that 46 bimonthly records out of 70 varied from the daily records by less than ± 5 per cent. Investigators comparing bimonthly records with monthly records obtained relatively similar results (1, 3, 4, 10). Alexander and Yapp (1) found that only 49 out of 684 bimonthly fat-corrected milk records varied more than ± 10 per cent from the monthly records. No important biases were found concerning the accuracy of bimonthly testing.

Alexander and Yapp (1) also compared the use of a quarterly test with monthly testing. When the three tests were taken on the second, sixth and tenth months of lactation, only 53 out of 684 quarterly records erred from the monthly records by more than ± 10 per cent.

Two investigations (4, 10) were reported which included correlation coefficients as a basis for comparing records computed from varying testing intervals. McCarthy and Boyle (7) pointed out that the correlation coefficient measures the extent to which the types of records fluctuate together, rather than measuring the extent to which they are identical.

The primary objective of the study reported in this paper was to estimate

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the comparative usefulness of quarterly and monthly tests for individual lactation records, for sire provings and as a means of gathering production data concerning the results of artificial breeding. Comparisons of bimonthly records with monthly records also were carried out to determine if the results from Dairy Herd Improvement Association data obtained from several herds would be comparable to the results of investigations already reported.

PROCEDURE AND RESULTS

The production records used in this study were made during the period from 1945 to 1949. They were obtained on 1,255 Holstein-Friesian cows in 42 herds of Dane and Dodge counties, Wisconsin. All the Holstein herds on standard

TABLE 1

A comparison of bimonthly and quarterly testing with monthly testing for estimating dairy cattle production (1255, 305d., 2×, actual milk records)

Intervals of testing and month of first test	Av. milk yield	Relative variability of records (% of monthly)	Av. difference from monthly milk record	Av. % error (sign ignored)	Frequency of errors larger than ±10%
	(lb.)		*(lb.)*		
MONTHLY	9,169	100			
BIMONTHLY					
1st mo. of lactation	9,238	101.0	+69 ± 2.8[a]	2.8	1 in 78
2nd mo. of lactation	9,151	104.0	−18 ± 3.4	3.2	1 in 32
Av.	9,194	102.5	+25	3.0	1 in 46
QUARTERLY					
1st mo. of lactation	9,241	100.3	+72 ± 4.7	4.5	1 in 10
2nd mo. of lactation	9,394	116.4	+225 ± 5.1	5.1	1 in 8
3rd mo. of lactation	9,022	109.3	−147 ± 5.4	5.5	1 in 7
Av.	9,219	108.9	+50	5.0	1 in 8

Range of differences from monthly record:
For bimonthly, −1,640 to +1,610 lb.; for quarterly, −2,620 to +2,650 lb.
[a] Standard error of the average difference.

DHIA tests in Dane County were included, with the exception of herds where information was incomplete. Herds were selected at random from Dodge County. The record of every milking cow in each herd was included if the lactation period was 150 days or more.

Lactation records for milk and butterfat were computed for the first 305 days, using the centering day method and monthly, bimonthly and quarterly tests. Computations were carried out on the tabulating card equipment of the Computing Service at the University of Wisconsin.

The 305-day milk records based on monthly tests averaged 9,169 lb. and ranged from 2,680 to 16,950 lb. The yearly fat yield based on monthly tests averaged 315 lb. and ranged from 89 to 605 lb.

The criteria used for comparing bimonthly and quarterly records to monthly records were variability, average difference in yield, average per cent error and

the frequency of errors larger than ± 10 per cent. The results of these comparisons are shown in tables 1 and 2. In general, the results were similar for milk and butterfat, except that the per cent errors and frequency of large errors were slightly greater for fat yield.

The relative variability of the records was measured by the ratios of their mean squares. Records computed from bimonthly tests were similar in this respect to those computed from monthly tests. The quarterly records varied slightly more than either of the other two. Although the average differences in yield of the monthly, bimonthly and the quarterly records were statistically significant, they were not economically important. The average per cent error without regard to sign was 3.0 for the bimonthly milk records and 4.0 for the fat

TABLE 2

A comparison of bimonthly and quarterly testing with monthly testing for estimating dairy cattle production (1255, 305d., 2×, actual butterfat records)

Intervals of testing and month of first test	Av. fat yield	Relative variability of records (% of monthly)	Av. difference from monthly fat record	Av. % error (sign ignored)	Frequency of errors larger than ±10%
	(*lb.*)		(*lb.*)		
MONTHLY	315.1	100			
BIMONTHLY					
1st mo. of lactation	319.3	104.7	+4.24 ± .40[a]	3.7	1 in 22
2nd mo. of lactation	311.2	102.2	−3.82 ± .48	4.4	1 in 12
Av.	315.3	103.6	+0.21	4.0	1 in 16
QUARTERLY					
1st mo. of lactation	321.6	107.3	+6.53 ± .59	5.3	1 in 7
2nd mo. of lactation	318.2	114.8	+3.42 ± .65	5.8	1 in 6
3rd mo. of lactation	304.2	102.9	−10.83 ± .69	6.8	1 in 5
Av.	314.8	108.3	− 0.30	6.0	1 in 6

Range of differences from monthly record:
For bimonthly −63 to +74 lb. For quarterly −106 to +156 lb.
[a] Standard error of the average difference.

records. The average per cent error for the quarterly records was 5.0 for milk yield and 6.0 for fat yield. Errors which were larger than ± 10 per cent of the corresponding monthly records occurred with a frequency of one out of 46 bimonthly milk records. They occurred in one out of 16 bimonthly fat records. The frequency of errors larger than ± 10 per cent was one out of eight for milk yield and one out of six for fat yield quarterly records.

Efforts were made to determine the amount of association between the per cent errors and certain factors which might affect them. Two groups of bimonthly records were computed. One group started with the first monthly test in the lactation and the other started with the second. The three sets of quarterly records started with the first monthly test, the second and the third. In both bimonthly and quarterly records, the errors were smaller and less

frequent when computation was started with the first test than when started with later tests.

Correlation coefficients were obtained between per cent error and yield, based on monthly tests, length of lactation, length of the first test period, and length of the last test period. The coefficients are presented in table 3. In general, per cent error in both the bimonthly and quarterly methods tended to decrease as the production of the monthly record increased. Also, as the length of the lactation increased, the per cent error tended to decrease. The similarity of these two associations with per cent error may be a reflection of increased production resulting from longer lactations.

The association of length of the first test period with per cent error was negligible when the first monthly test was used for starting the bimonthly and

TABLE 3

The association of per cent error of bimonthly and quarterly records with yield of monthly records, length of lactation, length of the first test period, and length of the last test period

Intervals of testing and month of first test	Correlation (r)[a] of per cent error with:							
	Yield of monthly record		Length of lactation		Length of 1st test period		Length of last test period	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
BIMONTHLY:								
1st mo. of lactation	−0.12	−0.13	−0.06	−0.15	−0.07	−0.09	0.12	0.17
2nd mo. of lactation	−0.18	−0.19	−0.15	−0.23	0.16	0.12	0.12	0.05
QUARTERLY								
1st mo. of lactation	−0.16	−0.14	−0.19	−0.25	−0.03	−0.06	0.31	0.28
2nd mo. of lactation	−0.06	−0.14	−0.05	−0.13	−0.11	−0.03	0.14	0.02
3rd mo. of lactation	−0.28	−0.19	−0.35	−0.32	0.10	0.08	0.08	0.04

[a] r = ± 0.055 is significant at the 5% level.

quarterly records. This association assumed some importance when later tests were used for starting computations. The results concerning the correlation between length of the last test period and per cent error were approximately the converse of those obtained with the length of the first test period.

DISCUSSION

In many respects the results of this study are comparable to those previously reported. However, the DHIA data from several herds yielded slightly greater per cent errors than previous studies which used data from single herds. Also, in the quarterly records, the frequency of errors larger than ± 10 per cent was greater in the present study than reported by Alexander and Yapp of Illinois (1). The explanation of this latter difference may be found in the different procedures used. The Illinois study eliminated all records less than 305 days in length. The procedure reported here utilized all records which were from 150 to 305 days in length. The shorter records were included in order to obtain lactations comparable to those used in DHIA sire provings. As mentioned previously, the per cent errors tend to decrease as lactation length increases.

Comparisons of bimonthly and quarterly records with records computed from monthly measures will yield smaller errors than comparisons of bimonthly and quarterly records with records computed from daily measures. As mentioned previously, monthly records tend to vary from records computed from daily measures by about 2 per cent for milk and 3 per cent for fat yield. By adding these amounts to the error values obtained in the present study, the bimonthly records may be considered as varying from records computed from daily measures by about 5 per cent for milk and 7 per cent for fat yield. The respective errors for quarterly records may be approximated at 7 and 9 per cent.

Judgment regarding the usefulness of the bimonthly and quarterly tests should consider all the criteria of comparison and also the purpose for which the records are to be used. While the differences in variability, the biases and the per cent errors are small, the frequency of errors larger than ± 10 per cent indicates that bimonthly and quarterly records may not be sufficiently accurate for estimating individual lactation records. However, when several of these records are averaged, the plus errors will tend to cancel the minus errors. Consequently, in sire provings and population studies, the frequency of errors larger than 10 per cent should be extremely rare.

