JOURNAL OF

DAIRY SCIENCE

Contents

Vol. XXXIV, No. 5, May, 1951

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

How Duraglas milk bottles help Dairies to greater profits!

✓ At the forming machines . . . at the lehr . . . at the raw material location—strict QUALITY CONTROL rules in the manufacture of Duraglas milk bottles.

✓ The result is *dependably* strong, high-trippage bottles of uniform capacity, with high resistance to impact, heat, cold, and washing solutions.

✓ In addition, Duraglas bottles have a "High-Luster" finish that keeps bottles glistening trip after trip. Dairies are assured of the finest bottles *always* because continuing research at the Duraglas Center *keeps* Duraglas bottles in the quality lead.

✓ High-quality, returnable glass bottles are unequaled for keeping container costs low. So remember, whatever your dairy container needs may be, look to one dependable source for glass containers—Owens-Illinois Glass Company.

ACL—Each bottle can be a "talking salesman" for the dairy with Applied Color Lettering designs. Stock designs are always available, and our artists are ready to develop special designs.

Duraglas Dairy Containers

TRADE MARK REG. U.S. PAT. OFF.

OWENS-ILLINOIS GLASS COMPANY • TOLEDO 1, OHIO • BRANCHES IN PRINCIPAL CITIES

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

F. E. NELSON, *Editor*
Ames, Iowa

Subscriptions. Price, $10.00 per volume in North and South America; $10.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising copy should be mailed to P. R. Ellsworth, The Ohio State University, Columbus 10, Ohio. Advertising plates or cuts should be mailed direct to the Business Press, Inc., N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to P. R. Ellsworth at the address above stated.

Entered as second-class matter April 13, 1934, at the postoffice at Lancaster, Pa. under the act of March 3, 1879.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXIV MAY, 1951 NUMBER 5

CERTAIN TRACE ELEMENTS IN THE FEEDS, ORGANS, AND TISSUES OF A SELECTED GROUP OF REPEAT BREEDING COWS IN NORTHEASTERN WISCONSIN[1]

O. G. BENTLEY, G. V. QUICKE, J. KASTELIC AND PAUL H. PHILLIPS
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

In laboratory animals sub-minimal ingestion of certain trace elements, especially manganese, has led to infertility and reproductive failures (13, 4, 10, 3, 8). The possibility of a trace element deficiency as a likely cause of infertility in cattle is pertinent in view of the variable concentration of some of the trace elements present in feeds. Unpublished data have shown that samples of Wisconsin grown alfalfa contain from 7 to 72 ppm. of manganese. Often farmers producing low-manganese hay are troubled with repeat breeding cows; hence, an effort was made to ascertain the concentrations of trace elements in the feeds of troublesome repeat breeding cows from a widely scattered area of northeastern Wisconsin. It also was found possible to estimate liver and blood concentrations as well as a few vitamin determinations on the blood samples.

METHODS AND PROCEDURES

The farms from which these cattle came were classified by the inspector as two poor, eleven fair, thirteen good and five excellent. The majority of these farms used pastures of fair to good quality. Their hay was fair to good while the silage was good to excellent in most instances. Samples of hay, silage and grain mixtures were obtained from 33 farms in ten northeast counties of Wisconsin. These were analyzed for their cobalt and manganese content. Repeat breeding cows were selected from these farms on the basis of (a) a minimum of four infertile services, (b) at least had given birth to one living calf, (c) the cattle were under 10 yr. of age, (d) not more than two cows from any given farm, (e) cattle with no genital abnormalities, (f) cows free from purulent discharges, (g) normal estrus cycles and (h) normal intervals between breeding. These cattle were handled and slaughtered as described by Tanabe and Casida (11) for those cattle obtained in 1947 and 1948. A second lot of cattle obtained in 1948 and 1949 were similarly selected and experimentally treated as in the first lot of cattle.

Liver and ovarian tissues were analyzed for manganese, while blood plasma was analyzed for vitamins A and C and carotene. The method used for the

Received for publication Oct. 5, 1950.

1 Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from The Tennessee Eastman Corp., Kingsport.

ascorbic acid analyses was that of Mindlin and Butler (7), while the Kimball procedure (6) was used for vitamin A determinations. Manganese determinations were made by the AOAC periodate method when samples were ample and the microbiological method of Bentley *et al.* (2) was used for blood and ovarian analyses.

RESULTS

Manganese and cobalt content of the feeds on these farms which had repeat breeder cow difficulties are presented in table 1. The data from 29 farms

TABLE 1

Data on the average manganese and cobalt of feeds fed repeat breeder cows and liver manganese concentrations from cows selected from farms with the repeat breeder problem

	Mn content (ppm.)			Co content (ppm.)		
	No. of samples	Range	Av.	No. of samples	Range	Av.
Hay	29	8.9–48.5	23.3	18	0.02–0.08	0.042
Silage	26	18.8–66.0	31.7	18	0.03–0.16	0.09
Grain	18	.....	.....	17	0.02–3.28	0.63
				13	.....	0.12
Liver	29	6.5–11.3	8.6	.....	.....	.....

showed average values of 23.2 ppm. as the manganese content of the hays with a range of 8.9 to 48.5 ppm. Twelve farms had hay below 20 ppm. which seems to be on the low side. Since a variable amount of silage is fed in this area, these hays might reasonably expect to have an influence upon the manganese ingestion and subsequently upon the breeding performance of the cattle fed thereon.

Twenty-six samples of silage from 26 farms were obtained and the manganese content ranged from 18.8 to 66 ppm. with an average of 31.7 ppm. Only eight of these samples were below 20 ppm. of manganese. It is quite evident from these figures that the manganese content of the feeds on farms from which these repeat breeder cows came was quite variable.

The cobalt content of 18 samples of hay gave an average of .042 and .09 ppm. in silage with a variable content of cobalt in the grain portion of the ration. Estimating a third of the total digestible nutrients from hay, silage and grain each we find that three farms in Door County were definitely low in cobalt intake and five were definitely low in Shawano County. The others were marginal or adequate. Four samples of grain mixtures contained 1 to 3.38 ppm. cobalt. If these four high values are eliminated on the assumption that they were the result of cobalt supplements the average content of the grain rations fed by 13 farms was 0.12 ppm. of cobalt. Apparently, the grain portion of the ration supplied many of the supplements needed to support these rations. It has been reported that 0.05 ppm. of cobalt (12, 5) was necessary for normal performance of cattle grazing on herbage low in cobalt. From these observations it would seem that cobalt was a more critical element in this region of Wisconsin than manganese.

It is interesting that 29 livers from these cattle coming to slaughter averaged 8.6 ppm. of manganese with a very narrow range in variation—namely 6.5 to 11.3 ppm. The livers were rather constant in their manganese content irrespective of the dietary intake. It is evident that the manganese content of the liver is difficult to influence by dietary means or else this organ harbors its manganese very tenaciously.

The ascorbic acid content of the blood plasma was on the low side, averaging .296 mg. per cent. This value is slightly low for Holstein cattle (9) and since the group of experimental cattle used in these studies was composed roughly of 50 per cent Guernseys and 50 per cent Holsteins, the value would be still further reduced since normal Guernseys averaged 0.4 to 0.5 mg. per cent. As would be expected, variation in the ascorbic acid content of these blood plasmas, varied from the extreme of .1 to .8 with the majority of them confined to the narrow limits of .2 to .4 mg. per cent. The blood plasma vitamin A and carotene contents averaged 0.30 to 6.02 γ per gram, respectively, values which are adequate.

Analyses of the liver, ovary and blood for manganese content indicate that there was breed difference in the manganese content of these tissues and organs

TABLE 2

Manganese content of certain tissues of repeat breeder cows

Breed	No. of animals		Range	Av.
H	41	Liver	6.4–23.0	9.5 μg./g.
G	18	"	6.1–11.3	8.8 "
H	8	Blood	2.5–13.0	6.7 μg./100 cc.
G	8	"	2.5– 9.0	5.2 "
H	5	Ovary	1.5– 1.9	1.6 μg./g.
G	3	"	1.8– 3.1	2.3 "

as shown in table 2. Liver consistently presented a higher average manganese content than the other organs studied—averaging 9.5 μg. per gram in the Holstein as against 6.7 μg. per 100 cc. in blood and 1.6 μg. per gram in the ovary. The manganese values for similar tissues in Guernsey cattle ran lower except in the ovary. In the ovary the manganese content was higher than that of the Holsteins.

The manganese content of the ovaries of these cattle are below the normal levels found by Bentley (1) in experimental animals fed known quantities of manganese supplements. In Bentley's experiments where adequate manganese was fed, a content of 2 to 3 μg. of manganese or more per gram was found in the ovary, whereas ovaries from the cattle used in these studies contained on the average distinctly less.

SUMMARY

In an effort to determine the relationship of trace minerals to the repeat breeding cow problem, a study of the distribution of manganese and cobalt in the feeds fed the cows from problem herds and the distribution of cobalt and manganese in certain tissues and organs of problem cows has been made. It is

evident that no single cause was responsible for the repeat breeder cow from these herds. Low blood plasma ascorbic acid values were found to exist. There was definite evidence of low or marginal cobalt intake associated with many of these problem herds. The manganese concentrations of the feeds from these farms was on the low side of the normal range, while ovarian concentrations were definitely low. It appears from these data that trace minerals have little if any direct relationship to the repeat breeding cow problem, although this possibility is not precluded by the data.

REFERENCES

(1) BENTLEY, O. G., Ph.D. Thesis, University of Wisconsin, page 82, 1950.
(2) BENTLEY, O. G., SNELL, E. E., AND PHILLIPS, P. H. A Microbiological Method for the Determination of Manganese. J. Biol. Chem., **170**: 343. 1947.
(3) BOYER, P. D., SHAW, J. H., AND PHILLIPS, P. H. Studies of Manganese Deficiency in the Rat. J. Biol. Chem., **143**: 417. 1942.
(4) KEMMERER, A. R., ELVEHJEM, C. A., AND HART, E. B. Studies on the Relation of Manganese to the Nutrition of the Mouse. J. Biol. Chem., **92**: 623. 1931.
(5) KILLHAM, B. J. Cobalt Deficiency in Some Michigan Cattle. J. Am. Vet. Med. Assoc., **99**: 279. 1941.
(6) KIMBALL, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. J. Lab. and Clin. Med., **24**: 1055. 1939.
(7) MINDLIN, R. I., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma. J. Biol. Chem., **122**: 673. 1937.
(8) ORENT, E. R., AND MCCOLLUM, E. V. Effects of Deprivation of Manganese in the Rat. J. Biol. Chem., **92**: 651. 1931.
(9) PHILLIPS, P. H., LARDY, H. A., BOYER, P. D., AND WERNER, G. M. The Relationship of Ascorbic Acid to Reproduction in the Cow. J. Dairy Sci., **24**: 153. 1941.
(10) SKINNER, J. T., VAN DONK, E. C., AND STEENBOCK, H. Manganese as a Factor in Reproduction. Am. J. Physiol., **101**: 581. 1932.
(11) TANABE, T. Y., AND CASIDA, L. E. The Nature of Reproductive Failures in Cows of Low Fertility. J. Dairy Sci., **32**: 237. 1949.
(12) UNDERWOOD, E. J., AND HARVEY, R. J. Enzoötic Marasmus: The Cobalt Content of Soils, Pastures, and Animal Organs. Austral. Vet. J., **14**: 183. 1938.
(13) WADDELL, J., STEENBOCK, H., AND HART, E. B. Growth and Reproduction on Milk Diets. J. Nutrition, **4**: 53. 1931.

UDDER DEVELOPMENT IN YOUNG HEIFER CALVES

DONALD C. BROWN[1]

Department of Animal Production, Wyoming Agricultural Experiment Station, Laramie

The stage of udder development in young heifer calves may be a fairly reliable index of their future milk-producing ability. Swett (6) has demonstrated that the udder development of the heifer calf at 4 mo. of age has a direct relationship with her later productive ability.

Schabinger and Knodt (4), experimenting with ten Holstein and nine Guernsey heifer calves, found a highly significant relationship to exist between mammary development and body weight of both Holstein and Guernsey calves at 3 mo. of age. Highly significant correlations also were found between udder development and gains in body weight from 8 days to 3 mo. of age for the heifer calves of both breeds. They found no significant correlation between the width of mammary tissue and body weight of either breed at 6 mo. of age nor between the gains in body weight from 8 days to 6 mo. and mammary development of either breed.

Since it seemed desirable to obtain additional information on various stages of udder growth and because published data on the subject are meager, the present study was undertaken.

EXPERIMENTAL PROCEDURE

Data were collected during the period from November, 1947, to April, 1950, at the University of Wyoming dairy farm and a nearby commercial dairy. A total of 35 Holstein heifer calves from 3 to 6 mo. of age were studied. Not all of the data were used because of death losses and culling to barn capacity and also because some initial data were taken at the wrong age for comparisons.

The calves were weighed and udder measurements taken for each calf at monthly intervals from 3 to 6 mo. of age. Weights for the nine calves from the commercial dairy were estimated by taking chest-girth measurements, since numerous experiments (1, 3) have shown their method to differ from the actual live weight by not over 7 per cent. Age data used in the summaries varied by $\pm$ 7 days in a few cases at the 3, 4, 5 and 6 mo. stages; however, a majority of the measurements were taken at $\pm$ 3 or 4 days.

Palpation examinations were made by using the method developed by Swett (6). In addition to weight and palpation, data also were recorded for stage of growth. Udders of heifer calves pass through a number of changes during their first 6 mo. These stages were identified as early quarter, late quarter, early half and late half at the 3- to 6-mo. level. Udders are in the quarter stage when each quarter can be felt separately and at the half stage when the front and rear glands on each side of the udder are attached. Length measurements were

Received for publication Oct. 9, 1950.

[1] The experimental data in this paper are taken from a thesis submitted by the author in partial fulfillment for the degree of Master of Science in Agriculture, 1950.

taken only at this stage. At the 6-mo. stage and older, the glandular tissue of well-conditioned calves grows into the fatty pad of the udder; hence the individual stages were difficult to determine. Great differences also were observed between calves as to the age at which their udders reached a given stage and size; this accounts for the differences in calf numbers used when summarizing the data.

All of the calves used in the study, with the exception of the nine commercial heifers, were on feeding experiments. Several planes of nutrition were tried during the period in which measurements were taken.

The statistical methods and analyses used were those of Snedecor (5).

RESULTS AND DISCUSSION

Considerable similarity of growth was observed between average width of the front and rear quarters and also between the average width and length of the quarters. Correlations between the mean width of the front and rear quarters were found to be highly significant. From these results it may be concluded that there is a tendency for the front and rear quarters to vary together, but the extent of variation depends on age. The highest correlation was found

TABLE 1

Summary of correlation coefficient (r) and linear regression (b) of mean width of udder and body weight and mean length and body weight

Age	Mean width of udder and body wt.			Mean length of udder and body wt.		
	No. calves	*r*	*b*	No. calves	*r*	*b*
(*mo.*)						
3	26	0.42**	0.0026	.....[a]	.....	.....
4	30	0.55**	0.0035	24	0.634**	0.0121**
5	28	0.55**	0.00061	28	–0.015	–0.000138
6	29	0.33*	0.0011	29	–0.141	–0.00218

[a] Omitted because of too few measurements at this stage.
* Significant.
** Highly significant.

to be at the 4-mo. level and since the quarters tend to vary together, the data indicate that at 4 mo. of age, a mean measurement of the front quarters could accurately indicate the relative development of both front and rear quarters.

Conclusions similar to the above can be made for the correlations between mean width and length of quarters, except at the 3-mo. level.

In order to determine the relationship between size of the udder and body weight, the data were further analyzed statistically and are summarized in table 1.

The highly significant correlations for mean width of the udder and body weight at 3 to 5 mo. of age may be interpreted to show that apparently the size of gland depends on size of animal. r^2 indicates that 20–25 per cent of the variance of udder size can be accounted for by difference in body weight for this age group. When tested statistically, the linear regression was found to be

non-significant. This further suggests that the unit change in size of gland per pound of difference in animal weight is so small that it has no practical value.

Statistical analysis of the mean length of glands and body weight shows a highly significant correlation coefficient and linear regression at the 4-mo. level and is non-significant at the 5- and 6-mo. levels. These data indicate that at 4 mo. the gland length has kept in step with body weight and also with gland width as indicated in table 1. At the 5- and 6-mo. levels it may be assumed

TABLE 2

Summary of correlation coefficients (r) and linear regression (b) of gain in mean width and length of udder and gain in body weight

Age	Mean gain in width of udder and gain in body weight			Mean gain in length of udder and gain in body weight		
	No. calves	*r*	*b*	No. calves	*r*	*b*
(mo.)						
3–4	24	0.18	0.002	.....	.....	.....
4–5	29	0.19	0.0012	22	0.095	0.0013
5–6	28	0.21	0.00058	27	0.0004	0.00004
3–6	21	0.0012	0.000054	22	0.138	0.002

that this relationship does not hold. Data presented in table 3 help to substantiate these assumptions.

Since all the data in table 2 are non-significant, it may be assumed that the amount the calves gain is not associated with gain in size of udder. These results correspond with the results of Schabinger and Knodt (4), who found

TABLE 3

Summary of gain in body weight and gain in udder size from 3 to 6 mo. of age

Age	No. calves	Mean gain body weight	Mean gain udder width	No. calves making no gain in udder width	No. calves	Mean gain body weight	Mean gain udder length	No. calves making no gain in udder length
(mo.)		*(lb.)*	*(in.)*			*(lb.)*	*(in.)*	
3–4	24	37.1	0.152	2	.....	.....	.....	.....
4–5	29	47.9	0.16	6	22	50.6	0.49	2
5–6	28	48.6	0.06	8	27	50.5	0.24	8
3–6	21	141.6	0.39	1[a]	22	103.0	0.70	2[a]

[a] Two of the calves were 100 and 103 lb., respectively, below normal weight at 6 mo. of age and very low in physical condition; the other calf was above normal size with large initial width and length measurements, showing slight increase in gland size with increases in age and body weight.

no significant relationship between gain in body weight from 8 days to 6 mo. and udder development at 6 mo.

To determine the effect of different breeding, feeding and environment on udder growth from 3 to 6 mo., a group of seven heifers from a commercial herd was compared with a group of 14 heifers from the University herd. The

TABLE 4

Summary of measurements and stage of growth for calves grouped as normal and above normal size on a weight basis

Age	No. calves	Average weight	Normal[a] or above weight (large calves)						
			Stage of growth[b]				Average gland size		
			C	D	E	F	Width front	Width rear	Length
(*mo.*)		(*lb.*)	(%)	(%)	(%)	(%)	(*in.*)	(*in.*)	(*in.*)
3	8	211	25.0	37.5	37.5	.....	0.61	0.62	1.62[c]
4	8	254	.....	25.0	75.0	.....	0.86	0.78	2.03
5	7	321	.....	.....	100.0	.....	0.92	0.88	2.30
6	9	377	.....	.....	44.0	56.0	0.98	0.97	2.62
			Below normal weight (small calves)						
3	20	176	45.0	25.0	30.0	.....	0.52	0.56	1.21[d]
4	23	217	.....	39.1	60.9	.....	0.72	0.74	1.60
5	22	262	.....	9.0	73.0	18.0	0.94	0.88	2.34
6	15	303	.....	7.0	33.0	60.0	0.95	0.96	2.50
			Average of all calves compared with BDI[e]						
3	28	186	67.8 (78)		32.2 (17)		0.55 (0.54)	0.58 (0.62)	1.40 (1.79)
4	31	226	35.5 (44)		64.5 (55)		0.75 (0.67)	0.75 (0.75)	1.74 (2.33)
5	29	276	7.0 (19)		93.0 (81)		0.94 (0.78)	0.87 (0.87)	2.33 (2.78)
6	24	332	4.0 (6)		96.0 (95)		0.96 (0.84)	0.96 (0.96)	2.55 (3.33)

[a] Interpolated from Morrison standard.
[b] C = early quarter stage.
D = late quarter stage.
E = early half stage.
F = late half stage.
[c] 4 calves.
[d] 5 calves.
[e] Bureau of Dairy Industry in parenthesis.

University heifers gained an average of 61 lb. more per animal than those in the commercial herd, while the difference in gain of udder size was only 0.02 in. When tested statistically, these differences were found to be non-significant, which further indicates that gland growth appears to be independent of the plane of nutrition. More data will be necessary to measure the effects of environment and breeding.

The data summarized in table 3 substantiate the assumptions made for table 2. The data further indicate that the greatest udder growth is made during the 3- to 5-mo. level period and slows up with increase in age.

In order to compare the amount of udder growth of small and large calves of the same age, they were grouped according to weight based on the Morrison normal growth standard (2). The summary of data in table 4 shows little significant difference in udder size at different ages between the two groups.

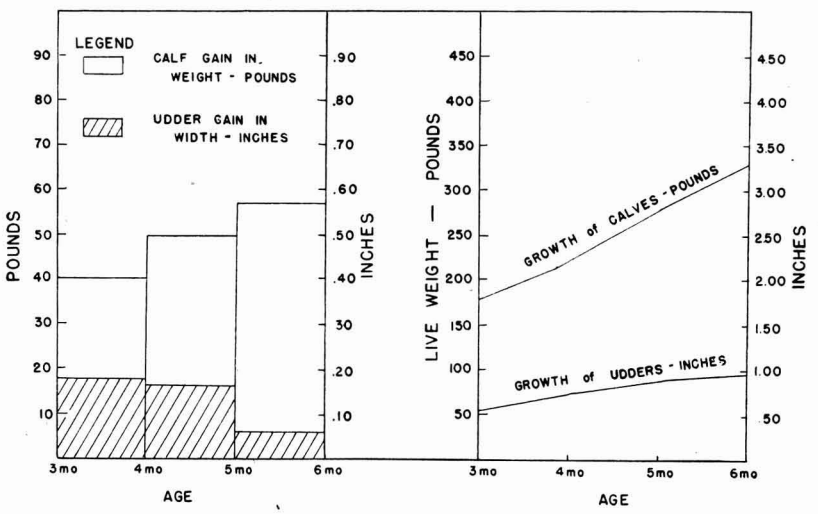

FIG. 1. Comparison of growth of udders and growth of calves.

The average of all mammary tissue when compared with those reported by Swett (6) also was very close for width measurements, but decidedly smaller for length size; the discrepancy may be explained by palpating technique or by population differences. Identification of stage of growth also was patterned after Swett (6); the data show considerable discrepancy from the 3–5-mo. levels and may be accounted for by differences of interpretation or in actual growth rates.

Previous analyses (tables 2 and 3) have indicated that gain in body weight is not associated with gain in size of udder and that the greatest udder growth is made during the 3- to 5-mo. period slowing down at the 5- to 6-mo. level. Graphic comparison of growth of udder and growth of calves is presented in fig. 1. Table 5 verifies the above assumptions and also shows the rate of growth of the small and large udders grouped independently of body weight. The

TABLE 5

Comparison of growth of small and large udders from 3 to 6 mo., grouped independently of body weight

Group	3 mo.		4 mo.		5 mo.		6 mo.		3–4 mo.		3–5 mo.		3–6 mo.	
	Width	Wt.	Width	Wt.	Width	Wt.	Width	Wt.	Width	Wt.	Width	Wt.	Width	Wt.
	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)	(*in.*)	(*lb.*)
I[a]	0.67	194	0.83	235	0.93	286	0.97	340	0.16	41	0.26	92	0.30	146
II[b]	0.46	183	0.65	214	0.87	259	0.96	304	0.19	31	0.41	74	0.50	121
Difference	0.21	11	0.18	21	0.06	27	0.01	36	0.03	10	0.15	18	0.20	25

[a] Calves having gland width above average of all calves at 3 mo. of age.
[b] Calves having gland width below average of all calves at 3 mo. of age.

main points brought out by these comparisons are that large calves have larger udders at the 3-mo. level by 0.21 in. but that at the 6-mo. level both groups had approximately the same gland size. Assumptions made from table 1 indicated that apparently size of gland is associated with size of calf for the 3- to 5-mo. period.

The udders that were smaller initially made 40 per cent more growth during the 3- to 6-mo. period than did those of the larger group. Similar results were obtained for udder length from the 4- to 6-mo. level.

SUMMARY AND CONCLUSIONS

A study has been conducted to obtain information on various stages of udder growth of young dairy heifers from 3 to 6 mo. of age. Most of the data obtained were treated statistically in an attempt to evaluate them better.

Highly significant correlation coefficients were obtained for the mean width of front and rear quarters and mean width and length of udders.

Statistical analysis of the relationship between udder size and body weight was found to be more significant at the 4-mo. level for both width and length of udder than at the other ages. These results were interpreted to mean that at the 4-mo. age, size of udder is somewhat dependent on size of animal.

No significant correlation was found between gain in udder size and gain in body weight for any of the age groups. From these results it was assumed that gain in body weight was not associated with gain in size of the udder.

The data indicated that the greatest udder growth was made during the 3- to 5-mo. period and slowed up with increase in age.

No significant difference was found between the udder size of small calves and of large calves at the various age levels.

The average width of front and rear quarters compared favorably with averages obtained in the herd of the Bureau of Dairy Industry, Maryland. Average length of udder was found to be considerably lower and may be accounted for by palpating technique or by population differences.

When mammary glands were grouped by size independent of body weight, the data indicate that large calves had larger udders at the 3- and 4-mo. levels but at 5 and 6-mo. of age both groups had udders of approximately the same size. It was concluded that mammary tissue tends to reach a certain growth, independently of body growth, by 6 mo. of age.

REFERENCES

(1) DAVIS, H. P., MORGAN, R. F., BRODY, S., AND RAGSDALE, A. C. Relationship of Height at Withers and Chest Girth to Live Weight of Dairy Cattle of Different Breeds and Ages. Nebr. Research Bull. 91. 1937.

(2) MORRISON, F. B. *Feeds and Feeding,* 21st ed., p. 766. The Morrison Publishing Co., Ithaca, N. Y.

(3) RAGSDALE, A. C., AND BRODY, S. Estimating Live Weights of Dairy Cattle. Mo. Bull. 354. 1935.

(4) SCHABINGER, J. R., AND KNODT, C. B. The Relationship of Mammary Development and Body Weight. J. Dairy Sci., 31: 95–98. 1948.

(5) SNEDECOR, G. W. *Statistical Methods.* Iowa State College Press, Ames. 1948.

(6) SWETT, W. W. A Cow a Calf Will Be. Yearbook of Agriculture 1943–47. pp. 197–200.

BIACETYL PRODUCTION BY CULTURES OF LACTIC ACID-PRODUCING STREPTOCOCCI[1]

M. A. KRISHNASWAMY AND F. J. BABEL

Purdue Agricultural Experiment Station, West Lafayette, Indiana

When a large number of lactic acid-producing streptococci are isolated and examined for flavor, variations are noted within the group. Many cultures give a flavor that has been designated as "green." The term "green" has been used to describe the flavor produced by a butter or buttermilk culture during the early stages of ripening when appreciable lactic acid is produced but only relatively small amounts of flavor compounds (biacetyl, volatile acids and CO_2). Some cultures of lactic acid-producing streptococci produce malty and unclean flavors. Occasionally, a culture is isolated which produces a flavor resembling a mixed culture of lactic acid-producing streptococci and citric acid-fermenting streptococci; such a culture has a flavor and aroma suggesting biacetyl, volatile acids and CO_2. It appeared desirable to determine the rate and amount of biacetyl produced by various cultures of lactic acid-producing streptococci at different incubation temperatures and to determine whether cultures producing appreciable amounts of biacetyl could be classified as *Streptococcus lactis* or *Streptococcus cremoris* by using accepted methods of classification.

HISTORICAL

The investigations of Van Niel *et al.* (18), which showed that biacetyl either was responsible for the aroma of butter or was the principal component of the aroma, led to considerable research on the production of this compound by butter cultures. Studies on the production of acetylmethylcarbinol and biacetyl by butter cultures indicated that citric acid was the source of these compounds (10) and that they were produced by the citric acid-fermenting streptococci. It was noted that butter cultures produced large amounts of acetylmethylcarbinol and biacetyl only when the pH of the milk was comparatively low (4).

Although some investigations have been conducted on the production of biacetyl by cultures of *S. lactis* and *S. cremoris,* the data available are not sufficient to draw definite conclusions. Schmalfuss and Barthmeyer (16) found biacetyl present in a mixed culture of *S. lactis* and *S. cremoris* grown in milk. The amounts produced were so small that accurate analyses were not possible. Michaelian *et al.* (10) studied the production of acetylmethylcarbinol plus biacetyl by 34 strains of *S. lactis* isolated from butter cultures and sour cream. Determinations made on milk cultures grown for 3 days at 21° C. showed that only four of the strains produced acetylmethylcarbinol plus biacetyl and these gave only traces. Palladina *et al.* (12) tested 380 strains of *S. lactis* and 150

Received for publication Oct. 29, 1950.

[1] Published with the approval of the Director of the Purdue Agricultural Experiment Station as Journal Series paper no. 500.

strains of *S. cremoris* for biacetyl production and found that the compound was not produced by *S. lactis* but was produced by a few strains of *S. cremoris*. Pien *et al.* (13) stated that lactic acid organisms which produced the most biacetyl were the most active in production of lactic acid. Experiments conducted by Rumments (15) indicated that *S. cremoris* did not ferment the citric acid of milk at pH 4.2 in 9 days and that no acetylmethylcarbinol was formed. A trace of acetylmethylcarbinol was produced by *S. lactis*. Wiley *et al.* (19) stated that the metabolism of *S. cremoris* varied with the temperature of incubation. At 20° C., cultures produced acid rapidly and biacetyl production was low. At 7° C., acid production was greatly restrained but biacetyl production was barely affected and was occasionally greater than at 20° C. Further experiments by Wiley *et al.* (20), with one strain of *S. cremoris* held at 21 and 7° C., showed that the production of biacetyl in milk was approximataely the same at both temperatures at 24 hr. After 24 hr., the rate of production of biacetyl at 7° C. was greater (1.8 ppm. in 96 hr.) than at 21° C. (1.0 ppm. in 96 hr.).

Microorganisms having the ability to ferment lactose with the production of lactic acid and citric acid with the production of acetylmethylcarbinol and biacetyl have been studied by several investigators. Wolff (21) described *Bacterium diacetylicum* (Voss) which formed *l*- and *d*-lactic acid as well as biacetyl. Van Beynum and Pette (17) described two organisms (*Streptococcus aromaticus* and *Streptococcus citrophilus*) capable of producing lactic acid and biacetyl in milk. Joshi and Ram Ayyar (8) isolated an organism similar to *Streptococcus citrovorus* and *Streptococcus paracitrovorus* which produced both lactic acid and aroma. The culture was named *Streptococcus lactis aromaticus*. Matuszewski *et al.* (9) isolated five strains of an organism which produced lactic acid as well as acetylmethylcarbinol. The organisms were named *Streptococcus diacetilactis*. Davis *et al.* (2) found that only the fecal streptococci (*Streptococcus fecalis* and *Streptococcus liquefaciens*) produced considerable quantities of biacetyl.

METHODS

Sources of cultures. All of the cultures except no. 232 and K were isolated from a commercial multiple-strain culture by plating on tryptone glucose beef extract agar (1). Isolated colonies were picked into litmus milk and purified by plating. Culture 232 was obtained from Iowa State College and culture K was obtained from the Kraft-Walker Cheese Co., Melbourne, Australia. The cultures were classified according to the method proposed by Yawger and Sherman (22).

Preparation of cultures. Cultures employed in these experiments were grown in skimmilk that had been heated at 15 lb. pressure for 15 min. The skimmilk was cooled to 21° C. and inoculated with 1 per cent of a 12 to 18 hr. culture. In certain experiments, a sterile citric acid solution was added to the milk just prior to inoculation with a test culture.

Biacetyl and acetylmethylcarbinol determinations. Biacetyl and acetylmethylcarbinol determinations were made according to the method of Prill and Hammer (14), as modified by Hoecker and Hammer (7). Color intensity was

measured with a Coleman Universal Spectrophotometer, using a wave length of 530 mμ.

Acidity determinations. Titrable acidity was determined by titrating a 9-g. sample of culture with 0.1 *N* NaOH, using phenolphthalein as indicator. Results were expressed as per cent lactic acid.

pH determinations were made electrometrically using a Leeds and Northrup Type K potentiometer with quinhydrone electrode and saturated calomel cell.

Volatile acidity was determined according to the procedure outlined by Hammer and Bailey (5).

EXPERIMENTAL

In order to determine whether cultures of lactic acid-producing streptococci were capable of producing biacetyl, 16 cultures were added to individual lots of sterile skimmilk at the rate of 1 per cent, and biacetyl, titrable acidity and pH determinations were made after incubation for 24 hr. at 21° C. Results of these determinations are shown in table 1. The titrable acidity and pH deter-

TABLE 1

Biacetyl production by cultures of lactic acid-producing streptococci grown in milk for 24 hr. at 21° C.

Culture no.	Titrable Acidity	pH	Biacetyl	Identification
	(%)		(*ppm.*)	
4	0.93	4.40	2.26	*S. lactis*
5	0.92	4.25	0.20	*S. cremoris*
9	1.03	4.40	1.80	Not identified
12	1.17	4.40	1.16	*S. cremoris*
12A	1.04	4.15	0.30	*S. cremoris*
15	1.00	4.45	0.20	Not identified
17	0.99	4.25	0.24	*S. cremoris*
23	0.88	4.25	0.20	*S. lactis*
25	0.96	4.20	2.40	*S. cremoris*
27	1.10	4.30	0.24	*S. cremoris*
34	1.00	4.35	0.20	*S. lactis*
36	0.94	4.25	<0.20	*S. cremoris*
38	0.95	4.25	<0.20	*S. cremoris*
232	0.97	4.30	0.30	*S. cremoris*
K	1.05	4.25	0.40	*S. cremoris*
K4	1.10	4.50	0.44	(?)

minations indicate that the entire group of cultures produce acid rather rapidly. Of the 16 cultures studied, the titrable acidities ranged from 0.88 to 1.17 per cent and the pH values from 4.15 to 4.50. Biacetyl determinations on these cultures showed values from less than 0.20 to 2.40 ppm. Four of the 16 cultures produced appreciable amounts of biacetyl (2.26, 1.80, 1.16 and 2.40 ppm.), while 12 cultures produced little or none of the compound (0.44 ppm. to less than 0.20). Two of the cultures producing appreciable quantities of biacetyl were classified as *S. cremoris,* one was classified as *S. lactis* and one was not identified. Strains of both *S. lactis* and *S. cremoris* were present in the group of cultures producing little or no biacetyl.

Studies on mixed cultures of lactic acid-producing and citric acid-fermenting streptococci have shown that the fermentation of citric acid in milk results in

the production of acetylmethylcarbinol, biacetyl, volatile acids and CO_2. With cultures of this type, the addition of citric acid to milk results in increased production of the compounds (10). It seemed advisable to determine whether the addition of citric acid to milk would result in the production of increased amounts of acetylmethylcarbinol, biacetyl and volatile acids by the lactic acid-producing streptococci. Data presented in table 2 show that the addition of citric acid to milk resulted in a slight increase in the production of biacetyl with three of the four cultures studied; one culture produced traces of biacetyl with or without added citric acid. Acetylmethylcarbinol production was not materially influenced by the addition of citric acid to milk. Two cultures (4 and 12) produced the same quantity of acetylmethylcarbinol in plain milk as they did in milk plus 0.15 per cent citric acid and two cultures (12A and 25) produced slightly more. Cultures 4 and 25 were tested for volatile acid production in plain milk and milk plus 0.15 per cent citric acid; addition of citric acid materially increased the production of volatile acids by both cultures.

TABLE 2

The Effect of addition of citric acid to milk on the production of biacetyl, acetylmethylcarbinol and volatile acids by cultures of lactic acid-producing streptococci (10 hr. incubation)

Culture no.	Citric Acid added	Titrable acidity	pH	Biacetyl	Acetylmethyl-carbinol	Volatile acidity
	(%)	(%)		(*ppm.*)	(*ppm.*)	(*ml. 0.1 N NaOH*)
4	0	1.27	4.40	2.18	298	27.80
	0.15	1.10	4.45	2.78	297	39.90
12	0	1.03	4.45	2.58	297	—
	0.15	0.95	4.50	2.92	297	—
12A	0	0.88	4.40	<0.20	200	—
	0.15	1.05	4.25	<0.20	218	—
25	0	0.88	4.75	2.40	267	13.10
	0.15	0.95	4.50	2.80	291	69.00

Data showing the rate of acid and biacetyl production by a culture of *S. cremoris* (no. 25) grown in milk without and with added citric acid and incubated at 7, 21 and 30° C. are presented in table 3. In milk without added citric acid and an incubation temperature of 7° C., *S. cremoris* (no. 25) produced acid and biacetyl slowly. The highest biacetyl content obtained at 7° C. was 0.54 ppm. after incubation for 72 hr.; later, the biacetyl content decreased. When the culture was incubated at 21° C., acid and biacetyl were produced rapidly and in greater amounts. The highest biacetyl content obtained at 21° C. was 2.54 ppm. after 12 hr. incubation and the compound gradually decreased thereafter. *S. cremoris* (no. 25) grown in plain milk at 30° C. produced more biacetyl (2.85 ppm.) than when incubation temperatures of 7 or 21° C. were employed. At 30° C., the biacetyl content reached a maximum at 12 hr. incubation and thereafter decreased. The addition of 0.15 per cent citric acid (calculated as anhydrous) to milk resulted in the production of more biacetyl at 7° C. than was produced without such an addition. Culture 25 produced 1.14 ppm. of biacetyl in milk plus 0.15 per cent citric acid after incubation at 7° C. for 84 hr.

TABLE 3

Rate of acid and biacetyl production of a culture of S. cremoris (no. 25) grown in milk, without and with added citric acid and incubated at 7, 21 and 30° C.

Incubation time	7° C.			21° C.			30° C.		
	Titrable Acidity	pH	Biacetyl	Titrable Acidity	pH	Biacetyl	Titrable Acidity	pH	Biacetyl
(*hr.*)	(%)		(*ppm.*)	(%)		(*ppm.*)	(%)		(*ppm.*)
				No citric acid added to milk					
12	0.21	6.15	0.20	0.84	4.55	2.54	0.85	4.70	2.85
24	0.47	5.10	0.44	0.97	4.30	1.28	0.97	4.45	1.28
48	0.84	4.60	0.48	1.01	4.25	0.86	0.95	4.50	0.96
72	0.87	4.50	0.54	0.99	4.30	0.44	0.95	4.40	0.85
96	0.85	4.45	0.24	0.92	4.30	0.28	—	—	—
				0.15% citric acid added to milk					
12	0.36	5.50	0.44	0.87	4.45	2.90	0.83	4.53	1.44
24	0.38	5.50	0.44	1.10	4.40	2.40	0.89	4.48	0.80
36	0.40	5.30	0.44	0.98	4.50	1.60	0.92	4.35	0.72
60	0.42	5.27	0.56	1.06	4.40	0.92	0.93	4.30	0.44
84	0.48	5.20	1.14	1.05	4.25	0.80	0.92	4.30	0.36
132	0.63	5.10	0.94	1.10	4.30	0.84	0.89	4.25	0.28
156	0.70	4.80	0.56	1.00	4.20	0.74	0.88	4.20	—

TABLE 4

Rate of acid and biacetyl production by a culture of S. lactis (no. 4) grown in milk, without and with added citric acid and incubated at 7, 21 and 30° C.

