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THE RELATIONSHIP OF MAMMARY DEVELOPMENT AND BODY WEIGHT[1]

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A method of selecting dairy calves on the basis of mammary development has been presented by Swett and Matthews (1). The extent of the mammary development of the heifer calf at 3 to 4 months of age has been demonstrated to be in direct relationship with the heifer's later productive ability (1).

This report deals with a study of the relationship of the mammary development to the body weight of Holstein and Guernsey heifer calves at 3 and 6 months of age.

EXPERIMENTAL PROCEDURE

Nineteen heifer calves (10 Holsteins and 9 Guernseys) were fed six grain rations which resulted in various rates of body growth. The original study involved an investigation into the possibilities of utilizing distillers dried solubles and distillers dried solubles with grains in the rations of dairy calves. While there were no statistically significant differences in the rates of growth of the several groups of calves, considerable variations existed in this respect between calves within the groups. The mammary development of all heifer calves was measured by the technique set forth by Swett and Matthews (1) at 3 and 6 months of age. Body weights were determined at the same ages.

Measurements were made of the width and length of each quarter at 3 months of age. Since the udders were in the half stage when the calves were 6 months of age, only the length of each half and the width of each quarter were measured at this age.

The mean width of the mammary tissue of the four quarters was correlated with body weight at 3 and 6 months. The mean length of the mammary tissue of the four quarters was correlated with body weight at 3 months. However, at 6 months it was necessary to use the mean length of

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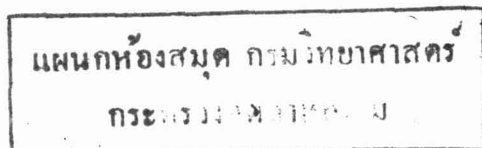

TABLE 1

Summary of udder measurements of Holstein calves at 3 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	*(lb.)*	*(mm.)*	*(lb.)*
285	20.25	194	20.75	94
170	15.25	176	17.25	83
270	17.75	171	22.75	73
186	16.25	165	16.25	80
207	16.50	163	14.75	65
176	14.25	155	15.25	71
187	14.25	153	15.00	68
231	11.75	144	12.75	56
205	15.75	139	15.50	61
212	11.50	123	10.50	46

the mammary tissue of the two halves because the udder development had reached the half-stage. Similar correlations also were made with gain in body weight from 8 days to 3 and to 6 months.

EXPERIMENTAL RESULTS

The udder measurements and body weights of the ten Holstein calves at 3 months of age are summarized in table 1. These data are presented in order of body weights. The correlation coefficient between the average width of the quarters and body weight was + 0.844**.[2] A correlation coefficient of + 0.821** was determined for the average length of quarters and body weight for these same ten calves. In table 2 are the data for nine Guernsey calves. A correlation coefficient of + 0.909** was found for body weight and the average width of the quarters. For the average length of the quarters and body weight a correlation coefficient of + 0.917** was

TABLE 2

Summary of udder measurements of Guernsey calves at 3 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	*(lb.)*	*(mm.)*	*(lb.)*
200	24.25	191	27.00	99
199	19.5	188	23.5	93
169	12.5	133	12.75	53
227	10.25	131	14.25	52
158	13.00	128	11.25	52
226	8.5	117	9.5	42
153	7.75	109	10.5	46
181	13.75	107	14.5	55
178	6.75	98	..........[a]	34

[a] The glandular tissue was too small to measure accurately.

[2] * = significant.
** = highly significant.

TABLE 3

Summary of udder measurements of Holstein calves at 6 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(*mm.*)	(*lb.*)	(*mm.*)	(*lb.*)
207	46.5	396	77.0	298
270	45.25	378	82.0	280
186	41.25	371	53.0	286
187	31.5	349	67.0	264
231	45.75	340	70.0	252
205	47.5	337	93.0	259
285	41.5	334	69.5	234
170	40.5	333	73.5	240
176	33.75	319	70.5	235
220	51.5	309	75.0	210
212	41.5	273	86.5	196

found. The correlation coefficients were statistically significant for both length and width of the glandular tissue for the Holstein and Guernsey calves.

The summary of udder measurements and body weights for 11 Holstein calves at 6 months of age is presented in table 3. The correlation coefficient for the average width of the glandular tissue and body weight was found to be + 0.0836 and − 0.29 for the average length of the half stage of the udder and body weight. When tested statistically neither of these correlation coefficients was significant.

The summary of the measurements for eight Guernsey calves at 6 months of age is presented in table 4. The correlation coefficient for the average width of each quarter with body weight was + 0.444. However, this coefficient was not significant. The average length of the half stage of the udder and the body weight had a correlation coefficient of + 0.834*. A t value of 3.707 was necessary to be highly significant with six degrees of freedom. A value of 3.701 was obtained when the t value was determined. Thus the length of the udder and body weight were significantly related.

TABLE 4

Summary of udder measurements of Guernsey calves at 6 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(*mm.*)	(*lb.*)	(*mm.*)	(*lb.*)
200	41.5	357	95.0	265
226	53.5	293	89.0	218
227	36.0	286	73.0	207
153	31.75	281	63.5	218
158	31.0	275	60.5	199
169	37.25	274	64.0	194
178	31.25	272	72.0	208
181	32.75	266	58.5	214

Mammary development was correlated with rate of growth as determined by gain in body weight from 8 days to 3 months of age. A coefficient of + 0.812** and + 0.7707** was found for width and length, respectively, for the Holstein calves. Also, with the Guernsey calves, a correlation coefficient of + 0.9659** was calculated for width and + 0.9756** for length of the secretive tissue, and gain in body weight.

No significant relationship was shown to exist between gain in body weight from 8 days to 6 months and udder development at 6 months of age. Correlation coefficients of – 0.006 for width and – 0.2676 for length of mammary secretive tissue were determined with the Holstein calves, while correlation coefficients of + 0.345 (width) and + 0.2386 (length) were determined with the Guernsey calves.

SUMMARY

Nineteen calves of the Holstein and Guernsey breeds were used to determine the relationship of mammary development to body wight. A highly significant statistical relationship was found to exist between the development of the mammary secretive tissue and body weight of both the Holstein and Guernsey heifer calves at 3 months of age. Highly significant correlations also were found between mammary tissue development and gains in body weight from 8 days to 3 months of age of the heifer calves of both breeds.

The lengths of the secretive tissues of Guernsey calves were related significantly to body weight at 6 months of age; however, there was no correlation in this respect with the Holstein calves at this age. There was no significant correlation between the width of the mammary secretive tissue and body weight of either breed at 6 months of age. No significant correlation was found between the gains in body weight from 8 days to 6 months and mammary development of either breed.

REFERENCE

(1) SWETT, W. W., AND MATTHEWS, C. A. Dairy Cow's Udder Studied to Establish Development Standards. Yearbook of Agriculture. Pp. 175–181. 1934.

PERMANENCY OF SYNTHETIC ASCORBIC ACID ADDED TO MILK[1]

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Hand (3) and others have shown that the reduced ascorbic acid content of raw, commercial cow's milk decreases rapidly during the first week after it is drawn from the cow. In his study, the average ascorbic acid value for 12 samples of milk was 19 mg. per l. at the beginning of storage at 1° C. and 7 mg. per l. 6 days later. Thus about 63 per cent of the original reduced ascorbic acid had disappeared from the milk during a 6-day storage period. Subsequently, Holmes and Jones (5) determined the loss of reduced ascorbic acid in mare's milk. They found that the rate of disappearance of ascorbic acid from mare's milk was only about one-seventh that reported by Hand for cow's milk. Since the composition of cow's milk and mare's milk is dissimilar in various respects, it is possible that a number of factors may influence the rate of loss of reduced ascorbic acid from the two types of milk. One obvious difference in composition is the amount of reduced ascorbic acid in the original milk. Hand reported that his samples of cow's milk contained from 14.8 to 22.8 mg. of ascorbic acid per l., whereas Holmes and Jones used samples of mare's milk that contained from 86 to 161 mg. of ascorbic acid per l. Accordingly, it was decided to determine the rate of loss of reduced ascorbic acid from cow's milk to which a sufficient amount of synthetic ascorbic acid had been added so that the ascorbic acid content of the milk approximated that of the mare's milk referred to above.

EXPERIMENTAL PROCEDURE

Since the stability of reduced ascorbic acid had been determined for raw mare's milk, raw cow's milk was used in this study. Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of synthetic ascorbic acid to a liter of milk. After the ascorbic acid was added, the milk was shaken thoroughly. One sample each of milk containing 75 mg. and 150 mg. of added ascorbic acid per l. was prepared per day. The enriched milk was placed in 500-cc. flasks and stored in the dark at 10° C. When the samples were prepared, the flasks were completely filled, but as aliquots were taken day by day for assay, the volume of milk decreased and the volume of atmosphere increased correspondingly. These conditions were the same as for the study of the stability of ascorbic acid in mare's milk and they were similar to the conditions in the average household where milk is stored in

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the refrigerator and at irregular intervals variable amounts are removed from the milk bottles.

The storage period was 10 days and only one sample of each series was placed in storage at a time. The amount of reduced ascorbic acid in all cases was determined by the method described by Holmes and Jones (5), and bentonite was used for clarification.

RESULTS AND DISCUSSION

The average values for the ascorbic acid assays of the two series of samples are reported in figure 1. The samples of the original milk before

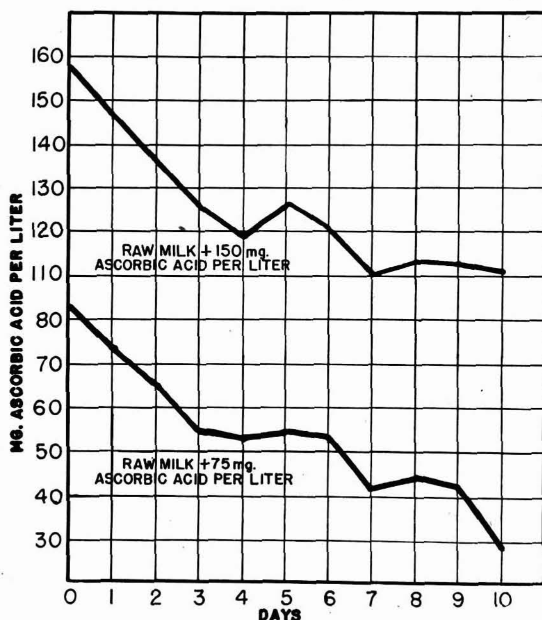

FIG. 1. Rate of loss of synthetic ascorbic acid from cow's milk.

the addition of the synthetic ascorbic acid contained, on an average, 9.5 mg. of reduced ascorbic acid per l. This value is in agreement with 7.5–9.2 mg. per l. reported by Christen and Virasoro (1), 10.8 mg. per l. by Lojander (9), and 12.2 mg. per l. by Mosonyi and Polónyi (10). However, these values for the ascorbic acid content of raw cow's milk definitely are less than those usually reported for milk—*i.e.*, 16.4 mg. per l. reported by Holmes *et al.* (7), 17.1 mg. per l. by Stewart and Sharp (12), 17.4 mg. per l. by Woessner *et al.* (13), 19.7 mg. per l. by Holmes *et al.* (6) and 22.2 mg. per l. by Sharp *et al.* (11).

When the two series of samples of ascorbic acid-enriched milk were placed in storage, their average reduced ascorbic acid contents were 83.0 mg.

and 157.5 mg. per l., respectively. Thus, losses of about 1.7 per cent and 1.2 per cent, respectively, occurred while the milk was being enriched and prepared for study. At this time the milk was exposed to laboratory temperature and full daylight but not to sunshine.