The results favor the use of the "combination" testing program, in which the tester takes the samples every other month and the farm operator takes the samples for the intervening months. The combined monthly samples are used for the farm operator's information regarding his cows' lactations. The lactations computed from the tester's bimonthly tests are submitted for use in sire provings.

SUMMARY

Milk and butterfat records for 1,255 Holstein-Friesian cows in 42 herds were computed for the first 305 days of lactation. The centering day method and monthly, bimonthly and quarterly tests were used. The bimonthly and quarterly records were compared to the monthly records according to their variability, average difference in yield, average per cent error and frequency of errors larger than ± 10 per cent.

The results were similar for both milk and butterfat yield. The differences in variability between the monthly, bimonthly and quarterly tests were small. The average differences in yield were significant but unimportant. The average per cent errors from milk yield were 3.0 for the bimonthly records and 5.0 for the quarterly records. The average per cent errors for fat yield were 4.0 and 6.0, respectively, for the two methods. The frequency of errors larger than 10 per cent was one in 46 regarding milk yield in bimonthly records and one in eight in quarterly records. Errors of this size occurred in the butterfat comparisons at the rate of one in 16 bimonthly records, and one in six quarterly records.

The frequency of the large errors indicates that bimonthly and quarterly records should be satisfactory for sire provings and population studies, but they may be unsatisfactory when used as individual lactation records.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. VII. pH, BUFFER CAPACITY AND OSMOTIC PRESSURE[1]

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Although many reports on the composition of colostrum and transitional milk of the dairy cow have appeared during the last century, investigations of pH, buffering and osmotic pressure have received relatively little attention (3). Van der Burg (9) obtained data on pH of 84 samples of first colostrum and Caulfield and Riddell (1) studied changes in pH of milk from ten cows at selected periods for 30 days following parturition. Koestler (4) investigated the buffering action of the mammary secretions from four cows during the colostral and transitional stages at the beginning of the lactation period. The investigation reported herein pertained to a study of changes in the three aforementioned properties of colostrum and milk obtained during the first 2 wk. after calving.

EXPERIMENTAL PROCEDURES

Feeding of cows. Colostrum and milk for these studies were obtained from cows of the Holstein, Ayrshire, Jersey and Guernsey breeds in the college herd. All of the cows were fed a typical barn ration (grain concentrate mixture, Atlas sorgo silage and hay) and/or pasture when available. The level of grain feeding both before and after calving was according to accepted herd practices.

Collection of samples. In studies of the pH of the first colostrum obtained, samples were collected from 57 cows of the herd that calved during a period of 18 mo.

From each of 20 cows calving during the first 6 mo. of the first year of the study, samples of the first, second, third and fourth milkings and composites of the fifth, sixth, seventh and eighth, 15th and 16th, and 27th and 28th milkings were obtained for determination of changes in pH, buffering capacity and osmotic pressure. Analyses were made on all of the first five samples from each of the 20 cows, but the last three composites were from 18, 16 and 15 cows, respectively. The cows were milked twice daily, as completely as possible, either by hand or machine, the first samples being taken shortly after parturition. In order that representative samples could be obtained, the calves were not allowed to nurse. Colostrum or milk obtained at each milking was thoroughly mixed before samples were taken for analysis. When daily composites were made, they were based on the weights of milk obtained. Samples that could not be analyzed immediately were stored in a refrigerator at 4° C. for a period not exceeding 3 days.

Analytical procedures. Samples were warmed in a water bath to 20° C. and

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poured several times to insure thorough mixing before portions were removed for analysis. The pH was determined using a Beckman pH meter with glass electrode. Buffering capacity at pH values lower than the initial pH was obtained by titration of 100 ml. of the sample with 0.10 *N* HCl. Small increments of the acid were added to the sample and, after thorough mixing each time, the pH was read. Similarly, another 100 ml. of sample was titrated with 0.10 *N* NaOH to study buffering effects at pH values above the original. Titration curves were prepared by plotting the milliliters of acid or of base added against the pH reading. Osmotic pressure was studied by the method developed by Lillie (5) wherein collodion bags of 50-ml. capacity are prepared and used as osmometer membranes. The difference in the initial and final manometric readings was recorded as the measure of osmotic pressure.

RESULTS AND DISCUSSION

pH. The average pH of the first postpartum colostrum from 57 cows (four breeds) was 6.28, range 6.00 to 6.61. Van der Burg (9) of the Netherlands reported only slightly different results, the average pH of 84 first-colostrum samples being 6.22, range 5.95 to 6.88. In the present study, the average pH of first colostrum from Holsteins was 6.25 (19 samples), Ayrshires 6.29 (10 samples), Jerseys 6.31 (18 samples) and Guernseys 6.26 (10 samples). Samples collected from each of the four breeds in the warm months had slightly lower average pH values than did those collected in the cool months of the year; this might have resulted from a small amount of acid formation during handling of samples, from environmental, or from feed effects.

TABLE 1

The average pH of colostrum and transitional milk collected from 20 dairy cows during the first 2 wk. postpartum

	No. milking postpartum							
	1	2	3	4	5 + 6	7 + 8	15 + 16	27 + 28
Av.	6.32	6.32	6.33	6.34	6.33	6.35	6.45	6.50
Low	6.00	6.11	6.09	6.08	6.08	6.13	6.24	6.30
High	6.61	6.59	6.62	6.67	6.58	6.64	6.59	6.71

The average pH of samples collected from 20 cows during the first 2 wk. of lactation increased from 6.32 to 6.50 (table 1). Caulfield and Riddell (1) reported the average pH of first colostrum from 10 cows was 6.25, increasing gradually to 6.57 by the 15th day after calving. The changes in pH during the first few days of lactation are in harmony with observations on titratable acidity (2).

Buffering capacity. Colostrum and early transitional milk had a greater buffering effect than did secretions obtained at later milkings. The data are shown graphically in fig. 1, and a part of them, including the low and high values for indicating variability in samples, are in table 2. Average buffering of the first samples collected postpartum was higher than that of succeeding sam-

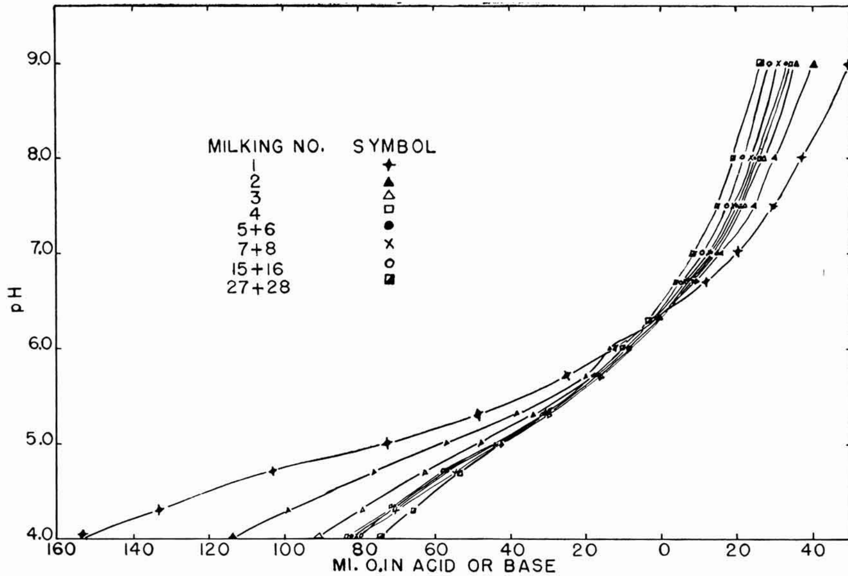

FIG. 1. Curves obtained on titration of 100-ml. samples of colostrum and transitional milk with 0.1 N acid or 0.1 N base (each point on each curve represents the average of individual samples from 20 cows, except samples 7 + 8, 18 cows; 15 + 16, 16 cows; and 27 + 28, 15 cows).

TABLE 2

Ml. of 0.1 N acid or of 0.1 N base added to 100 ml. of colostrum or transition milk in order to obtain various pH values

pH		No. milking postpartum							
		1	2	3	4	5 + 6	7 + 8	15 + 16	27 + 28
4.0[a]	Av.	153.4	114.2	91.6	82.8	83.0	80.7	79.8	75.4
	Low	91.0	67.0	66.5	65.5	67.0	69.0	69.5	66.0
	High	199.0	157.0	139.5	107.5	104.5	92.0	95.0	90.0
5.0[a]	Av.	72.6	57.1	48.1	44.1	43.2	42.6	43.6	42.6
	Low	41.0	36.0	35.0	32.0	31.0	33.0	33.0	35.5
	High	108.0	87.0	68.0	63.0	61.5	50.0	52.0	54.0
6.0[a]	Av.	12.2	13.4	8.7	8.5	8.6	9.3	9.8	11.0
	Low	0.0	2.0	2.5	2.0	3.0	4.0	3.5	6.0
	High	26.0	21.0	15.0	18.0	15.5	17.0	14.0	18.0
7.0[b]	Av.	20.4	16.0	14.3	13.6	13.3	12.7	10.9	8.4
	Low	15.0	9.0	8.0	6.0	8.5	8.0	7.0	5.5
	High	31.5	21.5	20.5	18.5	17.0	16.0	12.5	13.0
8.0[b]	Av.	37.5	30.2	26.9	25.7	25.3	23.9	21.6	19.1
	Low	27.5	22.0	21.0	16.5	22.0	18.0	17.5	14.5
	High	50.0	39.0	31.0	32.5	29.5	27.5	27.0	25.0
9.0[b]	Av.	49.2	40.5	35.6	34.0	33.2	31.4	28.6	26.6
	Low	36.0	25.0	25.0	26.0	29.5	27.0	24.0	22.0
	High	66.0	49.5	41.0	40.5	39.5	36.0	31.0	31.0

[a] Titration with 0.1 N acid.
[b] Titration with 0.1 N base.

ples throughout both acid and base ranges. Progressively decreasing values were obtained in the samples from the first to the fourth milking. The sample representing the fourth milking and composites of the fifth and sixth, seventh and eighth, and 15th and 16th milkings had approximately the same buffering effect in the acid range. The composite sample of the 27th and 28th milkings had a slightly smaller buffering action than did any of the other samples. Koestler (4), using a different method, also noted that buffering capacity of colostrum was greater than that of milk and that buffering capacity tended to decrease gradually during the transition.