Incubation time	7° C.			21° C.			30° C.		
	Titrable Acidity	pH	Biacetyl	Titrable Acidity	pH	Biacetyl	Titrable Acidity	pH	Biacetyl
(*hr.*)	(%)		(*ppm.*)	(%)		(*ppm.*)	(%)		(*ppm.*)
				No citric acid added to milk					
12	0.21	5.95	0.24	0.84	4.60	1.96	0.79	4.65	0.44
24	0.25	5.90	0.28	0.93	4.40	2.26	0.91	4.50	0.20
48	0.29	5.85	<0.20	0.98	4.30	1.04	0.90	4.55	0.20
72	0.37	5.40	<0.20	1.00	4.20	1.32	0.85	4.40	0.20
96	0.48	5.15	0.20	0.99	4.20	1.16	0.90	4.40	0.20
120	0.70	4.75	0.20	0.95	4.25	0.80	0.88	4.35	0.20
				0.15% citric acid added to milk					
12	0.40	5.40	1.24	0.96	4.40	2.72	0.80	4.50	0.44
24	0.62	4.80	1.32	1.10	4.35	1.16	0.85	4.45	0.48
48	0.70	4.75	1.48	1.08	4.35	0.68	0.83	4.45	0.82
72	0.75	4.60	2.08	1.06	4.32	0.70	0.90	4.40	0.56
96	0.80	4.45	1.28	1.03	4.30	0.64	0.85	4.30	0.20
120	0.75	4.50	0.50	1.03	4.30	0.64	0.85	4.30	0.20
144	0.70	4.45	0.30	1.10	4.35	0.45	0.80	4.40	0.20

Thereafter, the biacetyl content decreased. Slightly more biacetyl was produced in milk plus citric acid at an incubation temperature of 21° C. than when no citric acid was added. Also, the biacetyl content of the culture grown in milk with added citric acid did not decrease as rapidly as when no citric acid was added. At 30° C., the addition of citric acid to milk decreased biacetyl production by culture 25.

The rate of acid and biacetyl production by a culture of *S. lactis* (no. 4) grown in milk without and with added citric acid and incubated at 7, 21 and 30° C. is presented in table 4. Acid production by culture no. 4 was slow at 7° C. and little biacetyl was produced in plain milk. At 21° C., the culture produced 2.26 ppm. of biacetyl in plain milk after an incubation period of 24 hr. Further incubation of the culture resulted in a decrease in biacetyl content. At 30° C., the culture contained 0.44 ppm. of biacetyl in 12 hr. and further incubation resulted in destruction of the compound. The addition of 0.15 per cent citric acid to milk resulted in increased biacetyl production at 7° C.

TABLE 5

Biacetyl production by a series of cultures isolated from S. lactis (no. 4) and grown in milk for 12 hr. at 21° C.

Culture no.	pH	Biacetyl	Culture no.	pH	Biacetyl
		(*ppm.*)			(*ppm.*)
1	4.30	0.24	13	4.25	0.32
4A	4.35	0.32	14	4.20	0.44
5A	4.35	0.20	15	4.25	0.28
7	4.30	0.28	17A	4.30	0.24
9	4.35	0.28	18	4.25	0.20
10	4.35	0.56	21	4.30	0.36
11	4.20	0.48	22	4.25	0.30
12	4.30	4.00			

The culture increased in biacetyl content for 72 hr. and produced 2.08 ppm. of the compound. Also, slightly more biacetyl was produced in milk plus citric acid at 21° C. than in plain milk (2.72 ppm. in 12 hr.). Biacetyl production by *S. lactis* (no. 4) was only slightly greater in milk plus citric acid with an incubation temperature of 30° C. than in plain milk, but the amount produced was much less than at 21° C.

S. lactis (no. 4), which was originally obtained from the commercial multiple-strain culture by plating and picking isolated colonies and further purified by plating, was used to determine the constancy of biacetyl production among various cultures obtained by further plating and picking isolated colonies. Data presented in table 5 show the amount of biacetyl produced by 15 cultures obtained from *S. lactis* (no. 4) and grown in plain milk for 12 hr. at 21° C. Of the 15 cultures isolated, 14 produced biacetyl in amounts ranging from 0.20 to 0.56 ppm. and one culture produced 4.00 ppm.

Three cultures of lactic acid-producing streptococci which formed small amounts of biacetyl when grown in milk for 24 hr. at 21° C. (table 1) were used to study biacetyl production at 7° C. The rate of acid and biacetyl production by these cultures is given in table 6. Culture 12A produced

acid slowly at 7° C. and required 5 days to decrease the pH to 5.0. Biacetyl production by culture 12A was slow, but the compound increased gradually and after 10 days of incubation amounted to 1.10 ppm. Culture 23 produced acid somewhat faster than culture 12A and the reaction of milk was lowered to pH 5 in 1 day. Culture 23 showed a maximum biacetyl content (1.08 ppm.) after inculation for 5 days at 7° C.; further incubation resulted in a decreased biacetyl content. Culture 36 decreased the pH of milk rather rapidly and also produced appreciable amounts of biacetyl; biacetyl production reached 1.86 ppm. after incubation for 10 days.

Since biacetyl appeared to be produced by cultures of lactic acid-producing streptococci only after the pH of milk had been reduced somewhat, a series of experiments were conducted to determine the influence of a short incubation at 21° C. followed by incubation at 7° C. on biacetyl production. Table 7 gives the results of these experiments. Cultures 11, 17 and 23 produced little biacetyl

TABLE 6

Biacetyl production in milk by cultures of lactic acid-producing streptococci incubated at 7° C.

Incubation time	Culture no. 12A		Culture no. 23		Culture no. 36	
	pH	Biacetyl	pH	Biacetyl	pH	Biacetyl
(*d.*)		(*ppm.*)		(*ppm.*)		(*ppm.*)
0.5	6.15	0.20	6.10	0.56	6.10	0.20
1	5.80	0.40	5.00	0.60	5.25	0.38
2	5.70	0.44	4.70	0.68	4.35	0.52
3	5.60	0.48	4.45	1.00	4.30	0.56
5	5.00	0.56	4.30	1.08	4.25	1.00
7	4.88	0.83	4.30	0.84	4.20	1.60
10	4.75	1.10	4.20	0.65	4.20	1.86

in milk under the conditions of the experiment. Culture 23 formed less biactyl when grown in milk at 21° C. for 12 hr. and thereafter at 7° C. than it did with continued incubation at 7° C. (see table 6). Cultures 4 and 25 were included in these trials since previous results (table 1) indicated that they produced appreciable amounts of biacetyl at 21° C. In this experiment, cultures 4 and 25 produced 2.60 and 2.54 ppm. of biacetyl, respectively, after incubation for 10 hr. at 21° C. Further incubation of cultures 4 and 25 at 7° C. for 2 days did not materially influence biacetyl production. When the cultures were held for 10 days at 7° C. the biacetyl contents of both cultures decreased materially.

The effect of bi-weekly transfers and storage at low temperature on the stability of biacetyl production was studied with cultures 4, 12A and 25. Biacetyl production by these cultures was determined shortly after the cultures were isolated and again after being transferred bi-weekly in milk for 6 mo. Each transfer consisted of inoculating sterile milk with 1 per cent of the previous transfer, holding the culture at 21° C. until coagulation occurred and then holding the culture at 7° C. until the next transfer. Culture 4 produced as much as 4.0 ppm. of biacetyl in 12 hr. at 21° C. when first isolated, and after bi-weekly transfers for 6 mo. it produced 2.86 ppm. when ripened to the same

pH as the previous determination. Culture 12A produced biacetyl at 7° C. but not at 21° C. when first isolated. After bi-weekly transfers for 6 mo. the culture produced as much as 2.68 ppm. biacetyl in 10 hr. at 21° C. in plain milk and 3.0 ppm. in milk plus 0.15 per cent citric acid. Culture 25 produced 2.26 ppm. of biacetyl in plain milk when first isolated with incubation at 21° C. After bi-weekly transfer for 6 mo. the culture produced 1.48 ppm. Culture 25

TABLE 7

Biacetyl production of several cultures of lactic acid-producing streptococci grown in milk at 21° C. for 10 or 12 hr. and thereafter at 7° C.

Culture no.	Incubation temperature	Incubation time	pH	Biacetyl
	(°C.)			(ppm.)
11	21	12 hr.	4.35	0.20
	7	1 d.	4.30	0.20
	7	6 d.	4.25	<0.20
	7	16 d.	4.30	0.40
	21	12 hr.	4.35	0.20
17	7	1 d.	4.40	0.20
	7	6 d.	4.30	0.30
	7	16 d.	4.25	0.68
	21	12 hr.	4.35	0.20
23	7	1 d.	4.30	0.20
	7	6 d.	4.25	0.30
	7	16 d.	4.10	0.40
	21	10 hr.	4.53	2.60
4	7	2 d.	4.47	2.68
	7	10 d.	4.50	0.60
	7	15 d.	4.43	0.50
	21	10 hr.	4.52	2.54
25	7	2 d.	4.53	2.58
	7	10 d.	4.43	0.20
	7	15 d.	4.32	<0.20

originally produced 2.74 ppm. of biacetyl in milk plus citric acid and after bi-weekly transfers for 6 mo. produced 2.54 ppm.

DISCUSSION

The production of biacetyl is evident with certain cultures of *S. lactis* as well as *S. cremoris* (table 1) and is not restricted to *S. cremoris* as Palladina *et al.* (12) have reported. Also, production of biacetyl is not limited to the group of fecal streptococci as shown by Davis *et al.* (2).

It has been pointed out by Hammer and Baker (6) that *S. lactis* should be considered as a group of organisms rather than an individual species because of variations encountered with different strains. Much of the data presented in this paper further emphasize the variations among strains. The data also indicate a close similarity between *S. lactis* and *S. cremoris,* since cultures of both species could be isolated which produced relatively small and large amounts of biacetyl. Variations appear to exist with cultures of *S. lactis* as well as *S. cremoris.* Culture K (table 1) was classified as *S. cremoris* according to the method of Yawger and Sherman (22), but culture K4, which was an isolant

from culture K, had characteristics of both *S. lactis* and *S. cremoris*. Similar observations have been made with other cultures.

Certain variations encountered among strains of lactic acid-producing streptococci in respect to biacetyl production have been encountered previously with this group of organisms. Hammer (3) plated ropy cultures of *S. lactis* and obtained both ropy and non-ropy strains when colonies were picked into litmus milk. Results presented in table 5 show that a culture of *S. lactis* which produced an appreciable amount of biacetyl contained strains which formed large and small amounts of biacetyl when propagated under similar conditions. One culture isolated from the parent culture produced about twice as much biacetyl as the parent culture.

The cultures of *S. lactis* and *S. cremoris* which produced appreciable amounts of biacetyl differed from *S. aromaticus* (17) in that they curdled milk rapidly. They differed from *S. citrophilus* (17) and *S. diacetilactis* (9) in that they did not vigorously ferment citric acid and large increases in biacetyl were not obtained by the addition of citric acid to milk. It is suggested that cultures which correspond to the classification of *S. lactis* and which produce appreciable amounts of biacetyl be recognized as variants of *S. lactis* rather than assign a new species name to them. Since Hammer and Baker (6) have classified one variant of *S. lactis* on the basis of a difference in flavor (*S. lactis* var. *maltigenes*), it might be appropriate to classify *S. lactis* organisms which produce relatively high biacetyl contents in the same manner and designate them *S. lactis* var. *aromaticus*. Joshi and Ram Ayyar (8) suggested the name *S. lactis aromaticus* for an organism which produced both lactic acid and aroma.

The optimum temperature for production of biacetyl by cultures of lactic acid-producing streptococci varied with individual cultures. When a group of high biacetyl-producing cultures were incubated at 7, 21 and 30° C., highest biacetyl contents were obtained at 21° C. Certain other cultures produced more biacetyl at 7 than at 21° C.

SUMMARY

Certain cultures of *S. lactis* and *S. cremoris* grown in milk produced a flavor resembling a mixed culture of lactic acid-producing and citric acid-fermenting streptococci. The flavor and aroma of these cultures suggested formation of biacetyl, volatile acids and CO_2.

The incubation temperature, incubation time and pH required for maximum biacetyl production were variable.

The greatest amount of biacetyl produced by a lactic acid-producing streptococcus was 4.0 ppm. Many cultures produced little or no biacetyl in milk.

No direct relationship existed between the amount of acid produced by a culture and its ability to produce biacetyl. However, there was a correlation between the pH of milk and biacetyl production by cultures. Cultures of lactic acid-producing streptococci formed the largest amounts of biacetyl in milk after they had decreased the pH to 5 or less. With most cultures, maximum biacetyl production was evident in the range of pH 4.2 to 4.75.

A culture of *S. lactis* obtained from a commercial mixed culture by plating

and further purified by plating and picking isolated colonies contained both high and low biacetyl-producing strains.

The addition of citric acid (0.15 per cent) to milk cultures of lactic acid-producing streptococci generally resulted in slightly higher biacetyl production, although the increases were not as conspicuous as those obtained with mixed cultures of lactic acid-producing and citric acid-fermenting streptococci.

Acetylmethylcarbinol production was not increased appreciably by addition of citric acid to milk cultures of lactic acid-producing streptococci but volatile acid production was increased considerably.

When citric acid was added to milk, the biacetyl content did not decrease as rapidly as it did in plain milk after maximum production was reached.

It is suggested that cultures of *S. lactis* which produce appreciable amounts of biacetyl be recognized as a variety of *S. lactis* and be designated *S. lactis* var. *aromaticus* in accordance with the method of classification of this group proposed by Hammer and Baker (6).

REFERENCES

(1) American Public Health Association. *Standard Methods for the Examination of Dairy Products.* 9th ed. 1948.

(2) Davis, J. G., Rogers, H. J., and Thiel, C. C. Production of Diacetyl by Faecal Streptococci. Nature, **143**: 558. 1939.

(3) Hammer, B. W. Studies on Ropiness in Cultures of *Streptococcus lactis.* Ia. Agr. Expt. Sta. Research Bull. 74. 1923.

(4) Hammer, B. W., and Babel, F. J. Bacteriology of Butter Cultures: A Review. J. Dairy Sci., **27**: 83–168. 1943.

(5) Hammer, B. W., and Bailey, D. E. The Volatile Acid Production of Starters and of Organisms Isolated from Them. Ia. Agr. Expt. Sta. Research Bull. 55. 1919.

(6) Hammer, B. W., and Baker, M. P. Classification of the *Streptococcus lactis* Group. Ia. Agr. Expt. Sta. Research Bull. 99. 1926.

(7) Hoecker, W. H., and Hammer, B. W. Flavor Development in Salted Butter by Pure Cultures of Bacteria: Preliminary Results. Ia. Agr. Expt. Sta. Research Bull. 290. 1941.

(8) Joshi, N. V., and Ram Ayyar, C. S. An Aroma-producing Lactic Acid Organism Isolated from Indian Dairy Products. Indian J. Vet. Sci. and Animal Husb., **6**: 141–150. 1936.

(9) Matuszewski, T., Pijanowski, E., and Supinska, J. *Streptococcus diacetilactis* n. sp. i jego zastosowanie przy wyrobie masla. Polish Agr. Forest Ann., **36**: 1–24 (in English, 25–28). 1936.

(10) Michaelian, M. B., Farmer, R. S., and Hammer, B. W. Relationship of Acetylmethylcarbinol and Diacetyl to Butter Cultures. Ia. Agr. Expt. Sta. Research Bull. 155. 1933.

(11) Michaelian, M. B., Hoecker, W. H., and Hammer, B. W. Effect of pH on the Production of Acetylmethylcarbinol Plus Diacetyl in Milk by the Citric Acid Fermenting Streptococci. J. Dairy Sci., **21**: 213–218. 1938.

(12) Palladina, O. K., Mazjukewitsch, W. A., Milowa, E. L., and Grigorjewa, N. S. Über die Aromabildung gegorener Milch. Milchw. Forsch., **17**: 222–243. 1935.

(13) Pien, J., Baisse, J., and Martin, R. Contribution a l'étude du diacétyle. Lait, **16**: 119–138, 243–255. 1936.

(14) Prill, E. A., and Hammer, B. W. A Colorimetric Method for the Microestimation of Diacetyl. Ia. State Coll. J. Sci., **12**: 385–395. 1938.

(15) Rumments, J. Über den zitranensäuregehalt der Milch in Zusammenbang mit der

Acetylmethylcarbinolgarung. XI. Milchwirtschaftlicher Weltkongress, Ber., **2**: 107–111. 1937.

(16) SCHMALFUSS, H., AND BARTHMEYER, HELENE. Diacetyl ein Stoffwechselprodukt? Ztschr. Physiol. Chem., **176**: 282–286. 1928.

(17) VAN BEYNUM, J., AND PETTE, J. W. De beteekenis der melkzuurbacteriën bij de boteraromavorming. Verslag Hoorn Vereeninging Tot Exploitatie Eener Proefzuivel-Boerderij. 1935. pp. 205–236.

(18) VAN NIEL, C. B., KLUYVER, A. J., AND DERX, H. G. Über das Butteraroma. Biochem. Z., **210**: 234–251. 1929.

(19) WILEY, W. J., COX, G. A., AND WHITEHEAD, H. R. The Formation of Diacetyl by Starter Cultures. I. A Comparison of Single Strain and Mixed Culture Starters for Butter Manufacture. J. Council Sci. Ind. Research, **12**: 232–238. 1939.

(20) WILEY, W. J., COX, G. A., AND WHITEHEAD, H. R. The Formation of Diacetyl by Starter Cultures. II. Rate of Diacetyl Production by Lactic Streptococci. J. Council Sci. Ind. Research, **12**: 239–249. 1939.

(21) WOLFF, A. Das Bacterium diacetylicum (Voss). Zentr. Bakt., Parasitenk., II., **86**: 413–414. 1932.

(22) YAWGER, E. S., JR., AND SHERMAN, J. M. *Streptococcus cremoris*. J. Dairy Sci., **20**: 205–212. 1937.

EFFECT OF VITAMIN A DEPLETION ON LIVEWEIGHT, PLASMA AND LIVER LEVELS OF VITAMIN A AND MICROANATOMY IN YOUNG DAIRY CALVES

H. D. EATON, C. F. HELMBOLDT, E. L. JUNGHERR AND C. A. CARPENTER
Animal Industries and Animal Diseases Departments, Storrs Agricultural Experiment Station, University of Connecticut, Storrs

AND

L. A. MOORE
Bureau of Dairy Industry, Agricultural Research Administration, U. S. D. A., Washington, D. C.

A quantitative assay of the vitamin A stores, not involving sacrifice nor causing clinical or pathological changes is needed to evaluate different dietary sources and ways of administration of vitamin A and carotene fed to young dairy calves. The possibility of feeding calves a vitamin A-free ration for relatively short periods of time, accompanied by determinations of blood plasma levels of vitamin A to predict vitamin A stores has not been fully explored. Moore and Berry (9) observed differences in plasma vitamin A levels in two groups of calves placed on a carotene-free ration at 90 days of age and previously fed either clover and timothy or lezpedeza hays. Blood plasma vitamin A levels have been used as criteria to determine the utilization of fetal storage of vitamin A in newborn calves fed a ration of skimmilk (2). In addition, the time for the blood plasma vitamin A to reach 4 γ per cent in calves placed on a vitamin A-deficient ration after 90 days of age has been correlated with the vitamin A intake from birth to 90 days of age by Jacobson *et al.* (6). A relatively constant percentage decrease in liver stores of vitamin A in yearling steers fed a low carotene ration was demonstrated by Frey and Jensen (5).

The objectives of this experiment were to study in particular the effect of feeding a vitamin A depletion ration on the blood plasma levels of vitamin A and carotene and, secondarily, the effect of such a ration on liver levels of vitamin A, liveweight changes and microscopic anatomy in young rapidly growing dairy calves.

EXPERIMENTAL

Animals. Fourteen male and three female Holstein calves and four male Guernsey calves at 106 days of age were placed on experiment during the months of February through May, 1950. Fifteen of these calves, all Holsteins, had previously been fed one of three types of alfalfa hay. The detailed dietary and managerial history of these calves up to 106 days of age appeared elsewhere (3). For purposes of this experiment, those calves previously fed field-cured and field-baled alfalfa hay were designated group 1, those fed artificially-dried and ground alfalfa hay, group 2 and those fed artificially-dried and

Received for publication Nov. 2, 1950.

[1] Supported in part with funds provided by the Research and Marketing Act of 1946 through a contract between the Storrs Agricultural Experiment Station and the Bureau of Dairy Industry and also by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass.

pelleted alfalfa hay, group 3. Group 4 (controls) consisted of two Holstein and four Guernsey calves raised under similar conditions except that they had received, instead of one of the alfalfa hays, U. S. no. 1 extra green clover and timothy mixed hay which contained 17.71 mg. carotene per pound.

All calves were fed the depletion ration[2] at the rate of 2.67 lb. per 100 lb. of body weight, which was based on Morrison's mid-interval T. D. N. requirement for 300-lb. growing dairy cattle (10). The amounts fed were adjusted to the weight of individual calves at successive 7-day intervals. In addition, each calf in group 4 (control) received 250,000 USP units of vitamin A in the form of shark liver oil[3] three times in each 7-day period or a daily equivalent of 107,143 USP units of vitamin A.

Calves in groups 1 through 3 were continued on experiment until the blood plasma vitamin A level had reached a point below 4 γ per cent for 2 consecutive wk., as defined by Jacobson *et al.* (6). At that time, three calves from each of these groups were slaughtered. Calves in group 4 were continued on experiment in pairs to comparable average ages of those in groups 1, 2 and 3, and slaughtered.

Observations and analyses. Daily feed intakes and refusals were recorded. Liveweight records were made and venous blood samples for each calf were taken on the 105th day and at 7-day intervals thereafter until the calf was removed from the experiment. After the venous blood samples were obtained, they were chilled, centrifuged and a measured amount of plasma held at –18° C. for carotene and vitamin A analyses.

Daily clinical observations were recorded and every 2 days all calves were allowed in a dry lot for a 30-min. period. Spinal fluid pressure readings (9) were recorded at the start of the experiment, at 4-wk. intervals thereafter and at the termination of the experiment.

At slaughter, tissues were taken for histopathological examination as well as the liver for carotene and vitamin A analyses.

Analytical, histological and statistical procedures were similar to those previously reported (2, 7).

RESULTS

Feed consumed. At the level fed, all calves readily consumed the depletion ration allowance. However, those calves in groups 1, 2 and 3 began to refuse some of the feed allowed when the blood plasma level of vitamin A decreased to 4 γ per cent or during the last 2 wk. on experiment. This was particularly noticeable in six out of the fifteen calves (no. 5, 6, 8, 13, 14 and 15) in

[2] A mixture by weight of one-third of dried beet pulp and two-thirds of a grain mixture composed of the following: 419.5 lb. ground barley, 500 lb. crimped oats, 500 lb. wheat bran, 150 lb. linseed oil meal (expellar process), 150 lb. soybean oil meal (expellar process), 200 lb. cane molasses, 40 lb. 500-potency B-Y dried fermentation solubles, 20 lb. steamed bone meal, 20 lb. iodized salt, and 0.5 lb. irradiated yeast (Standard Brands type 9-F) per ton of mixture. This depletion ration contained $<$ 0.25 mg. of carotene per pound.

[3] The shark liver oil contained 25% by weight of crude soybean lecithin and 25,750 USP units of vitamin A per gram as assayed spectrophotometrically against the USP vitamin A reference standard. It was generously supplied by the Nopco Chemical Co., Harrison, N. J.

these three groups. Another calf, no. 11, refused a considerable portion of the feed allowed; however, a severe enteritis observed on necropsy might have contributed to this, rather than a deficiency of vitamin A.

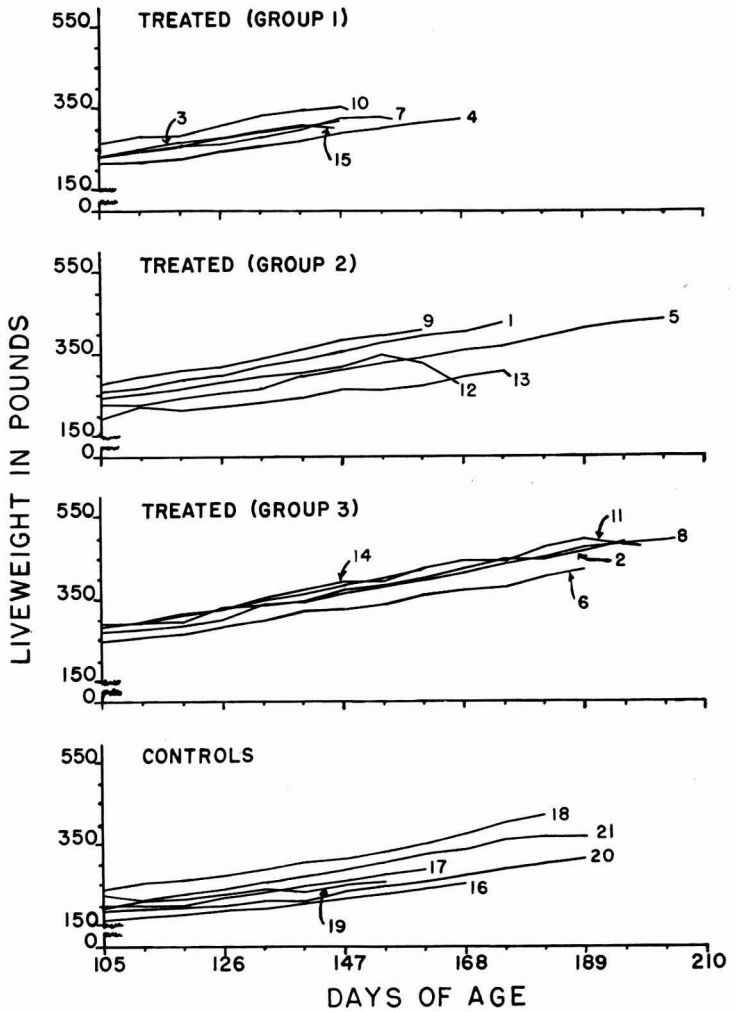

FIG. 1. The effect of a vitamin A depletion ration on liveweight in young calves.

Liveweight. All calves, with the exception of no. 13, were equal to or above Ragsdale's standards (11) for their respective breeds at the start and termination of the experimental period (fig. 1). An analysis of the liveweight data, which did not include those values for the last 2 wk. because of feed refusals in groups 1, 2 and 3, demonstrated more rapid rates of gain in those calves fed only the depletion ration. Since large calves usually make relatively greater

gains in liveweight than small calves when both are fed at the same level per unit of body weight, adjustment of the data for differences between calves in initial weight at 105 days of age was made by covariance. This analysis showed no significant differences in liveweight changes between groups of calves. Apparent feed utilization as measured by liveweight appeared not to have been affected, at least until the blood plasma level of vitamin A reached 4 γ per cent. This may appear to be in opposition to the depression in digestibility, absorbability and metabolizability of energy observed in vitamin A-deficient calves by Ritzman *et al.* (12); however, it was probable that the calves used in the Ritzman *et al.* experiment were exhibiting more pronounced and possibly secondary symptoms of vitamin A deficiency as compared to relatively early symptoms of A-hypovitaminosis observed with the calves reported herein.

Vitamin A metabolism.[4] Blood plasma carotene (table 1) levels decreased quite rapidly in all calves and the rate of decrease appeared to be related to the initial value at 105 days of age. The relatively rapid early rate of decrease and the later marked leveling off in the rate of decrease would indicate that plasma carotene was not as sensitive a measurement of the vitamin A metabolism as plasma vitamin A, as noted below.

In contrast to the plasma carotene values, the plasma vitamin A values of groups 1, 2 and 3 (table 2) decreased quite uniformly after 1 wk. on the depletion ration, and the values of group 4 were between 30 and 40 γ per cent for the entire experimental period. A linear regression analysis of the plasma vitamin A values with time for groups 1, 2 and 3 showed that the rate of decline was uniform between groups of calves. Therefore, within the levels observed, the rate of decrease in plasma vitamin A was independent of the level. This relationship, if confirmed by subsequent experimentation, would lend itself to a rapid means of prediction of depletion time by relatively short periods of feeding a depletion ration and might possibly meet the requirements of a quantitative assay of vitamin A stores.

The liver, carotene and vitamin A values (table 3), with the exception of those of calf 14, indicate that the liver reserves of these substances have been essentially depleted when the blood plasma level reaches less than 4 γ per cent. The continuation of calves on a depletion ration for a longer period than for 2 consecutive wk. after the blood plasma vitamin A level has reached less than 4 γ per cent does not seem to be necessary. Other calves fed the same depletion ration for 6 wk. after the blood plasma vitamin A levels were less than 4 γ per cent (4) had liver stores of vitamin A of the same magnitude as those observed in this experiment. These data appear to be evidence that the method used for determining liver carotene and vitamin A (1) was not sensitive to such minute changes or that calves were unable to further mobilize liver stores. The former appears to be the more reasonable explanation, if the low values and those substances which interfere with the Carr-Price reaction are considered.

Spinal fluid pressures and clinical observations. Spinal fluid pressures

[4] Since the trends and levels of both blood plasma carotene and vitamin A for the control calves were similar to those previously reported (7), they will not be cited here.

TABLE 1

The effect of a vitamin A depletion ration on plasma carotene in young calves

Expt. no.	Age in days														
	105	112	119	126	133	140	147	154	161	168	175	182	189	196	203
	(γ%)														
Group 1 (Treated)															
3	63	22	11	7	7	3	2	...	...	...	...	...	...	...	...
4	66	33	15	10	9	7	4	2	3	7	...	...	...	...	...
7	53	32	13	9	5	5	3	4	...	...	...	...	...	...	...
10	70	28	8	7	4	3	6	...	...	...	...	...	...	...	...
15	99	44	22	15	11	7	7	...	...	...	...	...	...	...	...
Group 2 (Treated)															
1	182	93	34	24	15	9	7	6	3	3	2	...	...	...	...
5	187	82	35	25	17	10	9	3	5	5	8	5	7	5	7
9	253	130	68	27	18	13	7	7	5	...	...	...	...	...	...
12	249	107	41	27	22	12	7	7	3	1	...	...	...	...	...
13	186	66	24	15	11	8	7	10	7	6	4	...	...	...	...
Group 3 (Treated)															
2	242	114	51	30	20	16	13	9	9	8	3	6	6	6	...
6	146	65	35	22	11	13	8	10	6	7	7	5	3	4	...
8	215	101	58	29	22	13	11	7	7	5	3	5	5	5	3
11	205	125	61	35	22	19	13	12	8	5	6	8	5	3	...
14	329	134	66	35	22	15	10	7	8[a]	...	...	...	...	...	..

[a] Calf accidentally strangled on neck strap on 162nd day.

TABLE 2

The effect of a vitamin A depletion ration on plasma vitamin A in young dairy calves

Expt. no.	Age in days														
	105	112	119	126	133	140	147	154	161	168	175	182	189	196	203
	(γ %)														
Group 1 (Treated)															
3	29.4	9.9	8.1	7.1	5.3	3.6	2.4	....	....	....	....	....	....	....	....
4	18.6	17.9	12.8	9.9	9.6	7.1	6.9	4.7	3.5	2.1	....	....	....	....	....
7	17.0	9.9	6.5	7.4	5.9	4.2	3.6	2.6	....	....	....	....	....	....	....
10	17.1	14.4	8.3	8.7	4.5	2.9	3.2	....	....	....	....	....	....	....	....
15	15.2	11.1	11.9	5.6	5.4	2.1	0.9	....	....	....	....	....	....	....	....
Group 2 (Treated)															
1	15.8	20.6	14.7	15.2	16.8	12.6	8.7	6.9	6.3	3.8	3.5	....	....	....	....
5	27.3	24.6	23.3	23.3	21.2	16.1	11.7	11.0	11.0	9.2	8.6	6.5	5.4	2.9	3.2
9	21.0	18.3	19.5	14.4	8.3	6.5	6.8	3.0	2.6	....	....	....	....	....	....
12	19.5[a]	21.5	15.9	18.6	14.0	11.1	8.4	6.5	3.5	2.7	....	....	....	....	....
13	16.8	16.1	11.7	10.1	10.5	7.8	5.3	5.6	4.1	3.6	1.8	....	....	....	....
Group 3 (Treated)															
2	26.4	32.6	28.2	24.0	21.6	18.9	19.1	15.8	11.7	10.1	9.6	5.4	3.8	3.5	....
6	21.0	18.3	18.2	16.4	14.6	15.9	13.7	11.3	7.8	9.0	6.3	4.2	3.6	1.8	....
8	23.7	28.4	32.0	20.0	17.9	13.8	11.6	11.9	8.0	8.0	6.9	6.2	4.5	3.6	1.2
11	27.5	21.0	17.9	18.0	17.9	13.1	14.9	14.6	11.9	9.5	8.7	7.8	3.6	3.6	....
14	24.3	24.8	23.9	17.0	21.8	14.4	13.7	11.9	10.5[b]	....	....	....	....	....	....

[a] Calculated missing value.

[b] Calf accidentally strangled on neck strap on 162nd day.

(table 4) were elevated in all calves receiving only the depletion ration, with the exception of calf 4, as contrasted to normal values being maintained throughout the experimental period by those calves receiving supplemental vitamin A. This is in agreement with previous reports (7, 9). Excitement readily caused an elevation in pressure and, in certain calves, it was extremely difficult to obtain a satisfactory spinal puncture or reading; this accounts for the many missing values indicated by lines in table 4.

TABLE 3

The effect of a vitamin A depletion ration on liver carotene and vitamin A and on the microanatomy of the parotid gland in young calves

Expt. no.	Liver			Squamous metaplasia in the parotid gland ducts
	Weight	Carotene	Vitamin A	
Group 1 (Treated)	(*g.*)	(γ/*g.*)		
7	2445	0.30	0.16	+
10	2591	0.25	0.14	+
15	2198	0.28	0.19	+
Mean	2411.3	0.277	0.163	
Group 2 (Treated)				
9	3112	0.33	0.22	+
12	2441	0.36	0.92	−
13	1910	0.35	0.22	+
Mean	2487.7	0.347	0.453	
Group 3 (Treated)				
8	4117	0.36	0.44	+
11	3559	0.36	0.42	+
14[a]	4917	0.62	2.79	+
Mean	4197.7	0.447	0.430[b]	
Group 4 (Controls)				
16	2165	0.26	153.80	−
17	2304	0.50	221.00	−
18	3492	0.40	121.60	−
19	1820	0.40	171.60	−
20	2260	0.40	143.60	−
21	3170	0.40	123.20	−
Mean	2535.2	0.393	155.80	

[a] Calf accidently strangled on neck strap on 162nd day; on 161st day blood plasma vitamin A was 10.5 γ per cent.

[b] Value for calf no. 14 not included in the mean.

The clinical symptoms observed in order of declining frequency were exophthalmos, muscular incoordination and diarrhea. The animals were observed during 30-min. exercise periods in a dry lot on alternate days to detect incoordination. Only one calf, no. 13, had a convulsion which was used as a criterion of vitamin A deficiency in an earlier study (7).

Histopathology. In a previous study on bovine A-hypovitaminosis (7), it was reported that the lesion generally considered pathognomonic for A-hypo-

TABLE 4

The effect of a vitamin A depletion ration on spinal fluid pressure and clinical appearance in young calves

Expt. no.	Spinal fluid pressures				Clinical observations
	Initial at 17 wk.	Second at 21 wk.	Third at 25 wk.	Terminal at end of depletion period	
			(*mm. H_2O*)		
Group 1 (Treated)					
3	<95	110	....	— (147)[a]	145th day diarrhea
4	<95	<95	....	95 (168)	None
7	<95	205	....	110 (155)	154th day muscular incoordination
10	110	122	....	185 (148)	118th day diarrhea
15	<95	....	....	120 (146)	140th day muscular incoordination
Group 2 (Treated)					
1	<95	<95	....	180 (175)	None
5	<95	135	135	— (203)	171st day exophthalmos, 196th to 203rd days diarrhea
9	<95	135	....	240 (166)	122nd-124th days temperature 102.0° F., 164th day muscular incoordination
12	<95	<95	....	— (169)	154th-169th days diarrhea
13	95+	95	165	195 (176)	119th day diarrhea, 155th day exophthalmos, 173rd day convulsion, muscular incoordination thereafter
Group 3 (Treated)					
2	<95	—	110	260 (196)	196th day exophthalmos
6	95+	—	115	— (196)	189th day exophthalmos, 196th day muscular incoordination
8	<95	—	205	215 (205)	196th day exophthalmos
11	95+	160	....	— (199)	None
14	180	207	....	— (162)	154th day exophthalmos, 162nd day accidentally strangled on neck strap.
Group 4 (Controls)					
16	<95	<95	120	— (178)	None
17	95+	105	....	125 (164)	108th day temperature 104.4° F.
18	95+	137	95	<95 (185)	None
19	....	....	....	95+ (156)	None
20	....	<95	<95	— (190)	None
21	....	<95	95	<95 (189)	None

[a] Values in parenthesis are days of age at termination of depletion period.

vitaminosis in animals, namely squamous metaplasia, was prominently observed in the interlobular ducts of the parotid gland.

For the purpose of this paper, only the changes in the parotid gland were reported. Other organs such as the adrenals, eye, heart, kidney, liver, pancreas, pituitary, spleen, testes and thyroid were examined without revealing constant diagnostic lesions. In the evaluation of the micropathology, it should be considered that the depletion was not as great as in the previous study (7).

Normally, the interlobular ducts and the main parotid duct of this gland are lined by pseudostratified columnar epithelium often containing intracytoplasmic colloid cysts near the free border. At the termination of the parotid duct, known as the papilla, in the mouth, there is found a true mucocutaneous junction between the columnar epithelium of the parotid duct and the normal stratified squamous epithelium of the oral cavity, according to a recent study by the authors (4).