During the first 3 or 4 days of storage, reduced ascorbic acid was lost more rapidly and more consistently than during the remainder of the experimental period. For the series of samples of milk to which 75 mg. per l. of ascorbic acid was added, the loss of ascorbic acid was 34 per cent during the first 3 days or 11 per cent per day, and 33 per cent during the remaining 7 days or 5 per cent per day, with an average loss of 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid was added per l., the loss was 24 per cent for the first 4 days or 6 per cent per day, and 5 per cent for the next 6 days or 1 per cent per day, with an average loss of 3 per cent per day for the 10 days the milk was in storage. These losses are decidedly less than those reported by Gunsalus and Hand (2), who noted a reduction of reduced ascorbic acid of from 14.9 mg. to 1.7 mg. per l. or an average loss of 14.7 per cent per day during 6 days' storage of raw cow's milk. Hand (3) observed a loss of from 19.0 mg. to 7.1 mg. per l. of milk stored 6 days at 1° C., averaging over 10 per cent per day. Kothavalla and Gill (8) reported a loss of 26 per cent of ascorbic acid from cow's milk (Indian) stored at 45° F., or an average of over 8 per cent per day. Thus it appears from the data assembled here that when considerable amounts of synthetic ascorbic acid are added to raw cow's milk, the percentge of loss of ascorbic acid during storage is smaller than for the reduced ascorbic acid naturally occurring in raw cow's milk. It should be noted that, except for the period while the samples were being prepared at room temperature and for short intervals while the aliquots for assay were being withdrawn, the milk was stored in the dark at 10° C. Consequently, in this study as well as in the study of the stability of ascorbic acid in mare's milk, the effect of light and elevated temperatures upon the destruction of the ascorbic acid was kept at a minimum. Holmes and Jones (4) have shown that these factors cause exceedingly rapid destruction of reduced ascorbic acid in cow's milk. Obviously the data assembled here, together with those reported by the cited investigators, are not sufficient to provide a complete understanding of the factors and conditions that influence the rapid destruction of reduced ascorbic acid occurring naturally in cow's milk, or to provide means for preventing the unfortunate loss of this essential vitamin from one of the most valuable human foods.

SUMMARY

Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of ascorbic acid to a liter of raw cow's milk. The samples were stored in 500-cc. flasks in the dark at 10° C. As aliquots were removed day by

day for analysis, the volume of milk decreased and the volume of air in the flasks increased correspondingly. For the series of samples of milk to which 75 mg. of ascorbic acid per l. was added, the loss was 11 per cent per day for the first 3 days and 5 per cent per day for the remaining 7 days, or 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid per l. was added, the loss was 6 per cent per day for the first 4 days and 1 per cent per day for the remaining 6 days, or an average of 3 per cent per day for the 10 days of storage.

REFERENCES

(1) CHRISTEN, C., AND VIRASORO, E. Determination of Ascorbic Acid by the Methylene Blue Method. Anales asoc. quím. argentina, **30**: 197–211. 1942. (Chem. Abs., **37**: 3839. 1943.)

(2) GUNSALUS, I. C., AND HAND, D. B. The Use of Bacteria in the Chemical Determination of Total Vitamin C. J. Biol. Chem., **141**: 853–858. 1941.

(3) HAND, D. B. Reduced and Total Vitamin C in Milk. J. Dairy Sci., **26**: 7–12. 1943.

(4) HOLMES, A. D., AND JONES, C. P. Effect of Sunshine upon the Ascorbic Acid and Riboflavin Content of Milk. J. Nutrition, **29**: 201–209. 1945.

(5) HOLMES, A. D., AND JONES, C. P. Stability of Reduced Ascorbic Acid in Mare's Milk. J. Nutrition, **34**: 113–119. 1947.

(6) HOLMES, A. D., JONES, C. P., WERTZ, ANNE W., AND KUZMESKI, J. W. The Ratio of Ascorbic Acid, Riboflavin, and Thiamin in Raw and Pasteurized Milk. J. Nutrition, **26**: 337–345. 1943.

(7) HOLMES, A. D., LINDQUIST, H. G., JONES, C. P., AND WERTZ, ANNE W. Effect of High-Temperature-Short-Time Pasteurization on the Ascorbic Acid, Riboflavin, and Thiamin Content of Milk. J. Dairy Sci., **28**: 29–33. 1945.

(8) KOTHAVALLA, Z. R., AND GILL, H. S. The Effect of Various Factors on the Vitamin C Content of the Milk of Cows and Buffaloes. Indian J. Vet. Sci., **13**: 35–43. 1943. (Chem. Abs., **39**: 2346. 1945.)

(9) LOJANDER, W. Variations in the Vitamin C Content of Milk and Factors Contributing to Such Variation. Acta Soc. Med. Fennicae Duodecim, **27**: 1–10. 1939. (Chem. Abs., **35**: 5579. 1941.)

(10) MOSONYI, J., AND POLÓNYI, P. Vitamin C Content of Cow's Milk. Kisérletügyi Közlemények, **44**: 97–106. 1941. (Chem. Abs., **38**: 2757. 1944.)

(11) SHARP, P. F., GUTHRIE, E. S., AND HAND, D. B. Deaeration as a Means of Retarding Oxidized Flavors and Preserving the Vitamin C of Milk. Ann. Rept. N. Y. State Assoc. Dairy & Milk Inspectors, **14**: 63–76. 1940.

(12) STEWART, A. P., JR., AND SHARP, P. F. Vitamin C Content of Market Milk, Evaporated Milk, and Powdered Whole Milk. J. Nutrition, **31**: 161–173. 1946.

(13) WOESSNER, W. W., WECKEL, K. G., AND SCHUETTE, H. A. The Effect of Commercial Practices on Ascorbic Acid and Dehydroascorbic Acid (Vitamin C) in Milk. J. Dairy Sci., **23**: 1131–1141. 1940.

SULFAMETHAZINE BLOOD AND MILK CONCENTRATIONS IN DAIRY COWS

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Bovine mastitis continues to be of great economic importance to all concerned with the production of milk. Reliable statistics on the losses attributed to the infection are unavailable, but it is considered to be the most important disease problem of the dairy industry (7). Various sulfonamides have been used in the treatment of mastitis. On the basis of present knowledge concerning the action of the sulfonamides, it would appear that the effectiveness of a sulfonamide depends upon adequate blood levels. The present study was initiated to determine the relationship between blood and milk concentrations of sulfamethazine.

CAUSES OF MASTITIS

Several species of bacteria may be associated with mastitis, but *Streptococcus agalactiae* has been recovered in the majority of cases. Staphylococci are considered to be second in importance, followed by other species of streptococci, coliform organisms, and corynebacteria (12). The exact manner of transmission of the disease is not known, but the most probable route of infection is through the teat duct. Environmental factors, repeated exposure to highly infective organisms, and injury to the udder, or a combination of factors, all have been held responsible for spread of the disease.

COMMONLY USED THERAPEUTIC AGENTS

Therapeutic agents have been relied upon to a great degree in the control of mastitis. While immediate infection can be corrected, re-infection cannot be prevented by these measures. However, correct herd management, in conjunction with good treatment procedures when disease does occur, will maintain a productive herd.

In vitro and *in vivo* studies have shown that sulfonamides are active against the streptococci, staphylococci, and other species of bacteria occurring in mastitis. In the early days of sulfonamide therapy, sulfanilamide was administered by mouth in the treatment of mastitis, and it continues to be

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[1] The authors are indebted to Mr. S. C. Griffith, of the Animal Industry Section, for aid in the conduct of the program, and to Mr. S. Senn and Mr. D. W. Gorton, of the Analytical Section, Lederle Laboratories Division, American Cyanamid Company, for sulfonamide analyses.

used by many veterinarians. The reported results (10, 14) are conflicting. However, subsequent experience with sulfanilamide has shown its clinical use to be limited by its rather narrow range of bacteriostatic activity against those organisms other than streptococci which are pathogenic for man and animals.

It has been reported (3, 5, 13) that therapeutic agents for intramammary infusion, with the possible exception of penicillin in sterile water or physiological saline, frequently result in varying degrees of udder irritation, and, in some cases, cause permanent damage, as evidenced by decrease in milk flow and production of abnormal milk. Several investigators attempted to find an agent which would be effective in mastitis when administered parenterally, inasmuch as unsatisfactory results too frequently followed treatment by udder infusion. Because of the success of the therapeutic use of penicillin in human streptococcic and staphylococcic infections, and the successful use of intramammary infusions of penicillin in mastitis (1, 9, 11), work was undertaken to determine the permeability of the bovine mammary gland to penicillin parenterally administered. Such trials have been disappointing (2, 6, 15, 17), and it was found that penicillin was not present in the milk in detectable amounts or amounts sufficient to affect existing mastitis infection. In this work, however, dosages of penicillin used in cows were not sufficiently great, as judged by amounts needed to control human disease effectively. Watts and McLeod (17) reported the use of doses of 1,000,000 Oxford units, with no diffusion of penicillin in the milk. Obviously, larger dosages, and the frequent administration necessary, generally would be economically unsound.

Welsh *et al.* (18) showed that sulfamethazine, the dimethyl derivative of sulfadiazine, maintained the highest blood concentration of seven sulfonamides tested, over a 24-hour period on a fixed intake. It has been reported to be among the least toxic of the sulfonamides in therapeutic dosages, and its action against both Gram-negative and Gram-positive organisms frequently has been shown. Lately, evidence has been presented that sulfamethazine therapy alone, or in combination with penicillin (8, 16), can correct immediate infection and keep cows in the milking string.

EXPERIMENTAL PROCEDURE

Four normal cows were used in these experiments. To permit correct comparisons, the same cows were used in all three tests, with rest periods of 12 and 5 days, respectively, after each trial. Freedom from clinical mastitis at the time of the trial was determined on the basis of udder palpation and physical appearance of the milk by strip cup test.

The cows were maintained under conditions comparable to those of the average farm. Water was continuously available at automatic foun-

tains. Cows regularly were turned out to pasture. They were milked at 12-hour intervals, and, at the time of the trial, they were producing a minimum of 40 lb. of milk per day.

The dosage used throughout the experiment was 1.5 grains per lb. of body weight on the first day, and 1 grain per lb. of body weight on the second day. This dosage was administered in three ways: (a) One daily

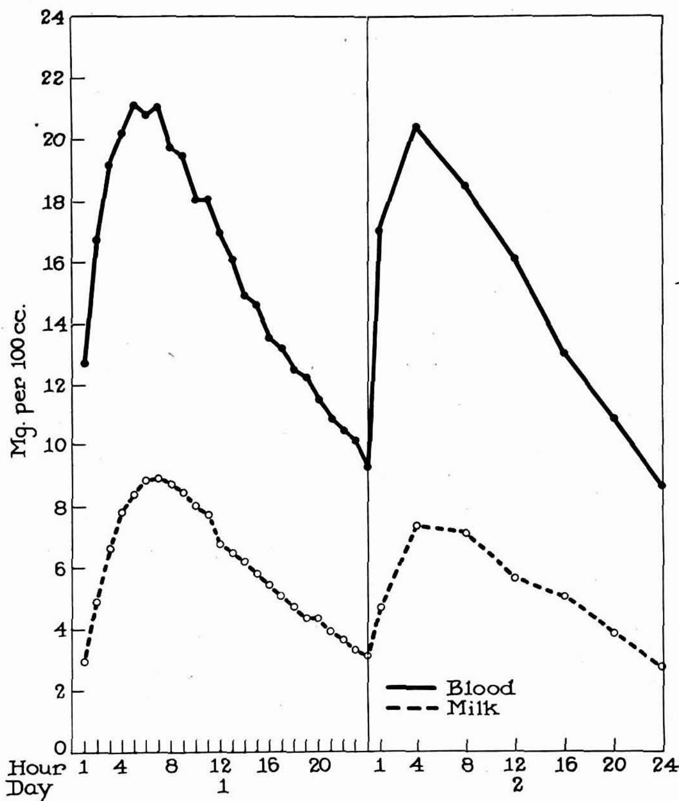

FIG. 1. Free sulfamethazine blood and milk concentrations in cows.
(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, subcutaneously; 2nd day, 1 gr./lb. body weight, subcutaneously. Milked at 0 and 12 hr.)

dose of sodium sulfamethazine 25 per cent w/v sterile solution was injected, subcutaneously, into each of four cows. (b) Sulfamethazine powder, in 1-ounce capsules, was administered orally, once a day, to two cows. (c) Sodium sulfamethazine 10 per cent w/v sterile solution was infused into the udders of four cows. Half of each total daily dose was administered immediately following complete morning and evening milkings, and equal amounts of each dose were infused into each quarter.

Sulfonamide analyses were made according to a modification (18) of the Bratton-Marshall method (4). Blood and milk samples were taken every hour for the first 24 hours, and at 1 hour, 4, 8, 12, 16, and 24 hours for the second 24 hours. Sulfamethazine determinations in milk were made for each period on each cow, using a composite sample from all quarters. Accurate records of milk production in pounds were kept for 9 days before the start of, as well as for the duration of, the experiment.