Colostrum from Holsteins had the smallest buffering capacity, whereas that from Jerseys had the largest (data not shown). Differences, however, were relatively small. Colostrum from Guernseys and Ayrshires had approximately the same buffering capacity, which was greater than that from Holsteins and less than that from Jerseys. Although the data are limited, a tendency was observed for the buffering of colostrum and transitional milk to be higher during the winter and early spring than during the remainder of the year. Watson (11) noted that herd milk from Jerseys had a greater buffering effect than milk from Holsteins; but, unlike present observations on colostrum and transitional milk, his findings did not show that season and pasture grazing affected this property.

Van Slyke (10) suggested the use of the ratio $\frac{dB}{dpH}$ as a numerical measure of buffer value. The values obtained for $\frac{dB}{dpH}$ may be plotted against pH, producing a curve of buffering intensity. Data obtained on first colostrum and on composites of the 27th and 28th milkings were used for calculating $\frac{dB}{dpH}$ by Whittier's formula (12) (fig. 2). The shape of the curve representing whole milk obtained 2 wk. after calving is similar to that given by Whittier for skim-milk, except that his values for $\frac{dB}{dpH}$ are slightly larger (probably resulting from concentration of buffering substances by removal of fat), his maximum is shifted to a slightly higher pH, and he did not show an inflection between pH 6 and pH 7. In the present study, maximum buffering of milk was approximately at pH 5.25. Above pH 8 the buffering index of milk again increased. Maximum buffering of colostrum was found at about pH 5. The colostrum curve was similar to that of milk, except the peaks of the curve for the former were more marked and the single inflection in the milk curve between pH 6 and 7 seemed to have resolved into two parts in the colostrum curve.

Van Slyke (10) stated that, in studies of buffering, the effect of dilution by the acid or base used for titration was small if the volume increase did not exceed 50 per cent. A 50 per cent average increase in volume was not exceeded by any samples representing milkings after the second at pH values of 5 to 9. In several samples titrated to pH 4, the volume of acid added exceeded 50 per cent, in which cases the buffering effect resulted from sample plus solution.

The difficulties of identification of the buffering systems of milk have been

pointed out (8). The greater complexity and variable composition of colostrum and early transitional secretions render even more difficult identification of the buffering systems of these secretions. It is of interest, however, that the peaks and inflections in the buffer index curve of colostrum at approximately pH 4.9, 6.2 and 6.8 correspond, respectively, to maximum buffering of calcium phosphate, bicarbonate and sodium and potassium phosphate (8) systems.

A test was made of the relative buffering of some colostrum constituents in the following manner: Colostrum was diluted and centrifuged to remove fat and adjusted to pH 4.5 to precipitate casein, which was removed; the solution was adjusted to the original pH and dialyzed to remove salts. It was found by

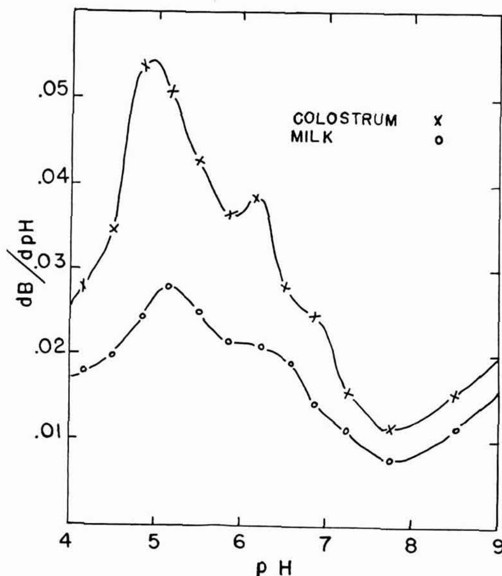

FIG. 2. Buffer index of first colostrum and 14th-day milk (27th + 28th milkings) plotted against pH.

titration of the various fractions to pH 4 that the defatted and decaseinated colostrum had only approximately one-half the buffering value of whole colostrum, and that the sample that also was dialyzed retained only about one-seventh of the original buffering capacity. At pH 9, however, the removal of casein caused only about one-fourth as great a decrease in buffering as did removal of casein plus dialyzable salts. The titration curves of whole colostrum and of defatted, decaseinated colostrum were practically identical from the initial pH of 6.4 to pH 5.7, which suggests the possibility that in this range salts and/or whey proteins are the principal buffering agents, and that casein had relatively little effect. The foregoing interpretations of the colostrum components responsible for buffering at various pH values are valid only to the extent that the methods

for preparing the various fractions may be assumed to leave them unaltered with respect to buffering capacity.

Osmotic pressure. The average osmotic pressure of the mammary secretions (table 3) decreased rapidly in the first four postpartum milkings; only relatively small changes were noted thereafter. The osmotic pressure of the 27th and 28th milking composite was about two-thirds that of first colostrum. The small but perceptible increase in osmotic pressure of the 15th and 16th milking composite was observed on samples from many cows. No explanation, however, can be offered for this apparent tendency, but it is of interest that buffering at pH 5 changed similarly (table 2). The larger protein and mineral content of colostrum as compared to milk (7) probably was the principal cause of the increased osmotic pressure of the former secretion. The complexity of a heterogenous system such as colostrum and its changing composition make it difficult to identify the contribution made by each component to osmotic pressure.

Although differences due to breed were small, colostrum of Jerseys had the highest osmotic pressure, whereas that of Ayrshires was lowest, and that of Holsteins and Guernseys was intermediate (data not shown). With the exception of samples from Jerseys, it was found that osmotic pressure of samples collected in winter and early spring, before pasture grazing, was slightly higher than that of samples collected during the pasture-grazing season.

TABLE 3

The osmotic pressure of colostrum and transitional milk obtained during the first 2 wk. postpartum (pressure in mm. of mammary secretion)

	No. milking postpartum							
	1	2	3	4	5 + 6	7 + 8	15 + 16	27 + 28
Av.	530	461	387	379	378	370	389	350
Low	342	304	277	290	323	189	329	297
High	643	725	535	493	439	457	484	402

Thus, changes in buffering and osmotic pressure not only follow trends somewhat similar to changes in several other properties and components of colostrum and milk during the transition period (6, 7), but the season during which the samples were collected, which appears to have some relationship to buffering and osmotic pressure of colostrum, also seems to have had a similar influence on viscosity (6), specific gravity and content of solids and ash (7).

SUMMARY

A study was made of changes in pH, in buffering capacity and in osmotic pressure of colostrum and transitional milk produced during the first 2 wk. postpartum. The average pH of first colostrum from 57 cows was 6.28, range 6.00 to 6.61. The pH of the early postpartum mammary secretions from 20 cows increased gradually from an average of 6.32 in first colostrum to 6.50 in milk of the 14th day (composites of 27th and 28th milking). Buffering capacity of

early postpartum secretions was greater than that of normal milk and decreased rapidly during the first four milkings. Buffer indices $\left(\frac{dB}{dpH}\right)$ were calculated at various pH values. The buffer index of first colostrum at maximum buffering (approximately at pH 5) was double that of 14th-day milk and shifted to a slightly lower pH value. Osmotic pressure of colostrum was higher than that of milk collected on the 14th day. The decrease in osmotic pressure was the most marked in the first four postpartum milkings. Milk collected on the 14th day had about two-thirds the osmotic pressure of first colostrum.

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THE EFFECT OF THE VARIOUS STEPS IN THE MANUFACTURE ON THE EXTENT OF SERUM PROTEIN DENATURATION IN NONFAT DRY MILK SOLIDS[1]

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There is a continually increasing market for "tailor-made" nonfat dry milk solids, that is, products processed to suit the specific requirements of the consumer. One of the more recent of these special products, "low-heat" nonfat dry milk solids, is manufactured for beverage purposes and cottage cheese making. Although standards of quality for this type of dry milk have not been formulated, proper control of the preheating, condensing and drying operations is necessary to produce a satisfactory product. Adequate control of these processes requires the aid of an objective method of evaluating the heat treatment of milk. Since it has been demonstrated that the heat denaturation of the serum proteins is a function of the time and temperature (3, 4), this property of milk was selected as a basis for measuring the heat effects of the several steps in the drying of skimmilk.