In this experiment, the normal epithelium of the interlobular ducts of the parotid gland was found to be replaced in eight out of nine calves (table 3) by a thick stratified squamous epithelium infrequently accompanied by hyperkeratinization; this condition is known as squamous metaplasia. More recently (4), it was found that the lining of the entire parotid duct may undergo squamous metaplasia. Since this lesion was never observed in the interlobular ducts or in the main duct of the controls, it was considered to be specific for A-hypovitaminosis in the bovine.

SUMMARY

The effect of a vitamin A-depletion ration on liveweight, carotene and vitamin A levels in the blood and liver, clinical appearance and microscopic anatomy was studied in 21 young calves beginning at 106 days of age. All calves were fed a vitamin A-depletion ration of 2.67 lb. per 100 lb. of liveweight, with six of the calves receiving in addition a daily equivalent of 107,143 USP units of vitamin A. Observations on the daily feed intakes, clinical appearance, weekly liveweight, plasma carotene and vitamin A were made and tissue samples for histological study and liver samples for carotene and vitamin A analyses were obtained at slaughter. The experimental period was terminated for those calves fed only the depletion ration when the blood plasma level was less than 4 γ per cent for 2 consecutive wk., and for those calves fed the supplemental vitamin A at average ages comparable to those fed only the depletion ration.

Until the blood plasma level decreased to 4 γ per cent, all calves readily consumed the vitamin A-depletion ration. At this level of plasma vitamin A, seven of the calves fed only the depletion ration began refusing some feed. Liveweight was not affected and both the gain in liveweight and rate of gain were not significantly different between groups of calves.

Blood plasma carotene decreased in all calves with successive weeks on experiment and was characterized by a relatively rapid decrease early in the experimental period with a marked decline in rate of decrease occurring during the middle and end of the experimental period. In contrast, plasma vitamin A values decreased in a uniform manner regardless of the level and, therefore,

indicated a possible means of measuring vitamin A stores by prediction, using this relatively uniform rate of decrease as a standard. The liver stores of vitamin A were considered depleted in those calves receiving only the vitamin A depletion ration.

Spinal fluid pressures at the termination of the experimental period were elevated in the calves receiving only the depletion ration, while those receiving the supplemental vitamin A remained essentially the same as at the start of the experiment. Exophthalmos was observed in six calves, muscular incoordination in five, diarrhea in four and convulsions in one, all symptoms occurring toward the termination of the experiment for the individual calves.

Squamous metaplasia of the interlobular and main ducts of the parotid gland was observed in eight of nine depleted calves and in none of the six controls.

ACKNOWLEDGMENT

The authors are most grateful to R. J. Caverno, M. W. Dicks and B. A. Donahue for technical assistance during the course of the experiment and to C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for suggestions as to the statistical analyses of the data.

REFERENCES

(1) DAVIES, A. W. The Colorimetric Determination of Vitamin A by Alkali Digestion. Biochem. J., **27**: 1770–1775. 1933.

(2) EATON, H. D., SPIELMAN, A. A., JOHNSON, R. E., AND MATTERSON, L. D. The Utilization of Fetal Stores of Vitamin A by the Newborn Calf. J. Dairy Sci., **32**: 764–768. 1949.

(3) EATON, H. D., CARPENTER, C. A., CAVERNO, R. J., JOHNSON, R. E., ELLIOTT, F. I., AND MOORE, L. A. A Comparison of U. S. No. 2 Field-cured Field-baled Alfalfa Hay with Artificially Dried and Ground and Pelleted Alfalfa Hays as a Source of Carotene and Roughage for Holstein Calves. J. Dairy Sci., **34**: 124–135. 1951.

(4) EATON, H. D., ELLIOTT, F. I., MATTERSON, L. D., DECKER, L. M., HELMBOLDT, C. F., AND JUNGHERR, E. L. Unpublished Data, Storrs Agr. Expt. Station 1950.

(5) FREY, P. R., AND JENSEN, R. Depletion of Vitamin A Reserves in the Livers of Cattle. Science, **105**: 313. 1947.

(6) JACOBSON, W. C., CONVERSE, H. T., AND MOORE, L. A. Effects of Vitamin A and Carotene Intake on Depletion Time of Young Dairy Calves. J. Dairy Sci., **32**: 418–428. 1949.

(7) JUNGHERR, E. L., HELMBOLDT, C. F., AND EATON, H. D. Parotid Gland Lesions in Experimental Bovine Vitamin A Deficiency. J. Dairy Sci., **33**: 666–675. 1950.

(8) MOORE, L. A., AND BERRY, M. H. Vitamin A and Carotene Content of the Blood Plasma of Dairy Calves from Birth up to Four Months of Age. J. Dairy Sci., **28**: 821–826. 1945.

(9) MOORE, L. A. Vitamin A Ascorbic Acid and Spinal Fluid Pressure Relationships in the Young Bovine. J. Nutrition, **31**: 229–236. 1946.

(10) MORRISON, F. B. *Feeds and Feeding.* 21st ed., p. 1148. The Morrison Publishing Co., Ithaca, N. Y. 1948.

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

(12) RITZMAN, E. G., COLOVOS, N. F., KEENER, H. A., AND TEERI, A. E. Influence of Vitamin A on the Utilization of Energy and Protein by Calves. N. H. Agr. Expt. Sta. Tech. Bull 87. 1945.

THE EFFECT OF LOW MANGANESE RATIONS UPON DAIRY CATTLE[1, 2]

O. G. BENTLEY AND P. H. PHILLIPS

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

A nuisance sterility exists and represents about 10 per cent of the cattle of certain herds where low manganese rations are fed. This type of sterility is persistent and recurs from year to year on farms where the forage manganese content falls below 20 ppm. Hay samples in certain areas of Wisconsin have been shown, by Bentley *et al.* (1), to vary in manganese from 8 to 50 ppm. Repeat breeder cows coming from farms where low manganese hays were fed invariably carried reduced blood vitamin C levels. In many herds the injection of ascorbic acid has been helpful in getting such cattle to conceive. It has been shown by Waddell *et al.* (12), Kemmerer *et al.* (3), Skinner *et al.* (7) and Boyer *et al.* (2) that the oestrus cycle of the rat is disturbed when low manganese diets are fed. Literature on the effect of manganese deficiency symptoms and its relationship to dietary intake has been amply reviewed by Von Oettingen (10), Schaible *et al.* (6) and Underwood (9). In view of the demonstrated effect of manganese upon sterility and its effect upon growth and other phenomena in different species, it seemed desirable to study the effect of low manganese rations formulated from practical feeds available to Wisconsin farmers to determine the effect of such low manganese rations upon milk production, growth and the reproductive performance of dairy cattle.

EXPERIMENTAL

Experiment 1. Eighteen grade Holstein heifer calves were purchased in the fall of 1942 and placed in a semi-isolated area of the Station dairy barn. The calves were equally allocated to dietary groups designated as lots 1 and 2, of experiment 1. These animals were fed a basal ration composed of a low-manganese (less than 10 ppm.) hay (usually a mixture of clover, timothy and alfalfa), corn silage (5.7 ppm.) and a mixed grain supplement (8.5 ppm.). The ingredients of the supplement were 71.7 per cent ground yellow corn, 25 per cent corn gluten meal, 1 per cent bone meal and 1.3 per cent salt. The animals in lot 2 received the basal ration plus a manganese supplement which increased the manganese ingestion from 4.0 to 17.0 mg. per kg. of body weight, by the addition of a commercial feeding grade of $MnSO_4$. The manganese content of the basal ration, hay silage and grain, ranged from 7 to 10 ppm., while the ration for the lot 2 animals averaged 30 ppm. of manganese. An intake of 4 lb. of the grain supplement, 10 lb. of hay and 30 lb. of silage by the lot 1 cows supplied 100 mg. of manganese, while the lot 2 cows obtained 300 mg.

Received for publication Nov. 11, 1950.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] These studies were supported in part by a grant from the Tennessee Eastman Corporation, Kingsport, Tennessee.

The customary management practices were followed in caring for these animals. The heifers were artificially bred at 15 mo. of age and the first calves were born in 1943–44. A second calf crop was obtained in 1944–45. Data pertinent to the breeding performance of these heifers and the vigor and appearance of their offspring were kept.

Samples of liver, pancreas, spleen, heart, adrenal and ovary for manganese analysis (periodate AOAC method) were obtained when these cows were slaughtered at the close of experiment 1 in the spring of 1946. (The experiment was terminated because several animals did not become negative to the Bang's test after a late vaccination.) Other samples were taken from the liver and kidney to be used for histological study. Throughout the experiment blood was taken at regular intervals for vitamin C, vitamin A and carotene analyses. The method of Kimble *et al.* (4) was used for the vitamin A determinations. Mindlin and Butler's method (5) for vitamin C was used. Blood manganese was determined microbiologically (1) on two different series of blood samples.

TABLE 1

The growth of heifers fed low manganese rations

Lot no.	Ration manganese[a]	No. of animals/lot	Av. weight		
			Initial	12 mo.	24 mo.
	(*ppm.*)		(*lb.*)	(*lb.*)	(*lb.*)
Expt. 1					
1	7–10	9	171	749	1290
2	30	9	175	745	1282
Expt. 2					
3	7–10	4	188	754	1086
4	40	4	184	749	1110
5	60	4	183	742	1090

[a] The manganese content based on the analysis of the feeds used. The values given are averages.

Experiment 2. In the fall of 1945 12 calves were purchased to be used in studies designed to determine the effect of higher levels of manganese in the ration of young dairy cattle. The basal ration used in experiment 1 was fed to four of the calves (lot 3) while two other lots (4 and 5) of four calves each were fed varying levels of manganese. The levels chosen were 40 ppm. for lot 4 and 60 ppm. for lot 5. In terms of daily intake, lot 3 cows received roughly 100 mg., lot 4, 350 mg. and lot 5, 550 mg. of manganese.

At 15 mo. of age these heifers were bred and the first calves were born in 1947. These cows were continued on experiment until the spring of 1949; thus each cow had at least two calves and in some instances three calves before the end of the experiment.

The level of manganese in the blood from these cows was determined frequently in order to study the effect of increased manganese feeding on the concentration of this element in the blood. Numerous blood samples were taken for vitamin A and C analysis as well.

RESULTS

Inspection of the data summarized in table 1 indicates that feeding a manganese supplement with the basal ration had no effect on the growth of young heifer calves during the first 12-mo. period. The average weights reported were obtained for the period of active growth before the heifers were bred, thus avoiding differences in weight caused by pregnancy or lactation. That feeding a manganese-low ration does not adversely affect growth is not surprising, since diets for rats must be extremely low in this element before the growth rate is reduced. Chicks, whose manganese requirement is relatively high, grow quite well when fed rations low in manganese.

Data on the reproduction performance of the cows from both experiments 1 and 2 are summarized in table 2.

TABLE 2

The effect of supplemental manganese in the ration on reproduction in dairy cows

	Ex. 1				Ex. 2					
	Lot 1 (Basal)		Lot 2 (30 ppm. Mn)		Lot 3 (Basal)		Lot 4 (40 ppm. Mn)		Lot 5 (60 ppm. Mn)	
	1944	1945	1944	1945	1947	1948	1947	1948	1947	1948
Age at first heat period (*mo.*)	9.56	....	7.44	....	9.0	....	8.5	....	8.0	....
Conception rate (% first service)	52.6	62.5	59.	62.5	50.	50.	80.	67.	33.3	57.1
No. of cows with calf	9	6	9	7	3	2	4	4	4	4
No. of calves born[a]	8	6	7	7	2	2	3	3	4	4
No. of calves with weak legs and pasterns	4	1	1	0	1	0	1	0	0	0
No. of days calves born early	11.7	9.1	7.1	4.1	10.7	10.	6.8	9.	13.2	9.8
Av. weight of calves at birth	88.5	85.7	88.8	97.	89.7	88.	95.	100	82.3	93.5

[a] Considerable difficulty was experienced during calving and some calves were lost at birth. These losses were not considered to be directly caused by the rations fed.

Heifers in the manganese-supplemented group (experiment 1) came into heat earlier (roughly 2 mo.) than the heifers in lot 2, which suggests a stimulating effect upon sexual maturity. The breeding records indicated that for both lots 1 and 3 the number of breeding services per conception was slightly higher for the animals fed the manganese-low ration, as compared to those receiving the same ration adequately supplemented with manganese. Otherwise, the reproductive performances of these cattle were very similar. One of the cows in lot 5 was hard "to settle" in 1947 and 1948 and failed to breed in 1949. The number of calves born and the number of calves born dead was not altered by feeding the low-manganese ration. There was a slight trend to heavier calves at birth from manganese-supplemented cows. This was noticeable in the second calf crop. Four of eight calves from the cows in lot 1 had weak legs and pasterns, whereas six of seven calves from the manganese-supplemented cows of lot 2 were normal in this regard. A typical example of the condition as seen in these calves is shown in figure 1.

Analyses were made of the levels of vitamins A and C and carotene in blood

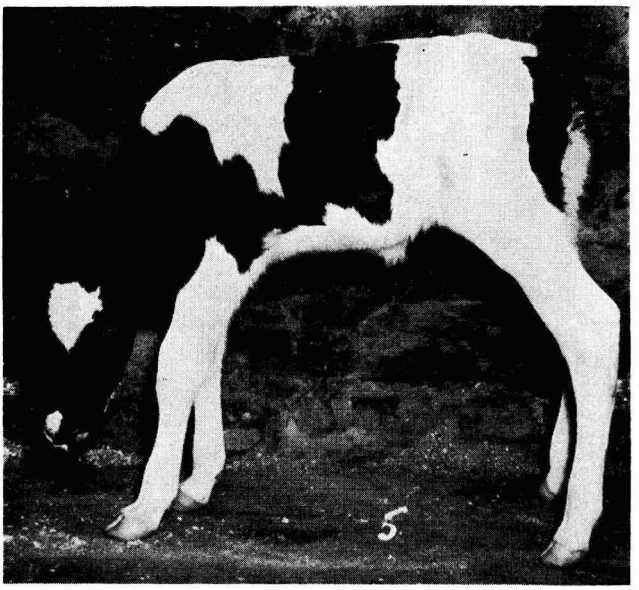

FIG. 1. Weak pastern calf from a cow on low-manganese ration.

plasma from cows maintained on the various experimental rations. Blood was collected at monthly intervals from the cows of lots 1 and 2, thus the averages presented in table 3 are for 34 different samplings for vitamin C and 29 for vitamin A and carotene. It was found that the addition of manganese to the ration failed to raise the level of these vitamins in the blood plasma. It has been suggested that the synthesis of vitamin C *in vivo* was dependent upon an adequate supply of manganese in the diet. Boyer *et al.* (2) were unable to verify this observation with the rat.

The report by Wachtel *et al.* (11) suggested that feeding a low manganese ration would result in alterations in the distribution of phosphorus in the blood.

TABLE 3

Vitamin A and C concntrations in the blood plasma of cows fed adequate and low levels of manganese

	Lot no.[a]	No. of cows	Vitamin content	
			Vitamin C	Vitamin A
			(mg./100 ml.)	*(γ/100 ml.)*
Expt. 1				
	1	9	0.46	17.6
	2	9	0.50	17.
Expt. 2				
	3	4	0.47	17.
	4	4	0.44	19.
	5	4	0.45	17.

[a] Manganese intake for lot 3, 7–10 ppm. in the ration; lot 2, 30 ppm.; lot 4, 40 ppm.; lot 5, 80 ppm.

This was checked by bleeding cows from lots 3 and 5 of experiment 2. The level of inorganic phosphorus and total phosphorus was the same in both groups of animals. (The total phosphorus was 25–37 and the inorganic phosphorus was 5.0 to 5.5 mg. per cent.)

Experiment 1 was terminated in the spring of 1946. The cows were slaughtered and the desired organs and tissue samples obtained for analysis. The dressing percentage for seven cows of lot 1 was 46.5 per cent and for nine cows of lot 2 it was 49.7 per cent.

In table 4 the data on the manganese content of the various tissues are presented. By comparing the average values of the liver, kidney, heart, pancreas and adrenals, it is evident that the low-manganese ration employed did not reduce the level of manganese present in these organs. The liver and kidney are chief storage places for manganese and it would be expected that these organs would reflect low dietary intakes of manganese. The ovary appeared, however,

TABLE 4

Manganese content of tissues from cows fed varying levels of manganese in the ration[a]

Tissue or organ	Lot 1 (Low Mn)	Lot 2 (Mn added)
	γ/g. dry wt.	γ/g. dry wt.
Liver	9.8	10.2
Kidney	4.4	5.0
Pancreas	7.6	7.2
Heart	1.4	1.7
Adrenal	4.0	3.7
Ovary	.85	2.0
Blood[b]	4.6±1.46	6.6±2.03

[a] Sample from 7 cows of lot 1 and 9 cows of lot 2.

[b] Values given in γ/100 ml. of blood with standard deviation. Determined by microbiological assay.

to be irregular in that the level of manganese in this organ from cows fed the low-manganese ration was reduced by more than 50 per cent.

The level of manganese in the blood was determined by microbiological assay, as described by Bentley *et al.* (1). The deviation from sample to sample was within the range found for normal samples of blood. Hence, it was concluded that the manganese in the diet did not influence the manganese content of the blood.

The manganese content of the liver and blood from calves born of dams maintained on the manganese-low ration for as long as 3 yr. was not reduced significantly and averaged 6.1 ppm.

Manganese had no effect upon milk production. The control animals (lots 1 and 3) with low manganese produced, during the first lactation, between 8,500 and 9,000 lb. of milk, while those receiving 30, 40 and 60 parts per million produced 7,522, 8,942 and 9,031 lb. of milk, respectively. While complete second lactation records were not obtained in the first experiment because of the reactors in the herd, second lactation records were obtained with the cattle in experiment 2. The controls (lot 3) produced 8,329 lb., while lots 4 and 5 pro-

duced 8,864 and 9,734 lb., respectively. These milks tested between 3.3 to 3.5 per cent butterfat. From these records it is clear that low manganese had no detrimental effect upon milk production and that the feeding of as much as 60 ppm. of added manganese did not inhibit milk secretion.

Detailed histopathological observations were made upon the liver and the kidney from the cattle on experiment 1. The data on the incidence of pathological changes in the liver are compiled in table 5. The kidneys were mostly normal. There was some hemosiderin and some interstitial infiltration of lymphocytes, but in the main, one would interpret these observations as representing normal cattle kidneys. In the liver there were characteristic changes which seemed to occur more frequently in the low-manganese lot 1 than in those receiving additional manganese supplements. The outstanding feature of the histopathology observed in the livers of the low-manganese cattle was bile duct proliferation. This change occurred in six out of eight animals in lot 1, while no changes were observed in the cattle fed supplemental amounts of manganese. Likewise, necrotic tissue occurred in a high proportion of the livers from the

TABLE 5

The occurrence of histopathology in the liver of cows from experiment 1

	No. of occurrences	
	Lot 1[a] (Low Mn)	Lot 2[a] (Mn added)
Bile duct proliferation	6	0
Abscesses	3	0
Atrophy	1	1
Fatty degeneration	2	0
Hyalinized blood vessel	4	1
Bile volume, av.	195 ml.	511 ml.

[a] Eight animals in each lot.

low-manganese group and resulted in well-defined abscesses in three out of eight cases. There was some fatty degeneration in these livers. An interesting feature of the pathology of these livers was the hyalinized blood vessels which occurred in 50 per cent of the cattle on low manganese.

At post mortem one of the gross and striking observations was the bile volume in the gall bladder. There was practically no bile in the gall bladders of the cattle receiving the control ration low in manganese. The average amount of bile present in the gall bladder of the control animals was 195 ml. while those which received manganese averaged 511 ml. of bile. Low manganese apparently is concerned with liver damage and specifically affects production of bile and the proliferation of the bile ducts.

DISCUSSION

These experiments seem to indicate that rations made up of mixed hay, corn silage and a grain mixture centered chiefly around corn and its by-products, may be marginal in their manganese content since there was some delay in the onset of the first evidence of oestrum, a slightly reduced conception rate and a greater

number of calves born with weak legs and pasterns at the first calving. Manganese had no effect upon the vitamin A, C or carotene concentrations of the blood plasma of these cattle, either in the growing stage or when they were fully matured.

It was very difficult with these rations to influence tissue manganese in any way, particularly in the liver. Apparently, cattle hold tenaciously to the manganese content of the liver under most conditions. The only organ or tissue affected by low concentrations of dietary manganese was the ovary, and, in this case, the low-manganese fed cattle contained 0.85 γ per gram of dry weight, as compared to 2 γ+ for the supplemented animals. This again would tend to support the suggestion that low manganese, of the order of 10 ppm. in the ration, may be marginal or border line as far as optimum reproductive performance is concerned. It is equally apparent from these experiments that manganese has little effect upon growth when as little as 10 ppm. are included in the ration, and that cattle can withstand supplementary amounts of manganese up to 60 ppm. There was some evidence to indicate that there was inferior body weight gain, as far as growth was concerned, at the higher level. In these experiments it is evident that low manganese affects the structural pattern of the liver and the concentration of manganese in the ovary. The latter may have an effect upon ovulation or the vitality of the ova produced from such an ovary.

The low-manganese cows aborted three calves with no brucella organisms recovered from the fetus, indicating that it was not due to a brucella infection. The cows which received 30 or more ppm. of added manganese aborted only one calf, and this case was complicated by a severe kidney and bladder infection.

SUMMARY AND CONCLUSIONS

Experiments have been made with young Holstein heifers from calfhood (200 lb. live weight) through one, two or three lactations on rations composed of good legume hay low in manganese, corn silage and a grain mixture of corn and corn by-products.

(a) Rations with less than 10 ppm. manganese were adequate for growth in young Holstein heifers. (b) Addition of added manganese as a mineral supplement to provide 30, 40 or 60 ppm. did not favorably stimulate growth above that of the control or low manganese lot. (c) Heifers raised on the low manganese or control rations were slower to exhibit oestrus and were slightly and consistently slower to conceive upon breeding. (d) Manganese has no appreciable effect on either the vitamin A, C or carotene content of the blood, hence the synthesis of vitamin C is not contingent upon the manganese content of the ration provided 7 to 10 ppm. of maganese are present in the ration. (e) It was extremely difficult to affect the manganese concentrations of the organs and tissues of dairy cattle with the one exception of the ovary, which exhibited low manganese concentrations when the diet of the cattle contained only 7 to 10 ppm. of manganese in the ration. (f) That low dietary manganese caused abnormal structural changes found in the livers of cattle subsisting thereon.

Cattle readily tolerate 60 to 70 ppm. of manganese in the diet. Twenty ppm.

would seem to be a satisfactory level of manganese which provides a margin of safety to meet the requirements of cattle and 10 ppm. or less is distinctly in the marginal or deficient zone. Under practical conditions it would seem that the addition of supplemental manganese to cattle rations would be indicated where the manganese is below 20 ppm. in the ration.

REFERENCES

(1) BENTLEY, O. G., SNELL, E. E., AND PHILLIPS, P. H. The Microbiological Method for the Determination of Manganese. J. Biol. Chem. **170**: 343. 1947.

(2) BOYER, P. B., SHAW, J. H., AND PHILLIPS, P. H. Studies on Manganese Deficiency in the Rat. J. Biol. Chem., **143**: 417–425. 1942.

(3) KEMMERER, A. R., ELVEHJEM, C. A., AND HART, E. B. Studies on the Relation of Manganese to the Nutrition of the Mouse. J. Biol. Chem., **92**: 623–630. 1931.

(4) KIMBLE, M. S. Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. Gen. Lab. and Clin. Med., **24**: 1055. 1939.

(5) MINDLIN, R. I., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma, The Macro Method and a Micro Method. J. Biol. Chem., **122**: 673. 1937.

(6) SCHAIBLE, E. J., BANDEMAR, S. L., AND DAVIDSON, J. A. The Manganese Content of Feedstuffs and Its Relation to Poultry Nutrition. Mich. Agr. Expt. Sta. Tech. Bull. 159. 1938.

(7) SKINNER, J. T., VAN DONK, E. C., AND STEENBOCK, H. Manganese as a Factor in Reproduction. Am. J. Physiol., **101**: 591–595. 1932.

(8) SKINNER, J. T., PETERSON, W. H., AND STEENBOCK, H. The Manganese Metabolism of the Rat. J. Biol. Chem., **90**: 65–80. 1931.

(9) UNDERWOOD, E. J. The Significance of the Trace Elements in Nutrition. Nutrition Abstracts & Revs., **9**: 515–531. 1939–40.

(10) VON OETTINGEN, W. F. Manganese: Its Distribution, Pharmacology and Health Hazards. Physiological Revs., **15**: 175–178. 1935.

(11) WACHTEL, L. W., ELVEHJEM, C. A., AND HART, E. B. Physiology of Manganese in the Rat. Am. J. Physiol., **140**: 72–82. 1943.

(12) WADDELL, J., STEENBOCK, H., AND HART, E. B. Growth and Reproduction on Milk Diets. J. Nutrition, **4**: 53–65. 1931.

THE KEEPING QUALITY OF WHOLE MILK POWDER. I. THE EFFECT OF PREHEAT TEMPERATURE OF THE MILK ON THE DEVELOPMENT OF RANCID, OXIDIZED AND STALE FLAVORS WITH DIFFERENT STORAGE CONDITIONS[1]

L. J. CHRISTENSEN,[2] C. W. DECKER AND U. S. ASHWORTH
Dept. of Dairy Husbandry, State College of Washington, Pullman

Powdered whole milk is subject to a variety of off-flavors of which rancid, tallowy or oxidized and stale are influenced by the preheat treatment of the milk previous to concentrating and drying. Other factors which may influence the development of these off-flavors are the conditions of storage, such as temperature of storage and inert gas packing.

This work was undertaken principally to correlate flavor with initial and residual lipase activity in experimental powders. In a second paper (3), the development of peroxides and ferricyanide-reducing groups will be discussed.

REVIEW OF LITERATURE

Rancid flavor and lipolysis. Nair (15) found no lipase activity in whole milk powders made from milk preheated at 145 to 148° F. for 30 min. Greenbank and Wright (6) report that whole milk powders made from milk preheated at 142, 152 and 162° F. for 30 min. and stored at 86° F. developed a rancid flavor within 112, 126 and 140 days, respectively.

Hetrick and Tracy (9), in a study of time-temperature inactivation of lipase in milk with induced lipolysis (heated to 105° F. and homogenized), found that 137° F. for 30 min. would inactivate lipase. Extrapolation of the log curve they used indicates that 140° F. for 17 min. should inactivate lipase. Tarassuk (19) reported that 130° F. for 30 min. would prevent spontaneous lipolysis (unactivated) in milk. There is a slight discrepancy of 7 min. between these two references in the time required for induced lipolysis and that required for spontaneous lipolysis at 130° F., due possibly to methods employed or to inherent differences.

Krukovsky and Sharp (13) found that shaking probably induced lipolysis by activating or changing the protein adsorption film.

Oxidized flavor. Oxidized or tallowy flavor in whole milk powder has been considered a different phenomenon from oxidized flavor in fluid whole milk. Holm and Greenbank (11) found a tallowy flavor produced in butterfat was due to autoxidation of the unsaturated fats principally oleic, and reported a like off-flavor development in whole milk powder due to the same cause (12). Thurston *et al.* (22,23) believed that oxidized flavor in fluid whole milk was due to oxidation of the lecithin rather than the milk fat proper.

Received for publication November 13, 1950.

[1] Scientific Paper no. 971, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

[2] Data in part from a thesis submitted in partial fulfillment for the degree of Master of Science, State College of Washington.

Lea *et al.* (14) summarized the mechanism of oxygen absorption by whole milk powder as follows: Skim powders absorb oxygen at a slow, uniform rate which is practically linear. In whole milk powder there is imposed on this absorption of oxygen by nonfat dry milk solids, the oxygen absorption by the butterfat, resulting in an induction period followed by a rapid phase of fat oxidation. Once this rapid phase of fat oxidation is reached, there is little slackening in the rate, even when absorption approaches the limits set by the quantities of oxygen in the sealed can.

Gas packing in an inert gas has been widely used to prevent or reduce oxidized flavor. The oxygen level after desorption (equilibrium after diffusion) of the powders is of primary importance in preventing oxidized flavor. Coulter (1), Lea *et al.* (14) and Coulter *et al.* (2) state that tallowy or oxidized flavor does not appear in cans with less than 1 per cent oxygen in the headspace. Lea's powders were low in moisture content (1.4 to 1.6 per cent). Lea *et al.* (14) and Schaffer (18) found that 3 per cent oxygen is the upper limit for oxygen at ordinary storage temperatures. Tarassuk and Jack (21) reported whole milk powder and powdered ice cream mix packed in less than 2 per cent oxygen were acceptable after 7-mo. storage at either room temperature or 100° F. Greenbank *et al.* (7) found the whole milk powder manufactured from milk of good quality and under recognized practices would keep 6 to 8 mo. at 68° F. when air packed. The temperature coefficient was given as 1.6 for each increase of 18° F.

Findlay *et al.* (4) found gas packing more efficient at higher than at lower temperatures of storage for low-moisture powder. Coulter (1) dried whole milk powder in an atmosphere of inert gas and found no improvement over gas packing.

Stale flavor. Tarassuk and Jack (21) report that stale flavor is connected with materials associated with the fat, and preheating temperatures are of major importance in preventing stale flavor. Oxygen was the chief contributing factor to the development of stale flavor.

Remaley (17) reports the development of stale and heated flavor to be in direct relationship to the time and temperature of preheating, and staleness bears a direct relationship to increases of moisture content above 1.0 per cent. By reducing time and/or temperature of preheating, heated flavor can be eliminated almost entirely and stale flavor can be inhibited.

Whitney and Tracy (24) believed the stale flavor to be connected with the butterfat and extracted butteroil (25) from stale whole milk powder by organic solvents, using a Soxhlet-type extraction procedure, and found that 90 per cent of the butteroil was recovered from the powder and 90 per cent of the stale flavor component was extracted with the butteroil.

Coulter *et al.* (2) summarized by saying that the characteristic stale flavor developing in normal dry whole milk is probably a composite of flavors resulting from lactose-protein changes and oxidation of the lipids.

EXPERIMENTAL PROCEDURE

Milk from the college herd was given one of the following preheat treatments in a 30-gal. pasteurizer in individual 10-gal. lots and held for 20 min. at the

following preheat temperatures: none, 140, 150, 160 and 170° F. In all, 23 lots of milk were dried. From 12 to 16 min. were required to reach the desired preheating temperatures, and upon completion of the preheating time the milk was simultaneously drawn into a vacuum pan and cooling begun on the pasteurizer. Approximately 5 min. were required to cool the remaining milk in the pasteurizer to below pan temperatures (120 to 130° F.). The milk was condensed to approximately 40 per cent total solids in a 16-in. diameter Roger's vacuum pan at 24 to 25 in. of vacuum, and the concentrate was homogenized twice in a two-stage Manton-Gaulin homogenizer of 25-gal. capacity at 2,000 and 500 lb. All the processing equipment in contact with the milk was made of stainless steel, except for the tinned metal cans or buckets in which it was received or handled during processing.

The condensed milk was cooled and dried in an experimental pilot plant drier based on the design of one at the University of Minnesota, as given by Coulter (1). The concentrated milk flowing in with a gravity head of about 2 ft. was atomized through a spray nozzle 1 mm. in diameter centered within an air outlet 2 mm. in diameter, through which air at 60 lb. pressure was admitted. The drying air at about 255° F. flowed concurrently with the milk spray. The moisture-laden air was drawn from the drying chamber at about 160° F. through cloth powder collectors and then passed through cold water sprays for recirculation. The loss in moisture corresponded to the difference in saturation values for air at the two temperatures.

Within 1 hr. after drying, the powders were air packed by hand in no. 2 flat tins, about 120 g. per can, and sealed. The powders were stored at 45 and 85° F. To prevent masking of possible rancid flavors by oxidized flavors, half of the samples stored at 85° F. were double nitrogen packed as follows: The tins were punctured with a nail and subjected to a pressure of 1 mm. or less for 4 hr. in a vacuum chamber, and nitrogen was run back to a pressure slightly above atmospheric and the can soldered. The remaining entrapped air was allowed to desorb from the powder and the process repeated after 48 hr.

The moisture content of the powders was determined by the vacuum oven method.

The lipase activity of the raw milk and concentrate was determined by a method modified from that of Peterson *et al.* (14) in which 4 ml. of raw milk or concentrated milk diluted back to 12.5 per cent total solids were used. For residual lipase activity of the whole milk powders, free fatty acids were determined by the method of Hollender *et al.* (11).

Powder samples were removed for flavor and free fatty acid determinations after 2 wk., then each month up to 6 mo. and finally after 8 mo. Flavor only was determined after 12 mo. on samples with none, 160 and 170° F. preheat treatments.

Flavor scoring was done by a panel of six people, a majority of whom judged each sample, and four of whom had at least 2 yr. of previous experience in judging whole milk powders. The powders were reconstituted on the basis of one part of powder to seven parts of distilled water. The score card used was as

follows: 9–10, no defect, equivalent to best fresh whole milk; 8–9, no defect except possibly very slight typical heated flavor; 7–8 slightly heated or off flavor not readily definable; 6–7 slightly oxidized or stale or both; 0–6 progressively more oxidized, stale or rancid, foreign etc. Scoring was in half points above and in full points below 5. Averages were taken of the scores of the judges. Statistical analyses were made of the difference between means of flavor scores by the method of Fisher (5) as used by Hening (8).

RESULTS AND DISCUSSION

Lipase activity. Table 1 shows the lipase activity of the original milk, condensed milk and powder, as well as the residual lipase activity of the whole milk powders in storage, as measured by free fatty acid development. Results for free fatty acids were obtained at more frequent time intervals, but the ones presented are representative.

Only the powders made from milk without preheating showed residual lipase activity, as indicated by high free fatty acid values which increased with storage time. Preheating the milk at 140° F. for 20 min., combined with the drying treatment, was sufficient to destroy lipase activity. However, momentary exposure of the milk to spray-drying temperatures apparently does not destroy lipase or the rancid flavor developed in the milk dried with no preheat treatment. The free fatty acid content of powders made from milk with no preheat treatment increased slowly in storage, and was lower at 45° F. storage than at 85° F., and lower in air-packed samples at 45° F. than in nitrogen-packed samples at 85° F. storage.

Table 2 shows the free fatty acid values of two powders exposed to relative humidities of 20, 60 and 90 per cent for varying periods of time. It was thought that any residual lipase activity might be brought out if the powder were held at higher humidities. The powders were made from milk preheated at 140° F. for 20 min. and from unheated milk. Again, as with previous free fatty acid determinations, only powder made from milk not preheated showed free fatty acid values which increased with storage time and increased humidity.

Rancid flavor. The average flavor scores of the flavor panel for all samples are given in table 3. All whole milk powder samples made from milk having no preheat treatment were rancid in flavor. The rancidity developed in the milk during the approximately 45 min. that the milk was in the vacuum pan at 120 to 130° F. This represents an induced type of lipolysis brought about by the agitation of the milk.

In no case did powders having preheat treatment of 140° F. or higher for 20 min. show any rancid flavor during 8 mo. of storage at 45 or 85° F. in air- or nitrogen-packed samples. Slight rancid flavors may have been covered up by oxidized or stale flavors in some of the lower preheat temperature powders. However, it is not likely, since nitrogen packing would tend to prevent oxidized flavors and allow the rancidity to come through if present. Low fatty acid values were shown in all of the powders made from preheated milk, and these values did not increase on storage of the powders.

Oxidized and stale flavors. The chief flavor criticism for whole milk powders

TABLE 1

Lipase activity in whole milk powders with different preheat treatments and storage temperatures

Powder no.	Moisture content	Lipase units[a]			Free fatty acids[b] in powders					
		Orig. milk	Cond. milk	Powder	Initial	3 mo. storage			6-8 mo. storage	
						45° F.	85° F.	N_2	45° F.	85° F.	N_2
					No preheat treatment						
219	1.66	0.50	0.51	0.79	2.47	2.73	3.04	—	2.82	3.09	—
225	2.19	1.05	0.52	0.45	2.41	2.21	2.45	2.41	2.19	2.63	2.43
234	2.49	0.94	0.51	0.53	2.39	2.42	3.22	2.98	2.65	4.04	3.28
239	2.91	0.60	0.75	0.56	2.40	2.25	2.84	2.63	2.47	3.20	3.01
					140°F.—20min.						
220	2.49	0.99	0.54	0.00	0.96	0.76	0.78	—	0.74	0.78	—
226	2.55	0.71	0.10	0.02	1.02	0.90	0.82	0.88	0.81	0.87	0.86
233	3.18	0.71	0.04	0.00	0.72	0.80	0.87	0.81	0.80	0.96	0.95
238	2.64	0.61	0.11	0.03	0.74	0.88	0.89	0.90	0.77	0.79	0.76
240	2.53	0.71	0.00	0.00	0.79	0.62	0.63	0.65	0.72	0.75	0.69
					150°F.—20min.						
221	2.45	0.78	0.25	0.00	0.82	0.70	0.71	—	0.68	0.68	—
227	2.48	0.68	0.12	0.00	0.67	0.79	0.77	0.79	0.71	0.74	0.85
232	2.70	0.51	0.00	0.00	0.78	0.75	0.90	0.89	0.83	0.81	0.80
237	2.45	0.72	0.00	0.00	0.79	0.81	0.83	0.79	0.78	0.78	0.80
241	2.83	1.37	0.00	0.00	0.81	0.76	0.78	0.79	0.69	0.71	0.68
					160°F.—30min.						
222	2.35	—	0.00	0.00	0.76	0.82	0.76	—	0.75	0.77	—
228	2.18	0.94	0.00	0.00	0.67	0.72	0.71	0.69	0.67	0.65	0.65
231	3.63	0.97	0.00	0.00	0.67	0.86	0.86	0.82	0.74	0.79	0.80
236	2.32	—	0.00	0.00	0.67	0.75	0.74	0.74	0.70	0.74	0.75
242	2.40	0.79	0.00	0.00	0.81	0.76	0.78	0.68	0.69	0.71	0.68
					170°F.—30min.						
224	2.25	1.05	0.00	0.00	0.84	0.71	0.76	—	0.75	0.74	—
229	2.96	0.74	0.00	0.00	0.68	0.72	0.73	0.73	0.66	0.69	0.68
230	2.32	0.71	0.00	0.00	0.65	0.91	0.83	0.83	0.66	0.63	0.67
235	2.41	0.51	0.00	0.00	0.75	0.83	0.84	0.80	0.77	0.78	0.84

[a] Lipase units are defined as the ml. $N/10$ NaOH required to neutralize the free fatty acids liberated from tributryin in a buffered solution at 40° C. for 1-hr. incubation, using 4 ml. of milk containing 12.5% total solids.