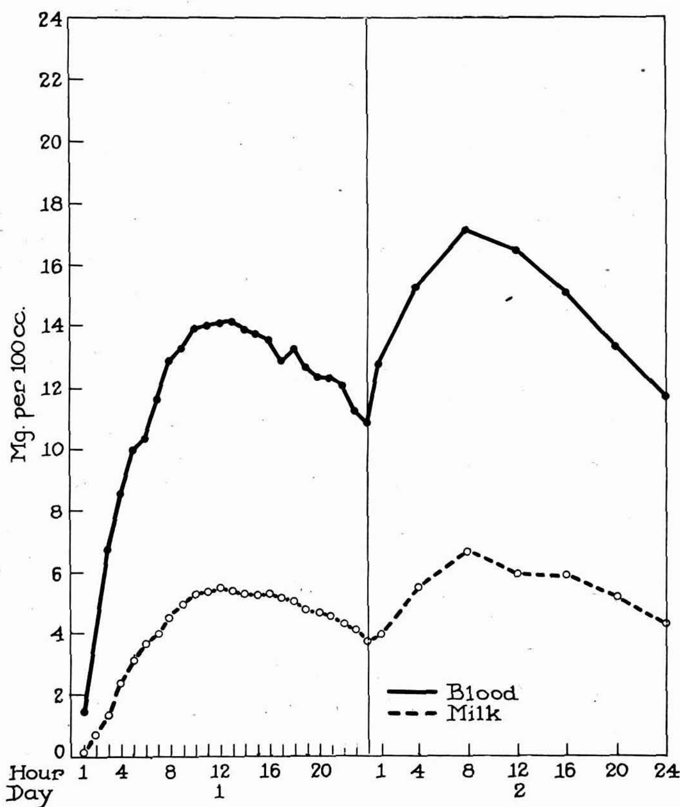

FIG. 2. Free sulfamethazine blood and milk concentrations in cows.
(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, orally; 2nd day, 1 gr./lb. body weight, orally. Milked at 0 and 12 hr.)

RESULTS

Average sulfamethazine blood and milk concentrations following subcutaneous administration are shown in figure 1. High blood levels were attained promptly, reaching a peak at about the fourth hour. The milk level curve closely followed the blood level curve, indicating that the

concentration in the milk is directly dependent on the blood concentration. The milk level was slightly less than half the blood level, which shows that an adequate milk level is dependent on a high blood level.

Figure 2 shows blood and milk concentrations after oral administration. The levels increased more slowly, reaching a peak on the first day between

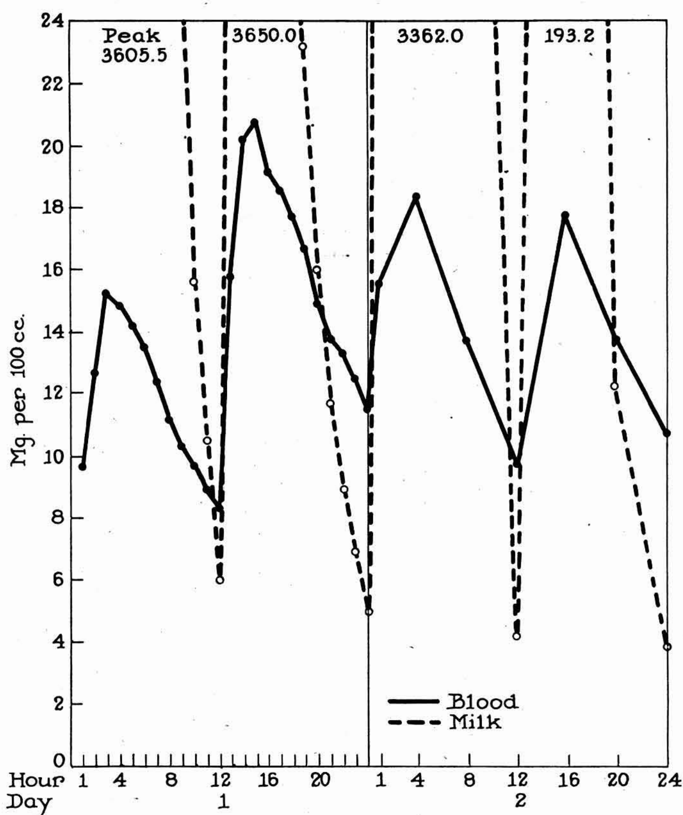

FIG. 3. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, intramammary infusion; 2nd day, 1 gr./lb. body weight, intramammary infusion. Half of each daily dose was administered immediately following the complete morning and evening milkings, and equal amounts of each dose were infused into each quarter. Milked at 0 and 12 hr.)

the eighth and twelfth hours and, on the second day, at about the eighth hour. The levels attained were not so high as after subcutaneous administration, but, likewise, did not decrease so rapidly. On the second day, the levels attained were higher than on the first day. This would indicate that, with an acutely ill animal, a prompt high blood level should be attained

by parenteral administration and maintained thereafter by oral dosing. Here, too, the milk level curve closely followed the blood level curve.

The concentrations attained by udder infusion are shown in figure 3. As might be expected, milk levels were extremely high after each infusion, rapidly decreasing from the second to the twelfth hour. Blood levels averaging between 10 and 15 mg. per 100 cc. of blood were attained within 3 hours after the first infusion, and gradually decreased to slightly more than 8 mg. per 100 cc. by 12 hours; they were maintained considerably higher after each of the next three infusions. It is evident, therefore, that sulfamethazine diffuses from udder to blood as well as from blood to milk.

Immediately following udder infusion, flakes were observed in the milk. This condition persisted for approximately 4 hours, when the milk again was normal in appearance.

In the normal cow, the decrease in milk production following administration of the drug by any of the routes described was not considered significant. The animals were being handled continuously during the 2-day trial periods, and this, in itself, would affect milk flow. During the three trials, average milk production decreased by 14 per cent, 16.5 per cent, and 18.5 per cent, respectively, from the average daily production during the 9 days preceding the experiment.

SUMMARY

1. Sulfamethazine was administered to cows parenterally, orally, and by infusion, and blood and milk determinations were made at frequent intervals after administration.

2. Sulfamethazine diffuses freely from blood to milk and from udder to blood.

3. Concentrations of 5 mg. or more of the drug per 100 cc. of milk throughout the day depend upon a persistently high concentration (more than 10 mg. per 100 cc.) of the drug in the blood.

4. All three methods of administration resulted in the attainment of blood and milk concentrations considered to be bacteriostatically effective.

5. No evidences of systemic toxicity of the drug were noted.

6. In this experiment, it has been shown that levels above 5 mg. per 100 cc. of milk can be achieved after oral or parenteral administration of the drug given once a day.

REFERENCES

(1) Barker, C. A. V. Observations on the Use of Penicillin in the Treatment of Bovine Mastitis. Can. J. Comp. Med. Vet. Sci., **9**: 235–243. 1945.

(2) Barker, C. A. V., and Dussault, H. P. Observations of Permeability of Lactating Bovine Mammary Gland to Penicillin. Can. J. Comp. Med. Vet. Sci., **9**: 332–335. 1945.

(3) Bean, C. W., Miller, W. T., and Heishman, J. O. Chemotherapy in Mastitis. Vet. Med., **37**: 401. 1942.

(4) BRATTON, A. C., AND MARSHALL, E. K. A New Coupling Component for Sulfanilamide Determination. J. Biol. Chem., **128**: 537–550. 1939.

(5) BRYAN, C. S. The Results Obtained with Tyrothricin in the Treatment of 157 Cows with Streptococcic Mastitis. Vet. Med., **37**: 364–369. 1942.

(6) BRYAN, C. S., HORWOODS, R. E., AND HUFFMAN, C. F. A Preliminary Report on Penicillin in the Treatment of Chronic Streptococcic Mastitis. Vet. Med., **40**: 87–89. 1945.

(7) EDITORIAL. Hoard's Dairyman, **92**: 638. 1947.

(8) JOHNSON, S. D., AND ROBERTS, S. J. Combination of Sulfonamide Compounds and Penicillin for the Treatment of Mastitis by Infusion. A Preliminary Report. Cornell Vet., **37**: 144–155. 1947.

(9) KAKAVAS, J. C. Penicillin in the Treatment of Bovine Mastitis. North Am. Veterinarian, **25**: 408–412. 1944.

(10) KAKAVAS, J. C. *In vitro* Studies of the Basis for Sulfanilamide Therapy in Bovine Mastitis. Am. J. Vet. Research, **6**: 9–16. 1945.

(11) KLEIN, L. A., CRISMAN, D. M., AND MOOR, J. W. Effect of Local Injections of Penicillin on Staphylococci in the Cow's Udder. Am. J. Vet. Research, **6**: 3–8. 1945.

(12) LITTLE, R. B., BROWN, J. H., AND PLASTRIDGE, W. N. Bacteriology of Mastitis; in Little & Plastridge: Bovine Mastitis. McGraw-Hill Book Co., Inc., New York, N. Y. Pp. 167–202. 1946.

(13) SCHALM, O. W. The Treatment of Chronic Bovine Mastitis. J. Am. Vet. Med. Assoc., **100**: 323–334. 1942.

(14) SCHALM, O. W., AND LITTLE, R. B. Treatment of Bovine Mastitis; in Little & Plastridge: Bovine Mastitis. McGraw-Hill Book Co., Inc., New York, N. Y. Pp. 363–416. 1946.

(15) SEELEY, H. W., JR., ANDERSON, E. O., PLASTRIDGE, W. N., AND PEARSON, P. Nonpermeability of the Lactating Bovine Mammary Gland to Penicillin. Science, **102**: 44–45. 1945.

(16) SLANETZ, L. W., AND ALLEN, F. E. Penicillin Therapy in Streptococcal Mastitis. J. Am. Vet. Med. Assoc., **111**: 125–127. 1947.

(17) WATTS, P. S., AND MCLEOD, D. H. The Estimation of Penicillin in Blood Serum and Milk of Bovines after Intramuscular Injection. J. Comp. Path. Therap., **56**: 170–179. 1946.

(18) WELSH, M., SCHROEDER, C. R., VROMAN, D. F., REDDIN, L., BURKHART, R. L., AND LANGER, P. H. The Fate of ½ gr./lb. Body Weight of 7 Sulfonamides in 7 Animal Species. U. S. Livestock San. Assoc., Proc. 50th Annual Meeting. Pp. 213–234. 1946.

THE UTILIZATION OF β-CAROTENE, VITAMIN A ALCOHOL, AND THE NATURAL ESTER OF VITAMIN A BY HOLSTEIN HEIFERS[1,2,3]

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Vitamin A is believed to be absorbed from the digestive tract in the alcohol form (1). The question has arisen as to whether vitamin A is most efficiently utilized when ingested as the alcohol form, as the natural esters of vitamin A or as β-carotene. A study was conducted to determine the relative efficiency of utilization of β-carotene, vitamin A alcohol, and the natural ester of vitamin A.

EXPERIMENTAL PROCEDURE

Six Holstein heifers between the ages of 12 and 15 months were placed on a low-carotene ration consisting of oat straw fed *ad libitum* and 10 lb. of a concentrate mixture low in carotene. The animals were maintained on this ration until the blood plasma vitamin A decreased to 6–8 γ per 100 ml. of blood plasma. They then were grouped into three pairs based upon age, body weight, and blood plasma vitamin A concentration. The three pairs of animals then received in rotation each of the three sources of vitamin A for a period of 20 days at the rate of 100 USP units of vitamin A per kg. of body weight per day. After the first and second feeding periods, the animals again were depleted to 6–8 γ of vitamin A per 100 ml. of blood plasma before starting the subsequent supplementary feeding. Thus, after three feeding periods of 20 days each, all six animals had received the three forms of vitamin A. In all instances the vitamin A supplement was administered daily in capsules during the feeding periods.

Blood samples were taken at weekly intervals during the depletion periods and daily during the feeding periods, except in the last half of the first 20-day test period; during this time, they were taken every other day. Blood plasma carotene and vitamin A were determined using the methods of Moore (3) and Kimble (2), respectively, while using an Evelyn photoelectric colorimeter.

Body weights were determined twice a month during the depletion periods and every 3 days during the feeding periods.

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[2] The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

[3] This work was supported in part by The Borden Company, New York, N. Y.

[4] Now associated with the University of Idaho, Moscow.