The amount of denatured serum proteins may be determined by the Kjeldahl nitrogen procedures of Rowland (5) or by the turbidimetric method of Harland and Ashworth (1). The latter was chosen for this problem because of its relative simplicity as compared to the Kjeldahl procedures.

MATERIALS AND METHODS

The skimmilk was prepared by centrifugation of fresh raw whole milk obtained from a bulked grade A supply. The samples were heat treated in 11×200 mm., thin-walled, stainless steel tubes immersed in a constant-temperature water bath at temperatures ranging from 155 to 175° F. for periods of from 2 to 120 min. The effective heating times were determined by graphical integration of the heating and cooling curves established for the apparatus used for these treatments. The rate of heat transfer through the steel tubes was so rapid that only approximately 1 min. of the total time was required for heating and cooling.

The condensed skimmilks were prepared by evaporation of raw skimmilk to 40 to 45 per cent total solids in a laboratory "Precision" evaporator at temperatures below 120° F. This milk then was diluted to yield a series of samples containing 9.0, 18.0, 27.0 and 36.0 per cent total solids. Aliquots of each of these were heated for 30 min. at 150, 160, 170 and 180° F., and the percentage of the serum proteins denatured was determined on each sample following dilution to 9.0 per cent solids.

The serum proteins were determined essentially according to the turbidi-

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[1] Paper no. 2696, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

metric method of Harland and Ashworth (1). The turbidity was developed by the addition of three drops of 10 per cent HCl to a cuvette containing 1 ml. or more of the non-casein filtrate and sufficient saturated NaCl solution to make 20 ml. and twice slowly inverting the stoppered cuvette. Finally the per cent transmittance at 420 mμ was determined within 5 min. without further agitation, using 19×150 mm. selected round cuvettes with a Coleman model 11 Universal Spectrophotometer adjusted to 100 per cent transmittance with distilled water.

RESULTS

The influence of the time of heating skimmilk at five temperatures on the percentage denaturation of the serum proteins is shown in figure 1. Each curve represents the average of three or more independent heat treatments.

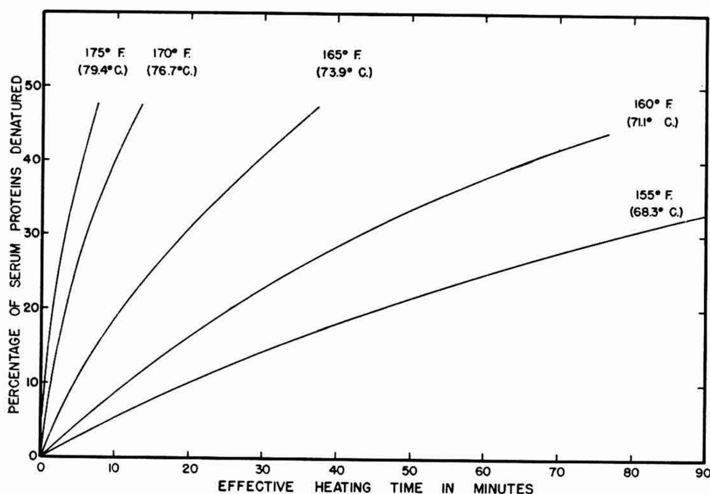

FIG. 1. The influence of the time and temperature of heating of skimmilk on the percentage of serum proteins denatured.

A more useful form of the data of figure 1 is presented in figure 2. This figure was constructed from a greatly expanded replica of figure 1 by plotting the logarithm of the time of heating necessary to denature a certain percentage of the serum proteins at each of the several temperatures against the temperature. A series of essentially straight lines resulted for the comparatively narrow range of temperatures studied. For every 13.5° F. increase in temperature, the time required for a given extent of denaturation decreases by ten-fold. Similarly, a pasteurization line is drawn through the points indicated by treatments at 143° F. for 30 min. and 160° F. for 15 sec. That the use of high-temperature, short-time pasteurization may be expected to cause less heat denaturation of the milk serum proteins than the holder method is evident from the difference in the slopes of the pasteurization line and the lines indicating protein denaturation.

It previously has been demonstrated by Krueger *et al.* (2) that concentration of the nonfat solids influences the reaction of the serum proteins to heat treatment. Since their data only cover the range up to about 23 per cent solids, information was secured on the effect of solids concentrations up to 40 per cent on the rate of denaturation. The results shown in figure 3 indicate that, in general, the effects of concentration on serum protein denaturation were not large. At 150° F. essentially the same amount of denaturation was secured at all concentrations studied. At 160 and 170° F. there was some decrease in serum protein denaturation with increases in solids concentration, but at 180° F. the effect

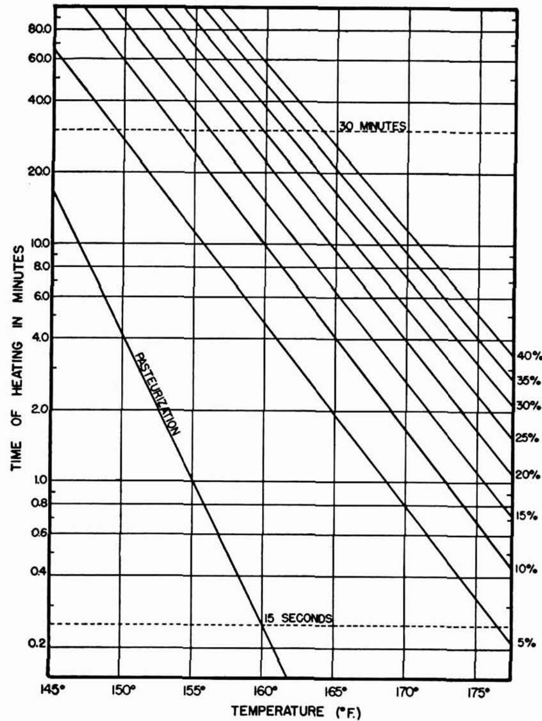

FIG. 2. The time-temperature relationships for heat denaturation of the serum proteins in fresh skimmilk.

of concentration again became negligible. Similar data were obtained when reconstituted freeze-dried milk was used for the preparation of the condensed samples. It may be concluded that no more serum protein denaturation for a given heat treatment may be expected in the vacuum pan than with the same treatment of the uncondensed product.

Although the time and temperature of heat treatment may be known for the pasteurization of milk, the time element is not so obvious in the operation of evaporators. Using the data of figure 2 as a guide, it is evident that excessive

denaturation of the serum proteins cannot be avoided if the first stage of a multiple-effect evaporator is operated at temperatures above 160° F. Furthermore, operation of even single-effect vacuum pans at above-normal temperatures will result in excessive heat treatment when low-heat powder is desired.

Data have been obtained from a commercial operation to show the heat damage that may occur when a triple-effect evaporator is used for the manufacture of nonfat dry milk solids. The percentages of the serum proteins of the raw milk denatured in the pasteurized, condensed and dry milk were determined. Pasteurization caused no measurable serum protein denaturation. However, 20 per cent of the serum proteins were denatured in both the condensed and dry milks. It is obvious, therefore, that most of the heat damage occurred in the evaporator. The first effect of the pan in question was operated at 17 in. of Hg gauge, which means that the temperature of the vapor was about 170° F. It

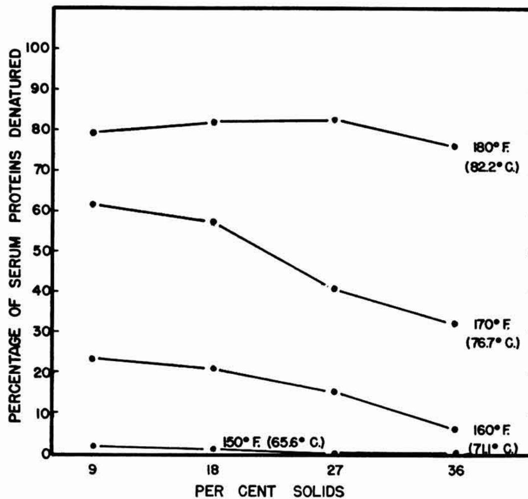

FIG. 3. The influence of the solids content on the percentage of the serum proteins denatured during 30-min. heating at various temperatures.

may be seen from figure 2 that only 2 min. exposure at this temperature is sufficient to denature more than 10 per cent of the milk serum proteins. The actual temperature of the milk would be higher than that of the vapor by the elevation in the boiling point due to dissolved solids and by an amount equivalent to the pressure differences in various parts of the evaporator. For any particular evaporator the approximate time and temperature of treatment under any given set of operating conditions could be computed. Obviously, the total time and temperature treatment in the complete system must be considered.

If the time and temperature at each stage of processing are known, the cumulative effect may be estimated with a fair degree of accuracy by converting all the treatments to the time equivalent at any one temperature. The approxi-

mate percentage serum protein denaturation to be expected may be read from figure 2, based on the estimated total time exposure at the temperature selected.

Data have been secured showing that the time and temperature of exposure of the condensed milk particles during the actual spray-drying operation are not sufficient to cause measurable denaturation of the serum proteins; the percentage denaturation in spray-dried milk is identical to that in the condensed milk from which it is made. Any heat exposure of the condensed milk prior to the actual spraying operation, however, must be considered in evaluating the total treatment. The effects of heat treatment of the powder itself have not been fully investigated.