[b] Free fatty acids are expressed as ml. $N/10$ alcoholic NaOH required to neutralize the free fatty acids extracted from 5 g. powdered whole milk by boiling with 95% alcohol.

TABLE 2

The fatty acid content of two powders stored at various relative humidities

Sample no. and preheat	Relative humidity	Storage age		
		0 d.	24 d.	60 d.
	(%)			
219	20	3.0	2.90	3.00
No preheat	60	3.0	3.04	3.27
	90	3.0	4.11	6.47
220	20	0.88	0.81	0.88
140°F.—20 min.	60	0.88	0.75	0.87
	90	0.88	0.80	0.91

which deteriorated when stored at 85° F. was "oxidized" and, to a lesser degree, "stale" (table 3). The two flavors sometimes are difficult to differentiate. The sequence of flavor deterioration is first a loss of the fresh milk flavor, resulting in a flat taste. Upon further aging, the samples may assume a typical

TABLE 3

Average[a] flavor scores of whole milk powder samples with different preheat temperatures and storage conditions

Storage Conditions	Age	Score and chief flavor criticism of material preheated at:				
		None	140°F.	150°F.	160°F.	170°F.
	(*mo.*)					
	0	1.3	7.7	7.9	8.2	7.9
	1		*7.2*	*7.6*	7.5	7.9
45°F.	2		*6.5*[b]	*6.9*	7.5	8.0
Air pack	3		6.2	6.2	*7.4*	8.0
	6		6.1	5.9	*7.3*	8.0
	12				5.7	8.2[c]
		Rancid	Oxidized	Oxidized	Stale & oxidized	Heated
	0	1.3	*7.7*	*7.9*	*8.2*	7.9
	1		*6.4*	*6.6*	*7.2*	7.4
85°F.	2		5.8	6.1	6.2	7.7
Air pack	3		5.7	5.9	6.0	7.1
	6		5.1	4.9	5.2	*7.4*
	12				3.1	*6.5*
		Rancid	Oxidized	Oxidized	Stale & oxidized	Heated to sl. oxidized
	0	1.3	*7.7*	7.9	8.2	7.9
	1		*6.7*	7.9	7.6	7.4
85°F.	2		6.7	7.7	7.7	7.8
Nitrogen pack	3		7.3	7.4	7.8	7.7
	6		5.6	7.5	7.9	7.6
	12				7.5	7.9
		Rancid	Sl. oxidized & Stale	Sl. chalky	Scorched sl. astringent	Heated & sl. chalky

[a] Average scores of panel for 5 replicate powders at 140 and 150° F. and 4 powders for 0, 160 and 170° F. for 20 min. preheat treatment.

[b] A flavor score loss of 1.0 in flavor score was found to be significant in all cases as determined by the method of Fisher (5) as used by Hening (8) for test of significance between means of samples.

[c] Flavor scores appeared to get better with age at 45° F. storage, due possibly to loss of slight heated flavor.

tallowy (oxidized) or a stale (astringent, chalky) taste which grows progressively worse up to a definite period of time and thereafter may slacken off. Not all samples show the same rate of flavor deterioration. Nitrogen-packed samples may go through only the first stage of flavor deterioration during 1 yr. in storage.

As measured by flavor scores, samples with preheat treatments of 140, 150 and 160° F. for 20 min. showed poor keeping quality. Slight variations exist between individual samples within a given preheat treatment, due possibly to slight individual differences in moisture contents of the powders and properties of the milks from which the powders were made. Preheating at 170° F. for 20 min. greatly prolongs the keeping quality of the powder. There was considerably more uniformity in the flavor scores and rates of deterioration of powders. Whole milk powder stored at 45° F. in air pack at the end of 1 yr. in storage was slightly superior in flavor to the same samples when fresh, as well as to the nitrogen-packed samples held at 85° F. The latter had shown little change in flavor score at the end of 1 yr. in storage. The superiority of flavor of the air-packed samples after 1 yr. of storage at 45° F. was thought to be due to the loss of the heated flavor, which did not take place to the same degree in the nitrogen-packed samples.

SUMMARY

Milk not preheated became rancid in the vacuum pan, and powder made from it was very rancid in taste. Preheating the milk to be dried at 140° F. or higher for 20 min. was sufficient to destroy lipase activity, as measured by flavor and free fatty acid development in powders stored at 45 and 85° F., including both air-packed and nitrogen-packed samples kept for periods of storage up to 8 mo. A slight progressive improvement was shown in the keeping quality of the resultant powders as the preheat temperature of the milk was increased from 140 to 160° F., and a large improvement was noted with an increase from 160 to 170° F.

Double nitrogen packing prolongs keeping quality of powders in relation to preheat treatment but is not a substitute for adequate preheat treatment (170° F. for 20 min.). Double nitrogen packing will prevent oxidized flavor in powders with adequate preheat treatment but not a slight flat (chalky) flavor at 85° F. storage. Storage of powders with adequate preheat treatment at 45° F. largely prevented stale as well as oxidized flavor defects in air or nitrogen pack for the duration of the experiment (1 yr.).

REFERENCES

(1) Coulter, S. T. The Keeping Quality of Dry Whole Milk Spray Dried in an Atmosphere of an Inert Gas. J. of Dairy Sci., 30: 995–1002. 1947.

(2) Coulter, S. T., Jenness, R., and Crowe, L. K. Some Changes in Dry Whole Milk during Storage. J. of Dairy Sci., 31: 986–1003. 1948.

(3) Decker, C. W., Ashworth, U. S., and Christensen, L. J. The Keeping Quality of Whole Milk Powder. II. The Effect of the Preheat Temperatures on the Development of Peroxides and Ferricyanide Reducing Groups under Different Storage Conditions. J. of Dairy Sci., 34: 412–418. 1951.

(4) FINDLAY, J. D., HIGGINBOTTAM, C., SMITH, J. A. B., AND LEA, C. H. The Effect of the Preheating Temperature on the Bacterial Count and Storage Life of Whole Milk Powder Spray-dried by the Krause Process. J. Dairy Research, **14**: 378–399. 1946.

(5) FISHER, R. A. *Statistical Methods for Research Workers.* Edinburgh and London; Oliver and Boyd, Ltd., ed. 9. 1944.

(6) GREENBANK, G. R., AND WRIGHT, P. A. The Heat Treatment Necessary to Prevent Lipolytic Action in its Dried Product (A Preliminary Report). J. Dairy Sci., **31**: 700. 1948.

(7) GREENBANK, G. R., WRIGHT, T., AND HOLM, G. E. Abs. of Paper Presented by G. E. Holm. "Tech. Conf. of the Dry Milk and Ice Cream Ind." 2–5, Q.M.C. Subsistence and Research Lab., Chicago. 1945.

(8) HENING, J. C. Flavor Evaluation Procedures. N. Y. Agr. Sta. Tech. Bull. 284. 1948.

(9) HETRICK, J. P., AND TRACY, P. H. Effect of High-temperature Short-time Heat Treatment on Some Properties of Milk. II. Inactivation of the Lipase Enzyme. J. Dairy Sci., **31**: 881–887. 1948.

(10) HOLLENDER, H. A., ROA, S. R., AND SOMMER, H. H. The Measurement of Free Fatty Acids in Dairy Products. J. of Dairy Sci., **31**: 719–720. 1950.

(11) HOLM, G. E., AND GREENBANK, G. R. Tallowiness in Butterfat. Proc. Soc. Exptl. Biol. Med., **20**: 176–177. 1922.

(12) HOLM, G. E., AND GREENBANK, G. R. The Keeping Quality of Butterfat, With Special Reference to Milk Powder. Proc. of the Worlds Dairy Congress. II. 1253–1265. 1923.

(13) KRUKOVSKY, V. N., AND SHARP, P. F. Effect of Shaking on the Lipolysis of Cows Milk. J. Dairy Sci., **21**: 671–682. 1938.

(14) LEA, C. H., MORAN, T., AND SMITH, J. A. The Gas Packing and Storage of Milk Powder. J. of Dairy Research, **13**: 162–215. 1943.

(15) NAIR, J. H. Lipase in Raw, Heated, and Desiccated Milk. Ind. Eng. Chem., **22**: 42–45. 1930.

(16) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Cheese Lipase. J. of Dairy Sci., **31**: 31–38. 1948.

(17) REMALEY, R. J. Fat Problems in Dairy Products. (Manual) QMC 17–7. 19–21. 1945.

(18) SCHAFFER, P. S. Abs. of papers presented by G. E. Holm. "Tech. Conf. of the Dry Whole Milk and Ice Cream Ind." 3, QMC Subsistence and Research Lab., Chicago, 1945.

(19) TARASSUK, N. P. Rancid Flavored Milk: Its Cause and Control. Bull. Inter. Assoc. Milk Dealers, **32**: 153–160. 1939.

(20) TARASSUK, N. P., AND HENDERSON, J. L. Prevention of Hydrolytic Rancidity in Milk. J. Dairy Sci., **25**: 801–806. 1942.

(21) TARASSUK, N. P., AND JACK, E. L. Biochemical Changes in Whole Milk Powder and Ice Cream Powder during Storage. J. Dairy Sci., **29**: 486–488. 1946.

(22) THURSTON, L. M., BROWN, W. C., AND DUSTMAN, R. B. Oxidized Flavor in Milk. I. The Probable Relation of Lecithin to Oxidized Flavor. J. Dairy Sci., **18**: 301–306. 1935.

(23) THURSTON, L. M., BROWN, W. C., AND DUSTMAN, R. B. Oxidized Flavor in Milk. II. The Effect of Homogenization, Agitation and Freezing on its Susceptibility to Oxidized Flavor Development. J. of Dairy Sci., **19**: 671–682. 1936.

(24) WHITNEY, R. M., AND TRACY, P. H. Stale Flavor Components in Dried Whole Milk. I. The Distribution of Stale Flavor between Fractions of Reconstituted Stale Whole Milk Powder. J. Dairy Sci., **32**: 383–390. 1949.

(25) WHITNEY, R. M., AND TRACY, P. H. Stale Flavor Components in Dried Whole Milk. II. The Extraction of Stale Butter Oil from Stale Dried Whole Milk by Organic Solvents. J. of Dairy Sci., **1**: 50–59. 1950.

THE KEEPING QUALITY OF WHOLE MILK POWDER. II. THE EFFECT OF PREHEAT TEMPERATURES ON THE DEVELOPMENT OF PEROXIDES AND FERRICYANIDE-REDUCING GROUPS UNDER DIFFERENT STORAGE CONDITIONS[1,2]

C. W. DECKER, U. S. ASHWORTH AND L. J. CHRISTENSEN
Washington State College, Pullman

Part I (3) of this study gave the flavor and residual lipase activity of milk powders made with different preheat treatments and stored air-packed at 45 and 85° F. and nitrogen-packed at 85° F. This paper reports the development of peroxide and potassium ferricyanide-reducing groups as measures of the keeping quality of these same powders.

REVIEW OF LITERATURE

Peroxide formation. There is general agreement that the first product formed in the oxidation of an unsaturated fat is a peroxide. However, the breakdown products formed when the peroxide is split at the double bond, and not the peroxides themselves, are responsible for the unpleasant oxidative rancidity (tallowy) flavors and odors. This theory holds true for butterfat according to Holm (10). He found tallowiness to occur in butterfat when the peroxide values reach 1.20 (m.e. of oxygen per kg. of fat) and are broken down by heating; however, values of 0.80 before destruction leave the butterfat still edible. Powick (15) believes the off-flavors and odors of oxidized fats are due primarily to medium molecular weight aldehydes (7 and 9 carbon) and the lower molecular weight aldehydes, acids, ketones, etc., do not play an important part. The amount of fat decomposed in the oxidation process may be not more than 0.1 per cent, according to Pritzer and Jungkunz (16).

Peroxide formation in whole milk powders. The use of the peroxide test to predict the onset or degree of oxidized flavor in whole milk powders is subject to differences of opinion by different authors.

Hollender and Tracy (9), using the peroxide test of Smith (19), conclude that taste is superior to the peroxide test as a means of detecting oxidized flavor at an early stage. Pyenson *et al.* (18), using the peroxide test of Chapman and McFarlane (2), analyzed 180 samples of spray-dried whole milk powder and reported that peroxide values are not a satisfactory criterion of keeping quality or palatability. Findlay *et al.* (6) using the test of Chapman and McFarlane (2), found that peroxide development was correlated with flavor scores when high preheat temperatures were used (190–200° F. for 20 sec.) and the lowest peroxide values were obtained at these high preheat temperatures. Greenbank

Received for publication Nov. 13, 1950.

1 Scientific paper no. 975, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

2 American Dairy Association Research Grant in co-operation with the Washington State Dairy Products Commission.

et al. (7) using a peroxide test of their own devising, found good correlation between flavor and peroxide values in air-packed samples up to 45° C. storage temperatures, but no correlation with nitrogen-packed samples.

A number of factors influence the development of peroxides in whole milk powder. Pyenson and Tracy (17) reported no significant difference in peroxide development between storage temperatures or between air and nitrogen pack. Lea *et al.* (13), however, found that increases in peroxide values were low in gas-packed samples.

Greenbank *et al.* (7) found the rate of peroxide formation with time increased logarithmically for powders at storage temperatures up to 37° C., but at temperatures up to 55° C. a progressive rate of peroxide deterioration took place. Tallowiness occurs at lower peroxide values when storage periods are long. The peroxides also decompose in some samples sooner than others.

The rate of formation of peroxides was found by Lea *et al.* (13) to increase rapidly until the oxygen was exhausted and then to fall off more or less rapidly. Pyenson and Tracy (17) reported that peroxide values remained low for the first 3 to 4 mo. of storage, increased sharply up to 6 to 9 mo., then started to decline and at 13 mo. decreased nearly to the values found at 3 mo. of storage.

Potassium ferricyanide-reducing groups in whole milk powder. Studies on acid ferricyanide-reducing groups formed in whole milk powder have been carried on by several investigators. Chapman and McFarlane (2) stated that heating increased the reducing power of protein groups in milk powder. Lea (12) suggests that the ferricyanide-reducing power of milk powder is an index of the formation and degradation of a protein-sugar complex, rather than a simple denaturation of the protein which makes sulfhydryls available as reducing groups. Glucose is much more reactive than lactose in undergoing such a reaction with protein, and sucrose is inert.

Coulter *et al.* (4) found that the moisture content (vapor pressure) of the powders was the primary factor in influencing the production of acid ferricyanide-reducing substances during storage, with the higher moisture content (1.32 to 4.78 per cent range) asociated with the greater reducing values. Oxygen appeared to be without effect on the production of substances reducing acid ferricyanide. Harland *et al.* (8) made a study of the factors influencing the production of acid ferricyanide-reducing substances and thiamin disulphide reducing substances during processing and drying of whole milk powder and found: (a) As the total solids content of the concentrate was increased from 40 to 90 per cent, the amount of acid ferricyanide-reducing substances formed during heating for 1 hr. at 85° C. increased rapidly during heating, reaching a maximum at 90 per cent solids, but above 90 per cent total solids the amount formed decreased rapidly as the system approached the moisture content of normal dry whole milk powder; (b) the amount of acid ferricyanide-reducing substances was not influenced significantly by preheating temperatures but was increased by increasing drying temperatures from 83 (normal) to 104° C.; (c) the presence of oxygen in the system decreased the amount of reducing substances produced and ascorbic acid had no effect; (d) thiamin disulphide reducing substances

(sulfhydryl groups) remained unchanged in storage of whole milk powders stored in oxygen for 4 wk. at 37° C.

EXPERIMENTAL PROCEDURE

The processing, handling and storage of the whole milk powder samples is described in the preceding paper (3). Preheat temperatures of 140, 150, 160 and 170° F. for 20 min. were used, along with controls receiving no preheat treatment; the powders were canned and stored at 45° F. in air packs, and at 85° F. in air and nitrogen packs.

The peroxide values were determined according to the method of Loftus Hills *et al.* (14). Ferricyanide-reducing values were determined by the method of Chapman and McFarlane (2), as modified by Crowe *et al.* (5). The values shown in table 2 and fig. 1 are representative of those obtained, and in fig. 1 where samples were not removed at uniform time intervals, the values were plotted against time.

RESULTS

When milk is preheated, a large increase is noted in the peroxide values of the resultant powders in storage as compared to powders made from milk with no preheat treatment (fig. 1). Once milk is preheated previous to drying, the critical preheat temperature with respect to peroxide development lies between 160 and 170° F. with a 20-min. holding period (fig. 1 and table 1). Preheat temperatures of 140 and 150° F. (not shown) give much the same picture as 160° F. preheat.

Storage of powder samples at 85° F. (fig. 1), as compared to 45° F., greatly accelerates the peroxide formation after 6 mo. in powders made from milk with an inadequate preheat treatment of 160° F. or lower. However, an adequate preheat temperature of 170° F. prevents this acceleration and gives lower peroxide values which are more nearly linear in formation and lower at 85 than at 45° F. storage. This effect is thought to be due to greater peroxide breakdown at the higher storage temperature. Irrespective of preheat treatment, nitrogen-packed samples at 85° F. storage gave very low peroxide values.

Aged powders (2 to 3 yr.) in table 1 bring out still more clearly the difference in peroxide development between powders preheated at 160 and 170° F. for 30 min., when held at a 45° F. storage temperature. The peroxide values range from 0.3 to 0.5 for the powders made from milk preheated to 170° F. and 0.83 to 1.50 for powder samples made from milk preheated at 160° F. In all cases peroxide values increased between 29 and 35 mo. of storage at 45° F., which would seem to indicate that the breakdown of peroxides is very slow at this temperature and that possibly free oxygen may still be present in the cans. Flavor scores were run in conjunction with peroxide studies and showed all samples preheated at 160° F. to be below 5.0 in score and to exhibit marked oxidized and stale flavors. However, powder samples preheated at 170° F. were above 7.0, indicating only a very slight stale flavor to be present.

This difference in ability of powders to resist peroxide formation when made from milk with different preheating temperatures does not appear to be due to

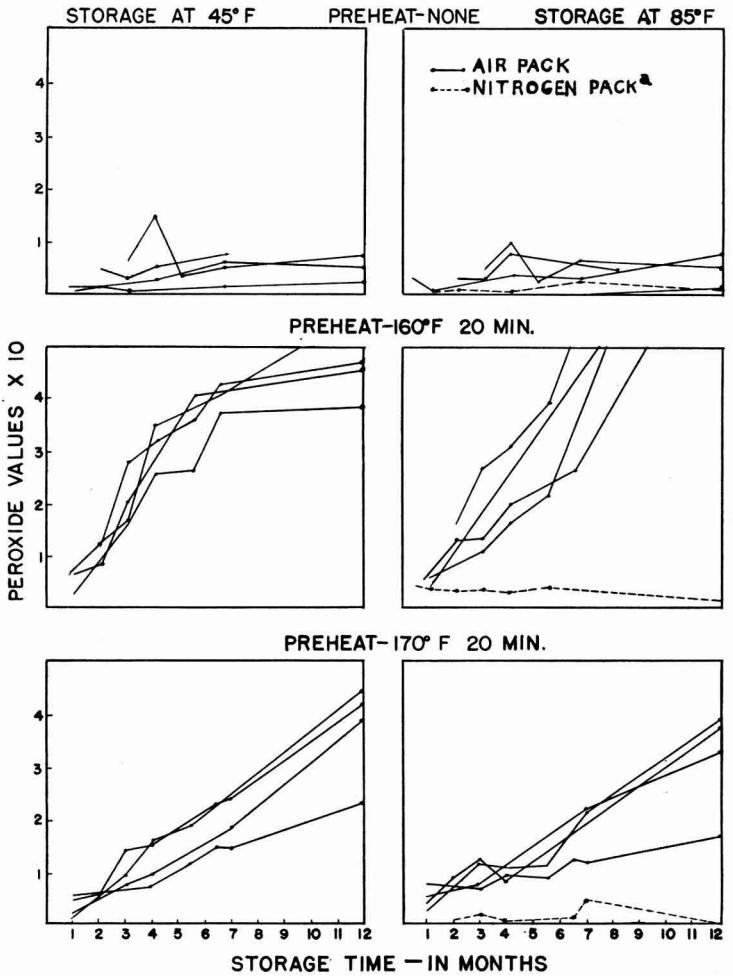

FIG. 1. Peroxide values (milliequivalents of oxygen per kilogram of powder) of 4 replicate powders at 3 preheat temperatures and stored air-packed at 45, 85° F. and nitrogen-packed at 85° F.

[a] Nitrogen-packed samples given represent an average of the 4 replicate samples in each case.

differences in the amount of acid ferricyanide-reducing substances formed (table 2), which apparently are not greatly influenced by preheat temperatures and may be as high or higher in powder with no preheat treatment. Storage at 85° F. gave higher acid ferricyanide-reducing values than storage at 45° F., and nitrogen-packed samples at 85° F. gave the highest acid ferricyanide-reducing values. The latter result probably is due to greater ascorbic acid retention in nitrogen-packed samples during storage (table 3). Ascorbic acid was found by Harland *et al.* (7) to be the greatest non-protein ferricyanide-reducing fraction of milk.

TABLE 1

Peroxide values and flavor scores of aged air-packed samples of whole milk powder with different preheat temperatures and stored at 45° F.

Sample no.	Preheat temp. for 30 min.	Flavor scores at:			Peroxide values at:	
		19 mo.	26 mo.	35 mo.	29 mo.	35 mo.
	(°F.)					
68	160	4.0	4.0	.....	1.13	1.50
70	160	5.0	4.3	.....	0.83	1.24
72	160	3.8	3.4	3.5	1.13	1.48
76	160	4.6	4.8	4.1	1.16	1.37
74	170	7.2	7.4	6.7	0.44	0.61
78	170	8.2	8.4	.....	0.36	0.44
80	170	7.7	8.0	7.6	0.36	0.47
82	170	7.8	8.1	7.5	0.34	0.39
84	170	7.3	7.3	7.5	0.41	0.43

DISCUSSION

Any method to successfully prevent oxidized flavor development at storage temperatures as high as 85° F. in air-packed samples must either (a) limit peroxide development below critical values, or (b) prevent the breakdown of peroxides if they should exceed critical levels. So far, the first objective has been accomplished by inert gas packing, but the second objective only by lowering the storage temperature. In no case in the present study was a maximum peroxide value reached in the powders during storage and then followed by a decline in values, as reported by Pyenson and Tracy (17).

The explanation of the prevention of oxidized flavor in whole milk powder by preheating the milk at 170° F. as compared to 160° F. for a period of 20 to 30 min., may lie in the destruction of an oxidizing enzyme or enzymes at the higher temperature. Krukovsky (11) believed that peroxidase in milk might be responsible for the quick conversion of ascorbic acid to dehydroascorbic acid by added hydrogen peroxide. Heat studies to 76.6° C. (170° F.) for 30 min. showed that

TABLE 2

Average ferricyanide values[a] of whole milk powders with different preheat and storage temperatures

Preheat temp. for 20 min.	Initial	Values at storage periods and temperatures of:					
		5–6 mo.			7–8 mo.		
		45° F.	85° F.	85° F. + N_2	45° F.	85° F.	85° F. + N_2
(°F.)							
None	7.85	7.53	8.28	8.34	7.73	8.27	9.02
140	6.80	7.70	8.46	8.60	7.42	8.00	8.35
150	6.86	7.00	7.34	7.95	7.08	7.88	8.40
160	6.73	7.33	7.60	7.87	7.13	7.80	8.00
170	7.50	7.40	7.95	8.40	7.50	8.30	8.77

[a] Moles $\times 10/-6$ of potassium ferricyanide reduced /g. of powder. Average of 5 replicate powders for 140 and 150° F. preheat, and 4 powders for 0, 160 and 170° F. preheating temperatures.

hydrogen peroxide would not oxidize ascorbic acid, while the addition of plant peroxidase would again induce the reaction. Further work on the effect of milk peroxidase on the keeping quality of whole milk powders is being carried on at this station.

The development of ferricyanide-reducing group values does not appear to be influenced greatly by preheat treatments. This is in agreement with the work of Harland *et al.* (8), who found that the acid ferricyanide-reducing groups are not influenced significantly by preheat treatments but are increased by higher drying temperatures. They also found that thiamin disulfide groups remained unchanged during storage of air-packed powders held at 37° C. for 4 wk. They believe thiamin disulphide and nitroprusside tests measure the same or parallel reducing systems and, therefore, sulfhydryl reducing groups probably would remain unchanged in storage. The role that heat-produced reducing groups, as measured by the above tests, may play in preventing the oxidized flavor bears further investigation.

TABLE 3

Average[a] ascorbic acid values of whole milk powder with different preheat and storage temperatures after eight months storage

Preheat temp. for 20 min.	Ascorbic acid content (γ/g. powder[b]) at storage temperatures of:		
	45° F.	85° F.	85° F. + N_2
(°F.)			
0	0.0806	0.0580	0.0776
140	0.0909	0.0636	0.0916
150	0.0795	0.0568	0.0922
160	0.0768	0.0559	0.0907
170	0.0859	0.0477	0.0711

[a] Average of 5 replicate powders at 140 and 150° F. preheat, and 4 powders at 0, 160, and 170° F. preheat.

[b] May be converted to milligrams of ascorbic acid per liter of reconstituted milk by multiplying by 125.

SUMMARY

With respect to peroxide development, the critical preheat temperature for milk to be dried lies between 160 and 170° F. for a 20-min. holding period. Storage at 85° F. tends to accelerate peroxide development in powder samples having an inadequate preheat treatment of 160° F. However, powder samples made from milk preheated at 170° F. gave slightly lower values at 85° F. than at 45° F. storage, due to greater breakdown of the peroxides at the higher temperature. Nitrogen-packed samples gave very low peroxide values, and samples made from milk with no preheat treatment gave quite low peroxide values which did not increase greatly during storage and were considerably lower than samples made from preheated milk, but were quite rancid in flavor, however.

The acid ferricyanide-reducing groups values were higher at 85° F. storage than at 45° F., and higher in nitrogen- than air-pack at 85° F. The latter result was thought to be due to greater ascorbic acid retention in the nitrogen-packed samples.

REFERENCES

(1) CHAPMAN, R. A., AND MCFARLANE, W. D. A Colorimetric Method for the Determination of Fat Peroxides and its Application in the Keeping Quality of Milk Powders. Can. J. Research, B, **21**: 133–139. 1943.

(2) CHAPMAN, R. A., AND MCFARLANE, W. D. A Colorimetric Method for the Estimation of Reducing Groups in Milk Powders. Can. J. Research, **23**: 91–99. 1945.

(3) CHRISTENSEN, L. J., DECKER, C. W., AND ASHWORTH, U. S.. The Keeping Quality of Whole Milk Powders. I. The Effect of Preheat Temperature on the Milk on the Development of Rancid, Oxidized, and Stale Flavors with Different Storage Conditions. J. Dairy Sci., **34**: 404–411. 1951.

(4) COULTER, S. T., JENNESS, R., AND CROWE, L. K. Some Changes in Dry Whole Milk During Storage. J. Dairy Sci., **31**: 986–1003. 1948.

(5) CROWE, L. K., JENNESS, R., AND COULTER, S. T. The Reducing Capacity of Milk and Milk Products as Measured by a Modified Ferricyanide Method. J. Dairy Sci., **31**: 595–609. 1948.

(6) FINDLAY, J. D., HIGGINBOTTOM, C., SMITH, J. A. B., AND LEA, C. H. The Effect of the Preheating Temperature on the Bacterial Count and Storage Life of Whole Milk Powder Spray-dried by the Krause Process. J. Dairy Research, **14**: 378–399. 1946.

(7) GREENBANK, G. R., WRIGHT, P. A., DEYSCHER, E. F., AND HOLM, G. E. The Keeping Quality of Samples of Commercially Dried Milk Packed in Air and in Inert Gas. J. Dairy Sci., **29**: 55–61. 1946.

(8) HARLAND, H. A., COULTER, S. T., AND JENNESS, R. Some Factors Influencing the Reducing Systems in Dry Whole Milk. J. Dairy Sci., **32**: 334–344. 1949.

(9) HOLLENDER, H. A., AND TRACY, P. H. The Relation of the Use of Certain Antioxidants and Methods of Processing to the Keeping Quality of Powdered Whole Milk. J. Dairy Sci., **25**: 249–274. 1942.

(10) HOLM, G. E. Fat Deterioration in Milk Products. Quartermaster Corps Manual 17–7. Deterioration of Fats and Oils: 151–153. 1945.

(11) KRUKOVSKY, V. N. Ascorbic Acid Oxidation in Milk by Preformed Hydrogen Peroxide. J. Dairy Sci., **32**: 163–165. 1949.

(12) LEA, C. H. The Reaction between Milk Protein and Reducing Sugar in the "Dry State." J. Dairy Research, **15**: 369–376. 1948.

(13) LEA, C. H., MORAN, T. AND SMITH, J. A. B. The Gas Packing and Storage of Milk Powder. J. Dairy Research, **2**: 162–215. 1943.

(14) LOFTUS HILLS, G. AND THIEL, C. C. The Ferric Thiocyanate Method of Estimating Peroxide in the Fat of Butter, Milk and Dried Milk. J. Dairy Research, **14**: 340–353. 1946.

(15) POWICK, W. C. Compounds Developed in Rancid Fats with Observations on the Mechanism of their Formation. J. Agr. Research, **26**: 323–362. 1923.

(16) PRITZER, J., AND JUNGKUNZ, R. Quantitative Aspects of the Kreis Rancidity Reaction. A. Untersuch. Lebensm., **57**: 419–429. 1929.

(17) PYENSON, H., AND TRACY, P. H. A Spectrophotometric Study of the Changes in Peroxide Value of Spray-dried Whole Milk Powder during Storage. J. Dairy Sci., **29**: 1–12. 1946.

(18) PYENSON, H., TRACY, P. H., AND TRIMBLE, J. M. A Study of the Peroxide Value of Stored Spray-dried Whole Milk Powder. J. Dairy Sci., **27**: 675. 1944.

(19) SMITH, J. A. B. The Rapid Determination of Peroxide Values for the Fat in Milk Powders. J. Dairy Research, **10**: 294–299. 1939.

RESIDUAL MILK FROM OXYTOCIN INJECTIONS THROUGHOUT THE LACTATION

ERIC W. SWANSON AND S. A. HINTON
University of Tennessee, Knoxville

An understanding of the physiology of the milking process in dairy cows has been considerably advanced in recent years. Ely and Petersen (4, 5), demonstrated the effectiveness of posterior pituitary extracts in producing milk let-down and also the antagonism of adrenalin, both injected and secreted naturally. The myoepithelium of the mammary gland has been photographed relaxed and contracted by Richardson (12), demonstrating the source of the milk let-down pressure. Although the amount of oxytocin secreted by cows in response to the milking stimulus has not been measured, there is indirect evidence to indicate that it may be variable. Knodt and Petersen (10) reported that cows which varied erratically in milk yield from milking to milking produced uniformly and averaged higher in yield following large oxytocin injections than before. Smith (14) found that cows did not give a normal let-down of milk when milked at 2-hr. intervals, but fairly uniform let-down and milk yield were obtained when oxytocin was injected intravenously. The amount of residual milk which can be obtained has been related to variations in milk let-down by Knodt and Petersen (9) and Miller and Petersen (11). It also is probable that other factors affect the amount of residual milk. The positive correlation between normal yield and amount of residual milk has been shown in the data of Dodd and Foot (2) and Johansson (8). The reported average amounts of residual milk secured from cows following a normal milking with proper let-down have varied from about 1 lb. (13) to nearly 6 lb. (2). This investigation was undertaken to determine the effect of stage of lactation and other related factors upon the residual milk obtainable by oxytocin injection.

EXPERIMENTAL

Cows for the experiment were selected from the dairy herd of the University of Tennessee as they freshened over a 2-mo. period. All cows had had at least one previous lactation and were selected upon the basis of past uniformity of milk let-down in order to exclude definitely abnormal individuals. Five Holstein-Friesians and five Jerseys were used in the monthly studies. Other animals in the herd also were used at intervals to compare with the ten regular experimental animals. The first sampling was made in the latter part of the first month of lactation and repeat samples were secured at 4- to 5-wk. intervals through the tenth month of lactation. Milking was conducted routinely in a milking parlor, using machines. Stripping was done largely by machine, but all cows were checked by hand-stripping before leaving the milking parlor. Once each month immediately following a normal evening milking the experimental animals were each given an intrajugular injection of 1 ml. of Pitocin

Received for publication Nov. 20, 1950.

containing 10 I. U. of oxytocic principles. Milking then was resumed by hand for approximately 5 min. to secure the residual milk. In order to check the completeness of let-down, a few cows were given a second injection of 10 I. U. of oxytocin following removal of the residual milk. In no case was any more milk secured.

All milking weights were recorded. Samples were taken of the normal evening milking, the residual milking and of the morning milking following the residual milking. The milk was analyzed for fat by the Babcock method and for total solids by use of the Mojonnier apparatus.

RESULTS

The average milk yields at each monthly sampling period are presented in figure 1. There was a gradual decline in the yield of residual milk from the

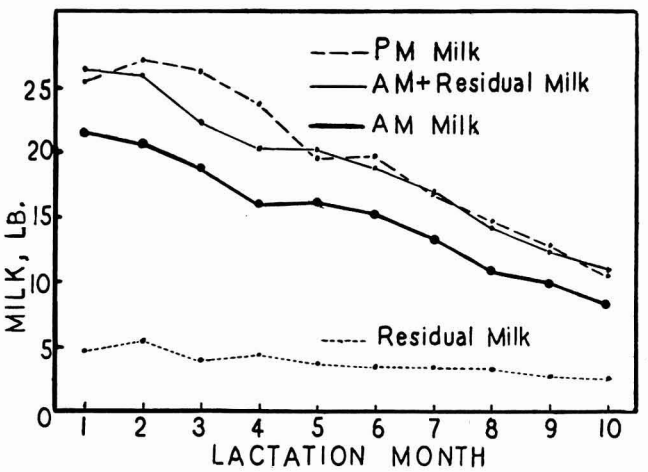

FIG. 1. Comparison of residual milk, normal p.m. milking and the post-injection a.m. milking throughout the lactation (average of 10 cows).

second month to the end of the lactation. The highest monthly average was 5.67 lb. and the lowest was 2.51 lb. The largest individual residual milking was 12.2 lb., the least, 0.9 lb. The average of all residual milkings was 3.85 lb. The amount of residual milk was directly related to the amount of the preceding normal milking. The correlation coefficient between normal p.m. milk and residual milk yields was 0.43. The curve for a.m. milk in figure 1 clearly shows that the residual milking reduces the amount of milk which will be secured at the next normal milking. Even adding the residual and the a.m. milking together barely equals the p.m. milking. Since the a.m. milking was normally the largest in this herd (milked at 12-hr. intervals) this indicates that the oxytocin injection resulted in a slight loss of milk production. This fact is demonstrated in another way by the data in table 1. These data show that the 24-hr. production, including the residual milk plus the a.m. milking, is below either that of the day before or the day after. The difference is statis-

TABLE 1

Comparison of average daily milk production the day of oxytocin injection with the preceding and succeeding days by lactation months

Month of Lactation	Pre-injection day	Day of oxytocin injections		Post-injection day
	A.m. and p.m. milking before oxytocin injection	P.m. milking before and a.m. milking plus residual milk after oxytocin injection	A.m. milking plus residual milk after oxytocin injection and p.m. milking following	P.m. milking 24 hr. after oxytocin injection and a.m. milking at 36 hr.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
1	53.9	52.4	53.8	55.0
2	53.9	53.8	53.0	54.7
3	51.4	49.2	48.0	52.5
4	45.6	44.4	43.5	45.2
5	39.8	39.6	39.9	40.1
6	38.9	38.7	37.8	37.8
7	34.9	34.0	34.4	35.5
8	29.9	28.9	29.2	30.5
9	25.8	25.3	24.8	26.4
10	21.2	21.3	21.5	21.7
Mean daily prod.	39.53	38.76	38.59	39.94

tically significant, the least significant mean difference being 0.64 lb.

One interesting observation was the similarity of residual milk yield between the breeds. The rate of decline of both normal and residual milk yield was greater with the Holstein-Friesians than with the Jerseys (fig. 2). From the fourth to the eighth month, the Jerseys actually averaged more residual milk than the Holstein-Friesians, although they were giving only about two-thirds as much milk at the normal p.m. milking. The percentage of fat in the monthly

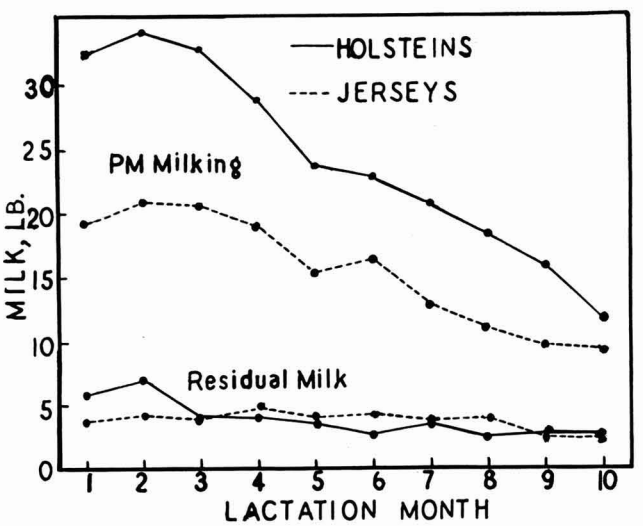

FIG. 2. Breed differences in the daily average amount of normal and residual milk secured throughout the lactation (average of 5 Jerseys and 5 Holsteins).