TABLE 1

The concentrations of blood plasma vitamin A of Holstein heifers fed β-carotene, vitamin A alcohol, and the natural esters of vitamin A[a,b]

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Period of β-carotene administration																			
717	5	6	6	5	6	11	11	9	10	9	10	8	7	8	7	8	8	7	7	7
718	13	9	9	11	10	8	10	9	11	*	12	*	8	*	10	*	11	*	9	9
724	15	6	9	7	4	5	4	5	4	5	5	6	4	4	5	10	4	6	7	7
725	4	4	4	4	1	10	7	6	7	9	6	4	5	2	5	7	5	5	5	9
726	11	9	10	6	6	8	8	8	10	*	8	*	6	*	6	*	8	*	8	8
730	10	6	8	7	5	2	1	2	3	2	5	4	4	2	4	5	4	4	2	5
Av.	9.7	6.7	7.7	6.7	5.3	7.3	6.8	6.5	7.5	6.3	7.7	5.5	5.7	4.0	6.2	7.5	6.7	5.5	6.3	7.5
	Period of vitamin A alcohol administration																			
717	10	9	9	10	8	8	8	9	10	12	11	12	13	10	11	13	12	10	13	12
718	9	12	15	14	14	17	18	17	20	21	23	15	12	13	14	14	14	13	12	14
724	11	11	12	12	7	8	9	10	12	*	12	*	8	*	13	*	12	*	11	12
725	11	6	8	6	4	4	4	6	4	7	7	7	9	10	7	8	9	7	11	10
726	5	9	8	10	4	12	10	11	12	11	13	11	10	10	11	11	10	12	10	10
730	8	10	9	10	10	12	17	11	14	*	13	*	10	*	11	*	12	*	12	11
Av.	9.0	9.5	10.2	10.3	7.8	10.2	11.0	10.6	12.0	12.8	13.2	11.3	10.3	10.8	11.2	11.5	11.5	10.5	11.5	11.5
	Period of administration of the natural esters of vitamin A																			
717	11	14	20	11	7	8	9	11	11	*	12	*	9	*	15	*	15	*	15	16
718	12	11	11	13	11	11	12	11	11	13	13	13	12	11	11	13	12	11	13	14
724	8	10	9	9	7	12	12	11	16	18	13	16	10	11	10	13	11	11	11	13
725	10	9	11	8	8	9	12	10	12	*	11	*	10	*	12	*	13	*	10	10
726	8	7	10	9	7	6	8	7	10	9	10	10	10	8	10	12	9	10	9	9
730	4	7	7	9	8	10	9	11	13	14	14	10	11	10	10	12	12	10	13	11
Av.	8.8	9.7	11.3	9.8	8.0	9.3	10.3	10.2	12.2	13.5	12.2	10.3	10.3	10.0	11.3	12.5	12.0	10.5	11.8	12.2

[a] Fed at the rate of 100 USP units per kilo of body weight per day.
[b] Expressed as γ of vitamin A per 100 ml. of blood plasma.
* No analysis.

TABLE 2

The blood plasma concentrations of carotene of heifers fed β-carotene, vitamin A alcohol and the natural esters of vitamin A[a,b]

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Period of β-carotene administration																			
717	52	58	56	58	69	67	69	69	82	80	91	94	89	85	85	100	103	94	85	98
718	40	44	46	50	52	50	52	48	48	*	61	*	67	*	78	*	78	*	73	78
724	63	65	76	65	69	80	85	80	76	80	85	91	94	96	96	94	96	96	96	98
725	33	35	37	42	44	50	50	52	58	63	59	61	58	56	59	63	56	61	61	63
726	50	54	58	54	58	56	69	65	65	*	86	*	96	*	86	*	86	*	89	87
730	42	44	54	52	56	58	58	54	54	58	63	65	61	61	63	65	65	65	59	61
Av.	46.7	50.0	54.5	53.5	58.0	60.2	63.8	61.3	63.8	70.3	74.2	77.8	77.5	74.5	77.8	80.5	80.7	79.0	77.2	80.8
	Period of administration of vitamin A alcohol																			
717	58	54	54	52	54	54	50	48	44	46	44	50	42	38	42	50	46	42	42	40
718	38	46	42	40	42	40	38	40	40	44	44	37	37	35	35	40	42	40	33	35
724	67	69	65	69	56	54	44	50	46	*	49	*	52	*	56	*	53	*	52	56
725	40	38	42	38	38	35	38	38	37	38	38	40	44	44	38	42	35	37	35	31
726	23	25	25	29	27	23	23	25	27	27	31	29	25	27	27	31	31	33	31	31
730	50	54	50	56	54	50	56	50	52	*	59	*	65	*	56	*	54	*	56	49
Av.	46.0	47.7	46.3	47.3	45.2	42.7	41.5	41.8	41.0	38.8	44.2	39.0	44.2	36.0	42.3	40.8	43.5	38.0	41.5	40.3
	Period of administration of the natural esters of vitamin A																			
717	76	78	78	74	78	65	61	67	65	*	70	*	68	*	76	*	75	*	66	67
718	48	54	48	50	48	44	44	44	42	48	46	52	52	46	50	50	50	48	48	44
724	67	67	63	63	67	61	59	58	61	69	56	65	54	54	52	59	54	54	58	59
725	42	48	42	42	38	37	35	35	33	*	43	*	48	*	42	*	46	*	42	39
726	50	50	56	50	48	48	46	40	44	48	50	48	48	46	46	46	46	46	42	38
730	33	33	31	29	29	27	27	27	29	31	31	27	25	38	25	27	29	27	31	29
Av.	52.7	55.0	53.0	51.3	51.3	47.0	45.3	45.2	45.7	49.0	49.3	48.0	49.2	46.0	48.5	45.5	50.0	43.8	47.8	46.0

[a] Fed at the rate of 100 USP units per kilo of body weight per day.
[b] Expressed as γ of carotene per 100 ml.
* No analyses made on first trial on these days.

RESULTS

Vitamin A values of 6 to 8 γ per 100 ml. of blood plasma appeared to be the critical level for these animals. When the concentration of vitamin A approached this level, the test animals stopped gaining in body weight. However, the animals usually resumed growth after about 10 days of supplemental feeding. The average time required to deplete the animals was 105 days following winter feeding, 24 days between the first and second feeding periods, and 30 days between the second and third feeding periods.

The data obtained relative to the analyses for vitamin A and carotene on the several trials are presented in tables 1 and 2.

In evaluating the blood plasma vitamin A data for the three supplementary treatments by an analysis of variance (table 3), a highly significant

TABLE 3

Analysis of variance of blood plasma vitamin A data

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total	179	2261	....
Treatments	2	593	296.50[a]
Individuals	5	393	78.60[b]
Days	9	189	21.00[a]
Interactions:			
Treatments × individuals	10	242	24.20[a]
Days × individuals	45	137	3.04
Days × treatments	18	134	7.44
Sampling error	90	573	6.37

[a] Significant at the 1% level.
[b] Approached significance at the 5% level.

difference was found between the treatments and between days of supplementation. The difference between heifers approached significance. On the basis of the least significant mean difference, it was determined that there was no significant difference in the blood plasma vitamin A concentrations of the heifers during administration of vitamin A alcohol or the natural esters of vitamin A. Both, however, produced a higher level (highly significant) of blood plasma vitamin A than did β-carotene. The following mean concentrations of blood plasma vitamin A (γ per 100 ml.) were found during the feeding periods: β-carotene feeding = 6.95, vitamin A alcohol feeding = 10.77, and the natural ester of vitamin A feeding = 10.83. The least significant mean difference at the 1 per cent level was 2.85.

In an analysis of variance of the determinations of blood plasma carotene of the heifers for the three treatments (table 4), a highly significant difference was found between treatments and between days on supplementation, but no significant difference was found between heifers. The following mean concentrations of blood plasma carotene (γ per 100 ml.) were determined

TABLE 4

Analysis of variance of blood plasma carotene data

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total	179	54,826	......
Treatments	2	18,607	9,303.50[a]
Individuals	5	13,997	2,799.40
Days	9	1,909	212.11[a]
Interactions:			
Treatments × individuals	10	10,837	1,083.70[a]
Days × individuals	45	839	18.64
Days × treatments	18	6,089	338.28[a]
Sampling error	90	2,548	28.31

[a] Significant at the 1% level.

during the feeding periods: β-carotene administration = 67.42, vitamin A alcohol administration = 43.57, and the natural ester of vitamin A = 49.28. The least significant mean difference at the 1 per cent level was 19.05. On this basis it was found that the feeding of β-carotene increased the blood plasma carotene concentration above (highly significant) that of the heifers receiving vitamin A. There was no significant difference in the blood plasma carotene concentrations when vitamin A alcohol or the natural ester of vitamin A was fed.

Linear regression lines were calculated for blood plasma vitamin A (Fig. 1) and carotene (Fig. 2) concentrations during the feeding periods. The regression equation for blood plasma vitamin A concentration when β-carotene was fed was $E = 7.422 - 0.0692\,X \pm 0.0442$ (not significant); when the natural ester of vitamin A was fed, $E = 9.403 + 0.1412\,X \pm 0.0384$

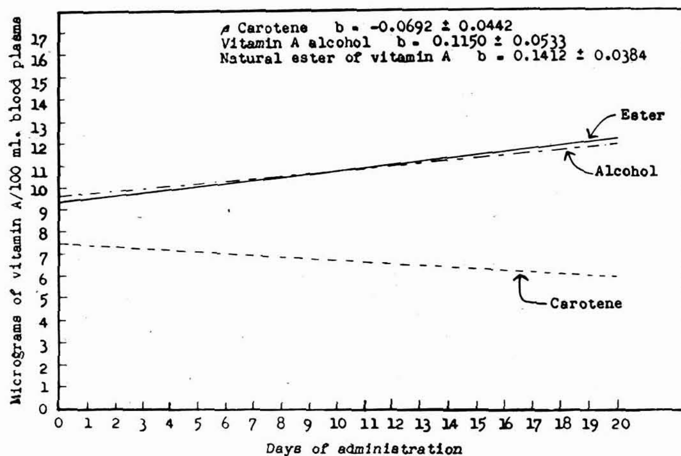

FIG. 1. The effect of the source of vitamin A upon blood plasma vitamin A levels.

(highly significant); and when the vitamin A alcohol was fed, $E = 9.61 - 0.1150\,X \pm 0.0533$ (significant). Therefore, there was a significant increase in blood plasma vitamin A concentration when vitamin A alcohol or the natural esters of vitamin A were fed, and there was a slight decrease when β-carotene was fed. This decrease, however, was not significant.

Regression equations for blood plasma carotene concentrations are as follows: when β-carotene was fed, $E = 48.70 + 1.8325\,X \pm 0.2186$ (highly significant); when the natural ester of vitamin A was fed, $E = 51.62 - 0.2934\,X \pm 0.2211$; and when vitamin A alcohol was fed, $E = 46.45 - 0.3313\,X \pm$

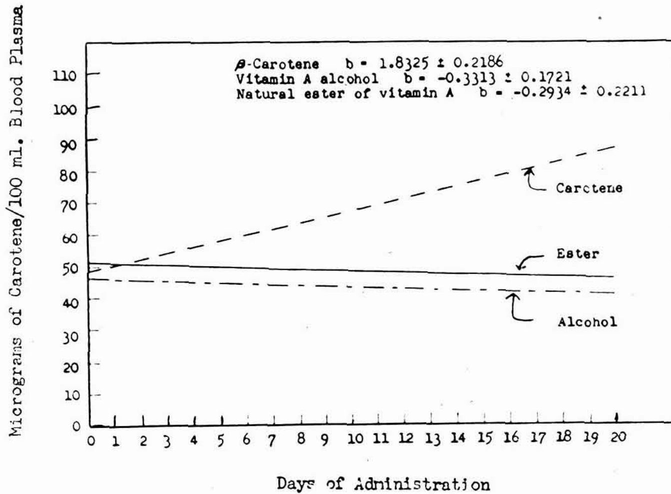

FIG. 2. The effect of the source of vitamin A upon blood plasma carotene levels.

0.1721. Therefore, feeding β-carotene increased the blood plasma carotene concentration significantly, but, when either of the two forms of vitamin A was fed, the blood plasma carotene concentration decreased slightly. This decrease, however, was not statistically significant.

CONCLUSIONS

1. The critical blood plasma vitamin A concentration was found to be 6 to 8 γ per 100 ml. blood plasma for Holstein heifers when gains in body weight were used as the criterion.

2. There was no significant difference in the efficiency of utilization of vitamin A alcohol and the natural esters of vitamin A by Holstein heifers when the blood plasma concentration of vitamin A was used as a criterion. However, both forms of vitamin A were utilized more efficiently than β-carotene.

REFERENCES

(1) GRAY, E. L., MORGAREIDGE, K., AND CAWLEY, J. D. Intestinal Absorption of Vitamin A in the Normal Rat. J. Nutrition, 20(1): 67–74. 1940.

(2) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. J. Lab. Clin. Med., 24: 1055–1065. 1939.