Data are being secured concerning the normal geographical and seasonal variability of the amount and heat lability of the serum proteins among milks. Although the results obtained to date indicate considerable variation among milks in this regard, this fact in no way detracts from the value of using serum protein denaturation as a means of controlling the various stages of manufacture of nonfat dry milk solids.

SUMMARY

The time and temperature relationships determining the extent of denaturation of serum proteins of fluid skimmilk have been established for temperatures ranging from 145 to 175° F. There is a ten-fold decrease in the time required for a given percentage denaturation of the serum proteins for each 13.5° F. increase in the temperature.

Any application of heat to milk may be detrimental to its utility, and since heat treatments are cumulative, the successful manufacture of a product such as low-heat nonfat dry milk solids depends not only upon adequate control of the time and temperature of pasteurization but also on each of the other manufacturing processes. Such control may be based upon the turbidimetric estimation of the milk serum proteins.

ACKNOWLEDGMENTS

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned no. 370 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to of the Army. The support of the Minnesota Institute for Research is gratefully acknowledged.

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ASSOCIATION ANNOUNCEMENT

FORTY-SEVENTH ANNUAL MEETING

UNIVERSITY OF CALIFORNIA

DAVIS

JUNE 24–26, 1952

REGISTRATION AND HOUSING

Registration and housing headquarters will be in the Administration Building from 10:00 a.m. to midnight June 23 and from 8:00 a.m. to 5:00 p.m. June 24–26. Housing facilities will be available in dormitories, hotels and tourist courts in or near Davis and Sacramento. The University cafeteria will be open from Monday noon, June 23, through Thursday, June 26. Other eating facilities in the vicinity of the University will be available.

Davis is on the main line of the Southern Pacific Railroad and on U.S. highways 40 and 99. It is served by United and Southwest Airlines at Sacramento (nearest airfield), which is 15 miles from Davis. Information on points of interest in California, scenic, historical and recreational, may be secured by writing the California State Chamber of Commerce, 350 Bush St., San Francisco, California.

Announcements of the meeting, including a card for advanced registration and room reservations, will be sent to individual members of the Association about April 25, 1952.

PROJECTION EQUIPMENT

Equipment will be available in all lecture rooms for the projection of 3.25 × 4 in. and 2 × 2 in. slides. Those wishing other projection equipment should notify their section chairman.

COMMITTEE AND SPECIAL MEETINGS

Groups wishing rooms for committee or other special meetings should contact Harold Goss, Animal Husbandry Division, College of Agriculture, Davis, California, stating the time and number of persons expected to participate. Provisions for special breakfasts, luncheons or dinners should be made by writing to P. T. Cupps, Animal Husbandry Division, College of Agriculture, Davis, California.

The following announcement was received in the editorial office on April 8:

XIIITH INTERNATIONAL DAIRY CONGRESS

Announcement has recently been made of the forthcoming XIIIth International Dairy Congress to be held at The Hague in the Netherlands June 22–26, 1953. The Congress is held under the direction of the International Dairy Federation. The Netherlands National Committee, with the approval of the Netherlands Government, will organize the 1953 Dairy Congress. Dr. J. Linthorst Homan will act as Chairman of the Committee on Management and Mr. G. H. Hibma as General Secretary of the Congress.

An invitation is extended to technical and scientific persons to present papers and be present for the discussion in the various sectional meetings of the Congress.

Anyone desiring to attend and prepare a paper is requested to signify his intention by April 1, 1952. All manuscripts are requested before September 1, 1952.

The Congress fee will be 50 guilders for each member. Tours will be arranged after the sessions. Anyone who desires to become a member of the Congress or present a paper (maximum 2000 words) for the program should write the undersigned or write directly to the General Secretary of the XIIIth International Dairy Congress, Mr. G. H. Hibma, The Hague, Netherlands.

O. E. REED, Chief, Bureau of Dairy Industry, USDA.

VOLUME XXXV APRIL, 1952 NUMBER 4

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

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

BOOK REVIEW

222. Zootecnica Speciale (Special Animal Husbandry). 2nd ed. T. BONADONNA. Istituto Editoriale Cisalpibo, Milan. 3 vol. 2,492 pp. 1,080 figs. 1350 lire. 1951.

The 1st vol. of this greatly expanded 2nd ed. contains chapters on distribution of livestock and the position of the industry in Italy followed by an account of reproduction in general and artificial insemination in particular. Half the volume is devoted to a detailed account of organization and policies of breed societies and of others for recording yields, etc. Much space is given to rules governing these societies and to sample reproductions of record sheets used by them, especially for European and American societies. It is specially useful for those who wish to compare the methods employed in various countries.

The 2nd vol. contains accounts of the principal breeds of dairy and other cattle, their distribution, milk yields and rates of growth. Historical notes on introductions, organization of breed societies, and of development in each country abound. The amount of space devoted to each is roughly proportional to the numerical importance of the breed. Most of the lesser breeds come in for brief notice. Horses also are included in this volume.

The 3rd vol. deals with sheep, swine, poultry and fur animals. In all classes of livestock, brief attention is paid to methods of management, hygiene, feeding, housing and disposal of the products. The book is well illustrated but the poor quality of the paper on which most are printed rather mars the quality of reproduction. It is a significant and comprehensive addition to the literature of dairy husbandry.

A. A. Asdell

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

223. The microscopical examination of milk powders. N. KING. Netherlands Milk & Dairy J., **2,** 3: 137–147. 1948.

Six samples of milk powder were examined microscopically, after mounting in liquid paraffin and glycerin, under ordinary light and in polarized light. No optically active areas showed up in spray-dried powders, but were numerous in roller-dried powders. Microscopic examination of reconstituted milks showed large fat spots on the surface of roller-dried, while spray-dried showed relatively small amounts. This indicated that destruction of the emulsion was more complete in roller-dried milk.

W. W. Overcast

224. Powder conveying method and apparatus. G. P. HENSLEY and D. O. JOHNSON (assignors to Golden State Co., Ltd.). U. S. Patent 2,583,-648. 5 claims. Jan. 29, 1952. Official Gaz. U. S. Pat. Office, **654,** 5: 1245. 1952.

A method and equipment are described for maintaining uniformity in powders, such as skimmilk and whole milk powder and moving same through a pipe line at a specified rate.

R. Whitaker

225. The use of dried skimmilk in breadmaking. B. VAN DAM, R. R. ABMA and J. G. REVALLIER-WARFFEMIUS. Netherlands Milk & Dairy J., **2,** 3: 148–161. 1948.

Several baking trials using dry skimmilk prepared in various ways are described. Substituting skimmilk powder for 5–6% of the flour produced a loaf equal in size to water bread and, in addition, stayed fresh longer, had higher nutritive value, was softer and had a finer brown color of the crust. By spray drying preheated skimmilk (120 or 110° C. for 20 min.) a suitable powder was prepared for use in breadmaking.

W. W. Overcast

226. Reconstituted dry milk for fluid purposes. R. F. MOORE, C. U. JENSEN and D. L. GIBSON. Can. Dairy and Ice Cream J., **31,** 11: 45–46, 76. Nov., 1951.

The fluid milk supply in many markets in Western Canada has deteriorated to such an extent that a large percentage of milk consumed has had to be supplemented by reconstituted dry milk. Spray-process skimmilk powder has produced the most satisfactory results for reconstituting milk for fluid purposes. Best results for standardization of reconstituted skimmilk are obtained by using 30% sweet cream. A good quality butter also can be used for the butterfat required, but it is suggested that a reconstituted cream of 30–35% butterfat be prepared from the butter for more accurate standardization. The process consists of adding 1 lb. of spray-process

skimmilk powder to each 10 lb. of water at a temp. of approximately 45° F., allowing 2- or 3-hr. agitation to rehydrate the powder. The milk is clarified and then placed in a holding vat where cream is added for standardization. The product then is forewarmed to 110–120° F., homogenized and pasteurized either by the vat or HTST system, is cooled to 40° F. or below and bottled. H. Pyenson

227. Dispensing holder for liquid containers with means for puncturing said containers. E. L. THOMAS (assignor to Clara Thomas). U. S. Patent 2,582,660. 4 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 833. 1952.

A plastic dispenser for evaporated milk in cans has a handle and 2 imbedded hollow punches, 1 for making an air vent and 1 for making a pouring outlet. R. Whitaker

228. Fresh concentrated milk. W. F. DONOVAN. Can. Dairy and Ice Cream J., **31,** 1: 42, 51. Jan., 1952.

Concentrated milk comprises a very small proportion of total sales of a large Boston dairy. The process consists of clarifying 3.7% milk, preheating in a barrel heater, condensing in a vacuum pan at low temperature to one-third volume. The milk then is homogenized, pasteurized, cooled and bottled in paper containers. The product tests 11% fat, 37% total solids and has a bacterial count of from 3,000–20,000. The flavor and keeping quality of this product has been excellent. In Sept., 1951, concentrated milk was selling to stores for 53–54¢ and to consumers for 61¢. The consumer was saving approximately 2¢/qt. on a reconstituted basis. H. Pyenson

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

229. Metylenblat—Reduktaseprøve? (Methylene blue reduction test or Resazurin test?) M. S. MOGENSEN. Nordisk Mejeri-Tidsskrift, **5:** 1–13. 1951.