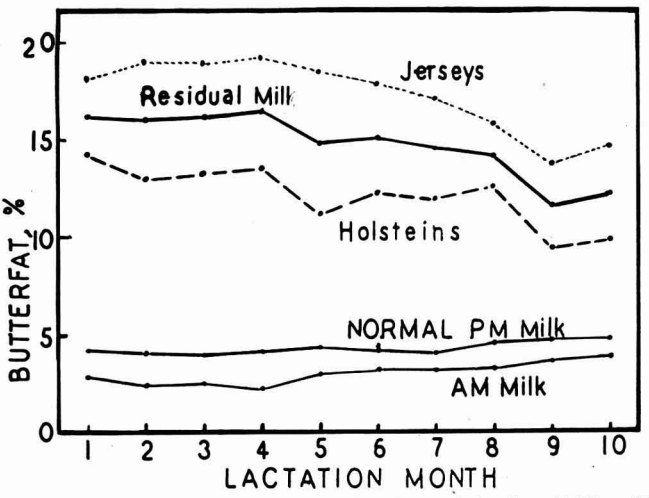

FIG. 3. Average fat tests of normal, residual and post-injection (AM) milk, and breed differences in the residual milk fat tests throughout the lactation (average of 10 cows, 5 Jerseys and 5 Holsteins).

milk samples is shown in figure 3. The fat test of the normal p.m. and the a.m. milking increased generally throughout the lactation, as should be expected. The fat test of the residual milk, on the other hand, was highest at the peak of lactation and decreased as lactation advanced. This was true of both breeds. The residual milk of the Jerseys had the highest test, corresponding to their superiority in fat test of normal milk. The relationship between quantity and test in residual milk was the reverse of that found with normal milk, *i.e.*, large yields of residual milk were associated with higher fat tests than were low yields. The

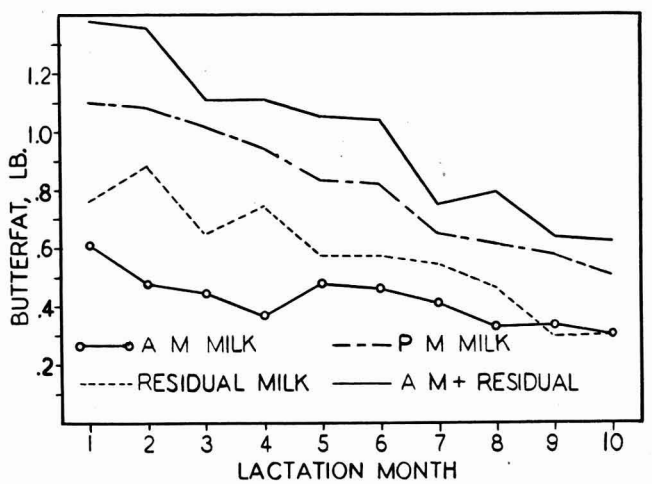

FIG. 4. Comparisons of amount of butterfat in normal, residual, and post-injection milkings throughout the lactation (average of 10 cows).

average of all residual milk samples was 14.75 per cent fat and the range was 23.5 to 6.8 per cent fat.

A combination of the milk-yield data and the fat tests produced the information on milk-fat secretion shown in figure 4. The amount of fat secured in the residual milk exceeded that secured at the next a.m. milking every month until the ninth and tenth when they were nearly equal. Adding the fat from the morning and the residual milkings produced significantly more than was secured at the normal p.m. milking in every month. This is in contrast to the milk yield relationships presented in figure 1 and table 1.

The monthly average changes in the non-fat solids of the various milk samples are plotted in figure 5. There was very little difference between the SNF of the p.m. and a.m. milkings. In all months the residual milk was lowest

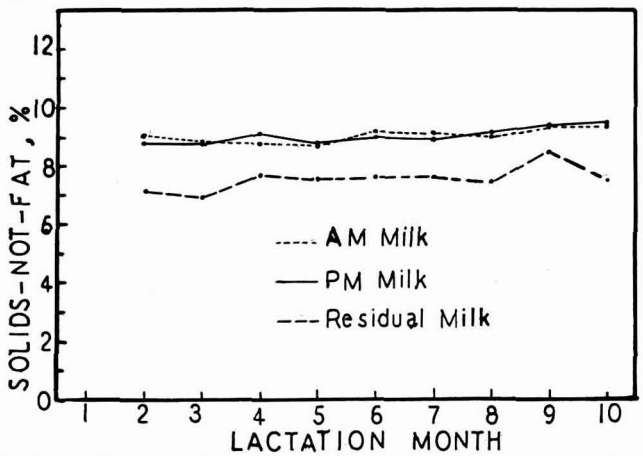

FIG. 5. Solids-not-fat percentage of normal milk, residual milk and the post-injection milking throughout the lactation (average of 10 cows).

in SNF percentage. There was a slight upward trend of all SNF values as lactation progressed.

Since the amount of residual milk from the ten regularly sampled cows had decreased so markedly during the last few months of lactation, the possibility of abnormal reaction due to anxiety and fright at the time of injection was raised. Some of the cows became agitated at the time of injection in the later months, although they had been relatively calm in the early months. To test the probable influence of this excitement, three cows in very advanced lactation which had received no previous intrajugular injections were submitted to the same routine as the 10 experimental cows. These cows were injected without undue excitement, following which each one gave 1.3 lb. residual milk, compared to 2.5 lb. average for the ten cows in the monthly sampling. Therefore, it was concluded that the drop in residual milk at that time was definite.

DISCUSSION

The amount of milk secured by stimulation with oxytocin after a normal milking will depend primarily on two factors: (a) the completeness of the nor-

mal milking and (b) the amount of active secretory tissue in the udder. The reason for the first factor is obvious. The explanation of the second factor is less simple. Part of the milk in the udder at milking time is easily secured because it can be squeezed or drained out of the teat and gland cisterns. The remainder is held in the gland as water in a sponge and is released only following an internal constriction of the glands (the letting-down phenomenon) due to contraction of the myoepithelium of the glands. With a carefully conducted milking, all of the milk made available by this process is removed from the udder. The remainder, or residual milk, is that which adheres to the surfaces of the ducts, ductules and alveoli. The amount therefore should be roughly proportional to the amount of these surfaces or the active secretory tissue of the gland. This would be especially true if the degree of myoepithelial contraction to the end of milking was the same at each milking. However, some variation must be expected in this natural phenomenon, and this is one of the most plausible explanations of the normal short-time day to day variation in milk yields and fat tests (1, 8). By using ten-cow averages the importance of these variations in completeness of milking was reduced, and the monthly average variations can be considered largely due to changes in the active secretory tissue in the glands. The observation of this study that the residual milk yield is significantly correlated with the normal yields is similar to reports by Dodd and Foot (2) and Johansson (8).

An alternative explanation of the decrease in residual milk with advancing lactation would be the assumption that the let-down stimulus is greater near the end of lactation than in early lacation and, thus, normal milking more complete. There is very little evidence to support this assumption. In fact, it frequently is noted that some cows become lax and erratic in milk let-down in late lactation and through this means hasten their drying up. Furthermore, studies of the rate of milk removal by Dodd and Foot (3) and Harshbarger (7) indicate that the rate of milking normally is reduced as the amount of milk per milking decreased.

Assuming that the amount of residual milk secured in these experiments reflects the amount of active secretory tissue in the udder, it can be assumed that there is a progressive decrease in such tissue from the peak of lactation to the end, with the most rapid changes in the last 2 mo. Many cows are in late pregnancy at this time and would be expected to have highly developed mammary glands (6). This interpretation of these experiments indicates that only a small part of the mammary gland is active at late lactation, even though it may be fully developed for the next lactation. Histological studies reported by Turner (15, 16) are in agreement with this interpretation.

The average 10-mo. decline of normal milk was greater than that of residual milk, being 61.7 per cent compared to 55.7 per cent. Although this difference is not marked, it may indicate that not all of the decline in milk production in late lactation is due to loss of active tissue, but some may be due to other factors, such as a lower metabolic rate, which result in less intensive secretion by the active tissue remaining.

The slight difference between breeds in amount of residual milk might indicate that the amount of secretory tissue in the udders of the Jerseys from mid-lactation on was as great as in the Holstein-Friesians. External examination of these cows indicated that with the exception of one small-uddered Jersey this quite possibly was true. Palpation indicated, however, that the teat and gland cisterns and large storage ducts in the Holstein-Friesian udders were decidedly larger.

Since the last milk to leave the udder is abnormally high in fat, it is indicated that the fat is more firmly held to the gland surfaces than the other milk constituents. Therefore, the larger the quantity of fat secreted per day in an udder, the higher will be the concentration of fat in any given amount of last-drawn milk. High milk and fat secretion and extensive mammary gland development in early lactation combine to produce high fat test along with high residual milk yield. The slight changes observed in non-fat solids of milk indicate that these constituents are excreted from the gland independently of, and more uniformly than the fat.

SUMMARY

The changes in residual milk during the first 10 mo. of lactation have been studied in ten cows of the Jersey and Holstein-Friesian breeds. The amount of residual milk obtained by injecting 10 I. U. of oxytocin intravenously varied roughly as the normal milk yield within breeds. The differences in residual milk yield between breeds were not significant. The fat test of the residual milk varied inversely as the fat test of the normal milk throughout the lactation within breeds, but Jerseys had higher fat tests than Holstein-Friesians. The results of these experiments are interpreted to indicate a progressive decrease in amount of active secretory tissue in the udder from the peak to the end of lactation, with a marked decrease during the last 2 mo.

REFERENCES

(1) ADAMS, H. P., AND ALLEN, N. N. The Value of Oxytocin for Reducing Fluctuation in Milk and Fat Yield during Experimental Periods. J. Dairy Sci., **31**: 662. 1948.

(2) DODD, F. H., AND FOOT, A. S. Residual Milk. Agr., **55**: 238–242. 1948.

(3) DODD, F. H., AND FOOT, A. S. Experiments on Milking Technique; 3. Combined Effect of Reducing the Milking Time and Washing the Udder with Hot Water; 4. Effect of Increasing the Milking Time. J. Dairy Research, **16**: 14–22. 1949.

(4) ELY, F., AND PETERSEN, W. E. The Ejection of Milk from the Mammary Gland. J. Dairy Sci., **23**: 536–537. 1940.

(5) ELY, F., AND PETERSEN, W. E. Factors Involved in the Ejection of Milk. J. Dairy Sci., **24**: 211–223. 1941.

(6) HAMMOND, J. *The Physiology of Reproduction in the Cow.* Cambridge Univ. Press. 1927.

(7) HARSHBARGER, K. E. Observations of the Rate of Milk Removal. J. Dairy Sci., **33**: 382–383. 1950.

(8) JOHANSSON, I. Variations in the Fat Content of Milk Caused by Varying Degrees of Udder Evacuation during Milking. XIIth Int. Dairy Congr., Stockholm pp. 171–181. 1949.

(9) KNODT, C. B., AND PETERSEN, W. E. The Effect of Continuous Injection of Pitocin upon Milk and Milk Fat Production. J. Dairy Sci., **25**: 709–710. 1942.

(10) KNODT, C. B., AND PETERSEN, W. E. The Effect of Complete Evacuation of the Mammary Gland by Pitocin upon Milk and Fat Production. J. Dairy Sci., **27**: 449–457. 1944.

(11) MILLER, K., AND PETERSEN, W. E. Some Factors Involved in Efficient Milking. J. Dairy Sci., **24**: 225–233. 1941.

(12) RICHARDSON, K. C. Contractile Tissues in the Mammary Gland with Special Reference to Myoepithelium in the Goat. Proc. Roy. Soc. B, **136**: 30–45. 1949.

(13) SHAW, J. C. The Effect of Oxytocin on Milk and Milk Fat Secretion. J. Dairy Sci., **25**: 1051–1055. 1942.

(14) SMITH, V. R. The Effect of Milking at Short Intervals with and without Injections of Oxytocin. J. Dairy Sci., **30**: 703–715. 1947.

(15) TURNER, C. W., AND REINEKE, E. P. A Study of the Involution of the Mammary Gland of the Goat. Mo. Agr. Expt. Sta. Research Bull. 235. 1936.

(16) TURNER, C. W. The Comparative Anatomy of the Mammary Glands. Univ. Coop. Store, Columbia, Mo. 1939.

DETERMINATION OF NON-CASEIN PROTEIN IN SKIMMILK AND NONFAT DRY MILK SOLIDS BY MONOLAYER FILMS

BURDET HEINEMANN AND O. B. PARKER
Producers Creamery Co., Springfield, Mo.

While performing experiments previously reported (2), it was noted that the alcohol-ammonia-water layer remaining in the Mojonnier flask after ether extraction of fat from skimmilk spread readily on a specially prepared oil surface, apparently in the form of a monolayer. The assumption was made and verified that the protein in the solution was responsible for this effect. The possibility of developing this observation into an analytical method was investigated and the results are presented below. Particular emphasis in experimental work was placed on the whey proteins of milk since the greatest usefulness of the method appeared to lie in this direction.

METHODS

The equipment and technique is the same as is employed for determining butterfat in skimmilk (2). A 0.2 per cent glacial acetic acid solution is poured into a prepared 8 in. pyrex pie plate. Specially prepared piston oil is slowly added to the surface of the acid solution until a second order green is obtained. (A third order green is used in the butterfat test, but the second order green is preferred for the protein test.) The surface is now ready for the deposition of the protein solution which is prepared as follows:

Weigh 20.0 g. of skimmilk or 2.00 g. nonfat dry milk solids into a 50 ml. volumetric flask. Add 10 ml. of 40° C. distilled water to the skimmilk and 30 ml. to the nonfat dry milk solids; a few drops of cream may be added to break the foam. After the foam has settled, add 1 ml. of 10 per cent acetic acid, a drop at a time, with gentle mixing. Let stand 10 min. at room temperature then add 1 ml. of *N*. sodium acetate solution and mix. Make to 50 ml. with distilled water, mix and allow to stand 5 to 10 min. Filter through No. 42 Whatman, discarding the first 5 ml. of filtrate. Transfer 5.00 ml. of the clear filtrate to a 50 ml. erlenmeyer flask. Add 1 ml. of concentrated NH_4OH and 4.00 ml. of ethyl alcohol and mix. Transfer 0.0072 ml. of the well mixed solution to the prepared surface. The technique of depositing the protein solution on the prepared oil surface was studied. The same solution was deposited eight times by blowing the 0.0072 ml. to the tip of the pipette to form a drop and then depositing the drop on the surface. The area of these spreads averaged 7.38 in.^{2} and the standard deviation was $\pm$ 0.46 in.^{2} In a second series, the tip of the pipette was held at the surface of the prepared oil surface. The 0.0072 ml. portion was blown gently and continuously until one or two bubbles indicated complete removal of the solution. The area of these spreads averaged 9.14 in.^{2} with a standard deviation of $\pm$ 0.31 in.^{2} In the first method, the protein solution creeps up on the outside of the tip of the pipette and is not deposited. The latter method is the correct technique to use. Trace the area of the spread three

Received for publication Nov. 24, 1950.

times as described in the method for butterfat. Three separate spreads should be made and each spread traced three times. The area of the nine tracings then is averaged and the average area multiplied by 0.0678 to obtain per cent non-casein protein in skimmilk and by 0.678 for nonfat dry milk solids. (The value of 0.0678 is obtained empirically as described below.)

Micro-Kjeldahl determinations follow the method of Ma and Zuazaga (3). The method of Rowland (4) is used for the separation of proteins of milk.

RESULTS

Table 1 presents data to show the spreading as monolayers of the various protein fractions of skimmilk. Since non-protein nitrogen does not spread, the spreading effect is due only to proteins. This table also illustrates the fact that lactose does not interfere, since the same sized aliquot of the original skimmilk was spread in the case of non-protein nitrogen, non-casein nitrogen, and proteose-peptone nitrogen. The method of preparation of these solutions is as follows: (a) For non-protein nitrogen, 20 g. of skimmilk is diluted to 50.0 ml.

TABLE 1

γ/in.^{2} of various protein fractions of skimmilk (average of 5 samples)

Fraction	% protein micro-Kjeldahl (N × 6.38)	γ protein delivered for spreading	Area of spread (in.^{2})	γ/in.^{2}
1. Total nitrogen	3.74	(see total protein, line 3)		
2. Non-protein nitrogen	0.21	3.02	0.00	.....
3. Total protein nitrogen (1 minus 2)	3.53	5.08	4.92	1.032
4. Non-casein nitrogen	0.88	(see non-casein protein, line 5)		
5. Non-casein protein (4 minus 2)	0.67	9.64	9.86	0.978
6. Proteose-peptone nitrogen	0.16	2.31	1.74	1.324

with 15 per cent trichloracetic acid and filtered. To 5 ml. of the filtrate is added 1 ml. of NH_4OH and 4 ml. of ethyl alcohol. After mixing, a 0.0072 ml. aliquot is spread. (b) For non-casein nitrogen, 20 g. of skimmilk is diluted with 10 ml. of 40° C. water and 1.0 ml. of 10 per cent acetic acid added. After 10 min., 1 ml. of 1*N* sodium acetate is added, the solution made to 50.0 ml. with water, filtered and a 5-ml. aliquot of the filtrate mixed with NH_4OH and ethyl alcohol as above. (c) For proteose peptone, 20 g. of skimmilk in a 50-ml. volumetric flask is allowed to stand in boiling water for 20 min. before following the procedure in (b).

To determine total protein, 20 g. of skimmilk is diluted to 50 ml. with water and mixed; 5 ml. of this dilution is made to 50 ml. with water and mixed. Then 5 ml. of this latter dilution is mixed with 1 ml. of NH_4OH and 4 ml. of ethyl alcohol as described above. The error, if any, due to the fat in the aliquot taken for spreading was calculated to be 0.13 in.^{2}, or less than the experimental error of the method.

A solution of 1:4 NH_4OH and ethyl alcohol containing 1 mg. of butterfat per ml. was prepared. This solution did not spread to a measurable extent. In another experiment, 1 ml. of 40 per cent cream was added to 20 g. of skimmilk and the fat and casein precipitated as in the monolayer method for non-

casein protein. The areas of the spreads made from this filtrate were not significantly different from those obtained on the same sample of skimmilk containing no added cream. These results show that the effect of the fat is negligible.

Non-casein nitrogen and nonprotein nitrogen were determined on 34 samples of skimmilk by micro-Kjeldahl. The difference between these two values multiplied by 6.38 is the non-casein protein (albumin plus globulin plus proteose-peptone). This value ranged from 0.60 to 0.68 per cent and averaged 0.642 per cent. Using the monolayer method described above, the area of the spread was determined on the same 34 samples. This area varied from 8.75 to 10.28 in.^{2} and averaged 9.48 in.^{2} The average non-casein protein divided by the average area equals 0.0678. In subsequent work, the area of the spread was multiplied by this factor to obtain per cent non-casein protein.

Using the above data, it was calculated that the non-casein protein spread on the prepared surface at the rate of 0.976 γ/in.^{2} as follows:

$$\frac{20 \times 0.00642 \times 0.0072 \times 10^6}{50 \times 2 \times 9.48} = 0.976 \text{ in.}^2,$$

or

$$\text{Area} \times 0.0678 = \text{per cent non-casein protein}$$

Attempts to verify this value directly were unsuccessful. Apparently, in the process of isolating and purifying albumin by chemical methods, the protein is partially denatured and does not spread to the extent naturally occurring albumin does. Values of two or three times the empirical value of 0.976 γ/in.^{2} are obtained with prepared albumin. Bull (1) states "most protein films exhibit remarkable similarity in dimensions. They all occupy in a compressed state from 0.8 to 0.9 square meter per milligram of protein and are from 9 to 10 Å thick." When this data is calculated on a γ/in.^{2} basis, the figures are 0.72 to 0.81 γ/in.^{2} These values were obtained with a film balance on a water surface yet are only slightly lower than the empirical value obtained with the method described above.

The same sample of nonfat dry milk solids was tested for per cent non-casein protein 29 times by the monolayer method. The mean was found to be 5.00 per cent, the standard deviation $\pm$ 0.26 per cent and the coefficient of variation 5.2 per cent.

Another sample of nonfat dry milk solids was tested for per cent non-casein protein 12 times by the micro-Kjeldahl method. The mean was 5.70 per cent, the standard deviation was $\pm$ 0.30 per cent and the coefficient of variation was 5.3 per cent.

Sixteen samples of whole milk were centrifuged and both methods were used to determine non-casein protein in the skimmilk. In the Kjeldahl procedure, non-casein protein is the per cent non-casein nitrogen minus the per cent non-protein nitrogen multiplied by 6.38. The mean Kjeldahl value of non-casein protein was 0.715 per cent, ranging from 0.51 to 0.90 per cent. The mean monolayer value was 0.735 per cent, ranging from 0.59 to 0.83 per cent.

The standard error of the difference between the two methods was ± 0.072 per cent. These results are presented in Table 2.

A total of 33 samples of nonfat dry milk solids was tested by both methods for non-casein protein. The mean Kjeldahl value was 4.63 per cent, ranging from 2.1 to 6.5 per cent. The mean monolayer value was 5.00 per cent ranging from 2.5 to 6.5 per cent. The standard error of the difference between the two methods was ± 0.44 per cent.

In a series of experiments dealing with the effect of heat on the non-casein protein content of skimmilk, 22 comparisons between the Kjeldahl and the mono-

TABLE 2

Comparison of the micro-Kjeldahl and the monolayer methods for non-casein protein on 16 samples of skimmilk

Sample	% non-casein protein by Kjeldahl method	Monolayer Method	
		Area (in.^{2})	% Protein (Area × 0.0678)
1	0.90	12.27	0.83
2	0.76	11.23	0.76
3	0.64	8.71	0.59
4	0.70	10.12	0.69
5	0.86	11.76	0.80
6	0.81	11.88	0.81
7	0.81	12.12	0.82
8	0.89	12.16	0.82
9	0.70	12.04	0.82
10	0.60	10.35	0.70
11	0.69	9.65	0.65
12	0.53	9.17	0.62
13	0.80	11.81	0.80
14	0.60	9.77	0.66
15	0.51	9.63	0.65
16	0.64	10.98	0.74
Av.	0.715		0.735

layer method were obtained. The Kjeldahl values ranged from 0.12 to 0.68 per cent, the monolayer values ranged from 0.14 to 0.78 per cent and the standard error of the difference between the two tests was ± 0.082 per cent.

CONCLUSIONS

A method for determining non-casein protein in skimmilk and nonfat dry milk solids is described. The method is based on the formation of a monolayer film of protein on a specially prepared surface of oxidized piston oil on 0.2 per cent acetic acid solution.

Results are presented which show that the monolayer method agrees well with the micro-Kjeldahl procedure.

REFERENCES

(1) BULL, H. B. *Outline of the Amino Acids and Proteins.* Ed. by M. Sahyun. Reinhold Publishing Co., New York City. 1944.

(2) HEINEMANN, B., AND ROHR, M. R. A Micromethod for Routine Determinations of Fat in Skimmilk and Nonfat Dry Milk Solids. J. Dairy Sci., **33**: 703–709. 1950.

(3) MA, T. S., AND ZUAZAGA, G. Micro Kjeldahl Determination of Nitrogen. Anal. Chem., **14**: 280–282. 1942.

(4) ROWLAND, S. J. The Determination of Nitrogen Distribution in Milk. J. Dairy Research, **9**: 30–46. 1942.

HEATING AS A MEANS OF PREVENTING AN OXIDIZED FLAVOR IN MILK DURING FROZEN STORAGE[1]

R. W. BELL AND T. J. MUCHA
Bureau of Dairy Industry, Agricultural Research Administration, U. S. D. A., Washington, D. C.

Evaporated milk does not develop an oxidized, tallowy, or cappy flavor similar to that encountered in market milk. During sterilization (115° C. for 15 min.), reducing substances are formed and a cooked or caramelized flavor is produced so that, even though the reactions leading to formation of the off-flavor could take place, the off-flavor would be masked. Furthermore, enzymes which might play a role in the development of this off-flavor would be inactivated by the high heat treatment (5). It follows that there is a heat treatment between that of pasteurization and sterilization below which an oxidized flavor may develop and above which it will not develop.

During the commercial preparation of homogenized market milk, the raw product may be held at temperatures as high as 155° F. (68.3° C.) for 30 min. instead of at 143° F. (61.6° C.) for 30 min., a minimum heat treatment recommended under the United States Public Health Service definition of pasteurized milk (7). This is possible because lack of creaming ability is not a consideration and the milk so treated does not taste heated.

This paper records the extent to which the development of an oxidized flavor in frozen beverage milk can be prevented by heating without impairing the flavor or body of the thawed product. The supplementary effect of adding ascorbic acid also was studied.

It has been shown (1) that fortification of milk with ascorbic acid greatly defers the development of an oxidized flavor in the frozen product. Earlier, others, notably Chilson (2), reported that cooled fluid milk to which ascorbic acid had been added, was free of this flavor defect longer than unfortified milk.

EXPERIMENTAL METHODS

About 2 gal. of fresh raw milk were heated in stainless steel containers that were set in suitable openings of a thick-walled chamber through which wet steam flowed. The milk was stirred during the heating and holding periods. At the end of each holding period it was poured into the stainless steel supply tank of an homogenizer of 125 gal. per hour capacity, homogenized at 2,500 lb. per square inch and cooled over a surface cooler to 15° C. or lower. To homogenize and cool the milk required at least 1 min. This treatment was given to all samples and should be considered in connection with each expressed holding period in order to obtain the approximate total holding time at or near the pasteurizing temperature. Each control sample was pasteurized by holding it at 71° C. for 15 sec. immediately prior to homogenization and cooling.

Received for publication Dec. 9, 1950.
[1] This work was done with funds from the Research and Marketing Act of 1946.

The ascorbic acid, in the form of a freshly prepared water solution, was added to the milk just before canning. The quantity of ascorbic acid in the milk was determined by the method described by Sharp (6).

The samples were not tasted until several hours after they were prepared. Since a heated or cooked flavor in freshly prepared milk diminishes in intensity, it is probable that some of the samples recorded as heated would have been described as cooked if they had been tasted immediately after they were prepared.

The milk samples were packaged in sealed cans of 160-ml. capacity and frozen in a room maintained at a temperature of −17° C. Samples were stored in this room and at −27° C. When samples were to be examined, the sealed

TABLE 1

Effect of time of heating at 71° C. and the addition of ascorbic acid on the flavor of milk when fresh and after 120 d. storage at −17° C.

Sample no.	Time held at 71° C.	Ascorbic acid in milk		E_h of milk		Flavor of milk	
		Fresh	120 d. Old	Fresh	120 d. Old	Fresh	120 d. Old
	(min.)	*(mg./l.)*	*(mg./l.)*	*(v.)*	*(v.)*		
1	0.25	13.0	0.0	0.249	0.281	....	st. ox.
2	1	13.0	0.0	0.212	0.275	....	ox.
3	3	14.8	0.0	0.206	0.260	heated	sl. ox.
4	5	15.1	0.0	0.182	0.254	heated	flat
5	0.25	62.2	25.2	0.171	0.208	....	clean
6	1	62.6	31.9	0.171	0.199	....	clean
7	3	64.4	35.7	0.159	0.196	heated	....
8	5	64.8	35.0	0.157	0.202	heated	flat
9	0.25	110.1	63.0	0.156	0.187	....	....
10	1	112.6	65.6	0.154	0.181	....	....
11	3	115.2	70.0	0.152	0.180	heated	flat
12	5	116.6	72.1	0.145	0.179	cooked	flat

cans were held overnight at 2° C. and then immersed in water at 20° C. in a 20° C. room until their contents had attained this temperature.

Data on body stability were obtained at the above temperature by whirling 50-ml. portions of samples in graduated 50-ml. tubes for 5 min. in a centrifuge that had a 10.5 in. head. Stability is expressed as milliliters of deposit in the tapered bases of the tubes.

EXPERIMENTAL RESULTS

It will be noted in table 1 that heating for 3 and 5 min. at 71° C. increased the ascorbic acid titre. This is believed to have been due to the formation of reducing substances which reacted with 2,6-dichlorophenolindophenol during titration (4). On the other hand, addition of a large volume of the dye during titration of the heavily fortified samples had a diluting effect that tended to cause a lower value to be obtained than was indicated by the sum of the quantity naturally present in the fresh milk (13.0 mg. per l.) and the ascorbic acid (50 and 100 mg. per l.) added to it.

The E_h (oxidation-reduction values) decreased as the holding time at 71° C. was increased. Ascorbic acid fortification of milk that had been held at 71° C.

for 15 sec. had a greater effect on the E_h than the additional heat. Most of this decrease in E_h was accounted for by the first 50 mg. of ascorbic acid per liter of milk; the addition of a second 50 mg. of ascorbic acid per liter had a smaller effect. Samples which were not fortified with ascorbic acid had a less stable E_h than those which were fortified and they had a strong tendency to develop the oxidized flavor, whereas the fortified samples did not. Lowering the E_h with heat alone was not sufficient to protect the milk against this flavor defect, except when the holding period at 71° C. was 5 min.

The fresh samples that tasted heated lost this property during storage. When these samples were 120 days old, most of those that had tasted heated when fresh now tasted flat. They were scored 18, 22 being the score of good

TABLE 2

Effect of increasing the temperature of heating above 71° C. and of added ascorbic acid on the resistance of milk to the development of an oxidized flavor during storage at −17° C.

Sample no.	Pasteurizing		Ascorbic acid in milk		Flavor of milk	
	Temperature	Holding time	Fresh	120 d. old	Fresh	120 d. old
	(° C.)	(min.)	(mg/l.)	mg/l.)		
1	71	0.25	14.0	0.0	....	st. ox.
2	71	0.25	64.4	19.6	....	sl. ox.
3	71	0.25	116.9	49.7	....	....
4	71	1	18.9	0.0	flat	st. ox.
5	71	1	68.3	21.0	flat	....
6	71	1	118.3	52.5	flat	flat
7	74	0.25	16.8	0.0	....	st. ox.
8	74	0.25	66.5	22.4	....	sl. ox.
9	74	0.25	123.2	54.6	....	flat
10	74	1	19.6	0.0	flat	sl. ox.
11	74	1	70.0	30.8	flat	....
12	74	1	124.1	57.4	flat	sl. heated
13	77	0.25	21.7	0.0	flat	ox.
14	77	0.25	71.4	27.3	flat	flat
15	77	0.25	126.1	60.9	flat	....
16	77	1	21.7	4.9	heated	flat
17	77	1	72.1	32.2	heated	flat
18	77	1	128.1	63.7	heated	sl. heated

grade fresh market milk. Numbers 5 and 6 had the best flavor and were given a numerical score of 20.

In this experiment, fortification of conventionally pasteurized milk with ascorbic acid was sufficient to prevent the development of an oxidized flavor for at least 120 days; holding the milk at the pasteurizing temperature until the cooled product had an objectionable heated flavor was not sufficient except when the milk was held at 71° C. for 5 min. The best combination of heating and fortification was represented by sample number 6.

In a similar experiment (table 2) in which the holding temperature was 74 and 77 as well as 71° C., the tendency of the samples to become oxidized was lessened by the higher heat treatment. However, these samples tasted flatter and more heated than did those heated to 71° C. Most of the thawed samples that were not oxidized tasted flat or heated to some degree. Number 5 had the best flavor. The period before the milk developed an oxidized flavor

was greatly increased by heating the milk more than is required in conventional pasteurization. The addition of 100 mg. of ascorbic acid per liter to milk which received the mildest heat treatment was sufficient to prevent the development of the off-flavor for at least 120 days and it did not noticeably affect the flavor or other general properties of the milk. However, even the best thawed samples were inferior to fresh market milk since they lacked a fresh and pleasing flavor. So far as their flavor was concerned, they could have been used if mixed with fresh milk of good quality.

In other experiments in which various temperatures and holding periods in this heat treatment range were employed, comparable results were obtained. The more heat that was put into the milk the more resistant the milk was to the onset of the oxidized flavor. Dahle and Palmer (3) and Gjessing and Trout (4) have shown this in their work on unfrozen milk. At the same time,

TABLE 3

Effect of forewarming treatment and boiling in a vacuum pan upon flavor and body stability of milk during frozen storage at −17° C. for 85 d.

Sample no.	Forewarming		Time under 28 in. vacuum	Flavor	Flocculated material /50 ml. of milk
	Temperature	Time			
	(° C.)	(*min.*)	(*min.*)		(*ml.*)
1	71.0	0.25	0.0	Strongly oxidized	7.0
2	76.5	0.25	0.0	Sl. oxidized	7.0
3	76.5	1.0	0.0	Flat	8.5
4	76.5	1.0	0.5	Sl. better than #3	8.5
5	76.5	1.0	3.0	Sl. better than #4	9.0
6	76.5	2.0	0.0	Flat	9.5
7	76.5	2.0	0.5	Sl. better than #6	10.0
8	76.5	2.0	3.0	Same as #6	12.0
9	76.5	3.0	0.0	Sl. heated, inferior to #3 and #6	12.0
10	76.5	3.0	0.5	Sl. better than #9	13.0
11	76.5	3.0	3.0	More heated than #9	14.0

there was a lowering in the flavor score due to different degrees of flatness or heated flavor.

In order to obtain data on the effect of the pasteurizing or forewarming treatment followed by boiling in a vacuum pan on the flavor and body stability of milk during frozen storage, fresh milk was heated at the temperature and for the times shown in table 3 and drawn into a stainless steel pan which was being operated at 28 in. of vacuum. In this experiment about 10 min. was required to attain these forewarming temperatures and 1 min. to draw the hot milk into the pan. The milk was boiled in the vacuum pan without additional heating for about 0.5 min., a portion of it was removed and the boiling was resumed for 3 min. Then each portion was homogenized, cooled and frozen. It was thought that quickly lowering the temperature of the hot milk by spraying the milk into the vacuum pan and allowing it to boil might remove volatile constituents and result in a better thawed product. Portions of the thawed samples were centrifuged as already described and the volume of flocculated material noted.

Boiling the hot milk briefly under a vacuum of 28 in. gauge pressure caused the flavor of the thawed milk at this stage to be slightly better than that of thawed samples of the same milk that was processed without this boiling. Boiling the milk for as long as 3 min. was beneficial or not depending upon the forewarming time at 76.5° C. However, a longer forewarming period than 0.25 min. and boiling in the vacuum pan caused the milk to be less stable in body, as indicated by the sedimentation data.

Portions of these eleven samples that were stored at −27° C. showed no flocculation and therefore no difference in body stability. Their flavor at 85 days of age was only slightly better than was that of those samples that were stored at −17° C. The flavor stability of the milk that was used in this experiment was excellent. After 220 days at −27° C., samples 3 to 11, inclusive, had not only a satisfactory body but also an acceptable market milk flavor. Samples 9, 10 and 11 no longer tasted heated.

DISCUSSION AND CONCLUSION

If milk solids that have been preserved in a frozen state should be used other than as a source of beverage milk, then a heated or even a cooked flavor might be tolerated. An example would be in the making of ice cream where other milk solids, sugar and flavoring agents would mask this property. Accompanying defects in body, which would make the milk unsuitable from a market-milk standpoint, also might not be objectionable because the unevenly dispersed solids could be redispersed by heat or homogenization or both. On the other hand, an oxidized flavor in the thawed milk would require rejection of the product not only as a beverage but also as a constituent of most food preparations.

Heat alone was not sufficient to prevent the onset of the oxidized flavor during storage at −17° C. without impairing the flavor and the body stability of the milk. This also was true of the flavor but not of the body of the milk stored at −27° C. for the same period. Under the conditions of these experiments best results were obtained when milk was heated at 71.0° C. for 1 min. and fortified with 50.0 mg. of ascorbic acid per liter and at 76.5° C. for 1 min. followed by brief boiling in a vacuum pan. The good results under the latter conditions were due primarily to the heat treatment. Boiling in the vacuum pan was a minor factor.

During frozen storage of milk, a heated flavor diminishes in intensity and finally may no longer be detectable. Therefore, milk that is to be stored a relatively long time can be given a more severe heat treatment than if it is to be consumed after a short storage period.

Milk that is to be preserved in frozen storage should be heated more than is required in conventional pasteurization in order to defer the development of an oxidized flavor but not heated to the extent that this desirable effect is offset by undesirable ones.

REFERENCES

(1) BELL, R. W. Retention of Ascorbic Acid, Changes in Oxidation Reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. J. Dairy Sci., **31**: 951–960. 1948.

(2) CHILSON, W. H. Off Flavors in Market Milk. Milk Plant Monthly, 24 (11): 24–28. 1935.

(3) DAHLE, C. D., AND PALMER, L. S. The Oxidized Flavor in Milk from the Individual Cow. The Pennsylvania State College School of Agr. and Expt. Sta. Bull. 347. May, 1937.

(4) GJESSING, E. C., AND TROUT, G. M. Ascorbic Acid and Oxidized Flavor in Milk. II. The Effect of Various Heat Treatments of Milk upon the Stability of Ascorbic Acid and upon the Development of Oxidized Flavor. J. Dairy Sci., **23**: 373–384. 1940.

(5) KRUKOVSKY, V. N. Ascorbic Acid Oxidation in Milk by Preformed Hydrogen Peroxide. J. Dairy Sci., **32**: 163–165. 1949.

(6) SHARP, P. F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. J. Dairy Sci., **21**: 85–88. 1938.

(7) U. S. Public Health Service Bull. 220, Milk Ordinance and Code.

SOME ADDITIONAL INFLUENCES AFFECTING THE STABILITY OF CONCENTRATED MILK IN FROZEN STORAGE[1]

H. L. WILDASIN[2] AND F. J. DOAN

The Pennsylvania Agricultural Experiment Station, State College, Pennsylvania

Many factors influencing the stability of the protein phase of concentrated milk under the conditions of frozen storage have been noted and studied (3, 4, 5, 6, 7, 8, 9, 12). Some of these are well established; others have been merely indicated. Because of the present interest in the possibility of marketing milk as a frozen concentrate, it was deemed important to study further some of the indicated influences and to investigate other factors which seem likely to affect the storage life of the product. Consequently, the investigation reported here was undertaken.

EXPERIMENTAL METHODS

Samples were prepared, frozen, stored and examined in a manner similar to that described by Doan and Warren (9), using a storage temperature of + 5° F. in order to accelerate flocculation of the protein and thereby obtain results in a shorter interval. A standard preheating temperataure of 170° F. was used prior to concentration unless otherwise noted. Protein separation for nitrogen distribution was accomplished as recommended by Rowland (14) with a few minor modifications. Total calcium was determined volumetrically according to the procedure of the AOAC (1) slightly modified for application to milk.

Cationic exchange treatment of fluid skimmilk was effected by passing the product through a 30-in. resin bed (I.R. 100), previously charged in the sodium cycle, contained in a glass tube 4 ft. long and 2 in. in diameter. Back washing was performed with tap water using an anionic detergent as an aid. When not in use, the resin bed was covered with a 0.25 per cent formaldehyde solution.

The dried ion-exchange treated skimmilk used in some of the studies was furnished by the M and R Dietetic Laboratories of Columbus, Ohio, and was reconstituted to the desired milk solids content with distilled water.

"Euglobulin-rich" and "euglobulin-poor" fluid skimmilks were prepared according to the method reported by Dunkley and Sommer (10).

EXPERIMENTAL RESULTS

Effect of homogenization on the protein stability of frozen milk. Fluid whole and skimmilk and concentrated whole and skimmilk were preheated to 90, 150 and 180° F. Half portions were homogenized immediately at 3,000 lb., after which all the samples were cooled, frozen and stored. Replicate samples were removed for analysis about every 10 days and the time required for significant flocculation (9) and for near maximum flocculation (14 volumes per cent) in the

Received for publication Dec. 17, 1950.

[1] Authorized for publication December 13, 1950 as Paper no. 1643 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Present address: Department of Animal Industry, University of Connecticut, Storrs.

defrosted samples was determined from interpolations of plotted data. Table 1 presents the results obtained.