(3) MOORE, L. A. The Determination of Blood Plasma Carotene in the Bovine Using a Photoelectric Colorimeter. J. Dairy Sci., 22(7): 501–511. 1939.

LIVEWEIGHT AND MILK–ENERGY YIELD AT VARIOUS FEEDING INTENSITIES[1]

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The data used in the present paper are extracted from the records of the Input-Output experiment conducted by the United States Bureau of Dairy Industry in cooperation with the Agricultural Experiment Stations of Delaware, Maryland, Mississippi, New York (Geneva), Pennsylvania, South Dakota, Indiana, Michigan, New Jersey, and Virginia. In the last four of these Stations, pasture was used, thereby upsetting the determination of digestible nutrients (D.N.) intake. A primary objective in the use made of the Bureau data at the Illinois Station is to allocate D.N. intake between maintenance and lactation by the procedure of fitting a suitable equation, for which purpose only the first six of the above-mentioned stations provide adequate data. Pursuit of the primary objective has provided, somewhat incidentally, valuable material on the relation between liveweight and milk-energy yield, which is reported in the present paper.

A detailed account of the Input-Output investigation has been published by Jensen *et al.* (2).

PROCEDURE

The first 35 full calendar weeks of each lactation are used in the present study. Lactations which do not provide such a period are not used. That is, the present paper deals with partial lactations, starting within 9 days after calving (the first 2 days after calving being rejected in the original records) and continuing through the following 35 calendar weeks. The records provide a total of 255 such partial lactations.

Each 35-week partial lactation is extracted in seven subperiods of 5 weeks each, and the sum of seven subperiods represents the 35-week partial lactation. For each lactation the following items, among others, are calculated:

$D.N.$ = digestible nutrient intake for 35-week period, lb./day

FCM_8 = milk-energy yield for 35-week period, lb. 4 per cent milk/day

W = average liveweight for 35-week period, lb.

W_1 = average liveweight for first 5-week subperiod, lb.

Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds are repre-

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[2] Now at Cornell University, Ithaca, New York.

sented in the present material (table 1). Perhaps the cows represent a cross section of dairy cows in American Experiment Stations.

RESULTS

Feeding intensity. Feeding intensity is calculated as (D.N. $-$ 0.008W) /FCM_8, *i.e.*, lb. D.N. for lactation per lb. *FCM*, allowing 8 lb. D.N. per day per 1,000 lb. liveweight for maintenance, in accord with the Haecker standard. The data are divided into feeding intensity classes as indicated in table 2. These classes are based on the actual rather than the intended feed intake. Consequently, the classes do not correspond strictly with the classes used by Jensen *et al.* (2), and there is the further important difference that the present analysis is based on a 35-week partial lactation, in contrast to a calendar-year record regardless of lactation status.

TABLE 1

The number of lactations and mean values by breeds

Breed	n	% Haecker	% Grain[a]	W_1	1000 FCM_8/W_1
Ayrshire	29	120	35	1035	25.7
Brown Swiss	25	107	52	1228	31.8
Guernsey	21	114	31	998	24.6
Holstein	104	114	46	1204	31.1
Jersey	76	123	45	844	31.0

[a] Per cent of digestible nutrient intake supplied by concentrates.

Liveweight and yield. It previously has been proposed that cows possess a certain inherent tendency to produce milk under conditions of the commercial dairy, a lactational drive which may be quantitatively measured as FCM_8/W_1. The immediate purpose of the present study is to see how FCM_8/W_1 is affected by feeding intensity.

In the light of previous work the postulate is advanced that FCM_8/W_1 fluctuates, as between cows, independently of W_1. A proper test of this postulate is to fit, by least squares, the equation, $FCM_8/W_1 = a + bW_1$ and find the value of b and its standard error. For the 255 lactations as a body, as shown in the last line and last column of table 2, b is even smaller than its standard error. Such a value of b readily could arise by chance if its true value is zero. Hence, the postulate is valid so far as this particular body of observations indicates.

By a similar equational procedure the value of b (in terms of 100,000 FCM_8/W_1) within feeding intensity class works out to be 0.21 ± 0.23 or essentially the same result as that in total.[3]

If it is desired to use the power equation $FCM_8 = aW_1^b$, the exponent b is 0.92 in total and 0.93 within feeding-intensity class. The exponent is derived from the means and linear regression. For example, in total, the ex-

[3] In similar manner: within Station, $b = -0.109 \pm 0.036$; within breed, $b = -0.109 \pm 0.039$; within times milked daily (2 or 3), $b = -0.061 \pm 0.024$.

TABLE 2

Feeding intensity and milk-energy yield per unit liveweight

Feeding intensity					Lactations	Mean		Value of b in the equation	
Class	Class limit	Mean	% Haecker	% grain[a]		W_1	$\frac{1000}{FCM_8/W_1}$	$FCM_8 = aW_1^b$	$\frac{100000}{FCM_8/W_1} = a + bW_1$
1	< 0.30	0.273	83	28	35	1074	26.2	+ 1.34	+ 0.83 ± 0.67
2	0.30	0.327	100	36	59	1078	27.3	+ 1.04	+ 0.10 ± 0.43
3	0.35	0.374	114	47	71	1088	32.0	+ 0.99	− 0.04 ± 0.50
4	0.40	0.422	129	52	54	1047	32.8	+ 0.95	− 0.17 ± 0.45
5	0.45	0.474	145	53	20	1031	28.6	+ 0.92	− 0.21 ± 0.94
6	0.50	0.600	183	53	16	968	30.3	− 0.07	− 3.34 ± 0.96
All		0.381	117	44	255	1063	30.0	+ 0.92	− 0.22 ± 0.25

[a] Per cent of digestible nutrient intake supplied by concentrates.

ponent = $1 + (-0.22 \times 1063/3000) = 1 - 0.08 = 0.92$. This is a valid approximation for the present material.

While the above procedure indicates no difference in total and within feeding-intensity class, it does not necessarily follow that feeding intensity is without influence on the relation of liveweight to milk-energy yield. Table 2 shows the weight-yield relation for each of the six classes separately. None of the b's is significant except the one for the 16 lactations of class 6. The W_1 distribution in class 6 is erratic and apparently the cows were more ravenous than representative.

A noteworthy feature is the rather consistent decrease in b as feeding intensity increases. The power-equation b shows this clearly. The data seem to suggest that FCM_8 tends to be proportional to W_1 under customary feeding intensity (100 per cent or 114 per cent of Haecker standard for the lactation fraction). W_1 appears still more influential on FCM_8 for underfeeding, which may trace back to the influence of fatness at calving.

DISCUSSION

The average of 255 35-week partial lactations (table 2) is 30.0 lb. of 4 per cent milk per day per 1,000 lb. W_1. Davis *et al.* (1) report for the Nebraska Station dairy herd average values for 1000 FCM_8/W_1 of 33.3 for 131 Ayrshire lactations, of 30.6 for 77 Guernsey lactations, of 39.1 for 367 Holstein lactations and of 34.5 for 171 Jersey lactations. Feeding intensity for the Nebraska data is not recorded but no doubt is above 100 per cent Haecker for all lactations. Control of feeding intensity was a major point in the Input-Output experiment. It is presumed this control does not bias the results as between breeds.

SUMMARY

The relation of liveweight in pounds within 5 weeks after calving, W_1, to milk-energy yield for the 35-week partial lactation in pounds of 4 per cent milk per day, FCM_8, is investigated by adjusting the equation, $FCM_8/W_1 = a + bW_1$, to observations from the Input-Output experiment of the Bureau of Dairy Industry. For all lactations (255), b is not significantly different from zero. The postulate that FCM_8 tends to be proportional to W_1 is valid so far as indicated by this body of observations on five breeds of dairy cows taken as a whole. Essentially the same relation holds for each feeding-intensity class (83 to 183 per cent of Haecker). However, as between feeding-intensity classes, there is a consistent tendency for b to decrease as feeding intensity increases. The consistency of this tendency may give it some meaning.

REFERENCES

(1) DAVIS, H. P., MORGAN, R. F., AND GAINES, W. L. Live Weight and Milk-Energy Yield in the Nebraska Station Dairy Herd. J. Dairy Sci., 26: 625–641. 1943.

(2) JENSEN, E., KLEIN, J. W., RAUCHENSTEIN, E., WOODWARD, T. E., AND SMITH, R. H. U. S. Dept. Agr. Tech. Bull. 815. 1942.

THE CHEMICAL COMPOSITION OF THE CRYSTALLINE DEPOSIT IN EVAPORATED MILK

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A white, crystalline deposit occasionally forms in evaporated milk during storage. It is found chiefly on the interior can surfaces but as the crystals increase in size and weight, agitation of the contents of the can gradually causes them to settle. The deposit does not appear in some samples of milk and, even when it is present, it remains unnoticed by most consumers. While the crystals are not harmful, they are at times a source of annoyance, especially in infant feeding when they obstruct the holes in nipples. Occasionally the crystal aggregates are mistaken for foreign particles.

The deposit cannot be redissolved in the milk after it has formed. The particles themselves vary from microscopic size to crystal aggregates $\frac{3}{8}$-inch in diameter. They are dense and hard and should not be confused with the insoluble protein deposit which sometimes is found in evaporated milk.

Sato was the first to investigate the salt crystals of concentrated milk. On the basis of his determinations of calcium, magnesium, and phosphorus in salt crystals found in sweetened condensed milk (9, 11), he reported calcium citrate to be the chief constituent of these crystals. Only qualitative determinations for citrates were reported. Crystals of tyrosin, leucin, and cysteine also were found. Later Sato (10) reported on the examination of the sediment obtained from one 2-year-old can of evaporated milk. A quantitative analysis of this deposit was stated to show that it contained tricalcium and trimagnesium phosphates and tricalcium citrate. The quantitative data and the relative proportions of these salts in the evaporated milk were not given.

Mojonnier and Troy (7) condensed unheated skim milk over sulfuric acid to one-third its volume, stored it at 85° F. for 3 months, and then found a considerable quantity of calcium citrate had crystallized in the milk.

While this manuscript was in preparation, Gould and Leininger (3) published the results of quantitative determinations made in duplicate on a composite sample of crystals separated from evaporated milk. They found that the crystals were largely composed of calcium citrate.

This study of the crystalline deposit that forms in evaporated milk was conducted to determine quantitatively the composition of the crystals and to define the conditions that favor and retard their formation. This paper is concerned with quantitative determinations.

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EXPERIMENTAL PROCEDURE

Crystals were obtained from several hundred cans of commercial evaporated milk. The sediment from each can was washed with 25 per cent alcohol, thoroughly agitated, and decanted a number of times until the wash alcohol showed no turbidity. The crystals then were assumed to be reasonably free from foreign matter. They were air dried at room temperature, ground in a mortar, redried at a low temperature under vacuum, and preserved in glass-stoppered bottles for analysis.

The method of McCrudden (4, 5) was used for the calcium determinations. The calcium oxalate precipitate was ignited and weighed as calcium oxide. Magnesium (4, 5) was determined on the filtrate from the calcium analyses. Nitric acid was added to this filtrate and the solution evaporated to dryness to expel the ammonium salts. The residue then was dissolved in a little hydrochloric acid. The usual procedure was carried out and the precipitate was weighed as magnesium pyrophosphate.

The official gravimetric method (1) was used for the determination of phosphorus.

Citric acid was determined by the pentabromoacetone method as modified by Deysher and Holm (2), with a few additional changes. The procedure is outlined here in some detail because citric acid determinations often have been found difficult to make and because good results were obtained with this modification.

Five-tenths gram of the material is dissolved in 40 ml. of sulfuric acid (1 to 1 by volume) in a 250-ml. volumetric flask and several milliliters of 10 per cent phosphotungstic acid are added to precipitate the small amount of protein material. The contents of the flask are made up to 250 ml., thoroughly shaken, and filtered. Fifty milliliters, which is equivalent to 0.1 g. of the sample, are taken for the determination. Five milliliters of 37.5 per cent potassium bromide are added, followed by 5 per cent potassium permanganate added dropwise until a brown precipitate remains for at least an hour. The mixture then is placed in a refrigerator over night, after which the excess potassium permanganate is discharged with 20 per cent ferrous sulfate. After filtering, drying in the vacuum desiccator, and weighing, the precipitate is dissolved with alcohol and ether. The crucible again is dried and weighed. The loss in weight represents the pentabromoacetone which, multiplied by 0.424, is equal to anhydrous citric acid.