The author bases his comparison on the report by P. Hempler on his studies of the resazurin test and on results obtained at the dairy research laboratory of the Royal Vet. and Agr. College, Copenhagen, Denmark.

The resazurin test will give a more rapid classification of the bacteriological quality of milk than the methylene blue test. The former is useful in detecting abnormal milk. The correct use of the resazurin test may be a helpful tool in improving the sanitary quality of milk.

Substitution of the methylene blue with the resazurin test in Denmark probably will be met with strong resistance. The classification by the resazurin test requires more critical judgment than with the methylene blue test. The staff at the research laboratory quickly obtained the necessary skill for accurately determining the resazurin test results. G. H. Wilster

230. The assay of the antibiotic nisin by means of a reductase (resazurin) test. BOSA FRIEDMANN and E. EPSTEIN, Fison's Research Lab., Loughborough. J. Gen. Microbiol., **5,** 5: 830–839. 1951.

A strain of *Streptococcus cremoris* was used as the test organism in a modified resazurin reduction procedure for nisin assay. The method saves time since results may be obtained in less than 1 hr. and the accuracy compares favorably with the many other methods which require more time. J. J. Jezeski

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

231. On the correction in the butyrometric determination of fat in milk powder by the Gerber-Van Gulik method. TH. BROUWER. Netherlands Milk & Dairy J., **2,** 4: 185–193. 1948.

A correction equation is derived from experimental data for use with the Gerber-Van Gulik method for determination of fat in milk powders. Also, for practical use, a correction table is given. Evidence shows a constant fat loss occurs, independently of the fat content, in both the Gerber and the Gerber-Van Gulik methods. W. W. Overcast

232. Methods for determining the fat content of milk. I. The Rose-Gottlieb method. L. RADEMA and H. MULDER. Netherlands Milk and Dairy J., **2,** 4: 204–209. 1948.

The principle on which the Rose-Gottleib method is based is discussed and a standard procedure for determining the fat content of milk by this method is presented. W. W. Overcast

233. Methods for determining the fat content of milk. II. The Gerber method. L. RADEMA and H. MULDER. Netherlands Milk & Dairy J., **2,** 4: 210–222. 1948.

The Gerber method is discussed on the basis of errors involved, reproducibility of results and correcting the results. The relation between the Gerber and Rose-Gottlieb methods is expressed as follows: Gerber = (1.040 × Rose-Gottlieb) – 0.070. It is shown that centrifuging twice for 3 min. at 65° C. is not necessary. W. W. Overcast

234. Standardization of methods for the analysis of milk and dairy products in the Netherlands. I. Determination of the percentage of fat in whole milk by the Gerber method. Govt. Dairy Sta. at Leyden. Netherlands Milk & Dairy J., **2,** 4: 194–203. 1948.

The standard method for determination of fat in whole milk by the Gerber method is presented. W. W. Overcast

235. The study of the Roeder method for the determination of fat in cheese. M. J. BERNAERTS. Netherlands Milk & Dairy J., **2,** 2: 99–107. 1948.

A comparison of the Roeder test for fat in cheese (using a stannous chloride in concentrated HCl as solvent) is made with the Weiball and Van Gulik test (which use dilute H_2SO_4 as solvent). In every case the Roeder test was from 1.57–2.84% higher than the other 2 tests. This was largely accounted for through formation of amyl esters with the lower fatty acids, which

formed more readily at the high temperature employed in the presence of tin chloride.
W. W. Overcast

236. Process for hydrolyzing lactalbumin. G. H. Carlson (assignor to R. P. Scherer Corp.). U. S. Patent 2,585,225. 5 claims. Feb. 12, 1952. Official Gaz. U. S. Pat. Office, **655,** 2: 413. 1952.

Dried hydrolyzed lactalbumin of high nutritive value is prepared by gelatinizing a slurry of lactalbumin with a non-toxic alkali in a pH range of 7.5–11.0 at 35–50° C., then hydrolyzing the gelatinized albumin with a lipolytic-free proteolytic pancreatic enzyme at 38–44° C. at pH 7 or above for not over 2 hr. Not over 19% of the total N content of the final product is in the from of α-amino N. After clarifying the effluent is dried.
R. Whitaker

237. Milk protein products and process. E. P. Partridge (assignor to Hall Labs., Inc.). U. S. Patent 2,582,353. 15 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 751. 1952.

A combination of casein and crystalline potassium metaphosphate is described.
R. Whitaker

238. Recovery of lactose from mother liquor. W. J. Pratt, H. F. Seibert and R. C. Stribley (assignors to Wyeth, Inc.). U. S. Patent 2,584,-158. 3 claims. Feb. 5, 1952. Official Gaz. U. S. Pat. Office, **655,** 1: 57. 1952.

Deproteinated whey is demineralized by resin ion-exchange material to remove about 85% of the ash. The lactose is crystallized from the concentrated solution. The resin bed rinse water, containing about 10% solids, is utilized by adding back to untreated lactose mother liquor.
R. Whitaker

239. The colorimetric determination of lactic acid in silage. A. J. G. Barnett, Univ. of Aberdeen. Biochem. J., **49,** 4: 527–529. 1951.

A method for determination of lactic acid (Barker & Summerson) in silage is described. The lactic acid is oxidized to acetaldehyde by H_2SO_4 and upon addition of p-hydroxydiphenyl (in presence of Cu) the color development is quantitative. Reported lactic acid in wet grass silages varied from about 1.6% at pH 3.9 – 0.1% at pH 5.6.
A. O. Call

240. Studies in vitamin A. 15. The variations in the serum β-carotene and vitamin A levels of cows near parturition. T. W. Goodwin and A. A. Wilson, Univ. of Liverpool. Biochem. J., **49,** 4: 499–503. 1951.

Nine cows kept under English farm conditions showed a drop in serum carotene and vitamin A levels at parturition. This is in agreement with earlier similar results reported in the U. S. The authors feel the changes are an indication of the concentration of blood constituents in general at parturition. No significant seasonal changes in serum concentration of vitamin A or β-carotene were noticed from May to Oct.
A. O.Call

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

241. Afprøving af Kolding pladepasteur, type 2 SKd (Testing of a high-temperature short-time pasteurizer, type 2 SKd). K. L. Andersen, Danish Expt. Sta., Copenhagen. Report no. 71. Oct., 1951.

A HTST pasteurizer type 2 SKd manufactured by the Danish Dairy Machinery Factory at Kolding was tested. The capacity of the pasteurizer was 3,600 l./hr. For the tests, whole milk was heated in 4 sec. to temperatures ranging from 80.9 – 65° C., followed by quick cooling to 65° C. and holding at this temperature for 16.5 sec. Milk inoculated with living tubercle bacteria was pasteurized. Guinea pigs were used for the tests. The bacteria were effectively killed when the milk was heated to 71° C. and above and afterwords held at 65° C. for 16.5 sec. A phosphatase-negative test was obtained when the milk was heated to 78° C. and above, and held at 65° C. for 16.5 sec. Milk pasteurized in this way had a satisfactory keeping quality; curd formation and the creaming property were only slightly affected. Steam consumption was 46.6 kg./1,000 kg. milk.
G. H. Wilster

242. Pasteurizing of milk by means of electrical energy of high frequency. S. G. Wiechers, H. De Zeeuw and J. Van Den Bosch. Netherlands Milk & Dairy J., **2,** 2: 59–69. 1948.

The specific effect of a high frequency electric current upon microorganisms in milk was not determined since the high frequency was not attained without an accompanying increase in temperature. The destruction of bacteria could be explained as due to temperature increase. The authors felt that there was no advantage in electrical pasteurization of milk and the cost was more than by conventional means.
W. W. Overcast

243. Rapid heat processing of fluid foods by steam injection. A. H. Brown, M. E. Lazar, T. Wasserman, G. S. Smith and M. W. Cole. Ind. Eng. Chem., **43,** 12: 2949–2954. 1951.

A pilot plant system for heat processing and concentrating liquid foods is described. The liquid is heated in a specially designed steam-injection heater and flashed into the steam-jacketed tube of a combination evaporator. Over-all heat transfer coefficients in this tube ranged from 510–600 b.t.u./hr./ft.^{2}/° F. with water, apple juice and berry puree. Fruit and vegetable juices were heated as high as 300° F. and cooled to 80° F. in a total time of less than 1 sec. with controlled amounts of concentration. Milk was the only liquid of the many tried which left an adhering deposit on all interior surfaces of the heater. Raw milk heated to 245° F. and cooled evaporatively to 80° F. in 0.7 sec. developed about the same flavor as normally pasteurized milk. Raw milk was heated to 244° F. and concentrated to 26% solids in a single pass through the evaporator without development of a perceptible cooked flavor and with loss of an undesirable feed flavor.
B. H. Webb

244. Food processing apparatus. F. W. KRUEGER (assignor to Food Machinery and Chemical Corp.). U. S. Patent 2,584,473. 11 claims; and U. S. Patent 2,584,474. 20 claims. Feb. 5, 1952. Official Gaz. U. S. Pat. Office, **655,** 1: 141. 1952.