Protein flocculation was hastened by increasing heat treatment of the samples, although the concentrated milks of table 1 do not show this because of a previous heat treatment incident to concentration. Homogenization also decreased the stability of all fluid products and all those containing fat but particularly the fluid whole milks and especially so at low preheating temperatures. In the latter case some of the effect may be due to lipolytic action, the flavor of these samples being noticeably rancid. The unhomogenized fluid whole milk suffered a loss of fat emulsion stability during storage. Some of the stability of

TABLE 1

Frozen storage life of fluid and concentrated milk as influenced by homogenization and heat

Product	Heated to 90° F.		Heated to 150° F.		Heated to 180° F.	
	Not homogenized	Homogenized	Not homogenized	Homogenized	Not homogenized	Homogenized
	Days required to obtain 4 volumes per cent of protein floc[a]					
Fluid skimmilk	70	40	60	50	45	40
Fluid whole milk	80	30	50	30	30	<20
27% T.S. skimmilk	22	32	32	32	26	26
27% T.S. whole milk	41	39	42	25	30	23
	Days required to obtain 14 volumes per cent of protein floc[a]					
Fluid skimmilk	95	75	95	85	75	75
Fluid whole milk	>120	45	>120	45	45	30
27% T.S. skimmilk	30	39	39	40	37	43
27% T.S. whole milk	50	48	58	53	56	43

[a] Values are the result of interpolation of graphical data. Analyses usually were made at 10-d. intervals.

the protein shown by the data for fluid whole milk resulted from the fact that on centrifuging the defrosted samples, a portion of the protein floc was carried upward into the fatty cream layer.

The results indicate rather definitely that fat has a destabilizing influence on the protein in frozen stored fluid milk, especially when homogenized. In concentrated milk this is not so apparent in the data, probably because the concentration of the whole milks was less than in the case of the skimmilks.

It might be mentioned that all of the samples heated to temperatures of 90 and 150° F. deteriorated in flavor during frozen storage. All became oxidized except those heated to 90° F. and homogenized, which developed rancidity. The concentrated milks (previously heated to 170° F.) and the fluid milks heated to 180° F. maintained a satisfactory flavor for the duration of the storage period.

Effect of sucrose on the protein stability of frozen milk. Two separate experiments utilizing three lots of concentrated skimmilk in each, were performed to study the effect of sucrose on the stability of the frozen product. Additions of sugar were made just after preheating and the products were concentrated and standardized to the following composition:

	Experiment A			Experiment B		
Lot no.	1	2	3	1	2	3
Per cent total solids	30.0	40.0	60.0	40.0	40.0	40.0
Per cent milk solids	30.0	26.7	30.0	40.0	26.7	20.0
Per cent sugar solids	0	13.3	30.0	0	13.3	20.0

After 110 days of storage, sample A-1 exhibited four volumes per cent of protein floc, while samples A-2 and A-3, even after 170 days when the experiment was discontinued, had developed no protein floc. Lactose crystallization was evident in sample A-3 after 10 days of storage and in sample A-2 after 30 days. Following 50 days of storage, sample B-1 had four volumes per cent of protein floc, while samples B-2 and B-3 had developed less than 1 per cent when the experiment was discontinued at 90 days. In all of the sugared products, lactose crystals were readily dissolved on reconstitution by a short period of agitation. As noted in both experiments, increased storage time, increased sugar concentration and increased total solids resulted in larger amounts of crystallized lactose. The results of these trials show that added sucrose delays the appearance of flocculated protein during frozen storage of concentrated milk.

Effect of calcium ion removal on protein stability of frozen fluid and concentrated skimmilk. Raw fluid skimmilk was treated with the calculated amount of potassium oxalate to precipitate all of the soluble calcium from solution, the titratable acidity being reduced from 0.150 to 0.055 per cent and the pH increased from 6.70 to 7.50. Control samples of fluid skimmilk developed four volumes per cent of protein floc in about 55 days storage, while the decalcified product showed no protein floc after 100 days of storage, when the experiment was discontinued. A portion of the stabilizing effect of the removal of soluble calcium in this case may be due to the elevated pH. Previous studies (9) showed, however, that in the range pH 6.30 to 7.00 little measurable effect of pH was observable on the frozen storage stability of concentrated skimmilk. These results indicate the importance of calcium and probably of certain other cations in relation to the sensitivity of protein in frozen milk. Additions of oxalates, of course, are not to be interpreted as possible commercial treatments because of their toxicity.

Studies were conducted wherein various proportions of ion-exchange treated dry skimmilk, previously reconstituted to the same solids level, were added to concentrated skimmilk containing 27 per cent solids. Similar samples to which commercial spray process dry skimmilk (of different brands) had been added were used as controls along with untreated samples.

Upon frozen storage, it was found that additions of reconstituted commercial dry milk have an accelerating effect on protein flocculation in concentrated milk. All lots employed showed this effect, the degree being roughly in proportion to the amounts added. Ion-exchange dry milks in amounts of 20 per cent or less (based on solids) accelerate flocculation compared with untreated samples but retard flocculation compared with samples containing commercial dry milks. Additions of ion-exchange dry milk amounting to 50 per cent of the solids of

concentrated milk cause a very noticeable retardation of flocculation compared with untreated samples. These results are interpreted to indicate that drying milk destabilizes the protein toward flocculation in frozen storage when reconstituted and that with small additions of ion-exchange dry milk this effect overshadows the stabilizing effect of the lowered calcium ion concentration.

The ion-exchange treatment described under Methods reduced the total calcium of fluid skimmilk by about one third and increased the pH to about 7.00. If the acidity of the milk was increased 0.13 per cent with 0.4 *N* HCl before treatment, the total calcium loss amounted to about two-thirds but the pH was not greatly affected. Experiments made by adding various proportions of these ion-exchange treated fluid skimmilks to concentrated skimmilk and adjusting the solids to comparable levels, demonstrated that little effect on protein stability in frozen storage is noted until the total calcium is reduced by ten per cent or more and at this level the flavor of the product is rendered objectionable, being variously described as salty, soapy, neutralized, etc. These findings are in substantial agreement with those of Haller and Bell (11) for frozen homogenized milk, but in their publication no mention was made of flavor changes.

The results obtained in the ion-exchange studies are disappointing inasmuch as the suppression or removal of calcium ions appears to be a very practicable method of stabilizing the protein of frozen milk but in these trials undesirable flavors appeared before the degree of treatment had reached a very effective point.

Nitrogen distribution in milk and its relation to protein stability under frozen storage conditions. Comparable lots of fluid skimmilk were obtained from the same whole milk source by the simple expedient of separating at 50° F. in one case and at 120° F. in the other. Nitrogen distribution determinations showed rather definitely, in several trials, that the globulin content (Rowland) is higher in the 120° F. skimmilk; this milk also resists protein flocculation for longer periods in frozen storage than does the 50° F. skimmilk. Upon pasteurization at 160° F. for 30 min., whey protein values are decreased for both euglobulin-poor and euglobulin-rich skimmilks, but the decrease in globulin is greater for the latter than for the former. Heat treatments of this degree seem to improve the stability of euglobulin-poor skimmilk and produce the reverse effect in euglobulin-rich skimmilk. The results obtained in one trial are shown in table 2.

The indicated relationship between globulin (by this method of separation) and resistance to flocculation in storage, of course, is not fully substantiated but it is interesting, particularly in view of recent findings that globulin is involved in ice cream shrinkage (13, 15). It also may point to a reason for some lack of uniformity in studies of the effects of heat on the frozen storage stability of skimmilk, namely, variations in temperatures of separation.

Data were obtained in several trials indicating that forewarming causes changes in the nitrogen distribution in skimmilk similar to those exhibited in table 2 for pasteurization, but that condensing to one-third volume and freezing, *per se,* do not alter the pattern subsequently.

Previous workers (9, 16) have shown qualitatively that casein is the principal

constituent of milk precipitated during frozen storage. Quantitative determinations were made of the distribution of nitrogen in raw fluid skimmilks and in the non-flocculated portions of the same milks after +5° F. storage for 1 yr., defrosting, reconstitution, centrifugation and washing. Comparison of these values shows conclusively that the preponderance of the nitrogen of the floc is casein nitrogen. The following values, representing the per cent of nitrogen not flocculated, are typical of the results obtained: casein, 5.6; albumin, 93.5; globulin, 87.2; proteose-peptone, 79.0; and non-protein, 83.3. By difference the amount of casein flocculated would amount to 94.4 per cent of that in the milk and by calculation it would comprise 96.0 per cent of the floc nitrogen. From these studies it is plain that casein (including, of course, any heat-denatured whey proteins which would be included as casein) is the only protein of milk which actually

TABLE 2

Nitrogen distribution and frozen storage life of euglobulin-rich and euglobulin-poor skimmilks

Sample	Storage life at +5° F.	Per cent distribution of nitrogenous constituents[a]					
	D.[b]	Casein	Albumin	Globulin	Proteose peptones	Non-protein	Total
Euglobulin-poor, separated at 50° F.	71	75.1	9.6	5.9	2.3	7.1	100
Euglobulin-rich, separated at 120° F.	128	76.0	8.2	6.4	1.8	7.6	100
No. 1 pasteurized at 160° F. 30 min.	100	80.8	3.7	5.7	2.6	7.2	100
No. 2 pasteurized at 160° F. 30 min.	78	80.8	5.8	4.1	1.9	7.4	100

[a] Separation according to Rowland.
[b] Time required to develop 5 volumes per cent of floc interpolated from graphical data.

flocculates under conditions of frozen storage; the very small quantities of other nitrogenous substances apparently are present in the occluded serum.

Effect of interval between concentrating and freezing on the protein stability of concentrated products. The effect of packaging concentrated skimmilk for frozen storage directly from the condensing equipment as compared with cooling the product and holding several hours before packaging was found to be very significant.

Preliminary studies indicated that high-solids products cooled very slowly in ice water and without agitation often showed signs of gelation even before freezing. One sample of 53 per cent total solids skimmilk after being cooled for 6 hr. in ice water could not be reconstituted with cold water. When held 6 hr. at 100 to 120° F., it could be reconstituted but exhibited 14 per cent by volume of protein floc in the cold. On the other hand, samples from the same batch when frozen immediately after condensing required 95 days of frozen storage to produce the same degree of flocculation.

Three portions of a lot of fluid skimmilk were concentrated to 30.4, 43.3 and 47.4 per cent of total solids (T.S.), respectively, and each portion was treated

in two or three different ways when drawn from the pan. Table 3 shows the effects of these different treatments on the susceptibility of the milk to flocculation under conditions of frozen storage.

In another case concentrated skimmilk containing 46.7 per cent T.S. developed four volumes per cent of floc in 17 days of frozen storage when cooled to 40° F. after condensing and when held at this temperature for 12 hr. prior to freezing. The same milk frozen immediately after condensing did not exhibit this degree of flocculation up to 45 days of storage.

From these results and other observations it is believed that holding concentrated milk after removal from the condensing equipment, particularly if it is first cooled, allows the casein micelles to aggregate into structures. In the case of high solids milk (over about 45 per cent), these actually may produce a gelation which appears as a flocculation upon reconstitution. In lower solids milk,

TABLE 3

Frozen storage life of concentrated skimmilk as influenced by the time and temperature of holding after concentration and before freezing

	Days required to obtain:					
	4 vols. % of protein floc[a]			10 vols. % of protein floc[a]		
	30.4% TS	43.3% TS	47.4% TS	30.4% TS	43.3% TS	47.4% TS
Frozen at once	80	71	47	105	87	55
Held 3 hr. at 40° F. before freezing	53			60		
Held 3 hr. at 110–120° F. before freezing	62	58	30	81	81	50

[a] Values are the result of interpolation of graphical data. The product testing 43.3% TS was preheated at 143° F. for 30 min.

though no gelation occurs, there is nevertheless an increase in viscosity which may be interpreted as indicating the same thing. It also is probable that, in high solids milk, holding at low temperature may cause the formation of lactose nuclei which might be expected to accelerate the formation of casein structures by actually becoming involved in them. An interesting observation bearing on this possibility was made in the case of a sample of concentrated skimmilk of 30.0 per cent total solids which was "seeded" with 0.1 per cent by weight of 250 mesh sand, held 3 hr., at 40° F., frozen and stored. The storage life (4 volumes per cent of floc) of this sample was 6 days compared with 23 days for a control sample containing no sand.

Babcock *et al.* (2) demonstrated that homogenized pasteurized fluid whole milk can be held cold for a matter of days previous to freezing without influencing the rate of protein flocculation in frozen storage. Concentrated milks, on the other hand, should be frozen without delay to obtain the maximum storage life and, if this is not possible, then the stability of the product is improved by holding without cooling.

Clarification as a possible influence on the stability of concentrated frozen milk. Inasmuch as evidence indicated that the presence of fine particles have

an unfavorable effect on the storage life of frozen concentrated milk, the possibility that leucocytes, bacterial cells and fine dirt also might have a similar influence was considered.

In two trials with average mixed milk efficient clarification did not show any effect on the rate of protein flocculation of the products after concentration and frozen storage. It seems unlikely, therefore, that this treatment would be of any benefit with milk of average satisfactory quality.

SUMMARY AND CONCLUSIONS

Homogenization of fluid whole milk, fluid skimmilk and concentrated whole milk increases the rate of flocculation of casein in these products during frozen storage. In general, the presence of fat and increases in preheating temperatures prior to homogenization lead to more rapid flocculation. The heat-plus-homogenization effects, however, are less than would be expected if they were additive. Concentrated skimmilk is not adversely affected by heat treatment and homogenization at temperatures that do not exceed any previous heat treatment.

Additions of sufficient sucrose to concentrated milk to affect significantly the hardness of the frozen product extends the satisfactory frozen storage period.

Soluble calcium probably is a prerequisite to the flocculation of casein in frozen milk products. The amount of reduction in total calcium required to produce a useful retarding effect on flocculation appears to be in excess of 10 per cent when accomplished by a cation exchange procedure. This amount of treatment has an adverse effect on flavor and limits the utility of the treatment.

The effect of heat on the protein stability of fluid skimmilk when stored in the frozen state is unpredictable unless the history of the product is known. The globulin (euglobulin) content appears to be an important variable in this connection.

The nitrogen distributions of a sample of fluid skimmilk before and after preheating, after condensing and after freezing and thawing are indicated and quantitative nitrogen determinations have substantiated the fact that the protein floc appearing in stored frozen milk products consists primarily of casein.

Concentrated milk products, especially those with high solids content, should be frozen as soon as possible after condensing, and if a time interval is unavoidable, the product should be held without cooling if maximum storage life is to be realized. This phenomenon seems to be attributable to a tendency for the casein to form structures on holding and, in some cases, for lactose nuclei to appear before the freezing is accomplished. That nuclei of any sort may act as a destabilizing influence on proteins in frozen milk was shown in experiments where very fine sand acted in this manner.

The clarification process applied to fluid milk before concentrating and freezing has no significant effect in retarding the flocculation of the protein of average quality milk during storage, even though the leucocyte count may be noticeably reduced.

REFERENCES

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. *Official and Tentative Methods of* Analysis. 6th ed. Washington, D. C. 1945.

(2) BABCOCK, C. J., STABILE, J. N., WINDHAM, E., EVANS, L. R., AND RANDALL, R. Frozen Homogenized Milk. V. Effect of Age before Freezing on the Keeping Quality of Frozen Homogenized Milk. J. Dairy Sci., **31**: 811–814. 1948.

(3) BELL, R. W. Effects of Cold Storage Temperature, Heat Treatment, and Homogenization Pressure on the Properties of Frozen Condensed Milk. J. Dairy Sci., **22**: 89–100. 1939.

(4) COLVARD, C. D., AND ROBERTS, W. M. Frozen Concentrated Milk. Milk Dealer, **38** (7): 46, 100–106. 1949.

(5) CORLEY, R. T., AND DOAN, F. J. A Study of Concentration and Freezing as a Means of Preserving Fluid Whole Milk. Food Research, **5**: 369–378. 1940.

(6) DOAN, F. J. Preserving Condensed Milk by Freezing. Natl. Butter and Cheese J., **28** (11): 24–26. 1937.

(7) DOAN, F. J., AND FEATHERMAN, C. E. Observations on Concentrated Frozen Milk. Milk Dealer, **27** (2): 33–34, 62. 1937.

(8) DOAN, F. J., AND WARREN, F. G. Frozen Milk with Special Reference to Protein Flocculation. Proc. 39th Annual Convention I.A.M.D. Lab. Sec. 1946.

(9) DOAN, F. J., AND WARREN, F. G. Observations on the Instability of the Protein Phase of Frozen Concentrated Milk. J. Dairy Sci., **30**: 837–848. 1949.

(10) DUNKLEY, W. L., AND SOMMER, H. H. The Creaming of Milk. Wisc. Agr. Expt. Sta. Research Bull. 151. 1944.

(11) HALLER, H. S., AND BELL, R. W. Improving the Keeping Quality of Frozen Homogenized Milk. The Milk Dealer, **40** (1): 98. 1950.

(12) LEEDER, J. G. Some Information on Frozen Concentrated Milk. Natl. Butter and Cheese J., **36** (9): 50–52. 1945.

(13) LANDO, J. C., AND DAHLE, C. D. Shrinkage. Ice Cream Rev., **33** (3): 150–154. 1949.

(14) ROWLAND, S. J. The Determination of the Nitrogen Distribution in Milk. J. Dairy Research, **9**: 42–46. 1938.

(15) TARASSUK, N. P., AND HUTTON, J. T. The Shrinkage of Ice Cream as Affected by the State of Milk Proteins. Proc. 45th Ann. Conv. I.A.I.C.M., **2**: 37–47. 1949.

(16) WEBB, B. H., AND HALL, S. A. Some Physical Effects of Freezing upon Milk and Cream. J. Dairy Sci., **18**: 275–286. 1935.

THE DISTRIBUTION OF CERTAIN AMINO ACIDS IN SOLUBLE FRACTIONS OF MILK CULTURES OF *STREPTOCOCCUS LACTIS*[1, 2]

M. E. MORGAN[3] AND F. E. NELSON

Department of Dairy Industry, Iowa State College

The literature contains numerous reports ascribing to *Streptococcus lactis* slight proteolytic action as determined by increases in soluble nitrogen in milk cultures. The results of these studies have been reviewed by Hammer and Babel (4). The proteolysis is carried to the amino acid stage, but no information has been available regarding the extent to which the various amino acids are liberated from milk proteins. Harper and Swanson (6) and Reihard and Garey (12) have recently demonstrated the presence of various free amino acids in cheddar cheese at different stages of ripening. The extent to which the proteolytic enzymes of *S. lactis* are involved in the liberation of free amino acids in cheese is not definitely known.

In the present investigation determination of the distribution of amino acids in some of the soluble fractions of *S. lactis* milk cultures was undertaken in hopes of obtaining further information concerning the proteolytic properties of this organism.

EXPERIMENTAL PROCEDURE

Preparation of S. lactis cultures. Colonies typical for *S. lactis* were picked from tomato juice-peptonized milk agar platings of different sour cream samples and transferred to litmus milk. After several transfers, cultures typical for *S. lactis* by their action in litmus milk, morphology, ability to grow at 10° C. and failure to grow at 45° C. were transferred in duplicate. These cultures were incubated at 30° C. for 24 hr., frozen and held for future use at –10° C.

Two hundred-ml. portions of fresh skimmilk were dispensed into pint screw-capped bottles; these were weighed to the nearest 0.1 g. Sterilization was accomplished by heating at 100° C. in flowing steam for 40 minutes on 3 successive days. After the last heat treatment the bottles were allowed to cool, then placed in a 21° C. incubator over night.

Frozen cultures of the *S. lactis* strains to be used as inoculum were allowed to thaw at room temperature and carried through four transfers at 21° C. before being used in the experiment. Duplicate 200-ml. quantities of sterile skimmilk were inoculated with four drops of a 24-hr. litmus milk culture of each of the strains of *S. lactis* to be studied. These large cultures and duplicate sterile controls were incubated at 21° C. for the desired length of time.

Preparation of culture filtrates. After incubation all cultures and sterile controls were made up to their original weight by addition of distilled water.

Received for publication Dec. 19, 1950.

[1] Journal paper no. J-1873 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 1050.

[2] This paper was prepared from data presented in a thesis by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Iowa State College.

[3] Present address: Department of Animal Industries, University of Connecticut, Storrs.

Each culture was agitated thoroughly by shaking and then mixed in a Waring blendor. Replicate 26-g. samples of each culture and the controls (equivalent to 25 ml. of skimmilk of sp. gr. 1.038) were weighed into 150-ml. beakers.

Tungstic acid filtrates of each of the cultures and the sterile control were prepared by a modification of the method employed by Hier and Bergeim (8) for preparation of protein-free filtrates of blood plasma. Each 26-g. sample was diluted with 20 ml. of distilled water and adjusted to pH 1.5 by addition of a measured quantity of 0.6 N H_2SO_4. The samples were brought to 68.7 ml. with water and 6.3 ml. of 10 per cent sodium tungstate added. After 3 min. of vigorous agitation, the material insoluble in tungstic acid was removed by filtration through dry, fluted Whatman no. 2 filter paper. All filtrates prepared in this manner had a pH of 2.4 to 2.5. This is below the limit of pH 2.85 found by Merrill (10) to be the point at which complete precipitation of blood proteins occurs with tungstic acid.

Preliminary trials indicated that if the pH of the milk cultures or sterile control were adjusted to 1.5 and sodium tungstate solution added to give a calculated final concentration of 0.7 per cent tungstic acid, the filtrates gave a minimum biuret reaction. These filtrates were less toxic in low dilutions to the assay organisms than were those obtained with concentrations of 0.8 and 1.0 per cent tungstic acid.

Whey filtrates of additional 26-g. samples from each culture were prepared by diluting to 75 ml. with water, heating on a boiling water bath with constant stirring for 5 min., then cooling in tap water. The coagulated protein was removed by filtration, as described for the tungstic acid filtrates. Since the pH of all the cultures after dilution was 4.2, the sterile control samples were diluted to 60 ml., adjusted to pH 4.2 with a measured quantity of 10 per cent lactic acid and made to 75 ml. From this point the sterile control samples were handled in the same manner as the culture samples. The whey filtrates prepared from both cultures and controls are referred to as the lactic acid filtrates.

Each milliliter of the above filtrates was equivalent to 0.33 ml. of the original skimmilk culture or control. In preparing the filtrates for assay, a 50-ml. quantity of each was adjusted to pH 6.8 with 0.1 N NaOH and made up to the desired volume.

Preparation of hydrolyzed filtrates. Fifty-ml. portions of tungstic and lactic acid filtrates of cultures and control were made 2N with H_2SO_4 by adding 6.4 ml. of 50 per cent H_2SO_4 and bringing the volume to 60 ml. with water. The acidified filtrates in 150-ml. beakers were covered with larger inverted beakers and hydrolyzed by autoclaving at 15 lb. pressure for 5 hr. While still hot, the hydrolyzed filtrates were transferred to large centrifuge tubes and treated with 18 g. of $Ba(OH)_2 \cdot 2H_2O$ in 25 ml. of boiling water to remove the excess sulfate. After thorough mixing, the precipitated $BaSO_4$ was removed by centrifugation. The supernatant was decanted and passed through sintered glass crucibles to remove any precipitate which was not thrown down in the centrifuge tubes. The precipitates were triturated with 50 ml. of boiling water, recentrifuged and the washings decanted. Washing of the precipitates was repeated a second time and the

washings combined with the first supernatants. The hydrolyzed sterile control filtrates were concentrated under vacuum. All the hydrolyzed filtrates were adjusted to pH 6.8 with 0.1*N* NaOH and made to the desired volume. All filtrates were stored under toluene in screw-capped bottles in the refrigerator.

Amino acid assay of filtrates. Assays for valine, leucine, isoleucine, threonine, arginine, methionine, histidine, tryptophan, tyrosine and phenylalanine were performed on the prepared filtrates by means of the microbiological method described by Stokes *et al.* (18, 3). *Lactobacillus delbrueckii* LD5[4] was employed as the assay organism for tyrosine and phenylalanine and *Streptococcus faecalis* was used for the other amino acids.[5] The material for standard curves and for assays was incubated 65 hr. at 37° C. Upon removal of the tubes from the in-

TABLE 1

Amino acid activity of tungstic and lactic acid filtrates prepared from 15-day skimmilk cultures of five strains of S. lactis

Amino acid	Filtrate	Amino acid equivalents as γ/ml. of culture						
		Control[a]	1[a]	2	3	4	5	Av. increase
Valine	T[b]	8	113	131	100	103	95	101
	L[c]	9	123	147	110	117	109	113
Leucine	T	5	122	147	111	110	111	115
	L	9	150	172	139	139	136	138
Isoleucine	T	5	65	81	78	70	46	63
	L	5	98	121	106	105	82	97
Threonine	T	3	74	71	61	61	51	61
	L	6	78	78	68	68	56	63
Arginine	T	7	77	80	59	55	65	60
	L	12	106	108	96	82	98	86
Methionine	T	4	33	30	24	24	18	22
	L	5	38	37	34	33	28	29
Histidine	T	2	30	38	26	26	28	28
	L	4	42	50	40	36	40	38
Tryptophan	T	3	14	15	12	11	13	10
	L	4	20	18	15	15	16	13
Tyrosine	T	4	79	30	55	49	44	58
	L	4	123	115	99	101	80	100
Phenylalanine	T	3	93	101	83	79	75	84
	L	5	175	173	163	174	137	158

[a] Uninoculated milk; culture no. indicate *S. lactis* strains.
[b] Tungstic acid filtrate.
[c] Lactic acid filtrate.

cubator, growth of the assay organisms was arrested by steaming for 15 min. at 100° C. The cultures were cooled to room temperature and titrated to pH 7.0 with standard alkali, using a potentiometric titration apparatus similar to that described by Rockland and Dunn (13).

RESULTS

Amino acid activity of unhydrolyzed filtrates. Assays for ten amino acids were made on unhydrolyzed tungstic acid and lactic acid filtrates of skimmilk cultures of five strains of *S. lactis* and a sterile skimmilk control, all of which had

[4] Rogosa (14) has shown this organism to be identical with *L. casei.*
[5] Cultures of *L. delbrueckii* LD5 #9595 and *S. faecalis* #9790 were obtained from the American Type Culture Collection.

been incubated at 21° C. for 15 days. The results of these assays, expressed as micrograms of amino acid per milliliter of original culture, are shown in table 1.

The values indicated for both the tungstic and lactic acid filtrates of the sterile control probably are not entirely accurate, as preliminary trials, in which such filtrates were added to a complete basal medium, revealed that the filtrates could not be diluted less than 1:6 without inhibiting the acid production of the assay organisms. Since the amino acid activities of the control filtrates were so low, nearly all of the titration values obtained at the various levels fell between the blank value and the second increment of amino acid on the various standard curves, an area of reduced accuracy.

The assay values for all ten of the amino acids on the tungstic acid filtrates from each of the five cultures revealed increases over those obtained on the sterile control. The average increases in leucine and valine were highest, followed by that for phenylalanine. Increases for isoleucine, threonine, arginine and tyrosine were approximately equal in magnitude. The increases in histidine, methionine and tryptophan were slight, with tryptophan showing the least increase.

All of the assay values determined on the lactic acid filtrates were higher than the corresponding values on the tungstic acid filtrates. With these filtrates, the average increase was greatest for phenylalanine, followed in order of decreasing magnitude by those for leucine, valine, tyrosine, isoleucine, arginine, threonine, histidine, methionine and tryptophan.

A comparison of the average increases of the tungstic acid and lactic acid filtrates reveals that the latter had 89 per cent more phenylalanine activity than the former. The tyrosine activity of the lactic acid filtrates was approximately 70 per cent greater, while that for threonine was only 4 per cent greater.

When a qualitative biuret test (7) was applied to the filtrates, the tungstic acid filtrate of the sterile control was negative, whereas the lactic acid filtrate gave a bright pink color, indicating the presence of proteose or peptone. A light pink color was obtained on the tungstic acid filtrates of all cultures, and the lactic acid filtrates of cultures gave a bright pink color.

There appears to be some variation in the ability of different strains of *S. lactis* to convert milk protein and protein intermediates into their component free amino acids or fractions containing amino acid residues which are microbiologically available. In most cases the tungstic and lactic acid filtrates of cultures 1 and 2 gave higher assay values than those of 3, 4 or 5.

Amino acid activity of unhydrolyzed and hydrolyzed filtrates after different periods of incubation. Assays for leucine and phenylalanine activity were made on both unhydrolyzed and hydrolyzed tungstic and lactic acid filtrates prepared from skimmilk cultures of two strains of *S. lactis* which had been incubated for 1, 3, 7 and 14 days at 21° C. Strains 2 and 4[6] were chosen for this experiment because of the apparent difference in their proteolytic activity as judged by the previous experiment.

Assays for these particular amino acids were made because it was believed

[6] Cultures of these strains were duplicates of the strains used previously and had been preserved in a frozen condition.

that they would provide the most information regarding the extent to which *S. lactis* liberates amino acids and protein degradation products containing residues of such amino acids from milk proteins. It has been shown that *S. lactis* definitely requires leucine in its metabolism, whereas phenylalanine has only a stimulatory effect on its growth (11). In the previous experiment the ratio of the average increase in the phenylalanine assays of the unhydrolyzed lactic acid filtrates over that for the corresponding tungstic acid filtrates seemed to be exceedingly high. Because of the rather marked upward drift in the phenylalanine values obtained in the assays of the lactic acid filtrates as levels of added diluted filtrate increased, it was suspected that the lactic acid filtrates contained factors stimulatory for *L. delbrueckii* LD5 which were not present in the basal medium.

The values for the leucine activity of unhydrolyzed and hydrolyzed tungstic

TABLE 2

Leucine activity of unhydrolyzed and hydrolyzed tungstic and lactic acid filtrates of skimmilk cultures of two strains of S. lactis after different periods of incubation

Period of incubation	Leucine equivalent as γ/ml. of culture			
	Tungstic acid filtrates		Lactic acid filtrates	
	Unhydrolyzed	Hydrolyzed	Unhydrolyzed	Hydrolyzed
(*d.*)				
		Strain 2		
0	3.0	15.8	7.1	154.5
1	48.0	66.0	57.0	189.9
3	89.0	109.0	119.0	265.5
7	102.5	146.4	137.7	252.5
14	135.0	159.0	177.0	298.5
		Strain 4		
	3.0	15.8	7.1	154.5
1	30.0	53.0	38.3	156.0
3	57.0	72.5	71.3	192.0
7	76.2	126.0	105.5	212.6
14	91.5	111.0	120.0	202.5

and lactic acid filtrates of cultures of the two strains after the various incubation periods are presented in table 2. There was a sharp increase in the leucine activity of the unhydrolyzed filtrates of the cultures of strain 2 during the first 3 days of incubation. The rate of increase was somewhat more rapid with the lactic acid filtrates, indicating that protein degradation products possessing leucine activity but insoluble in tungstic acid were being formed. From the third to the fourteenth day the leucine activities of both filtrates continued to increase gradually and at comparable rates. The change in rate shown in both cases after the third day probably followed attainment of the maximum stationary growth phase of the culture.

The relatively high leucine activity of the hydrolyzed lactic acid filtrate of the sterile control as compared to the low activity of the unhydrolyzed filtrate indicates that heat-sterilized skimmilk contains an intermediate fraction in which most of the leucine residues are bound in a microbiologically unavailable form.

The increase in the leucine assay value during the first 3 days of incubation indicates that some of the milk protein was hydrolyzed to a form soluble in lactic acid. The difference between the hydrolyzed and unhydrolyzed lactic acid filtrate values were much the same for every analysis period, indicating that nearly the same amount of inactive intermediate was present throughout the incubation period. The increase in leucine values between the third and fourteenth days indicates that the milk proteins continued to be attacked by the proteolytic enzymes of *S. lactis,* even though the number of viable cells undoubtedly was decreasing during this time.

The higher leucine assay values of the hydrolyzed tungstic acid filtrates as compared to the unhydrolyzed indicates that tungstic acid will not remove all of the amino acid in bound form from milk cultures in which partial protein

TABLE 3

Phenylalanine activity of unhydrolyzed and hydrolyzed tungstic and lactic acid filtrates of skimmilk cultures of two strains of S. lactis after different periods of incubation

Period of incubation	Phenylalanine equivalent as γ/ml. of culture			
	Tungstic acid filtrates		Lactic acid filtrates	
	Unhydrolyzed	Hydrolyzed	Unhydrolyzed	Hydrolyzed
(*d*)	Strain 2			
0	0.9	3.2	3.4	3.2
1	25.5	32.6	59.3	57.0
3	54.0	56.3	111.8	75.0
7	70.5	36.5	145.2	148.2
14	80.1	78.9	174.0	187.5
	Strain 4			
0	0.9	3.2	3.4	3.2
1	24.0	28.5	42.5	50.7
3	43.5	46.5	77.7	64.5
7	52.2	30.0	101.7	120.8
14	57.6	56.7	121.5	144.0

hydrolysis has taken place. This inactive bound form of leucine was negligible in the control milk but increased up to the third day of incubation and remained much the same through the fourteenth day.

The leucine activities of the various filtrates prepared from cultures of strain 4 indicate this strain was somewhat less active than strain 2 in liberating leucine or hydrolytic products possessing leucine activity. The hydrolyzed lactic acid filtrates from cultures of this strain reveal little or no increase in leucine activity during the first day of incubation, indicating that no appreciable attack on the milk protein was made during this time.

The phenylalanine activities of the various filtrates shown in table 3 reveal that the uninoculated sterile skimmilk contained very little free or combined active phenylalanine. The lower values for all filtrates from cultures of strain 4 again reveal its lesser proteolytic activity. Comparison of the unhydrolyzed tungstic and lactic acid filtrates shows that the latter had about twice the phenylalanine activity of the former throughout the 14-day incubation period. Apparently, the lactic acid filtrates contain some intermediate fractions which have

phenylalanine activity. This is demonstrated further by a comparison of the unhydrolyzed and hydrolyzed lactic acid filtrates. The activity of the unhydrolyzed filtrate approached that of the hydrolyzed filtrate throughout the incubation period and even surpassed it at the third day.

Evidence presented in table 4 indicates that milk cultures of *S. lactis* contain a substance stimulatory for *L. delbrueckii* LD5 which is not acid- or heat-coagulable but is inactivated by acid hydrolysis and is precipitated with tungstic acid. The data presented are values obtained at the various assay levels for all filtrates

TABLE 4

Phenylalanine assay values at various levels of added filtrates

Filtrate	Phenylalanine equivalent as γ/ml. of diluted filtrate			
	Tungstic acid filtrate		Lactic acid filtrate	
	Unhydrolyzed	Hydrolyzed	Unhydrolyzed	Hydrolyzed
(*ml.*)				
	3-d. culture of strain 2			
0.5	3.6	3.0	5.8	9.2
1.0	3.6	3.9	6.5	10.8
1.5	3.6	3.7	6.8	9.6
2.0	3.6	3.7	7.7	9.8
3.0	3.6	3.7	8.8	9.4
	7-d. culture of strain 2			
0.5	5.0	4.0	8.0	10.6
1.0	4.8	4.2	8.6	10.2
1.5	4.7	5.0	9.3	9.8
2.0	4.6	5.0	9.8	9.9
3.0	4.7	5.2	11.0	9.6
	14-d. culture of strain 2			
1.0	5.0	5.0	9.9	12.6
1.5	5.2	5.2	11.1	12.9
2.0	5.5	5.3	11.8	12.6
2.5	5.5	5.4	12.7	12.4
3.0	5.5	5.4	12.6	11.8

prepared after the 3, 7 and 14-day incubation periods on strain 2. The values for both the tungstic acid filtrates show little, if any, drift with added increments of filtrate. The values on unhydrolyzed lactic acid filtrates revealed a marked upward drift with each increment of added filtrate from the 0.5- to the 3.0-ml. levels. There was a slight drift at 0 and 1 days but not so marked as after the longer incubation periods. An identical drift was noted in the assays of the same filtrates prepared from cultures of strain 4. This apparent stimulatory effect never was noted in the leucine assays of any of the filtrates where the assay organism was *S. faecalis*.

DISCUSSION

There is little doubt that the unhydrolyzed filtrates prepared from *S. lactis* cultures contain intermediate products of protein degradation. In view of recent studies (2, 9, 16, 17), which reveal microbiological availability of some amino acids in peptide form, the assay values on such filtrates cannot be assumed to represent only the free amino content. Since the lactic acid filtrates gave higher

values than the tungstic acid filtrates for all amino acids for which tests were made, it is quite probable that the difference was due to intermediate fractions which were precipitable with tungstic acid and which contained microbiologically active amino acids in bound forms.

Because the tungstic acid filtrates gave higher assay values after hydrolysis in the case of leucine and phenylalanine, it was concluded that these filtrates also contained some bound amino acids. Some of the amino acids in this form no doubt were active in the assay procedure. Interpreted in this manner, the amino acid activity values for the unhydrolyzed filtrates are indicative of the degree to which both free amino acids and lower fractions possessing amino acid activity were liberated from milk proteins by the proteolytic action of *S. lactis*.

There appears to be a relationship between the average increase in the amino acid activity values obtained on the unhydrolyzed tungstic acid filtrates from the five 15-day cultures of *S. lactis* when compared with the relative amount of each amino acid present in milk proteins. The assay values for both milk and casein, as reported by Stokes *et al.* (18), indicate the following decreasing quantitative order of occurrence: leucine, valine, phenylalanine, isoleucine, tyrosine, threonine, arginine, histidine, methionine and tryptophan. The quantitative order of increase of values for these amino acids in the culture filtrates occurred in the same order, except that the value for tyrosine fell between those for arginine and histidine. This seems to indicate that the proteolytic enzymes of *S. lactis* liberate amino acids and their simpler peptides from milk protein in amounts which are related to the occurrence of such amino acids in the protein.

The increases in the leucine and phenylalanine assay values of the hydrolyzed lactic acid filtrates prepared from cultures of strains 2 and 4 after various periods of incubation indicate that intermediates containing these amino acids were being split from the milk protein. The over-all increase in these values from the first to the fourteenth day of incubation indicates that there must have been a decrease in total protein of the cultures. This is in opposition to the results of Braz and Allen (1) which indicated a measurable increase in the total protein nitrogen of *S. lactis* milk cultures after 6-wk. incubation. If the total protein of the present cultures was going to increase due to synthesis of cell protein, it seems as though such an increase would have occurred during the logarithmic and maximum stationary growth phases and would have been reflected in a decrease in the soluble products of protein hydrolysis. The protein content probably would not increase during incubation extended beyond 14 days, as the number of viable cells would be decreasing during this period. Contrarily, the cells would be autolyzing, thereby increasing the total soluble fractions.