This method was tried on C.P. calcium citrate in which the water of crystallization had been determined. The recovery on four determinations was 98.7, 101.7, 100.3, and 99.4 per cent.

RESULTS

The analytical results obtained on four groups of crystals gathered from cans of commercial evaporated milk are given in table 1.

TABLE 1

Analysis of crystals from 4 brands of evaporated milk[a]

Milk no.	Loss on ignition	CaO	MgO	P_2O_5	Anhydrous citric acid	Loss on ignition + CaO + MgO + P_2O_5
	(%)	(%)	(%)	(%)	(%)	(%)
1[b]	70.79	29.0	0.23	0.19	64.25[c]	100.21
2[b]	70.40	29.22	0.12	0.44	63.97	100.18
3	65.80	29.90	0.12	4.43	60.71	100.25
4[b]	70.95	29.10	0.31	0.19	63.60[c]	100.55

[a] Each group of crystals was collected from cans of milk produced in a single plant and processed on the same or on consecutive days.

[b] Only a trace (0.02%) of SiO_2 was found in a composite of these samples.

[c] Triplicate determinations; all others made in duplicate.

The calcium oxide was found to be about 29.0 per cent and the anhydrous citric acid 63.0 per cent. The composition of C.P. calcium citrate in terms of calcium oxide and anhydrous citric acid is 29.48 per cent and 67.33 per cent, respectively (6).

There was a 9 to 10 per cent loss in weight of the evaporated milk crystals at 120° C., which indicates that water of crystallization was present. According to Merck's Index (6) all the water of crystallization of calcium citrate is lost at 120° C.

The amounts of magnesium oxide in the different samples were fairly uniform but the quantity of phosphorus pentoxide in no. 3 was in large excess over the phosphorus pentoxide in the other three samples. This indicates the presence of a substantial quantity of calcium phosphate in no. 3.

Since the calcium in the calcium caseinate-calcium phosphate complex (8) of milk exists as tribasic phosphate, calculations were made to determine the quantities of tribasic magnesium and calcium phosphate and of calcium citrate that might be present in the crystals. Results of the calculations are presented in table 2. All the magnesium oxide was converted to trimagnesium phosphate, but this required more phosphorus pentoxide than was present in samples 1 and 4, giving the latter a negative phosphate balance.

TABLE 2

Calculated values[a] *for calcium and magnesium phosphates and for calcium citrate in the salt crystals of evaporated milk*

Milk no.	MgO as $Mg_3(PO_4)_2$	P_2O_5 balance	Remaining P_2O_5 as $Ca_3(PO_4)_2$	CaO balance	Remaining CaO as CaCit. $4H_2O$	Total $Mg_3(PO_4)_2$ + $Ca_3(PO_4)_2$ + CaCit.$4H_2O$
	(%)	(%)	(%)	(%)	(%)	(%)
1	0.50	− 0.08	....	29.00	98.33	98.83
2	0.26	+ 0.30	0.65	28.87	97.90	98.81
3	0.26	+ 4.29	9.37	24.82	84.18	93.81
4	0.67	− 0.17	....	29.10	98.68	99.35

[a] Calculated from the determined values shown in table 1.

The remaining phosphorus pentoxide in samples 2 and 3 was converted to tricalcium phosphate. The calcium balance remaining after the phosphorus pentoxide was exhausted was converted to calcium citrate. This required a little more citrate than was found in the samples. The sums of these calculated values for the phosphates and citrates are given in the last column of table 2. With the exception of sample 3, they are not far from 100 per cent.

There are other salt combinations that might be assumed to exist for the purpose of calculating the approximate composition of the crystals. However, in the absence of accurate data on the salt crystal structures, any determination of the manner in which the various components are combined must be deferred.

SUMMARY

Four groups of salt crystals that had separated from evaporated milk during storage were analyzed for calcium oxide, magnesium oxide, phosphorus pentoxide and citric acid. One group of crystals was high enough in phosphorus pentoxide to indicate the presence of almost 10 per cent tricalcium phosphate. Most of the crystals contained about 98 per cent calcium citrate and small but varying amounts of tricalcium and trimagnesium phosphates.

REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 4th Ed. Washington, D. C. P. 19. 1935.

(2) Deysher, E. F., and Holm, G. E. Determination of Citric Acid. Ind. Eng. Chem., Anal. Ed., **14**: 4–7. 1942.

(3) Gould, I. A., and Leininger, E. The Composition of the Solids which Deposit from Evaporated Milk during Storage. Mich. Agr. Expt. Sta. Quart. Bull. 30. Pp. 54–56. 1947.

(4) McCrudden, F. H. The Quantitative Separation of Calcium and Magnesium in the Presence of Phosphates and Small Amounts of Iron Devised Especially for the Analysis of Foods, Urine, and Feces. J. Biol. Chem., **7**: 83–100. 1909.

(5) McCrudden, F. H. The Determination of Calcium in the Presence of Magnesium and Phosphates. J. Biol. Chem., **10**: 187–199. 1911.

(6) Merck Index. Pub. by Merck and Co., Rahway, N. J. 5th Ed. P. 108. 1940.

(7) Mojonnier, T., and Troy, H. C. The Technical Control of Dairy Products. 1st Ed. Mojonnier Bros. Co., Chicago, Ill. P. 762. 1922.

(8) Ramsdell, G. A., and Whittier, E. O. Composition of Casein in Milk. J. Biol. Chem., **154**: 413–419. 1944.

(9) Sato, M. Untersuchüngen Uber Die Milchkrystalle in Kondensierter Milch Mit Zuckerzusatz. J. Coll. Agr., Tokyo Imp. Univ., **5**: 321–338. 1913–1914.

(10) Sato, M. Sediments of Evaporated Milk. Proc. World's Dairy Congr., **2**: 1284–1285. 1923.

(11) Sato, M. The Crystals Found in Sweetened Condensed Milk. Proc. World's Dairy Congr., **2**: 1285. 1923.

THE ISOLATION AND PROPERTIES OF THE IMMUNE PROTEINS OF BOVINE MILK AND COLOSTRUM AND THEIR ROLE IN IMMUNITY: A REVIEW

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In mammalian life, the mother supplies the fetus and the newborn offspring with all of the substances necessary for growth. However, in addition to providing the usual food factors, the offspring is given the antibodies necessary to resist infectious diseases. Since the work of Ehrlich (8), it has been known that antibodies may be transmitted through the colostrum or first milk, passively immunizing the offspring by means of immune bodies which are ingested by mouth and then pass from the digestive tract to the blood stream. In addition, Ehrlich discovered that, in some species, immune bodies also may be transmitted through the placenta directly from the blood stream of the mother to the circulation of the fetus.

In the ruminants, placental transmission does not occur, and the colostrum is the sole source of antibodies for the newborn animal (9, 13, 22). Some years ago, Smith and Little (32, 33) investigated the factors concerned with the survival of newborn calves and found that intestinal infections were among the major causes of death. Feeding of colostrum was found to decrease the mortality enormously. Obviously, the antibodies transmitted by the colostrum are of great importance in enabling the newborn animal to resist infection. At about the same time, Howe (10) and Orcutt and Howe (17) observed that, after the ingestion of colostrum, agglutinins appear in the calf serum associated with a globulin which is precipitable at low concentrations of sodium sulfate. Other investigators (11, 21) have since found by electrophoretic analysis that the serum of the newborn calf does not possess any γ-globulin and that the appearance of slow-moving globulin occurs only after the ingestion of colostrum.

In recent years it has been amply demonstrated by many investigators that antibodies are associated with globulin components of the serum, and much has been learned regarding their properties (12). Until recently, no attempts were made to isolate and study the proteins associated with immunity from milk or colostrum.

The immune proteins of bovine colostrum and milk have been isolated in order to determine their relationship to the immune proteins found in blood serum (24, 25, 26). It obviously is of some importance to ascertain

[1] The experimental work included in this paper was performed while the author was at the Biological Laboratories of E. R. Squibb and Sons.

[2] This laboratory is aided by a grant from the United States Public Health Service.

whether the immune bodies in the colostrum and milk are identical or similar to those in the maternal blood stream. It also is of interest to determine what happens to the colostrum antibodies during their passage into the blood stream of the newborn animal. The cow represents a good species for such a study because, in addition to the availability of milk and colostrum, the situation is not complicated by placental transmission of antibodies. It is convenient to refer to the colostrum and milk globulins which are associated with immunity as "immune lactoglobulins", although it is realized that the actual antibody content may account for only a very small portion of these fractions.

BOVINE IMMUNE PROTEINS

Colostrum obtained within a few hours after parturition was found to have a protein concentration between 15 and 26 per cent or, roughly, two to three times the concentration of blood plasma (25). By electrophoretic analysis, the immune protein may represent as much as 50 to 60 per cent of the total protein in colostrum, and as high as 85 to 90 per cent of the protein in colostrum whey. Therefore, it was a comparatively simple matter to isolate the immune protein in electrophoretically homogeneous form. After removal of the casein (Fraction *A*) by isoelectric precipitation at pH 4.5, the filtrate was brought to pH 6.0 with 0.5 M sodium hydroxide, and successive fractions were removed at 0.3 (Fraction *B*), 0.5 (Fraction *C*), and 0.9 (Fraction *D*) saturation with ammonium sulfate. After reprecipitation of each fraction within the same limits of salt concentration, the preparations were dialyzed and dried from the frozen state. Figure 1 shows the electrophoretic patterns obtained with the four fractions and with the original colostrum.

The electrophoretic pattern of the whole colostrum shows only a few components, with the slow-moving large peak due to the immune protein. The crude casein of colostrum (Fraction *A*) is complex in nature, like that of milk (15, 35), and contains at least two components (25). Fraction *B* consists entirely of a slow-moving globulin, and Fraction *C* of about 85 per cent of this protein. All of the immune activity of the colostrum was found to be associated with the lactoglobulin of low electrophoretic mobility (-1.8 to -2.2×10^{-5} sq. cm. per volt per second at pH 8.4).

The lactalbumin (Fraction *D*) is complex in nature and, like the similar fraction of milk whey, consists mainly of the β-lactoglobulin isolated by Palmer (18). The crystalline β-lactoglobulin of colostrum, as far as could be determined, is identical with that obtained from milk (25).

Quantitative isolation of the immune lactoglobulins was accomplished by precipitation at 0.4 saturation with ammonium sulfate after isoelectric precipitation of the casein. After this fraction was reprecipitated several

times under the same conditions, the material was homogeneous. Prolonged dialysis of the protein resulted in a separation of water-insoluble and water-soluble portions or eu- and pseudoglobulin fractions.

Figure 2 shows the electrophoretic patterns obtained with the normal whey of the later milk. Here the immune globulin represents only about 10 per cent of the whey protein in the normal animal, although the pro-

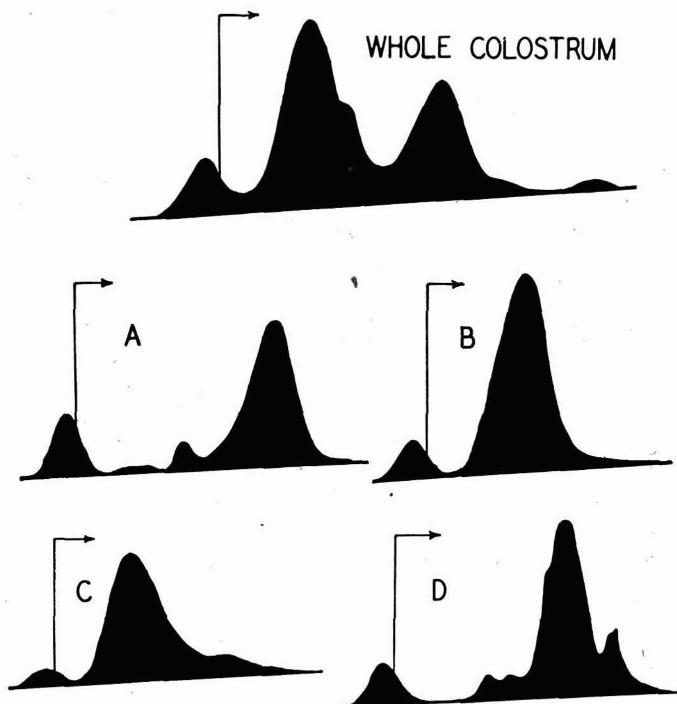

FIG. 1. Electrophoretic patterns of the descending boundaries of whole colostrum and of fractions derived from it. *A* is the casein; *B*, *C* and *D* are ammonium sulfate fractions obtained between 0 and 0.3 saturation (*B*), between 0.3 and 0.5 (*C*), and between 0.5 and 0.9 (*D*), respectively. Fraction *B* consists entirely of immune globulin and *C* mainly of this protein. The principal component of *D* is β-lactoglobulin, which could be obtained in crystalline form. Electrophoresis was for 200 minutes in veronal buffer at pH 8.3 to 8.4 at an ionic strength of 0.1. (Figure reproduced by permission of the Journal of Biological Chemistry.)

portion may increase somewhat in animals that have been hyperimmunized (26). The changes in protein composition during the transition from colostrum to milk have been studied by Crowther and Raistrick (5). More recently these changes also have been observed in the Tiselius apparatus (7, 14).