A sterilizer of the continuous agitating type is described in which the cans are introduced into a steam-heated pressure chamber through a revolving inlet lock, then through a tortuous pathway consisting of series of rotating pockets to an outlet lock. A similar piece of equipment is used for cooling. R. Whitaker

245. Method and apparatus for emulsifying fluids. E. M. IRWIN. U. S. Patent 2,577,247. 14 claims. Dec. 4, 1951. Official Gaz. U. S. Pat. Office, **653,** 1: 156. 1951.

Milk is homogenized by passing it continuously through this apparatus which consists of 2 parts: (a) a conventional pressure release valve and (b) a pair of electrodes in the milk stream between which a small electrical potential is maintained. Typical operating conditions resulting in efficient homogenization of milk are as follows for a milk flow of 10 gal./min.: (a) pressure drop of 500–600 psi., followed by application of (b) a direct current of 0.15 amp. between electrodes 2 in. apart, giving a voltage drop of 1.25 volts. The use of alternating current is indicated during a minor part of the cycle to maintain efficiency. R. Whitaker

246. Apparatus for homogenizing. A. G. BORCK and C. GRONNING (assignors to Aktiebolaget Separator). U. S. Patent 2,583,206. 10 claims. Jan. 22, 1952. Official Gaz. U. S. Pat. Office, **654,** 4: 1064. 1952.

Milk and other dispersions of oil-in-water type are homogenized by passage through a bed of closely packed balls of small diameter.
R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

247. The utilization of non-protein nitrogen in the bovine rumen. 7. A qualitative and quantitative study of the breakdown of carbohydrate which accompanies protein formation in bovine rumen contents during in vitro incubation. M. L. MCNAUGHT, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **49,** 3: 325–332. 1951.

Rumen liquid taken from a permanent fistula was centrifuged to remove protozoa and then incubated with addition of various substances as sources of bacterial energy. Urea (0.05%) was added to all samples and the amount of protein synthesized was taken as a measure of bacterial growth. A table of 30 comparisons is shown. Generally speaking, only boiled starches, raffinose, inulin, fructose, cellobiose, L-(+)arabinose and D-(+)xylose gave values as high as maltose controls. Apparently, a primary alcohol group and a potential aldehyde are both essential for maximum utilization. Pentoses gave no lactic acid as an endproduct, thus differing from maltose.

In quantitative measurements of endproducts, 90–96% of the total C was accounted for in the case of maltose, but with pentoses only 83–93% was accounted for. A. O. Call

248. On Ruminococcus flavefaciens, a cellulose-decomposing bacterium from the rumen of sheep and cattle. A. KAARS SIJPESTEIJN, Organisch Chemisch Inst. T.N.O., Utrecht, Holland. J. Gen. Microbiol., **5,** 5: 869–879. 1951.

A cellulolytic bacterium, *Rumincoccus flavefaciens,* was isolated from rumen of sheep and cattle in pure culture using the dilution method and agar media containing strips of filter paper. The organism is a gram positive streptococcus, an obligate anaerobe, which attacks cellulose and cellobiose but not maltose, starch, lactose and rarely glucose. Growth on cellulose increased in the presence of *Clostridium sporogenes.* The endproducts of the fermentation of cellulose and cellobiose included succinic, acetic and formic acids. J. J. Jezeski

249. The action of the ciliates of the sheep's rumen upon various water-soluble carbohydrates, including polysaccharides. F. M. MASSON and A. E. OXFORD, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., **5,** 4: 664–672. 1951.

Holotrichic ciliates from the rumen of hay-fed sheep were allowed to act on water-soluble carbohydrates under conditions presumably similar to those in the rumen; however, not in the presence of bacterial populations present in the rumen. Iodophilic polysaccharide granules possessing the properties of starch rather than glycogen were produced in the presence of a number of carbohydrates including glucose, fructose, sucrose, and cellobiose. Oligotrich ciliates were able to produce starch granules from various carbohydrates at a much slower rate. Similar starch granules were isolated from dried rumen contents of sheep that were fed on starch-free spring grass. J. J. Jezeski

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

250. Evaluating dairy sires. J. C. BERRY, Univ. of Brit. Columbia. Holstein-Friesian World, **49,** 4: 321–322. Feb. 16, 1952.

Equal-parent, regression and expectancy indexes are compared. A plan is proposed in which each record would be compared with the breed class average and expressed in percentage. The daughters of a bull could be compared with their dams on the basis of percentage above or below breed average. Sires could be compared on a similar basis. Correction factors for age, no. of milkings or length of record would not be used under this plan. A. R. Porter

251. A sire's transmitting ability. J. P. BEARDSLEY, Am. Jersey Cattle Club. Jersey Bull., **71,** 3: 154–155, 182, 184–186. Feb. 10, 1952.

A discussion is given of the number of daughters of a sire needed to estimate his transmitting ability. When records are made in the same herd, 5 unselected daughters are more reli-

able than 1 by 84%; 10 daughters are more reliable than 1 by 110%. Each additional daughter with a production record adds to the accuracy of the estimate, but at a diminishing rate.

When the bulls being compared have daughters in different herds, 5 unselected daughters are more reliable than 1 by 33%; 10 are 39% more reliable than 1.

Publishing a preliminary report when at least 5 daughters of a sire have completed records is suggested to supplement the present Tested Sire Ratings based on 10 or more daughters.

A. R. Porter

252. The cytological changes in the cervical mucosa of the cow (Bos taurus) throughout the estrous cycle. J. B. Herrick, Iowa State Coll., Ames. Am. J. Vet. Research, **12,** 45: 276–281. Oct., 1951.

In order to obtain information about the cellular changes in the cervix of the same cow at various stages of the estrous cycle, an instrument was designed for the recovery of daily biopsy specimens. Four cows with normal breeding histories were selected for the study. Photomicrographs of the epithelial changes in the cervix of 1 cow are presented. All of the epithelial cells lining the cervix were found to contain mucus (stained with Mayer's mucicarmine) at all stages of the cycle. Evidence of some secretion appeared at all stages, but was greatest during estrus when the cells at the apex of the folds appeared to rupture and sometimes disappear. During estrus the blood vessels also were larger, and there was a correlation between vascularity and character of the mucus secreted throughout the cycle. E. W. Swanson

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

253. Milk examining pan. R. L. Herring. U. S. Patent 2,582,432. 3 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 772. 1952.

A 4-compartment tray for collecting samples of milk from the 4 quarters of a cow's udder is described. R. Whitaker

245. Milker claw. H. A. Sherwood (assignor to Harold Ransier). U. S. Patent 2,585,178. 1 claim. Feb. 12, 1952. Official Gaz. U. S. Pat. Office, **655,** 2: 401. 1952.

A design for a 4-way manifold for attaching a vacuum milk line to tubes leading to each of 4 teat cups is described. R. Whitaker

255. Milk receiving and discharging unit. G. W. Berry (assignor to Berry Milking System). U. S. Patent 2,583,723. 1 claim. Jan. 29, 1952. Official Gaz. U. S. Pat. Office, **654,** 5: 1265. 1952.

A device is described for discharging milk from a pulsating vacuum-type milking machine into a collecting vessel. R. Whitaker

256. Milk strainer. F. G. Hodsdon (assignor to International Harvester Co.). U. S. Patent 2,584,206. 3 claims. Feb. 5, 1952. Official Gaz. U. S. Pat. Office, **655,** 1: 71. 1952.

A small enclosed disc-type filter for milk, suitable for installing in the milk line from a milking machine, is described. R. Whitaker

257. Raw milk aerator. L. C. Harp. U. S. Patent 2,584,202. 6 claims. Feb. 5, 1952. Official Gaz. U. S. Pat. Office, **655,** 1: 70. 1952.

A motor-driven agitator stirs milk in a can. The agitator is shaped like a coiled spring and is raised and lowered by a shaft through a hole in a lid which covers the can. R. Whitaker

258. Dehorning instrument. W. J. Thompson. U. S. Patent 2,583,347. 9 claims. Jan. 22, 1952. Official Gaz. U. S. Pat. Office, **654,** 4: 1102. 1952.

A mechanical dehorning tool for removing the horns of cattle, cows, etc., is described.

R. Whitaker

259. Dehorning device. S. S. Mims. U. S. Patent 2,582,450. 6 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 777. 1952.

A means of dehorning farm animals by heat is described. R. Whitaker

260. Animal restraining device. H. C. Krueger. U. S. Patent 2,582,339. 2 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 748. 1952.

A device for leading domestic animals by the nose is described. R. Whitaker

261. Animal controlled watering device. C. H. Harmon (assignor to Waterloo Foundry Co.). U. S. Patent 2,585,547. 5 claims. Feb. 12, 1952. Official Gaz. U. S. Pat. Office, **655,** 2: 497. 1952.

Water is admitted to this drinking vessel for farm animals as the result of pressure by the animal's nose. R. Whitaker

262. Sanitary bag for animals. W. G. Kahlert. U. S. Patent 2,585,251. 8 claims. Feb. 12, 1952. Official Gaz. U. S. Pat. Office, **655,** 2: 420. 1952.