The rather large amount of leucine present in the soluble portion of the sterile control milk, as shown by the high activity of the hydrolyzed lactic acid filtrates, indicates that sterilized milk contains some of the protein intermediates. It is quite likely that these intermediates constitute the proteose-peptone fraction which Rowland (17) reported to be present in normal milk. The very low phenylalanine activity of the lactic acid filtrates of the sterile control reveals

that the proteose-peptone and other non-protein nitrogen constituents contain little phenylalanine.

The lack of increase in the leucine content of the hydrolyzed lactic acid filtrate of the cultures of strain 4 during the first 24-hr. incubation is considered evidence of low proteolytic activity. During this lag before the organism had made any appreciable attack on the milk protein, the leucine activity of the unhydrolyzed tungstic acid filtrate was increasing at a rapid rate. This may be an indication that strains of *S. lactis* possessing low proteolytic activity derive amino acid for metabolism during their early phases of growth through hydrolysis of soluble protein intermediate fraction present in the milk, *e.g.*, proteose-peptone. This probably was true for the more active strain as well, but growth and metabolism were so rapid that any lag in proteolysis would have been negligible.

The drift observed in all phenylalanine and tyrosine assays of the unhydrolyzed lactic acid filtrates undoubtedly was due to some substance present in these filtrates which has a stimulatory effect on the growth of *L. delbrueckii* LD5. There is no evidence which would reveal the nature of this substance other than that it is not acid- or heat-coagulable but is precipitated by tungstic acid and is destroyed by acid hydrolysis. The phenylalanine activity of the unhydrolyzed lactic acid filtrates possibly was high due to the presence of active phenylalanine peptides. It is doubtful if such peptides would produce any stimulatory drift in assay values with increasing amounts of added filtrate.

The results of the present work appear to be directly applicable to further elucidation of the role of *S. lactis* in the ripening of cheddar cheese. Since a large proportion of *S. lactis* cells are trapped in the curd and remain viable in the cheese in large numbers for several days and may be present in decreasing numbers for months, it seems quite plausible that these organisms would bring about some increase in the soluble nitrogen. It would appear that this change would include some breakdown of the cheese protein to the various intermediate and smaller fractions and small quantities of most of the amino acids present in milk proteins. Even though the *S. lactis* cells in the cheese die off during the early stages of ripening, their proteolytic enzymes may continue active after death and autolysis of the cells. The lactobacilli which are important in the later stages of ripening are known to have complex amino acid requirements. The amino acids and smaller fractions of protein degradation provided by action of the proteolytic enzymes of *S. lactis* on the cheese protein should help provide the necessary stimulus for the development of the lactobacilli.

The factor stimulatory for *L. delbrueckii* LD5 present in the lactic acid filtrates of the *S. lactis* culture also may be present in cheese and enhance the growth of lactobacilli during ripening. It is believed that this effect may be similar to that observed by Hansen (5) when he demonstrated that extracts of *S. lactis* have a stimulatory effect upon the growth of *L. casei*.

SUMMARY

Microbiological assays for ten amino acids performed on tungstic and lactic acid filtrates of skimmilk cultures of five strains of *Streptococcus lactis* after ex-

tended incubation revealed marked increases over values for the uninoculated control filtrates. Higher assay values obtained on the lactic acid filtrates were attributed to the presence of a higher proportion of the lower fractions of protein degradation which contain amino acid residues available to the assay organisms.

Assays for leucine and phenylalanine on both unhydrolyzed filtrates prepared from cultures of two strains of *S. lactis* after various periods of incubation indicated that active strains are able to effect a marked increase in the microbiologically available forms of these two amino acids during the first 3 days of incubation and a slower increase through the fourteenth day. Simultaneous increases in the assay values of the hydrolyzed lactic acid filtrates indicated that the total concentration of protein degradation products increased throughout the 14-day incubation period.

Evidence is presented which indicates the presence of an intermediate fraction in sterilized skimmilk which is probably proteose or peptone in nature. This fraction contains a considerable amount of leucine but very little phenylalanine.

The role of *S. lactis* in cheese ripening is discussed in light of the present work.

REFERENCES

(1) BRAZ, M., AND ALLEN, L. A. Protein Metabolism and Acid Production by Lactic Acid Bacteria in Milk. Influence of Yeast Extract and Chalk. J. Dairy Research, **10**: 20–34. 1939.

(2) DUNN, M. S., AND MCCLURE, L. E. The Response of *Lactobacillus casei* to Partial Hydrolysis of Protein. J. Biol. Chem., **184**: 223–233. 1950.

(3) GUNNES, M., DWYER, I. M., AND STOKES, J. L. Microbiological Methods for the Determination of Amino Acids. III. Extension of the Uniform Assay Method for the Ten Essential Amino Acids to Include Tyrosine. J. Biol. Chem., **163**: 159–168. 1946.

(4) HAMMER, B. W., AND BABEL, F. J. Bacteriology of Butter Cultures: A Review. J. Dairy Sci., **26**: 83–168. 1943.

(5) HANSEN, P. A. A Study in Cheese Ripening. The Influence of Autolyzed Cells of *Streptococcus cremoris* and *Streptococcus lactis* on the Development of *Lactobacillus casei*. J. Dairy Sci., **24**: 969–976. 1941.

(6) HARPER, W. I., AND SWANSON, A. M. The Determination of Amino Acids in Cheddar Cheese and their Relationship to the Development of Flavor. Proc. World's Dairy Congr., 12th Congress, Stockholm, Sec. II, 147–155. 1949.

(7) HAWK, P. B., AND BERGEIM, O. *Practical Physiological Chemistry*. 11th ed., p. 149. The Blakiston Co., Philadelphia. 1939.

(8) HIER, S. W., AND BERGEIM, O. Microbiological Determination of Free Leucine, Isoleucine, Valine and Threonine in Dog Plasma. J. Biol. Chem., **161**: 717–722. 1945.

(9) KREHL, W. A., AND FRUTON, J. S. The Utilization of Peptide by Lactic Acid Bacteria. J. Biol. Chem., **173**: 479–485. 1948.

(10) MERRILL, A. T. A Note on the Relation of pH to Tungstic Acid Precipitation of Protein. J. Biol. Chem., **60**: 257–259. 1924.

(11) NIVEN, C. F., JR. Nutrition of *Streptococcus lactis*. J. Bact. **47**: 343–350. 1944.

(12) REIHARD, D. G., AND GAREY, J. C. Microbiological Determination of Free Amino Acids in Cheese during the Curing Period. Abs. of Papers, 49th meeting. Soc. of Am. Bacteriologists, p. 52. 1949.

(13) ROCKLAND, L. B., AND DUNN, M. S. Apparatus for Rapid Electrometric Titration of Acid. Ind. Eng. Chem., Anal. Ed., **19**: 812–813. 1947.

(14) ROGOSA, M. The Significance in Nutritional Research of Correct Identification of *Lactobacillus casei, Lactobacillus delbrueckii* and *Lactobacillus bulgaricus*. J. Bact., **51**: 575. 1946.

(15) ROWLAND, S. J. The Heat Denaturation of Albumin and Globulin. II. Denaturation and Degradation of Protein at Temperatures of 75–120° C. J. Dairy Research, **8**: 1–5. 1937.

(16) SIMMONDS, S., TATUM, E. L., AND FRUTON, J. S. The Utilization of Phenylalanine and Tyrosine Derivatives by Mutant Strains of *Escherichia coli*. J. Biol. Chem., **169**: 91–101. 1947.

(17) SIMMONDS, S., TATUM, E. L., AND FRUTON, J. S. The Utilization of Leucine Derivatives by a Mutant Strain of *Escherichia coli*. J. Biol. Chem., **170**: 483–489. 1947.

(18) STOKES, J. L., GUNNESS, M., DWYER, I. M., AND CASWELL, M. C. Microbiological Methods for the Determination of Amino Acids. II. A Uniform Assay for the Ten Essential Amino Acids. J. Biol. Chem., **160**: 34–49. 1945.

PROGRAM

FORTY-SIXTH ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF TENNESSEE

KNOXVILLE, TENNESSEE

JUNE 6–8, 1951

GENERAL PROGRAM COMMITTEE

G. M. TROUT, Michigan, *Chairman*

J. H. HETRICK, Rockford, Ill. — R. ALBRECTSEN, New York

L. O. GILMORE, Ohio — C. E. WYLIE, Tennessee

GENERAL PROGRAM

Tuesday, June 5, 1951

10:00 a.m. REGISTRATION, *Alumni Memorial Auditorium*

7:30 p.m. INFORMAL GET-TOGETHER, *Alumni Memorial Auditorium*

Wednesday, June 6, 1951

8:00 a.m. REGISTRATION, *Alumni Memorial Auditorium*

9:30–12:00 a.m. OPENING SESSIONS, *Alumni Memorial Auditorium*

C. E. WYLIE, *University of Tennessee,* presiding

Prelude

MRS. LOUIS O. BALL

National Anthem

Leader—RALPH W. FROST

Invocation

DR. CLIFFORD BARBOUR

Greetings

DR. C. E. BREHM, *President, University of Tennessee*

Presidential Address

DR. R. B. BECKER, *University of Florida*

Grasslands and Hydroelectric Power

DR. HARRY A. CURTIS, *Director, Tennessee Valley Authority*

Impact of Atomic Energy on Agriculture

DR. PAUL B. PEARSON, *Chief, Biology Branch, Atomic Energy Commission*

Announcements

1:30– 4:30 p.m. SECTION MEETINGS

Manufacturing Section

Symposium on Milk Fat

Court Room, Law Building

Production Section A
Genetics and Reproduction (Male)
Room 203, Dabney Hall
Production Section B
Roughage
Auditorium, Ferris Hall
Extension Section
Opening Business Session, Teaching Methods and Exhibits
Rooms 203 and 201, Physics and Geology Building

8:00 p.m. ENTERTAINMENT, *Alumni Memorial Auditorium*

Thursday, June 7, 1951

9:00–12:00 a.m. SECTION MEETINGS
Manufacturing Section A
Cheese
Court Room, Law Building
Manufacturing Section B
Cream, Butteroil, Chemistry
Room 54, Biology Building
Production Section A
Reproduction (female)
Auditorium, Ferris Hall
Production Section B
Physiology
Room 203, Dabney Hall
Extension Section
Dairy Herd Improvement Associations
Room 203, Physics and Geology Building

11:00 a.m. **Production Section Business Meeting**
Auditorium, Ferris Hall

11:15 a.m. **Manufacturing Section Business Meeting**
Court Room, Law Building

1:30– 5:00 p.m. JOINT SESSION OF EXTENSION AND PRODUCTION SECTIONS
Joint committee Reports
Symposium—The Role of Roughage in Milk Production
Auditorium, Ferris Hall

1:30– 4:30 p.m. MANUFACTURING SECTION MEETINGS
Manufacturing Section A
Microbiology, Pasteurization
Court Room, Law Building
Manufacturing Section B
Evaporated Milk, Chemistry
Room 54, Biology Building

6:30 p.m. BARBECUE
Association Announcements and Awards
Alumni Memorial Auditorium

Friday, June 8, 1951

9:00–12:00 a.m. SECTION MEETINGS

Manufacturing Section A
Cheese, Ice Cream
Court Room, Law Building

Manufacturing Section B
Dried Milk, Milk, Frozen Milk and Chocolate Milk
Room 54, Biology Building

Production Section A
Management
Auditorium, Ferris Hall

Production Section B
Calf Nutrition
Room 203, Dabney Hall

Extension Section
4-H Club Work, Dairy Cattle Breeding
Room 203, Physics and Geology Building

11:15 a.m. **Manufacturing Section Business Meeting**
Court Room, Law Building

1:30– 3:00 p.m. SECTION MEETINGS

Manufacturing Section
Symposium on Training of Students at the College Level for the Dairy Industry
Room 54, Biology Building

Production Section A
Mineral Metabolism
Auditorium, Ferris Hall

Production Section B
Feeding
Room 203, Dabney Hall

Extension Section
Papers, Committee Reports, Committee Appointments
Room 203, Physics and Geology Building

3:00 p.m. ASSOCIATION BUSINESS MEETING
Auditorium, Ferris Hall
Adjournment

ENTERTAINMENT FOR ALL

TUESDAY EVENING: Informal get-to-gether in Memorial Auditorium

WEDNESDAY EVENING: Entertainment for everybody

THURSDAY EVENING: Barbeque, Folk Dancing

ENTERTAINMENT FOR LADIES

WEDNESDAY: 1:00 P.M., Style Show—Luncheon at Andrew Johnson Hotel

THURSDAY: Trip to Clingman's Dome—Leave at 9:30 a.m.
—Box Lunch at Chimneys
FRIDAY: Local Tours
CHILDREN'S PROGRAM
AGE GROUP: 3 to 5—U. T. Nursery School
AGE GROUP: 6 to 12—U. T. Physical Education Department—Swimming—Tennis—Etc.

SPECIAL GROUP MEETINGS

(Those on which information was available at press time)

June 6

ECKLES CLUB BREAKFAST, *Cafeteria*
JOURNAL WORKERS LUNCHEON, *Cafeteria*
TENNESSEE ALUMNI DINNER, *McCord Hall*
PENNSYLVANIA STATE DINNER (*tentative*)

June 7

IOWA GROUP BREAKFAST, *Cafeteria*
SOUTHERN AGRICULTURE GROUP LUNCHEON, *Cafeteria*
MINNESOTA GROUP LUNCHEON (*tentative*)
OHIO GROUP, 4:30 p.m., *McCord Hall*

June 8

MISSOURI GROUP BREAKFAST, *Cafeteria*

PROGRAM OF PRODUCTION SECTION

Wednesday, June 6, 1951

1:30– 4:30 p.m. Section A. **GENETICS AND REPRODUCTION (MALE).** L. O. GILMORE, *Chairman*
Room 203, Dabney Hall

P1 A Factor Contributed by the Male Resulting in Early Bovine Embryonic Mortality. G. W. SALISBURY, *Univ. of Illinois,* AND R. W. BRATTON, *Cornell Univ.*

P2 Progress Report on Red Sindhi Crossbreeding. M. H. FOHRMAN AND R. E. MCDOWELL, *Bureau of Dairy Industry*

P3 Heritability of the Percentage of White Hair Area in Ayrshires. G. C. PICKARD AND W. J. TYLER, *W. Virginia Univ.*

P4 Prediction Value of Dairy Pedigrees. N. R. THOMPSON, *Virginia Polytechnic Inst.*

P5 Sex-influenced Color Inheritance in Cattle. C. S. BALDWIN, L. O. GILMORE AND D. C. RIFE, *Ohio State Univ.*

P6 Uniformity of Semen Production Behavior in Monozygous Triplet Bulls. H. H. OLSON AND W. E. PETERSEN, *Univ. of Minnesota*

P7 The Storage of Egg Yolk-Sodium Citrate Semen Diluter. V. HURST, *S. Carolina Agr. Expt. Sta.*

P8 Some Biases to Consider in Measuring Fertility in Artificial Insemination. R. H. FOOTE, C. R. HENDERSON AND R. W. BRATTON, *Cornell Univ.*

P9 Semen Production and Fructose Content of Semen as Related to Controlled Levels of Sexual Excitement of Dairy Bulls. C. BRANTON AND G. D'ARENSBOURG, *Louisiana State Univ.*

P10 The Fertility of Bovine Semen in Diluters Containing Varying Amounts of Egg Yolk. J. O. ALMQUIST AND D. L. THACKER, *Pennsylvania Agr. Expt. Sta.*

P11 The Effect of Dead and Immotile Sperm on the Motility and Fertility of the Stronger Sperm in Bovine Semen. H. J. BEARDEN AND E. W. SWANSON, *Univ. of Tennessee*

P12 Metabolic Response of Bovine Semen to Sodium Arsenite. D. MAHLER AND A. B. SCHULTZE, *Univ. of Nebraska*

1:30– 4:30 p.m. Section B. **ROUGHAGE.** N. N. ALLEN, *Chairman*
Auditorium, Ferris Hall

P13 A Comparison of U. S. Grades of Alfalfa Hay for Milk Production. O. H. HORTON AND K. E. HARSHBARGER, *Univ. of Illinois*

P14 "Ballast" and Calculated Net Energy as Related to the Unidentified Factor(s) Needed to Balance the Total Digestible Nutrients in Roughage. C. F. HUFFMAN, C. W. DUNCAN AND C. M. CHANCE, *Michigan Agr. Expt. Sta.*

P15 The Effect of Physical State on the Utilization of Dehydrated Alfalfa. T. H. BLOSSER, F. R. MURDOCK, R. E. ERB AND A. O. SHAW, *State Coll. of Washington*

P16 Comparative Digestion Studies on Orchard Grass. E. A. KANE, R. E. ELY, W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry*

P17 Metabolism of Lignin in Orchard Grass Hay Cut at Various Stages of Maturity. R. E. ELY, W. C. JACOBSON AND E. A. KANE, *Bureau of Dairy Industry*

P18 Rumen Digestion of Some Crude Fiber Constituents. J. T. MILES, *Univ. of Wisconsin*

P19 The Relation of Roughage Intake to the Fat Content of the Milk and the Level of Fatty Acids in the Rumen. W. TYZNIK AND N. N. ALLEN, *Univ. of Wisconsin*

P20 The Effects of Added Corn Meal and Dried Whey and of Moisture Level on the Preservation and Quality

of Alfalfa Silage Stored in Miniature Silos. T. G. MARTIN, A. R. PORTER AND G. E. STODDARD, *Iowa State College*

P21 A Comparison of Half-Dry and Slightly-Wilted Alfalfa Stored in Gas-Tight Steel Silos. C. H. GORDEN, J. B. SHEPHERD, H. G. WISEMAN AND C. G. MELIN, *Bureau of Dairy Industry*

P22 Sulfur Dioxide Silages. S. R. SKAGGS, *Pennsylvania State Coll.*

P23 Influences of Temperature and Atmosphere on Silage Preservation. A. O. SHAW, N. S. GOLDING AND U. S. ASHWORTH, *State Coll. of Washington*

P24 A Procedure for Measuring the Digestibility of Pasture Grass Under Grazing Conditions. J. T. REID, P. G. WOOLFOLK, W. A. HARDISON, C. M. MARTIN, A. L. BRUNDAGE AND R. W. KAUFMANN, *Cornell Univ.*

P25 Use of the Chromogen(s) Technique in the Determination of Seasonal Changes in Digestibility of Dry Matter and Protein of Pasture Herbage. M. MCCULLOUGH, R. F. ELLIOTT AND G. M. BASTIN, *Univ. of Kentucky*

Thursday, June 7, 1951

9:00–12:00 a.m. Section A. **REPRODUCTION (FEMALE).** L. O. GILMORE, *Chairman*
Auditorium, Ferris Hall

P26 Reproductive Efficiency in a Holstein Herd 1897–1950. H. P. DAVIS, *Univ. of Nebraska*

P27 Blood Changes in the Nymphomaniac Cow. O. WAYMAN AND S. A. ASDELL, *Cornell Univ.*

P28 A Comparison of the Effectiveness of Estrogens in Inducing Estrus in Dairy Cattle. R. P. REECE, *N. J. Agr. Expt. Sta.*

P29 Spectrophotometric Analysis of Neutral Steroids of Cattle Urine. J. P. MIXNER, *N. J. Agr. Expt. Sta.*

P30 Progesterone and the Corpus Luteum in Maintenance of Pregnancy in Dairy Heifers. J. L. RAESIDE AND C. W. TURNER, *Missouri Agr. Expt. Sta.*

P31 The Effect of Progesterone on Ovulation Time in Dairy Heifers. W. HANSEL AND G. W. TRIMBERGER, *Cornell Univ.*

P32 The Effect of Administering an Unfractionated Gonadotrophic Pituitary Extract During Estrus on the Time of Ovulation and Length of the Estrual Period of Dairy Heifers. G. B. MARION AND V. R. SMITH, *Univ. of Wisconsin*

P33 Stimulatory Action of Breeding on the Release of Oxytocin as Measured by Intramammary Pressure. R. L. HAYS AND N. L. VANDEMARK, *Univ. of Illinois*

P34 Pregnancy Interruption and Breeding Techniques in the Artificial Insemination of Cows. N. L. VANDEMARK AND L. E. BOLEY, *Univ. of Illinois*

P35 A Method for the Intrauterine Insemination of Cows. N. S. FECHHEIMER, T. M. LUDWICK AND F. ELY, *Ohio State Univ.*

P36 The Effect of Incipient Vitamin A Deficiency on Female Reproduction in the Rabbit. G. E. LAMMING AND G. W. SALISBURY, *Univ. of Illinois*

P37 A Meristic Mutation Involving Teat Number in the Cow. W. W. YAPP AND L. E. ST. CLAIR, *Univ. of Illinois*

9:00–12:00 a.m. Section B. **PHYSIOLOGY.** N. N. ALLEN, *Chairman. Room 203, Dabney Hall*

P38 Evaluation of Changes in Adrenal Cortical Secretion Rate by Measurement of Changes in Circulating Eosinophils of Dairy Cattle. J. E. JOHNSTON, L. L. RUSOFF AND G. D'ARENSBOURG, *Louisiana State Univ.*

P39 Further Studies on the Use of Cortisone in the Treatment of Ketosis in Dairy Cattle. J. C. SHAW AND B. C. HATZIOLOS, *Univ. of Maryland*

P40 Hormonal Development of Mammary Tissue in Dairy Heifers. J. F. SYKES AND T. R. WRENN, *Bureau of Dairy Industry*

P41 Trends in the Incidence of Mastitis Infections by Different Organisms. L. A. BURKEY, CECILIA R. BUCKNER, P. C. UNDERWOOD AND W. W. SWETT, *Bureau of Dairy Industry*

P42 Recent Developments in the Use of Vitamin D in the Prevention of Milk Fever in Dairy Cows. J. W. HIBBS AND W. D. POUNDEN, *Ohio Agr. Expt. Sta.*

P43 Effect of Environmental Temperature on Hunger and Thirst in Cows. S. BRODY, A. C. RAGSDALE, H. H. KIBLER AND C. R. BLINCOE, *Missouri Agr. Expt. Sta.* and H. J. THOMPSON AND D. M. WORSTELL, *BPISAE, USDA.*

P44 The Effect of Clipping on the Population of Chewing Lice (*Bovicola bovis*) on Dairy Heifers. N. N. ALLEN AND R. J. DICKE, *Univ. of Wisconsin*

P45 Inhibition of the Proteolytic Activity of Trypsin by Green Plant Extracts. K. A. KENDALL, *Univ. of Illinois*

P46 Effect of Previous Lactose Feeding upon Intestinal Absorption of Lactose in the Rat. JESSIE FISCHER AND T. S. SUTTON, *Ohio State Univ.*

P47 Antibiotic Studies with Dairy Calves. J. K. LOOSLI, R. H. WASSERMAN AND L. S. GALL, *Cornell Univ.*

P48 The Response to Aureomycin Supplementation of Young Dairy Calves Fed Various "Practical" and Restricted Diets. W. R. MURLEY, N. L. JACOBSON, J. M. WING AND G. E. STODDARD, *Iowa Agr. Expt. Sta.*

P49 Effect of Aureomycin on Growth of Young Calves Weaned from Milk at an Early Age. L. L. RUSOFF AND A. V. DAVIS, *Louisiana State Univ.*

P50 The Effect of a Terramycin Supplement on the Growth and Well-being of Dairy Calves. J. L. CASON AND H. H. VOELKER, *Univ. of Arkansas*

1:30– 5:00 p.m. **JOINT SESSION OF EXTENSION AND PRODUCTION SECTIONS**

L. O. GILMORE AND R. ALBRECTSEN, *Co-chairmen*
Auditorium, Ferris Hall

Joint Committee Reports

Breeds Relations. A. R. PORTER, *Chairman*
Dairy Cattle Health. W. D. KNOX, *Chairman*
Dairy Cattle Breeding. J. TAYLOR, *Chairman*
Type. M. J. REGAN, *Chairman*
Purebred Dairy Cattle Association. F. IDTSE, *Secretary*
Antibiotics. W. A. KRIENKE, *Chairman*

Symposium—The Role of Roughage in Milk Production

Milk Potentials of Roughage. J. B. KITCHEN, JR., *Rutgers Univ.*

Advantages of Roughage in Economical Milk Production as Shown by D. H. I. A. Records. W. T. CRANDALL, *Cornell Univ.*

Feeding Quality Forage to Dairy Cattle. L. A. MOORE, *Bureau of Dairy Industry*

Possibility of Unidentified Nutritional Factors in Hay. C. F. HUFFMAN, *Michigan State Coll.*

How Much Concentrates for Dairy Cows? F. B. MORRISON, *Cornell Univ.*

Friday, June 8

9:00–12:00 a.m. Section A. **MANAGEMENT.** L. O. GILMORE, *Chairman*
Auditorium, Ferris Hall

P51 The Effect of Environmental Temperature on the Composition of Milk. J. W. COBBLE, H. A. HERMAN AND A. C. RAGSDALE, *Missouri Agr. Expt. Sta.*

P52 The Influence of Season on Persistency of Milk Production. D. M. SEATH AND M. MCCULLOUGH, *Univ. of Kentucky*

P53 The Effects of Mastitis on Milk and Butterfat Production. M. C. HERVEY, *Univ. of Minnesota*

P54 Influence of Frequency of Milking Upon Milk Secretion. W. E. PETERSEN, T. W. GULLICKSON AND L. S. MIX, *Univ. of Minnesota*

P55 The Accuracy of Mammary Gland Measurements on 3-, 4- and 5-month-old Heifer Calves. R. W. TOUCHBERRY, *Univ. of Illinois,* AND K. A. TABLER, *Bureau of Dairy Industry*

P56 Some Causes of Variation in Type Ratings of Ayrshire Cows. R. H. BENSON, W. J. TYLER AND G. HYATT, JR., *West Virginia Univ.*

P57 Prepartum II. Some Effects on Production of Cows and Growth of Calves. R. A. ACKERMAN AND G. HYATT, JR., *West Virginia Univ.*

P58 Toxicological Effects of Toxaphene on Dairy Cows. R. E. LEIGHTON, K. A. KUIKEN AND H. A. SMITH, *Texas Agr. Expt. Sta.*

P59 Open Shed versus Conventional Housing for Dairy Calves. R. E. ERB AND F. R. MURDOCK, *State Coll. of Washington*

P60 Methods of Milking and Milk Handling as Factors Affecting the Quality and Economy of Milk Produced. M. H. ALEXANDER AND E. E. ORMISTON, *Univ. of Illinois*

P61 The Relationship Between Early Growth and Later Butterfat Production in Dairy Cows. B. N. SINGH AND M. PLUM, *Univ. of Nebraska*

9:00–11:00 a.m. Section B. **CALF NUTRITION.** N. N. ALLEN, *Chairman Room 203, Dabney Hall*

P62 Intravenous and Oral Administration of an Aqueous Suspension of Carotene to Calves Depleted of their Vitamin A Stores. H. D. EATON, L. D. MATTERSON, L. M. DECKER, C. F. HELMBOLDT AND E. L. JUNGHERR *Storrs Agr. Expt. Sta. Univ. of Connecticut*

P63 The Absorption of Vitamin A Natural Esters and Carotene from the Digestive Tract in Male Holstein Calves. M. RONNING AND C. B. KNODT, *Pennsylvania State Coll.*

P64 Manganese, Calcium and Phosphorus Interrelationships in the Nutrition of Dairy Calves. G. E. HAW-

KINS, JR., G. H. WISE, W. L. LOTT AND G. MATRONE, *N. Carolina Agr. Expt. Sta.*

P65 Distillers' Grain Solubles in Calf Starters. R. F. ELLIOTT, D. M. SEATH AND G. M. BASTIN, *Kentucky Agr. Expt. Sta.*

P66 The Nutritive Value of Dried Distillers' Corn Solubles for Dairy Calves as Determined by Feeding, Digestion, and Palatability Trials. S. T. SLACK AND K. L. TURK, *Cornell Univ.*

P67 Use of Activated Glycerol Dichlorohydrin in the Analysis of Dairy Calf Blood Plasma for Vitamin A. R. S. ALLEN, P. G. HOMEYER AND C. Y. CANNON, *Iowa Agr. Expt. Sta.*

P68 Effect of Glycerol-monostearate, an Emulsifying Agent, on Fat Absorption, Growth and Health of Calves. J. S. HUFF, R. K. WAUGH AND G. H. WISE, *N. Carolina Agr. Expt. Sta.*

P69 A Study of the Lipids in the Blood Plasma of Young Dairy Calves. J. H. ZALETEL, R. S. ALLEN AND N. L. JACOBSON, *Iowa Agr. Expt. Sta.*

P70 B-Vitamin Nutrition of the Dairy Calf Studied with "Synthetic Milk" Diets. B. C. JOHNSON, W. B. NEVENS AND H. H. MITCHEL, *Univ. of Illinois*

P71 Concentrations of Certain B Vitamins in the Digestive Tract Contents of Young Dairy Calves. E. M. KESLER AND C. B. KNODT, *Pennsylvania State Coll.*

P72 Apparent Digestion of Nutrients in Colostrum and Milk by Calves during the First 18 Days of Life. D. B. PARRISH, D. U. BURRIS, R. T. MCINTYRE AND E. E. BARTLEY, *Kansas Agr. Expt. Sta.*

11:00 a.m. **SECTION BUSINESS MEETING**

Auditorium, Ferris Hall

1:30– 3:00 p.m. Section A. **MINERAL METABOLISM.** L. O. GILMORE, *Chairman*

Auditorium, Ferris Hall

P73 Daily Fecal Excretion of Calcium, Sodium and Potassium and Fecal pH of Eleven Dairy Cows at the Time of Parturition. G. M. WARD AND M. F. ADAMS, *State Coll. of Washington*

P74 Some Changes in the Blood Levels of Pyruvic, Lactic and Citric Acids and Hematocrit Values in Cows Treated for Milk Fever. G. M. WARD, T. H. BLOSSER AND J. B. CRILLY, *State Coll. of Washington*

P75 Effects of Feeding Thyroprotein to Dairy Cows for Successive Lactations—a Report of Progress. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry*

P76 Metabolism of Radioactive Iodine in the Newborn Calf and in the Dam at Parturition. R. A. MONROE, E. W. SWANSON AND C. E. WYLIE, *Univ. of Tennessee*

P77 Behavior of Radiocalcium in the Lactating Goat after Oral and Intravenous Administration. W. J. VISEK AND J. K. LOOSLI, *Cornell Univ.*

P78 Integration of Concurrent Chemical and Radioisotope Balance Trials for the Interpretation of Calcium Metabolism Studies. S. L. HANSARD, C. L. COMAR, M. P. PLUMLEE AND C. S. HOBBS, *Tennessee Agr. Expt. Sta.*

P79 The Transfer of Calcium from Pregnant Heifers to Fetus Following Single Oral or Intravenous Dosage Using Calcium^{45}. M. P. PLUMLEE, S. L. HANSARD, C. L. COMAR AND C. S. HOBBS, *Tennessee Agr. Expt. Sta.*

1:30– 3:00 p.m. Section B. **FEEDING.** N. N. ALLEN, *Chairman*
Room 203, Dabney Hall

P80 Liveweight Gains of Yearling Dairy Heifers with Pasture as the Only Feed. W. B. NEVENS AND S. C. HOWERTER, *Univ. of Illinois*

P81 The Nutritive Value of Lespedeza Hay for Wintering Dairy Heifers. H. H. VOELKER AND O. T. STALLCUP, *Univ. of Arkansas*

P82 Detection of Adulteration in Alfalfa Meal by Means of the Phosphatase Test. G. P. SANDERS, J. A. HUPFER, JR. AND H. G. WISEMAN, *Bureau of Dairy Industry*

P83 Effects of Supplementing Dairy Cow Rations with Dehydrated Alfalfa. E. E. BARTLEY, D. B. PARRISH, F. C. FOUNTAINE AND C. H. WHITNAH, *Kansas Agr. Expt. Sta.*

P84 Nutritive Value of Cottonseed Meals for Dairy Cattle. W. A. KING AND D. B. RODERICK, *S. Carolina Agr. Expt. Sta.*

P85 Expeller vs. Extracted Linseed Oil Meal for Milk Production. A. D. PRATT AND C. F. MONROE, *Ohio Agr. Expt. Sta.*

P86 The Effect of Feeding Moderate Amounts of Ground Soybeans, Soybean Oil Meal and Linseed Oil Meal on the Level of Carotenoids and Vitamin A in the Milk and Blood of Dairy Cattle. C. F. MONROE, J. W. HIBBS AND R. G. WASHBURN, *Ohio Agr. Expt. Sta.*

P87 Progress Report on an Experiment to Determine the Effects of Feeding During Early Life Upon Subse-

quent Productive and Reproductive Performance of Dairy Cattle. J. T. Reid, G. W. Trimberger, S. A. Asdell, K. L. Turk and S. E. Smith, *Cornell Univ.*

P88 The Relationship of the Drying Temperature to the Nutritive Value of Corn. I. L. Hathaway, F. D. Yung and T. A. Kiesselbach, *Nebraska Agr. Expt. Sta.*

3:00 p.m. **ASSOCIATION BUSINESS MEETING**
Auditorium, Ferris Hall

PROGRAM OF MANUFACTURING SECTION

Wednesday, June 6

1:30– 4:30 p.m. **SYMPOSIUM ON MILK FAT.** J. H. Hetrick, *Chairman*
Court Room, Law Building

The Fat Emulsion in Milk from a Chemical Standpoint
H. H. Sommer, *Univ. of Wisconsin*
Discussion Leader: E. W. Bird, *Iowa State Coll.*

The Physical Behavior of Milk Fat
A. H. Rishoi, *Cherry-Burrell Corp., Chicago, Ill.*
Discussion Leader: G. C. North, *Beatrice Foods Co., Chicago, Ill.*

Oxidative Changes Which May Occur in Milk Fat
F. A. Kummerow, *Univ. of Illinois*
Discussion Leader: W. C. Brown, *Kraft Foods, Glenview, Ill.*

Nutritional Properties of Milk Fat
C. A. Baumann, *Univ. of Wisconsin*
Discussion Leader: E. L. Jack, *Univ. of California*

Thursday, June 7

9:00–11:15 a.m. Section A. **CHEESE.** O. F. Garrett, *Chairman*
Court Room, Law Building

M1 A Study of Changes in Cheese Protein during Ripening. H. J. Bassett, K. R. Spurgeon and A. M. Swanson, *Univ. of Wisconsin*

M2 The Order of Appearance of Amino Acids during Ripening of Cheddar Cheese as Determined by Paper Chromatography. C. J. Honer and S. L. Tuckey, *Univ. of Illinois*

M3 The Proteinases of *Streptococcus lactis* and *Lactobacillus casei* and their Relationship to Cheese Ripening. L. E. Baribo and E. M. Foster, *Univ. of Wisconsin*

M4 The Identification of Sugars in Cheddar Cheese during the Initial Stages of Ripening. H. J. Fagen, J. B.