Because of the low concentration of immune protein in the milk whey,

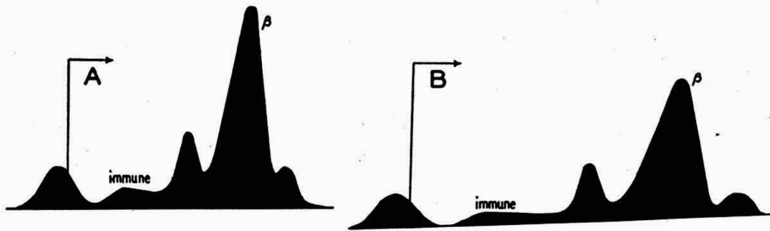

FIG. 2. Electrophoretic patterns of normal whey taken at 166 minutes (*A*) and 250 minutes (*B*). The principal component is β-lactoglobulin. The immune globulin represents about 10 per cent of the total protein. (Figure reproduced by permission of the Journal of Biological Chemistry.)

a somewhat different procedure from that used for the colostrum was adopted in order to isolate the immune lactoglobulin. This was accomplished

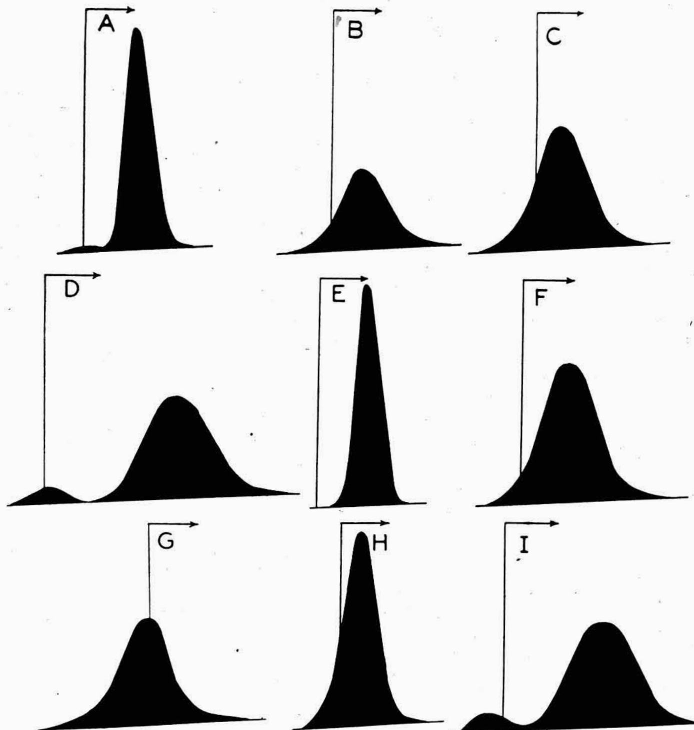

FIG. 3. Descending patterns of the purified immune lactoglobulins. The pseudoglobulin is shown in *A* (pH 3.90), *B* (pH 5.11), *C* (pH 6.81), and *D* (pH 8.55). The euglobulin is in *E* (pH 3.81), *F* (pH 5.12), *G* (pH 6.13), *H* (pH 6.82), and *I* (pH 8.65). These boundaries do not show the presence of the other milk proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

by ammonium sulfate fractionation involving isoelectric precipitations at different pH values (26). The immune lactoglobulins of milk and colostrum, as far as the authors have been able to determine, are extremely similar or, more probably, identical. Figure 3 shows the electrophoretic patterns obtained at various pH values with some of the purified immune lactoglobulins. These proteins are free from the other milk proteins. How-

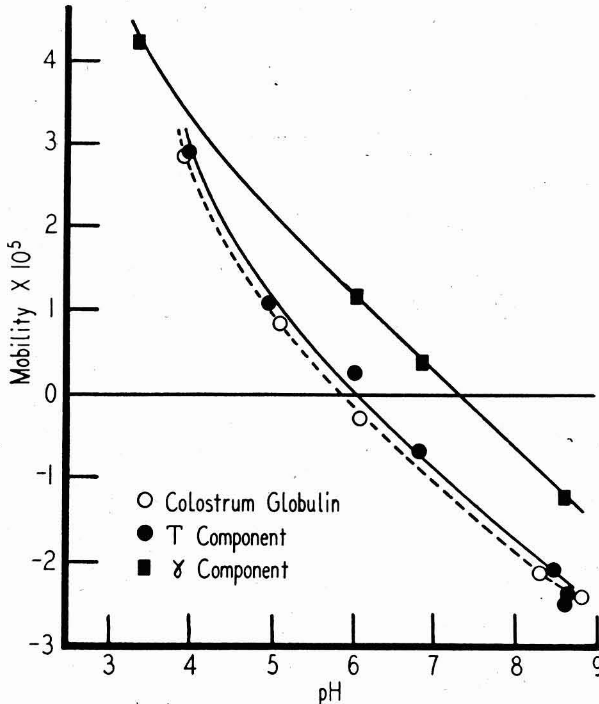

FIG. 4. Electrophoretic mobility as a function of pH for colostrum globulin, *T*-globulin, and γ-globulin. All of the values were calculated from descending migrations in univalent buffers at 1° C. The mobility is in sq. cm. per volt per second. The isoelectric point of the γ-globulin is at pH 7.2, that of the *T*-component at pH 6.15, and that of the total immune globulin of colostrum at pH 5.85. The separated lactoglobulins (not shown in the figure) gave for the pseudoglobulin an isoelectric point of pH 5.6, and for the euglobulin an I.E.P. of pH 6.05. (Figure reproduced by permission of the Journal of Biological Chemistry.)

ever, most of the boundaries show greater spreading than would be expected for a single molecular species. This is similar to the many observations that have been made with the γ-globulins of serum.

The animals from which samples of milk and colostrum were obtained had been hyperimmunized. All of the immune activity is associated with the specific lactoglobulins which were isolated and not with any of the other proteins of milk or colostrum, such as casein or β-lactoglobulin.

Since the immune proteins of bovine serum had not been previously isolated, it was necessary to develop a procedure for this purpose. As in the plasma of the horse (28, 34), immune activity is associated with the more rapidly migrating *T*-fraction as well as with the γ-globulin (25). The procedures developed by Cohn *et al.* (4) and Oncley *et al.* (16) for the fractionation of normal human plasma were adapted for the fractionation of hyperimmune bovine serum. This permitted the isolation of the γ- and *T*-globulins in electrophoretically homogeneous form (25). With these proteins in hand, it then was possible to compare the bovine immune proteins obtained from the different body fluids.

Figure 4 shows the electrophoretic mobilities as a function of pH for the bovine immune proteins. The γ-globulin possesses a much higher isoelectric point than the *T*-globulin or the total colostrum immune lactoglobulin. The eu- and pseudoglobulins of the colostrum or milk have slightly different isoelectric points, but these fall on either side of those of the *T*-globulin. From these facts alone it is possible to say that the immune proteins found in milk and colostrum are not γ-globulins as defined in terms of electrophoretic mobility and isoelectric point. However, it is not possible from these measurements to differentiate the lactoglobulins and the *T*-globulins.

While none of these proteins is completely homogeneous in the ultracentrifuge, they contain roughly 80–90 per cent of a component which sediments at 7 Svedberg units. The diffusion constants of these preparations range from about 3.3 to 3.9×10^{-7} sq. cm. per second. From these values it may be calculated that the principal components of the bovine immune lactoglobulins and serum globulins possess molecular weights in the neighborhood of 180,000. The ready diffusibility of the immune lactoglobulins of the colostrum through the intestine of the newborn animal cannot be due to any difference in size of these proteins as compared to the immune globulins of the serum.

Some studies have been made of the carbohydrate and amino acid content of the different bovine immune globulins (29, 30). All of these proteins were found to contain hexose and hexosamine in a ratio of about 2 to 1. These proteins, as shown in table 1, contain all of the amino acids known to be required in mammalian nutrition. In general, the data are similar to the analyses of human γ-globulin reported by Brand *et al.* (1). The immune globulins from horse and human serum also have been analyzed (29, 30); these greatly resemble the bovine proteins in their amino acid composition, although there are enough differences to indicate the different species from which the proteins are obtained.

It is rather striking that the immune proteins of different species appear to form a definite homologous group, with extremely similar physical and

chemical properties, in much the same way as do the serum albumins and the hemoglobins. It also is noteworthy that the immune globulins are quite different in amino acid content from any of the other proteins which are known to be present in mammalian milk or serum.

Although the bovine immune proteins greatly resemble one another, it is possible to distinguish between them. For example, attention may be called to the values for arginine and methionine of γ-globulin, which are much higher than those for the *T*-globulin or the lactoglobulins. The leucine content of the different proteins also shows marked differences. The phenylalanine content of the *T*-globulin is higher than that of the γ-globulin

TABLE 1

Amino acid and carbohydrate content of bovine immune proteins

(Data are averages derived from (25), (26), (29) and (30). All values are in terms of the anhydrous ash-free proteins.)

Constituent	Eu-lacto-globulin	Pseudo-lactoglobulin	*T*-globulin	γ-globulin
	(%)	(%)	(%)	(%)
Arginine	4.9	3.5	4.8	5.8
Histidine	1.89	2.14	2.01	2.05
Lysine	6.3	7.2	6.4	6.7
Isoleucine	3.1	3.1	3.0	3.2
Leucine	10.4	9.1	8.6	7.4
Valine	10.4	9.4	9.5	10.0
Threonine	10.5	10.1	9.5	10.0
Phenylalanine	3.6	3.8	4.5	3.2
Tryptophane	2.4	2.7	2.6	2.6
Cystine	3.2	3.0	2.8	2.9
Methionine	0.98	1.08	1.00	1.18
Sulfur	1.05	1.04	0.95	1.02
Hexose	2.9	2.8	2.5	2.05
Hexosamine	1.45	1.32	1.50	1.31

or of the immune lactoglobulins; this is strikingly reflected in the ultraviolet absorption spectra of these proteins (27). As shown in figure 5, bovine *T*-globulin possesses a much steeper end-absorption than the colostrum or γ-globulin. It thus appears that the lactoglobulins possess somewhat different protein moieties than the serum immune proteins.

However, the lactoglobulins and the serum globulins must be substances very closely related. All of these proteins will give quantitatively equivalent anaphylactic cross-reactions in guinea pigs sensitized to bovine immune proteins whether they are derived from milk or from serum (25). It is well known, particularly from the works of Wells and Osborne (36), that the globulin fractions of milk and serum are immunologically related. It now has been shown that these cross-reactions are due to the immune proteins.

The presence in the immune proteins of all the amino acids known to be required for the maintenance of nitrogen equilibrium in mammals raises

an important nutritional question. Obviously, for protection of the adult and the newborn against infectious disease, all of the so-called essential amino acids must be supplied in adequate amount. Evidence is available which shows that hypoproteinemia causes a decrease of antibodies and a lower resistance to infection. The synthesis of the globulins concerned with immunity is then a special problem in nutrition, as recently emphasized by Cannon (3).