A bag, supported by and easily detachable from a harness, collects excrement and urine from cows, etc. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

263. Shrinkage. E. L. Thomas, W. B. Combs and S. T. Coulter, Univ. of Minn. Ice Cream Trade J,. **48,** 1: 40–42, 82–87. Jan., 1952.

Ice cream mixes were heat-treated at 165 and 185° F. for 1–6 min. Maximum shrinkage occurred in samples processed at 185° F. for 10 min. and at 165° F. for 30 min. Heat treatment sufficient to coagulate an appreciable amount of the serum proteins increases the tendency of ice cream to shrink. Addition of colostrum milk before and after mix pasteurization in quantities sufficient to increase the globulin content 0.05 and 1.10% increased shrinkage regardless of the treatment of ice cream samples prior to storage.

Samples of previously hardened ice cream were exposed to dry ice in an insulated cabinet for 36 hr., also in a vented chamber at atmospheric

pressure for 48 hr. at 18 ± 2° F. and other samples were placed in a metal shotgun can, lid sealed air-tight with tin foil and scotch tape, can surrounded with dry ice in an insulated ice cream packer and held for 48 hr. The exposure of ice cream to CO_2 resulted in marked shrinkage of all samples, whereas the effect of exposure to low temperature alone was negligible. Exposure of ice cream samples to nitrous oxide was as effective in causing shrinkage as was exposure to CO_2.

It is believed that dry ice-induced shrinkage is caused by disruption of ice cream structure through internal pressure changes associated with alternate absorption and desorption of CO_2. The jolting of samples at the rate of 230/min. for either 4 hr. at 0 ± 3° F. or for 6 hr. during which time the temperature gradually was increased from – 18–6° F. did not accelerate shrinkage.

W. H. Martin

264. Candies for ice cream. A. Mann, Mann's Candy Co., Los Angeles, Calif. Ice Cream Field, **58,** 2: 54, 56. Aug., 1951.

The use of candy as a source of flavor in ice cream is gaining in popularity, it is claimed. Use of candy especially designed for ice cream will give best results and also will aid in eliminating difficulty with feeders.

The use of from 3–5 lb. of candy/5 gal. of mix is recommended. Peppermint stick, lemon stick, butter pecan crunch, almond, hazelnut, English toffee, butter brickle and midget marshmallows are listed as available for the ice cream trade.

W. C. Cole

265. The importance of insulated bags for the "carry home" ice cream business. P. H. Tracy, G. Edman and J. Kurmann, Univ. of Ill., Urbana. Ice Cream Trade J., **48,** 1: 74. Jan., 1952.

Samples of ice cream frozen in a continuous freezer and hardened at – 10° F. for a minimum of 24 hr. were placed in insulated bags at – 10° F. They then were prestored at – 10, 0 or + 10° F. for 24 hr. The bagged samples were held at 37, 72 and 90 ± 20° F. for periods varying from 1–5 hr. Each package was discarded after a temperature of the ice cream was measured. Ice cream was considered unsable when it was sufficiently melted to drip when the open package was inverted.

The ideal combination was a low pre-storage and holding temperature. Ice cream in insulated bags kept satisfactorily at 72° F. for 2 hr. when the previous storage temperature was – 10° F.; at 90° F., the ice cream was held satisfactorily for 1 hr. when the storage temperature was 0° F. or less. Replacing 1/3 of the sugar solids with corn syrup did not appreciably damage results obtained when storing all-sucrose ice cream in insulated bags. Ice cream with 100% over-run increased in temperature slightly faster than ice cream with 70% over-run. Dry ice (3.75 oz./qt.) delayed softening of the ice cream, extending storage time about one-third. Air movement over the bags hastened warning.

W. H. Martin

266. Delivering ice cream on retail routes. Anonymous. Ice Cream Trade J., **48,** 1: 48. Jan., 1952.

Trucks of the Country Club Dairy retail routes have been equipped with small insulated boxes measuring about 2 ft. in all dimensions. Refrigeration for the day's run is provided by 2 Dale hold-over cartridges with a – 9° eutectic hold-over Back in the plant the cartridges are placed in the racks and wheeled into the freezing room for – 25° F. charging. By morning they are ready to go back into the insulated boxes in the trucks which are used for carrying ice cream and frozen foods.

W. H. Martin

267. Ice cream pop manufacturing and packaging P. Schenk. U. S. Patent 2,582,655. 11 claims. Jan. 15, 1952. Official Gaz. U. S. Pat. Office, **654,** 3: 832. 1952.

A wrapper for a rectangular-shaped portion of ice cream, 2 flaps of which contain holes which coincide when the flaps are folded is described. A stick is inserted through the holes into the ice cream to hold the flaps closed and to serve as a handle while eating the ice cream.

R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

268. Better manufacturing milk results from sediment testing. C. E. Lackner. Can. Diary and Ice Cream J., **31,** 1: 33–36, 38–40, 51. Jan., 1951.

Previous to June 1, 1951, compulsory grading of milk was only for cheese factories in Ontario. Now grading includes milk manufacturing plants. Milk that is not sweet and clean in flavor and that is below grade "B" as determined by the sediment test shall be rejected. There has been marked improvement in milk quality since the new regulations went into effect. H. Pyenson

269. Milk or milks? G. A. Richardson, Oregon State College. Jersey Bull., **71,** 3: 150–151. Feb. 10, 1952.

The composition of Jersey milks of different butterfat percentages is presented. The combustible energy and nutrient energy/lb. is calculated for milk of different butterfat tests. Combustible energy is expressed as Cal./lb. of milk = 56.4 (fat %) + 114.2. The nutrient energy value is food energy; units/lb. of milk = 50.6 (fat %) + 107.26.

Charts show theoretical yields of evaporated milk, butter, condensed skimmilk, cheese, dry whey or buttermilk from 1,000 lb. of milks of different fat contents. A. R. Porter

270. Filter. J. P. O'Meara (assignor to W. M. Sprinkman Corp.) U. S. Patent 2,583,963. 3 claims. Jan. 29, 1952. Official Gaz. U. S. Pat. Office, **654,** 5: 1328. 1952.

Details are given for construction of a sanitary-

type filter for milk and similar fluids, consistmg of a series of horizontal plates pressed together by a spring and holding discs of filtering material.
R. Whitaker

271. Container for liquids. W. M. FLEMING (assignor to Eskimo Pie Corp.). U. S. Patent 2,583,211. 2 claims. Jan. 22, 1952. Official Gaz. U. S. Pat. Office, 654, 4: 1066. 1952.

A paper container for liquids such as milk is described; it is rectangular in shape and provided with a scored area which can be easily punched out and used for pouring of the contents.
R. Whitaker

272. Liquid packaging machine. E. KAYAT (assignor to Perga Containers, Ltd.). U. S. Patent 2,583,106. 15 claims. Jan. 22, 1952. Official Gaz. U. S. Pat. Office, 654, 4: 1037. 1952.

A machine is described in detail for filling paper containers with liquids, such as milk.
R. Whitaker

273. Apparatus for manufacturing whipped cream: F. F. SUELLENTROP (assignor to Lemay Machme Co.). U. S. Patent 2,584,063. 8 claims. Jan. 29, 1952. Official Gaz. U. S. Pat. Office, 654, 5: 1353. 1952.

Partially filled containers of cream arc charged wIth a gas such as nitrous oxide by this equipment.
R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

274. Mode of formation of milk fatty acids from acetate in the goat. G. POPJAK, T. H. FRENCH, G. D. HUNTER and A. J. P. MARTIN, Nat'l. Inst. for Med. Research, London. Biochem. J., 48, 5: 612-618. 1951.

In previously reported work (see abs. 592, Oct., 1951) 4 different butterfat fractions from goat's milk were shown to possess varying degrees of activity following injection of radioactive Na acetate. In thc present study the individual component fatty acids from the pooled milk samples taken (a) up to 12 hr. after injection and (b) 12-48 hr. after injection, were fractionated, identified and checked for activity. All even-numbered saturated acids from C_2 to C_{18} were found. Acetic, butyric and caproic acids were broken down chemically to determine which of the carbon atoms originated from the isotope. From evidence it is suggested that the short-chain fatty acids are intermediates in synthesis of higher ones. In synthesis of butyric acid, it is believed that not only acetate but also C_4 compound, possibly β-hydroxybutyric acid, acts as a precursor and that stearic and oleic acids originate mostly from a precursor other than acetate.
A. O. Call

275. Incorporation of blood amino acids into milk proteins in the rabbit. (abs.) P. N. CAMPBELL and T. S. WORK, Nat'l. Inst. for Med. Research, London. Biochem. J., 49, 3: xlvi-xlvii. 1951.

Isotopic valine in 1 case and isotopic lysine in another were injected intravenously into lactating rabbits. After 6 hr. the activity of the milk proteins in both cases was much higher than blood proteins, indicating amino acids of blood are being directly incorporated into milk proteins.
A. O. Call

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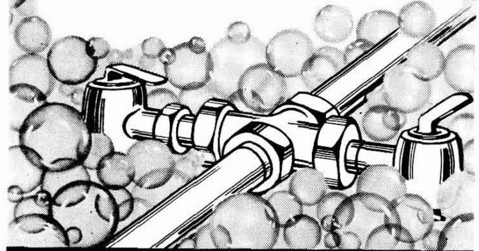

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