STINE AND R. V. HUSSONG, *Kraft Foods Co. Research Laboratories, Glenview, Ill.*

M5 A Chromatographic Method for the Determination of the Lower Fatty Acids in Cheese. W. L. HOCK, JR., O. J. KRETT AND R. V. HUSSONG, *Kraft Foods Co. Research Laboratories, Glenview, Ill.*

M6 The Role of the Lower Fatty Acids in Swiss Cheese. O. J. KRETT AND J. B. STINE, *Kraft Foods Co. Research Laboratories, Glenview, Ill.*

M7 A Preliminary Report on the Bacteriology of Provolone and Romano Cheese. K. T. MASKELL, R. E. HARGROVE AND R. P. TITTSLER, *Bureau of Dairy Industry, U.S.D.A.*

M8 Lipase System of Rennet Pastes with Reference to the Ripening of Italian Varieties of Cheese. W. J. HARPER, *Ohio State Univ.*

9:00–11:15 a.m. Section B. **CREAM, BUTTEROIL, CHEMISTRY.** E. L. JACK, *Chairman*
Room 54, Biology Building

M9 The Field Estimation of Water-insoluble Acids in Cream. R. A. GREENBERG, W. O. NELSON AND W. A. WOOD, *Univ. of Illinois*

M10 The Liberation of Water-insoluble Acids in Cream by *Geotrichum candidum*. M. PURKO AND W. O. NELSON, *Univ. of Illinois.*

M11 Butyric Acid and Water-insoluble Acid Values of Cream Held at Temperatures of 38 to 85° F. I. I. PETERS, L. T. KESTER AND F. E. NELSON, *Iowa Agr. Expt. Sta.*

M12 Studies on the Water-insoluble Acids, Butyric Acid and Fat Acidity of Butter. C. E. PARMELEE AND F. J. BABEL, *Purdue Univ.*

M13 A New Method of Manufacturing Butteroil. C. M. STINE AND S. PATTON, *Pennsylvania State Coll.*

M14 The Cryoscope as an Aid in the Detection of Neutralized Cream. W. A. KRIENKE AND E. L. FOUTS, *Florida Agr. Expt. Sta.*

M15 The Esterifying Enzyme(s) of Milk. A Preliminary Report. G. R. GREENBANK, *Bureau of Dairy Industry, U.S.D.A.*

11:15–12:00 a.m. **SECTION BUSINESS MEETING.**
Court Room, Law Building

1:30– 4:30 p.m. Section A. **MICROBIOLOGY, PASTEURIZATION.** P. R. ELLIKER, *Chairman*
Court Room, Law Building

M16 Effect of Penicillin on Certain Microorganisms in Milk. H. H. WILKOWSKE, W. A. KRIENKE AND E. L. FOUTS, *Univ. of Florida*

M17 Multiple Strain Bacteriophage Infection of a Lactic Culture used for Manufacture of Blue Cheese. D. D. DEANE AND F. E. NELSON, *Iowa Agr. Expt. Sta.*

M18 Mutation to Bacteriophage Resistance in Pure Cultures of Lactic Streptococci. L. E. MULL AND F. E. NELSON, *Iowa Agr. Expt. Sta.*

M19 Action of Certain Viricidal Agents on Lactic Streptococcus Bacteriophage. F. W. BENNETT, *Univ. of Georgia,* AND F. E. NELSON, *Iowa Agr. Expt. Sta.*

M20 Selective Sanitizing Effect of Dilute Lye Solutions as Shown by Recovery of *Streptococcus lactis* Types of Bacteria from Lye-treated Teat Cup Liners. T. J. CLAYDON, *Kansas Agr. Expt. Sta.*

M21 Some Factors Influencing the Isolation and Quantitative Estimation of *Pseudomonas putrefaciens.* R. O. WAGENAAR AND J. J. JEZESKI, *Univ. of Minnesota*

M22 The Effect of Low Temperatures on the Growth and Biochemical Activity of Certain Microorganisms Important to the Dairy Industry. V. W. GREENE AND J. J. JEZESKI, *Univ. of Minnesota*

M23 The Amino Acid Requirements of *Bacterium linens.* M. E. FRIEDMAN AND W. A. WOOD, *Univ. of Illinois*

M24 High-temperature Short-time Pasteurization of Dairy Products. K. M. SHAHANI, E. O. HERREID AND Z. J. ORDAL, *Univ. of Illinois*

M25 Bacteriological Aspects of High-temperature Short-time Pasteurization of Ice Cream Mix. C. A. GROSCHE, M. L. SPECK AND H. L. LUCAS, *N. Carolina State Coll.*

M26 The Effectiveness of the Cornell Phosphatase Test for Dairy Products. F. V. KOSIKOWSKY, *Cornell Univ.*

M27 Influence of Buffer Concentrations, pH Values and Incubation Temperatures on Sensitivity and Accuracy of Phosphatase Tests. O. S. SAGER, G. P. SANDERS AND J. A. HUPFER, JR., *Bureau of Dairy Industry, U.S.D.A.*

1:30– 4:30 p.m. Section B. **EVAPORATED MILK, CHEMISTRY.** J. H. HETRICK, *Chairman*

Room 54, Biology Building

M28 Effect of Storage Temperature on Properties of Evaporated Milk. B. H. WEBB AND E. F. DEYSHER, *Bureau of Dairy Industry, U.S.D.A.*

M29 Gel Formation and Fat Separation in Evaporated

Milk as Affected by the State of Milk Proteins. N. P. TARASSUK AND H. D. SIMONSON, *Univ. of California*

M30 Isolation and Characterization of a Whey Constituent Capable of Producing the Solar-activated Flavor. B. R. WEINSTEIN, C. W. DUNCAN AND G. M. TROUT, *Michigan Agr. Expt. Sta.*

M31 An Electrophoretic Examination of the Minor-protein Fraction Capable of Producing the Solar-activated Flavor. B. R. WEINSTEIN, H. A. LILLEVIK, C. W. DUNCAN AND G. M. TROUT, *Michigan Agr. Expt. Sta.*

M32 The Sulfhydryl Groups of Milk Serum Proteins. B. L. LARSON AND R. JENNESS, *Univ. of Minnesota*

M33 A Test of the Heat Stability of Milk Proteins for Use in Research. R. McL. WHITNEY, KATHERINE PAULSON AND G. K. MURTHY, *Univ. of Illinois*

M34 Studies Relating to Rennet Coagulation Time and Rennet Curd Tension. L. A. KELLEY, A. M. SWANSON AND W. V. PRICE, *Univ. of Wisconsin*

M35 Observations on the Effect of Heat Treatment upon the Dissolved Calcium and Phosphorus in Skimmilk. M. HILGEMAN AND R. JENNESS, *Univ. of Minnesota*

M36 Determination of Unoxidized Tocopherols in Milk Fat. R. L. HANDWERK AND E. W. BIRD, *Iowa Agr. Expt. Sta.*

M37 Detection of Vegetable Fat in Milk Fat by the Tocopherol Determination. E. W. BIRD, D. J. PATEL AND R. L. HANDWERK, *Iowa Agr. Expt. Sta.*

M38 Studies on 2-Thiobarbituric Acid as a Reagent for Detecting Milk Fat Oxidation. G. W. KURTZ, E. F. PRICE AND S. PATTON, *Pennsylvania State Coll.*

M39 Additional Biochemical Properties of Fresh Milk Influencing the Development of Oxidized Flavors. V. N. KRUKOVSKY AND B. YORK, *Cornell Univ.*

Friday, June 8

9:00–11:15 a.m. Section A. **CHEESE, ICE CREAM.** E. M. BARKER, *Chairman*

Court Room, Law Building

M40 A Study of the Microflora of Blue Cheese Slime. C. B. HARTLEY AND J. J. JEZESKI, *Univ. of Minnesota*

M41 The Effect of Bleaching with Benzoyl Peroxide on the Vitamin A Potency of Milk and Blue Cheese. J. J. JEZESKI, S. KURAMOTO, W. B. COMBS AND H. A. MORRIS, *Univ. of Minnesota*

M42 Partition of Lactose, Citric Acid and Biacetyl during

the Manufacture of Cottage Cheese. M. A. KRISHNASWAMY AND F. J. BABEL, *Purdue Univ.*

M43 A Study of Process Cheese Emulsifiers. A. F. HOLTORFF, VIRGINIA MULARZ AND E. TRAISMAN, *Kraft Foods Co. Research Laboratories, Glenview, Ill.*

M44 The Endoprotease Activity of Some Oxidative Type Yeasts Isolated from Trappist Cheese. S. A. SZUMSKI AND J. F. CONE, *Pennsylvania State Coll.*

M45 Brick Cheese. H. J. BUYENS AND W. V. PRICE, *Univ. of Wisconsin*

M46 Retarding Moisture Loss and Flavor Deterioration in Packaged Ice Cream. J. A. MEISER, JR., *Michigan Agr. Expt. Sta.*

M47 A Study of the Effect of Mix Composition and Freezing Conditions upon Properties of Soft Ice Cream. P. F. ROSSI AND J. J. SHEURING, *Univ. of Georgia*

9:00–11:15 a.m. Section B. **DRIED MILK, MILK, FROZEN MILK AND CHOCOLATE MILK.** E. L. JACK, *Chairman*
Room 54, Biology Building

M48 The Effect of Various Steps in Manufacture on the Extent of Serum Protein Denaturation in Nonfat Dry Milk Solids. H. A. HARLAND, S. T. COULTER AND R. JENNESS, *Univ. of Minnesota*

M49 Estimation of the Serum Protein Content as a Method of Evaluating Nonfat Dry Milk Solids for Use in Cottage Cheese Manufacture. H. A. MORRIS, S. T. COULTER, W. B. COMBS AND L. R. HEINZEL, *Univ. of Minnesota*

M50 An Evaluation of the Methods Used to Determine the Baking Quality of Nonfat Dry Milk Solids. B. L. LARSON, R. JENNESS AND W. F. GEDDES, *Univ. of Minnesota*

M51 A Tactual Flavor Defect of Dried Milk. J. T. HUTTON AND D. V. JOSEPHSON, *Pennsylvania State Coll.*

M52 Separation and Recombination as a Means of Deferring an Oxidized (Cardboard) Flavor in Milk During Frozen Storage. T. J. MUCHA AND R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*

M53 Preservation of Chocolate Drink by Freezing. C. J. BABCOCK AND D. R. STROBEL, *Production and Marketing Admin., U.S.D.A.*, R. H. YAGER, *VC, USA*, AND E. S. WINDHAM, *Army Medical Center, Washington*

11:15–12:00 a.m. **SECTION BUSINESS MEETING**
Court Room, Law Building

Friday, June 8

1:30– 3:00 p.m. **SYMPOSIUM ON TRAINING OF STUDENTS AT THE COLLEGE LEVEL FOR THE DAIRY INDUSTRY.** O. F. GARRETT, *Chairman*
Room 54, Biology Building
Speakers: P. H. TRACY, *Univ. of Illinois.* College Viewpoint
B. W. FAIRBANKS, *Director, American Dry Milk Institute, Chicago, Illinois*
Industry Viewpoint
Discussion

3:00 p.m. **ASSOCIATION BUSINESS MEETING**
Auditorium, Ferris Hall

PROGRAM OF EXTENSION SECTION

WEDNESDAY, JUNE 6

1:30– 4:00 p.m. **OPENING BUSINESS SESSION AND TEACHING METHODS AND EXHIBITS.** R. ALBRECTSEN, *Chairman*
Room 203, Physics and Geology Building

E1 Unique Teaching Techniques on Subject "Milking and Mastitis." W. A. DODGE, *Univ. of Vermont*

E2 Selection of Colored Slides for Effective Extension Teaching. C. F. JOHNSON, *Pennsylvania State Coll.* Report of Teaching Methods Committee and Explanation and Discussion of Exhibits. (Exhibits in room 201)
S. GAUNT, *Committee Chairman in Charge, Univ. of Massachusetts*

Thursday, June 7

9:00–12:00 a.m. **DAIRY HERD IMPROVEMENT ASSOCIATIONS.** R. ALBRECTSEN, *Chairman*
Room 203, Physics and Geology Building

E3 Variation in Fat Test of Milk due to Mixing Procedure. M. SENGER, *Univ. of Maryland*

E4 Culling Practices in Iowa DHIA Herds. D. E. VOELKER, *Iowa State Coll.*

E5 Why Some Farmers Use and Others do not Use DHIA Program in Howard County, Maryland. M. E. SENGER, *Univ. of Maryland*
Panel Discussion and Report of Dairy Records Committee. D. E. VOELKER, *Committee Chairman in Charge*

1:30– 4:00 p.m. **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS.**

Auditorium, Ferris Hall

(See Production Section Program)

4:00– 5:00 p.m. **BUSINESS AND COMMITTEE MEETINGS**

Friday, June 8

9:00–12:00 a.m. **4-H CLUB WORK. DAIRY CATTLE BREEDING**

R. LEIGHTON, *Chairman*

Room 203, Physics and Geology Building

E6 Presenting Materials on the Junior Dairy Project to Extension Leaders. W. A. DODGE, *Univ. of Vermont*

E7 The Basis of a Sound 4-H Dairy Program. H. A. WILLMAN, *Cornell Univ.*

Report of 4-H Club Committee. G. W. VERGERONT, *Committee Chairman, Univ. of Wisconsin*

E8 North Central and Southern Dairy Cattle Breeding Research Projects under the Research and Marketing Act. M. H. FORHMAN, *Bureau of Dairy Industry*

E9 Progeny Testing of Bulls for Use in Artificial Insemination in England. J. L. LUSH, *Iowa State Coll.*

E10 Temporary Revision of Proved Sire Program. J. F. KENDRICK, *Bureau of Dairy Industry*

PAPERS AND COMMITTE REPORTS

Room 203, Physics and Geology Building

1:00– 3:00 p.m. E11 Review of Artificial Breeding in the Southern States. J. B. FRYE, JR., *Louisiana State Univ.*

E12 Production Results of Three Generations of Artificial Breeding and of the Use of Analyzed Sires. R. ALBRECTSEN, *Cornell Univ.*

Committee Reports

Committee Appointments

3:00 p.m. **ASSOCIATION BUSINESS MEETING**

Auditorium, Ferris Hall

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

249. Milk and Milk Products. 4th ed. C. H. ECKLES, W. B. COMBES and H. MACY. McGraw-Hill Book Co., Inc., New York. 454 pages; illus. $5.00. 1951.

This latest edition will be of interest to the instructor and students of an introductory course in dairy manufacturing. While no new sections have been added, some of the chapters have been revised or rewritten. New material concerning developments in the dairy industry during World War II is included. The vol. contains 95 illustrations, many of them new, and a 22-page appendix of reference material and tables that should be valuable to the student and practicing dairyman. D. D. Deane

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

250. Pathology of necrobacillosis of the bovine foot. J. C. FLINT and R. JENSEN, Colo. Agr. Expt. Sta., Ft. Collins. Am. J. Vet. Research, **12,** 42: 5–13. Jan., 1951.

Foot rot was studied by serially sectioning 116 infected feet from slaughterhouse cases and 8 feet infected by injecting *Actinomyces necrophorus* into the digital artery. The natural port of entry in 53.6% of the cases was the skin and this produced dermatitis, interdigital necrosis and coffin joint arthritis. The blood was the port of entry in 21.5%, producing osteitis and laminitis. Trauma was responsible for 20.7% of the natural cases. The artificially-induced foot rot caused osteitis and arthritis, but not dermatitis or interdigital necrosis. Photographs of typical serial sections clearly show the pathology described.
E. W. Swanson

251. Hyaluronidase production in vitro by streptococci isolated from bovine mastitis cases. T. A. GOCHANAUER and J. B. WILSON, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **12,** 42: 20–22. Jan., 1951.

The role of hyaluronidase in mastitis inflammation and recovery following penicillin and sulfa infusions was studied in several dairy herds. Quarters with severe inflammation had no greater a proportion of hyaluronidase-producing bacteria than did infected quarters that showed no inflammation. Nearly half of the streptococci isolated from these mastitis cases did not produce hyaluronidase. Following chemotherapy, 43 of 47 quarters with hyaluronidase-positive streptococci and 19 or 21 quarters with hyaluronidase negative streptococci were freed of infection. These results indicated that the hyaluronidase did not increase the effectiveness of the antibiotics with *Str. agalactiae* and *Str. dysgalactiae.*
E. W. Swanson

Also see abs. no. 266.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

252. Nøytralisering av smør av stor betydning for kvaliteten (Neutralization of butter is important for quality). O. BENTERUD. Meieriposten, **39:** 23, 397–402. June, 1950.

In 1945 the Norwegian Dept. of Agr. undertook some experiments on neutralization of butter as developed in Finland (A.I.V. neutralizing salt: disodiumphosphate and soda), in order to reach a decision whether the method should be made legal in Norway. In the opinion of the author, neutralization of butter ought to be continued for the manufacture of high quality butter.

It was found advantageous to develop a high acidity and a pronounced flavor in pasteurized cream and then increase the pH of the butter by neutralization. The creamery operators would be free to churn without neutralization if they chose to, but the following reasons were given for using the butter neutralization method: (a) To produce butter having a pleasing aroma and keeping quality. The ripening of the cream to a high acidity served to cover up to some extent off-flavors which might be present in the cream used. This was not possible when the cream was ripened to a low acidity. (b) The method of high ripening resulted in increased efficiency of churning of butter.

In 1934 when storing quantities of butter became necessary, low acid cream for churning was used extensively. For this reason, 2 øre (1 øre now about 1/7¢) more per kg. of butter was paid to make up for the loss in yield when using low acid cream for churning. It was not possible to store salted butter made from high acid cream without experiencing a decrease in quality. Observations at 37 creameries, involving 4,610 churnings and reported in 1948, showed that by neutralization of the butter it was possible to manu-

facture and store salted butter made of high acid cream, without a decrease in butter quality. The butter was scored twice, first when fresh and again after 2 wk. in storage. High-acid cream and butter neutralization resulted in a higher scoring butter, when fresh, than low-acid cream and no neutralization. After the butter had been 2 wk. in storage the scores were identical. A number of creameries therefore changed to high-acid cream and butter neutralization in order to make high quality butter. They had no further difficulty with decreased butter quality when the high-acid cream and butter neutralization method was followed. The method became popular with 75 creameries, including the 37 participating in the experiments. Only in 1 creamery was the new method discontinued and this was due to lack of proper equipment for using the method.

If all creamery butter was made from well-ripened cream, considering an output of 10,500 tons, this would result in an increased yield of 105,000 kg., which would have a value of 560,000 Kroner, it was stated. G. H. Wilster

253. Recent advances in the butter industry. G. H. Wilster, Ore. State College, Corvallis. Am. Dairy Prod. Mfg. Rev., **13,** 2: 24–26, 28, 30–33. Feb., 1951.

The author reviews recent developments in programs, techniques and equipment that have contributed to advances in the butter industry. T. J. Claydon

CHEESE

A. C. DAHLBERG, SECTION EDITOR

254. Forsøk med maskinell forming av blandet geitmysost (Experiments with the mechanical moulding of goat whey cheese). I. Mork, A. Nilsen and O. Aasåren, Dairy Research Sta., Agricultural College, Norway. Report no. 39, 1947.

Experiments with the mechanical moulding and wrapping of goat whey cheese proved that a saving in labor of about 65% was possible when hand-moulding and wrapping of cheese was discontinued.

The moulding machine consisted of a cheese grinder, and an extruding section made of metal, equipped with a jacket through which water at 10° C. (50° F.) could circulate to cool the outer layer of cheese inside of the machine from 35–40° C. down to 30° C. (86° F.) This section of the machine was lined with plastic after finding this more satisfactory than stainless steel and linoleum. The cheese came through with a smooth firm surface, sliding on to the roller conveyor and cutting apparatus. The moulding machine worked as a simple semi-automatic power butter moulder. The whey cheese was cut into prints weighing 0.5–1 kg.

Cellophane and aluminum foil were satisfactory for wrapping whey cheese. The machine-moulded whey cheese was more compact than the hand-moulded cheese. Diagrams and photographs of the mechanical moulding equipment were included in the 40-page report.

255. California sells cottage cheese. G. Aughinbaugh, Cal. Dairy Ind. Advisory Bd. Am. Milk Rev., **12,** 7: 12, 14, 16. July, 1950.

The California dairy industry has promoted cottage cheese to such an extent that the state can now claim a per capita consumption of cottage cheese 230% greater than the country as a whole. To achieve this, members of the dairy industry have cooperated effectively with special emphasis on merchandising, quality control, education and research. D. J. Hankinson

Also see abs. no. 262.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

256. Etude de queleues milieux simples pour la conservation de ferments lactiques (Sudy of some simple media for carrying lactic cultures). C. Alais and J. Brigando. Lait, **31,** 301–302: 1–7. Jan.–Feb., 1951.

Three new media formulae suitable for the growth of lactic cultures were developed. Two of these utilized calcium caseinate as a source of nitrogen, while the third employed only ammonium phosphate. This latter medium is quite simple and gave promising results for the carrying of certain lactic acid stock cultures to be disseminated to dairies. S. Patton

257. Contribution au controle bactériologique du lait (Contribution to the bacteriological control of milk). C. G. Macris and C. C. Tzivanopoulos. Lait, **31,** 301–302: 7–15. Jan.–Feb., 1951.

It is proposed that the property of the *coli* group to decompose nitrates to nitrites be utilized as a basis of quality control for milk. In essence, the technique involves the addition of a given amount of 1% KNO_3 to the sample of milk under examination, holding the mixture at a temperature of 37–40° C. and following the formation of nitrite by a sensitive colorimetric reaction with sulfanilic acid-naphthyl amine reagent. The authors claim the procedure shows good selective measurement of contamination and that test results are correlated well with bacterial quality of the milk. S. Patton

258. Drugs, mastitis and acid starters. W. A. Krienke, Florida Agr. Expt. Sta. Am. Milk Rev., **12,** 6: 54, 56. June, 1950.

Sulfamethazine was found to inhibit buttermilk starters when 0.1 ml. of a 25% solution was added directly to 100 ml. of milk. Temperatures as high as 241° F. for 15 min. did not reduce the inhibiting effect of 5.0 ml. of 25% solution of sulfamethazine when added to 100 ml. of milk. Aureomycin at the rate of 0.0005 mg./ml. definitely retarded acid production in milk pasteurized with the antibiotic. Aureomycin still existed 12 milkings after treatment in sufficient quantity to retard acid production. D. J. Hankinson

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

259. Determination of lactose in biological materials. M. G. Horowitz, H. M. Davidson, F.

D. HOWARD and F. J. REITHEL, Univ. of Oregon, Eugene. Anal. Chem., **23,** 2: 375–377. 1951.

Three methods were tested for determining small changes in lactose concentration in metabolizing mammary gland homogenates when glucose and galactose were the only other sugars present. It was necessary to remove interfering substances not normally present in mammary gland when such substances were added with each substrate or coenzyme. The methods used were (a) differential fermentation, (b) manometric and (c) colorimetric. In *a* the reducing power of a preparation was determined before and after fermentation by an organism that fermented only glucose, one fermenting only glucose and galactose in the presence of lactose and one fermenting all 3 sugars. In *b,* lactose was hydrolyzed by the enzyme lactase and glucose was determined manometrically by yeast fermentation. Method *c* was based on the principle that compounds containing the 1,4 glucosidic linkage react with alkali and methylamine to yield a pink color. The colorimetric method was the most rapid and convenient. B. H. Webb

260. Le paiment du lait suivant sa richesse an matière grasse (Payment for milk based on its richness in fat). R. MOREAU. Lait, **31,** 301–302: 20–23. Jan.–Feb., 1951.

Detrimental aspects of sampling and calculating composite test results of patrons' shipments by conventional methods are discussed. Recommendations for simplifying and rendering the procedures more accurate are given. S. Patton

261. Le dosage de la matière grasse des crèmes par la détermination taux d'humidité (The amount of fat in creams by determination of moisture content). A. CAMUS, J. BURDIN and A. LE GUEN. Lait, **31,** 301–302: 15–20. Jan.–Feb., 1951.

The method of determining the fat content of creams by determination of their moisture content appears sufficiently reliable to meet the needs of industry providing the cream has not been watered in excess of 10% and the sample is conserved in good condition. Conversion of moisture content to fat content is accomplished through the use of a formula of the type $G = 100 - 1.1H$, where G = fat content and H = moisture content. The constant (1.1 in this case) is derived from average values for the solids-not-fat portion of milk. Advantages claimed for the method as compared to butyrometric methods include lower cost, less work, simpler materials and usefulness for purposes other than measuring fat content. S. Patton

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

262. Forsøk med anvendelse av vifte og propell ved inndamping av myse (Research to investigate the use of a fan and propeller to increase evaporation during whey cheese making). A. T. BERGUM and O. FRAMHUS, Dairy Research Sta., Agricultural College, Norway. Report no. 40, 1949.

Experiments were undertaken in an attempt to lower the time necessary for evaporation during whey cheese making. In order to control the foam cap usually forming over the whey pans, a strong current of air was directed by a fan toward it. The foam cap was crushed and evaporation during boiling-down increased by about 45%. Settling of the foam cap usually took place when the mixture had been concentrated to a dry matter content of about 47%. From the moment the boiling down was finished until the whey cheese had obtained the proper consistency, the time of evaporation was measured and recorded. Whey cheese of proper consistency had a dry matter content of 80%. Pans, 2.2 × 2.2 m. with a capacity of 1,100 l., were used. A 37% increase in pan capacity resulted from quick boiling down as compared with the usual procedure. No deleterious effects on quality of whey cheese resulted from quick boiling down of whey. In further trials some pans 2.2 × 2.2 m. in size were filled with 1,250 l. of whey and the contents boiled down quickly, a fan being used to speed evaporation. The whey cheese quality was not equal to that obtained in ordinary whey cheese making. It would be difficult, in practice, to carry out cooking in pans with such large fillings. In an attempt to increase the evaporation by means of an outlet fan for the escaping steam, when air was supplied artificially to the pan at the same time, the escaping steam was sucked away satisfactorily. However, the fan did not effect any increase in the evaporation. The final evaporation should lie between 20 and 30 kg. of water per sq. meter. For the ordinary type of pan, this would mean a steam pressure of about 30 lb. should be used. If the final evaporation was forced, a burnt flavor was apt to occur without any time being saved. Measurement of steam showed that if a fan were used while cooking the whey, the steam expenditure remained practically the same as in ordinary cooking. When the foam was crushed with a propeller, evaporation did not increase significantly (4.2%). A specially designed foam destroyer increased evaporation by 15%. A beater was employed instead of a propeller. G. H. Wilster

263. Experimental falling film evaporator for preparation of juice and puree concentrates at low temperature. L. H. WALKER and D. C. PATTERSON, Western Reg. Research Lab., Albany, Cal. Ind. Eng. Chem., **43,** 2: 534–536. 1951.

A diagram and description of an all-glass evaporator designed to operate at 57–175° F. in terms of the boiling temperatures of water is presented. The evaporation rate is 12 lb. of water/hr. and the apparatus is suitable for low temperature evaporation studies on liquids. B. H. Webb

264. Packaged steam generators. J. T. WINSLOW, Cherry-Burrell Corp. Am. Milk Rev., **12,** 6: 62, 63, 88. June, 1950.

Eleven advantages of packaged steam generators are listed. Important advantages are elimination of coal as fuel, elimination of stacks, high efficiency, fast steaming and reduction of fuel

costs. Two types of packaged steam generators are described. The fire-tube type has the hot gases inside the tubes, while the water-tube type has the water inside the tubes. Points which should be considered before purchasing such equipment are listed. D. J. Hankinson

265. Aeration gives low-cost waste disposal. W. A. HASFURTHER. Ill. Sanitary Water Board. Food Inds., **23,** 2: 90–93. 1951.

Aeration-type equipment for waste disposal is relatively inexpensive, operates at moderate cost and has certain advantages over the trickling filter and activated sludge methods of disposal. A description of an aeration plant layout is given. Three installations are in operation in Illinois. Following certain improvements in original design and equipment, these are giving satisfactory results. One of the installations is in a milk plant with 200,000 lb. daily milk intake. Some difficulty was experienced with clogging of air jets and from unsettling floc, causing low BOD reductions. This generally has been overcome through modifications and careful operation. BOD reductions up to 97.3% have been obtained.

T. J. Claydon

Also see abs. no. 254.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

266. Bacterial flora of the bovine male genitalia. H. J. RUEBKE, Iowa State College, Ames. Am. J. Vet. Research, **12,** 42: 14–19. Jan., 1951.

The genital tracts of 45 bulls and 5 steers secured at a slaughterhouse were examined carefully for the presence of a wide variety of microorganisms. Bacteria were not found in the testicles, epididymides, vasa diferentia (ampullae), seminal vesicles, prostate, or bulbo-urethral glands of any of the specimens. A progressively larger proportion of the genitalia yielded bacteria from the urethra to the glans. All specimens had bacteria at the prepuce and the glans penis. No *Brucella abortus* were found. *Corynebacterium* was the genus most frequently found, followed by *Micrococcus and Streptococcus.* A number of organisms considered to be pathogenic in the bovine were found, but pathologic changes were not noted in the infected tracts. E. W. Swanson

267. Physiology of fertilization in mammals. M. C. CHANG and G. PINCUS, Worchester Foundation for Exptl. Biol., Shrewsbury, Mass. and Tufts College Med. School, Boston. Physiol. Rev., **31,** 1: 1–26. 1951.

The authors review the field of fertilization in mammals including dairy cattle. 350 references.

E. G. Moody

268. Stumpy, a recessive achondroplasia in Shorthorn cattle. M. L. BAKER, C. T. BLUNN and M. M. OLOUFA, Univ. of Neb., Lincoln. J. Heredity, **41:** 243–245. Sept., 1950.

The dwarf syndrome described in Shorthorns is manifested particularly in the legs with enlarged hoodheads and knees. The cannon bones are twisted laterally and hoofs are turned outward. The switch is smaller than normal. Affected calves are born with curly coats, making it possible to detect them at birth. A metabolic disturbance prevents normal growth, keeping cattle in a thin condition. Body length and head dimensions are normal. During the last 9 yr. of a 15-yr. linebreeding program, the frequency of this trait was 4.6%. All but 1 of the 26 affected calves were traced 1 or more times to a common ancestor. Pedigree analyses and mating-type frequency (heterozygotes) yield the conclusion that a single autosomal recessive gene is the genetic cause. No matings with cattle known to carry the gene(s) for any of the other dwarf traits were carried out.

L. O. Gilmore

Also see abs. no. 279.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

269. Inflator. W. A. CYPHERS. U. S. Patent 2,541,988. 4 claims. Feb. 20, 1951. Official Gaz. U. S. Pat. Office, **643,** 3: 761. 1951.

A teat cup for a mechanical milker, consists of a rigid cup with a flexible inner liner. A pulsating source of vacuum causes the liner to contract, the milking action being caused by differences in the thickness of the liner wall.

R. Whitaker

270. Milking machine. C. A. FLOERKE. U. S. Patent 2,543,162. 1 claim. Feb. 27, 1951. Official Gaz. U. S. Pat. Office, **643,** 4: 1182. 1951.

A bar adjacent to the cow holds a teat cup claw beneath the udder and also supports a swinging pendulum which is attached to a piston for controlling pulsations of the vacuum system.

R. Whitaker

271. Milk can holder. W. L. CHRISTMAN. U. S. Patent 2,542,118. 1 claim. Feb. 20, 1951. Official Gaz. U. S. Pat. Office, **643,** 3: 798. 1951.

A frame designed to hold milk cans against a wall is described. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

272. Report on shrinkage. C. D. DAHLE and J. C. LANDO. Pennsylvania State College, State College. Ice Cream Rev., **34,** 6: 50, 52, 66, 68–72. Jan., 1951.

Ice cream shrinkage is accentuated by use of the continuous freezer, dry ice, paper containers, prolonged aging of ice cream mix, failure to maintain satisfactory temperatures on delivery trucks or in cabinets, high overrun, use of certain malt syrups, use of sweetened condensed milk, presence of free fatty acids and partial hydrolysis of certain milk proteins.

Experimental evidence is presented to show that hydrolysis of the whey proteins (albumin and globulin) will accentuate shrinkage to a greater extent than will hydrolysis of casein. Presence of native globulin in a mix reduced shrinkage, whereas partial hydrolysis of the globulin increased shrinkage. Agglutinin-rich mixes were found to shrink less than agglutinin-poor mixes.

Treating mixes at 185–190° F. resulted in reduced shrinkage when fresh dairy products were used. Such heat treatments did not denature a considerable portion of the globulin fraction, although the native albumin content was reduced to zero at 185° F.

Addition of unheated fruit juice to a mix for 30 min. or more prior to freezing increased shrinkage. W. J. Caulfield

273. Saving dollars in carton stamping costs. B. A. BEANE, The Borden Co., Pittsburgh, Pa. Ice Cream Rev., **34,** 6: 46, 53. Jan., 1951.

Model no. 240 Multigraph Printer with a metal slug insert which impregnates the lettering and ink into the paper has been used successfully to print the flavor identification on all 4 sides of an ice cream carton at the rate of 5400/hr. The machine costs from $1,100–$1,300; its use results in up to 50% saving in carton inventory, and also saves time and labor. The carton is neater and cleaner and the flavor identification can be read easily. W. J. Caulfield

274. Guinea pig of merchandising. R. T. SMITH, Robert T. Smith Dairy Lab., Scranton, Pa. Ice Cream Rev., **34,** 6: 43, 78, 80–83. Jan., 1951.

A consumer clinic, consisting of 20–25 representative adult consumers, has been used with success in evaluating ice cream quality. In the conduct of a taste panel, each of the participants is asked to rate the ice cream on color and appearance, body and texture, and flavor. The ratings for each characteristic are expressed as good, 90 points; fair, 80 points; and poor, 70 points. In addition, appropriate criticisms are recorded. It is the opinion of the author that in any attempt to determine consumer reaction to the quality of ice cream, the method of packaging or method of merchandising, a panel of representative consumers and not a staff of experts should be used. The plan described is one which might well be used to good advantage throughout the industry. W. J. Caulfield

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

275. Communities awarded milk sanitation ratings of 90 per cent or more during 1949 and 1950. Anonymous. Div. of Sanitation, Milk and Food Branch, Pub. Health Service. Pub. Health Reports, **66,** 8: 239–243. Feb. 23, 1951.

A list is presented of those Public Health Service Ordinance communities that received a market milk rating of at least 90% during the period from Jan. 1, 1949 to Dec. 31, 1950.

D. D. Deane

276. Wonders homogenization has wrought. G. M. TROUT, Mich. State College. Am. Milk Rev., **12,** 6: 21, 22, 98, 99. June, 1950.

The author describes (a) the history of homogenized milk, (b) 23 ways in which the process has influenced the dairy industry, (c) several problems concerned with processing, packaging, distribution, laboratory control, utilization of returns, cookery and product quality and (d) 8 public health values of the process and the product.

D. J. Hankinson

277. Modified milk product. F. K. DANIEL (assignor to Sun Chemical Corp.). U. S. Patent 2,542,633. 6 claims. Feb. 20, 1951. Official Gaz. U. S. Pat. Office, **643,** 3: 931. 1951.

A dry dairy product derived from milk is dispersible in water and suitable for dietetic purposes. The protein/lactose ration is the reverse of that in milk, the proportion of Na, K and Cl is less and the protein and Ca are greater than in the original milk. R. Whitaker

278. Sour cream—a natural. Anonymous. Am. Milk Rev., **12:** 7, 8, 10. July, 1950.

O. M. Richards, general manager of the American Dairy Association, points out that the name "sour" cream is a handicap in the marketing of this product. More should be done to bring to the consumer's attention the many possibilities of the use of sour cream and its real value and desirability. A pamphlet, "Sour Cream," published by the California Dairy Industry Advisory Board, consists of a few directions on how to use sour cream, as well as recipes. D. J. Hankinson

Also see abs. no. 260, 261.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

279. Secretory function of male accessory organs of reproduction in mammals. T. MANN and CECILLIA LUTWAK-MANN, Molteno Inst. and Biochem. Lab., Univ. of Cambridge. Physiol. Rev., **31,** 1: 27–55. 1951.

The functions of the secretions of the accessory sex organs in the mammalian male are reviewed. 226 references. E. G. Moody

280. Isopropyl alcohol in cows suffering from acetonaemia. A. ROBERTSON, C. THIN and A. M. STERLING. Nature, **166,** 4231: 954. 1950.

The blood, milk and rumen contents of cows suffering from acetonaemia contain isopropyl alcohol. A new approach to the problem of the development of ketone bodies in this disease is suggested. R. Whitaker

Also see abs. no. 267.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

281. Sanitation in the fluid milk processing plant. W. H. HASKELL, Klenzade Prod., Inc. Am. Milk Rev., **12,** 7: 42–45, 68. July, 1950.

Emphasis is made of the importance of thorough sanitation practices at all points from the cow to consumer. Special attention is given to use of milk cans, equipment in the processing plant with reference to its construction and repair and proper use of sanitizing cleansers and the importance of employee training. D. J. Hankinson

282. Special equipment for effective and economical cleaning. J. R. PERRY, Natl. Dairy Prod. Co., Inc. Am. Milk Rev., **12,** 6: 6, 30–32, 36, 90, 91. June, 1950.

The following pieces of special cleaning equipment are described in detail: (a) cleaning solution tank, both portable and stationary, (b) 1- and 2-handed solution-fed brushes, (c) compressed air-driven, solution-fed brushes for flat surfaces and for IT heaters and coolers, (d) solution hose for portable tanks and cleaning solution stations, (e) portable cleaner table equipped with electrically heated, thermostatically controlled solution tank, circulating pump and solution-fed brush, (f) spray sterilizing gun which can deliver a stream, a mist or a fog of sterilizing solution and (g) special light for cleaning.

The cleaning aids have been tested in plants and have effected savings in water consumption, fuel consumption, cleaning supplies and labor. As much as 48% saving in water consumption and 50% saving in fuel oil have been reported. D. J. Hankinson

283. The way to foolproof sanitation. J. C. DAWSON, J. C. Dawson and Assoc., St. Louis, Mo. Food Inds., **23,** 2: 99–100, 221. Feb., 1951.

The objectives of food plant sanitation must first be recognized and defined. Top management is responsible for sanitation conditions. Cooperation of all plant personnel is essential and a qualified plant sanitarian is required to supervise the program. An analysis should be made jointly by management and the plant sanitarian and should include all conditions relating to raw materials, storage equipment, etc. Pertinent check questions are suggested. General methods of insect and rodent control need to be decided. The biggest problem lies in the changing of old habit patterns. If success is to be achieved, all personnel must be sold on the program.

T. J. Claydon

NOTICE TO CONTRIBUTORS

Authorship of Original Articles and Reviews.—Space in the JOURNAL is reserved for the publication of original research voluntarily submitted by members of the Association to the JOURNAL and review articles by invitation. In the case of joint authorship, the membership ruling applies to one author only.

Papers that already have appeared in print or that are intended for simultaneous publication elsewhere will not be accepted.

Manuscripts.—Manuscripts should be submitted in double spacing on one side of suitable 8½" x 11" paper. The original copy should be furnished. All illustrative and tabular material should accompany the manuscript.

Except in cases of invited reviews, papers must be limited to 12 printed pages unless previous permission from the editor is obtained. When non-review articles exceed 12 pages, a charge of $5 per over page is made.

Manuscripts will be published in the order of their receipt. They should be sent to the Editor, F. E. Nelson, Dept. of Dairy Industry, Iowa State College, Ames, Iowa. In order to speed publication, one author should be designated to assume the responsibility of checking the galley on all papers of multiple authorship. All galleys should be returned in the minimum possible time to avoid delay in publication.

Figures.—Original drawings, diagrams and charts should be done in India ink on tracing cloth (or white board) not larger than standard letter size (8½" x 11"). All lettering should be inked in block style and be of such size that the lettering will be not less than ⅛ in. in height when the figure is reduced to 4 in. in maximum dimension. Typewritten labeling of axes and axis units is not acceptable. Original drawings should be submitted, rather than photographs of such drawings. When suitable drawings are not furnished, the author will be charged for the preparation of drawings of satisfactory quality by an independent agency.

Photographs.—Photographs for halftone reproduction should be glossy prints free of all imperfections.

Legends.—Legends for figures and photographs should be typed on a sheet separate from the illustrative material and should be made as concise as possible while retaining their descriptive character.

Tabular Material.—Tabular material should be clear, concise and accurate. Often data can be condensed and presented in summarized tabular form. Tables of only one or two lines should be avoided except in most unusual cases. Excessively large or complicated tables are almost impossible to print satisfactorily. Headings should be as concise as possible, yet descriptive in character. Data may be presented in either tabular form or in figures, but the same data must not be presented in both forms. Each table should be placed on a separate sheet and not in the body of the manuscript. The letters *a*, *b*, *c*, etc., should be used for footnote designations. If possible, tables should be so organized that they may be set across the page, rather than the length of the page.

References.—Literature reviews should be limited to only the most pertinent references. Reference lists should be double spaced and arranged alphabetically as to author and by chronological appearance of the journals cited under a given author. Papers by a single author always precede papers by that author and associates. References to multiple authors are arranged in the alphabetical order of the several authors. Give only initials rather than full first names of male authors. Citations in the text should be made by the number in parentheses, corresponding to the number in the reference list.

Each reference should contain the following: Reference number, author(s), title of article, name of journal, volume number, first and last page numbers, and year of publication. Titles of all articles should appear in complete untranslated form. Consult recent published articles in the JOURNAL for proper citation. Publications are abbreviated according to the form given in CHEMICAL ABSTRACTS, vol. 40, no. 24, part 2. 1946.

Sample of journal citation: (1) JONES, L. W., AND SMITH, J. D. Effect of Feed on Body of Butter. J. DAIRY SCI., 24: 550–560. 1941.

Sample of book citation: (1) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.

For Experiment Station publications, the citation should be as follows: (1) COULTER, S. T., AND JENNESS, R. Packing Dry Whole Milk in Inert Gas. Minn. Agr. Expt. Sta. Tech. Bull. 167. 1945.

The more common abbreviations used in the text are: cm., centimeter(s); cc., cubic centimeter(s); g., gram(s); mg., milligram(s); γ, microgram(s); ml., milliliter(s); mμ, millimicron(s); C., Centigrade; F., Fahrenheit; lb., pound(s); oz., ounce(s).

Where configurational structures of chemical compounds are used, drawings suitable for reproduction by photoengraving are to be furnished by the author.

In preparing manuscripts, use of first person should be avoided.