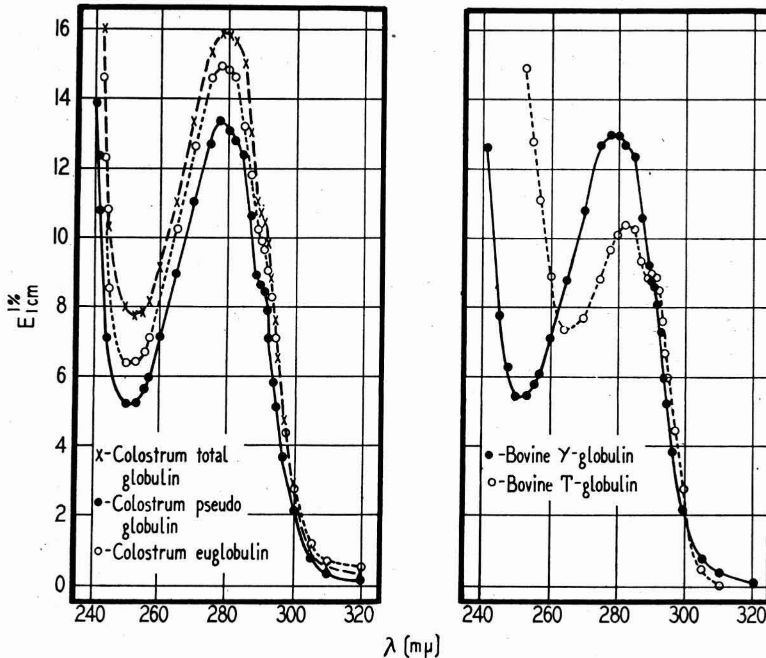

FIG. 5. Ultraviolet absorption spectra of bovine globulins. The absorption curve of the *T*-globulin appears to reflect the higher content of phenylalanine by the steeper end-absorption as compared to the other proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

PASSIVE IMMUNITY IN THE CALF

Since it already has been demonstrated that the colostrum immune globulin is different from γ-globulin in electrophoretic mobility and other properties, it is preferable not to refer to the globulin which appears in the blood stream of the newborn after the ingestion of colostrum as a γ-globulin. The protein which appears in the blood stream of the calf after ingestion of colostrum possesses the electrophoretic mobility of the immune lactoglobulin and not that of γ-globulin (31). Figure 6 shows the electrophoretic patterns obtained with the serum of the newborn calf at birth and

at various times later. The calf was fed colostrum only during the first day of life, and thereafter was isolated from the mother. The serum of the newborn calf did not contain any slow-moving globulin. However, the pattern of the serum obtained 2 days after birth showed a large amount of colostrum globulin (44 per cent of the total protein). Thereafter, the

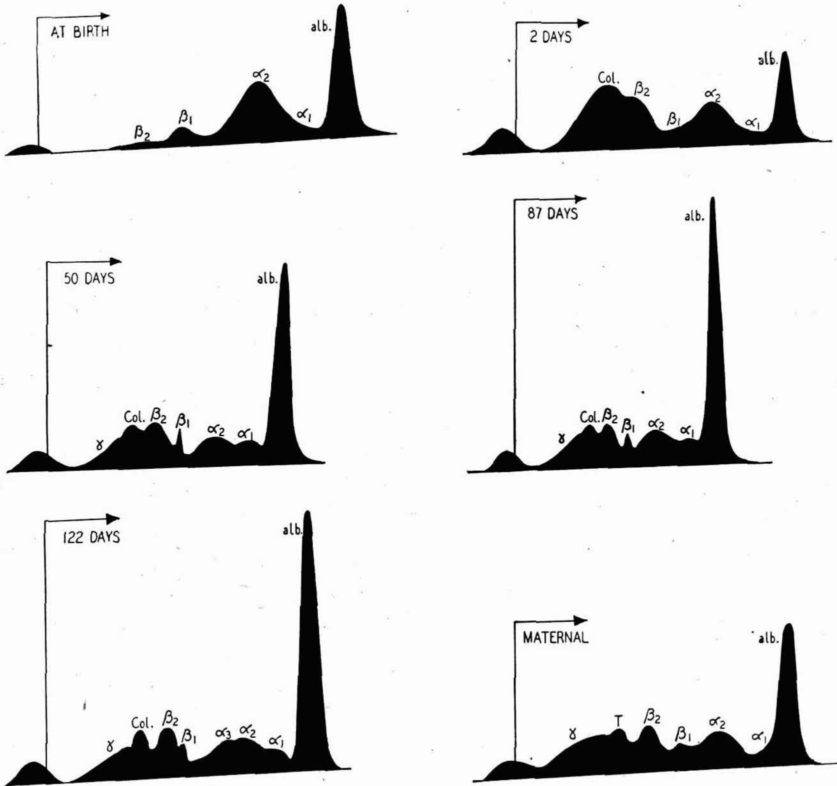

FIG. 6. Electrophoretic patterns of the descending boundaries of the serum of a newborn calf and of the same animal 2, 50, 87 and 122 days later. For comparison, the maternal serum obtained 3 days before term also is shown. The runs were performed at 1° C. in a veronal buffer of pH 8.4 to 8.6, and at an ionic strength of 0.1. The serum of the newborn is practically devoid of slow-moving globulins. The immune component (Col.) appears after feeding colostrum. The absolute heights of the different serum samples cannot be compared as the runs were made at somewhat different protein concentrations.

amount of colostrum globulin decreased steadily. From such data it is possible to estimate the time during which this protein remained in the blood stream of the calf. The immune component decreased to about one-half its initial concentration in about 20 days, and persisted for many

months. γ-globulin was hardly detectable in the blood stream of the newborn calf. Even after 4 months, it had not reached the normal adult level. It is obvious that the passively acquired immunity is of real importance to the health of the calf for the long period before it is capable of making antibodies of its own.

It also should be stated that the mobilities and relative concentrations of the various serum proteins in the newborn calf may be very different from those in the adult. This should be extremely useful in approaching some of the problems of the physiology and chemistry of the fetus. In fact, Pedersen (20) recently has reported the isolation of fetuin, an α-globulin, from the serum of the bovine fetus. Bovine fetal hemoglobin reportedly differs from that of the adult (37).

SUMMARY

Colostrum serves a special function in order to enhance the resistance of the newborn to infectious disease. This is shown by the extremely high concentrations of immune lactoglobulins in the colostrum. These globulins are passively transferred to the offspring, where they may persist in the blood stream for many months. The lactoglobulins which have molecular weights near 180,000 pass from the intestinal tract of the calf to its blood stream. The immune lactoglobulins of bovine milk and colostrum, and the γ- and T-globulins of bovine serum, have been isolated and compared with respect to their physical and chemical properties.

CONCLUSIONS

It is clear that the colostrum serves a special function in order to enhance the survival of the newborn animal. Not only is the colostrum richer than the milk in some of the vitamins, as demonstrated by various investigators (6, 19, 23), but it also contains a totally different distribution of proteins than the milk. The extremely high percentage of immune lactoglobulins, together with the fact that the colostrum may contain 25 per cent protein in the aqueous phase, demonstrates the extreme nature of this adaptation. It is not surprising that many investigators have found that colostrum generally possesses higher immune titers than the maternal blood (2). The long duration of the passively acquired immunity in the calf also emphasizes the great importance of colostrum to the health and well-being of the newborn.

The protein synthesis of the mammary gland presents an intriguing picture. The gland makes special proteins such as casein and β-lactoglobulin, which are not found elsewhere in the body, and even gives its own characteristic label to the immune bodies which must be drawn from the blood stream.

REFERENCES

(1) BRAND, E., KASSELL, B., AND SAIDEL, L. J. Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. III. Amino Acid Composition of Plasma Proteins. J. Clin. Invest., **33**: 437–444. 1944.

(2) BRAUN, H., HOFMEIER, K., AND HOLZHAUSEN, G. v. Die Vererbungsfrage in Der Lehre von Immunität Gegen Infektionskrankheiten. In Kole, W., and Wassermann, A. v., eds., Handbuch der Pathogenen Mikroorganismen. Aufl. 3, Bd. **1**: 1109–1146. 1929.

(3) CANNON, P. R. The Relationship of Protein Metabolism to Antibody Production and Resistance to Infection. In Anson, M. L., and Edsall, J. T., eds., Advances in Protein Chem., **2**: 135–154. 1945.

(4) COHN, E. J., STRONG, L. E., HUGHES, W. L., JR., MULFORD, D. J., ASHWORTH, J. N., MELIN, M., AND TAYLOR, H. L. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. J. Am. Chem. Soc., **68**: 459–475. 1946.

(5) CROWTHER, C., AND RAISTRICK, H. A Comparative Study of the Proteins of the Colostrum and Milk of the Cow and Their Relations to Serum Proteins. Biochem. J., **10**: 434–452. 1916.

(6) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. II. The Carotene and Vitamin A Content of Cows' Colostrum. Biochem. J., **27**: 1998–2005. 1933.

(7) DEUTSCH, H. F. A Study of Whey Proteins from the Milk of Various Animals. J. Biol. Chem., **169**: 437–448. 1947.

(8) EHRLICH, P. Ueber Immunität Durch Vererbung und Säugung. Z. Hyg. Infektionskrankh., **12**: 183–203. 1892.

(9) FAMULENER, L. W. On the Transmission of Immunity from Mother to Offspring. A Study upon Serum Hemolysins in Goats. J. Infectious Diseases, **10**: 332–368. 1912.

(10) HOWE, P. E. An Effect of the Ingestion of Colostrum upon the Composition of the Blood of New-Born Calves. J. Biol. Chem., **49**: 115–118. 1921.

(11) JAMESON, E., ALVAREZ-TOSTADO, C., AND SORTOR, H. H. Electrophoretic Studies on New-Born Calf Serum. Proc. Soc. Exptl. Biol. Med., **51**: 163–165. 1942.

(12) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.

(13) MASON, J. W., DALLING, T., AND GORDON, W. S. Transmission of Maternal Immunity. J. Path. Bact., **33**: 783–797. 1930.

(14) McMEEKIN, T. L., DELLAMONICA, E., AND CUSTER, J. H. Separation and Properties of Bovine Whey Proteins. Federation Proc., **6**: 277. 1947.

(15) MELLANDER, O. Elektrophoretische Untersuchungen von Casein. Biochem. Z., **300**: 240–245. 1939.

(16) ONCLEY, J. L., MELIN, N., RICHERT, D. A., CAMERON, J. W., AND GROSS, P. M. J. Am. Chem. Soc. In press.

(17) ORCUTT, M. L., AND HOWE, P. E. The Relation between the Accumulation of Globulins and the Appearance of Agglutinins in the Blood of New-Born Calves. J. Exptl. Med., **36**: 291–308. 1922.

(18) PALMER, A. H. The Preparation of a Crystalline Globulin from the Albumin Fraction of Cow's Milk. J. Biol. Chem., **104**: 359–372. 1934.

(19) PEARSON, P. B., AND DARNELL, A. L. The Thiamine, Riboflavin, Nicotinic Acid and Pantothenic Acid Content of Colostrum and Milk of the Cow and Ewe. J. Nutrition, **31**: 51–57. 1946.

(20) Pedersen, K. O. Ultracentrifugal Studies on Serum and Serum Fractions. Almqvist and Wiksells Boktryckeri, Upsala, Sweden. 1945.

(21) San Clemente, C. L., and Huddleson, I. F. Electrophoretic Studies of the Proteins of Bovine Serums with Respect to Brucella. Michigan Agr. Expt. Sta. Bull. 182. Pp. 3–44. 1943.

(22) Schneider, L., and Szathmáry, J. I. Ueber die Immunität der neugeborenen Säugetiere. Z. Immunitätforsch., **94**: 458–464. 1938.

(23) Semb, J., Baumann, C. A., and Steenbock, H. Fat-Soluble Vitamins. XLI. The Carotene and Vitamin A Content of Colostrum. J. Biol. Chem., **107**: 697–703. 1934.

(24) Smith, E. L. The Immune Proteins of the Cow. Federation Proc., **5**: 154. 1946.

(25) Smith, E. L. The Immune Proteins of Bovine Colostrum and Plasma. J. Biol. Chem., **164**: 345–358. 1946.

(26) Smith, E. L. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. J. Biol. Chem., **165**: 665–676. 1946.

(27) Smith, E. L., and Coy, N. H. The Absorption Spectra of Immune Proteins. J. Biol. Chem., **164**: 367–370. 1946.

(28) Smith, E. L., and Gerlough, T. D. The Isolation and Properties of the Proteins Associated with Tetanus Antitoxic Activity in Equine Plasma. J. Biol. Chem., **167**: 679–687. 1947.

(29) Smith, E. L., and Greene, R. D. Further Studies on the Amino Acid Composition of Immune Proteins. J. Biol. Chem., **171**: 355–362. 1947.

(30) Smith, E. L., Greene, R. D., and Bartner, E. Amino Acid and Carbohydrate Analyses of Some Immune Proteins. J. Biol. Chem., **164**: 359–366. 1946.

(31) Smith, E. L., and Holm, A. The Transfer of Immunity to the New-Born Calf from Colostrum. Unpublished.

(32) Smith, T., and Little, R. B. The Significance of Colostrum to the New-Born Calf. J. Exptl. Med., **36**: 181–198. 1922.

(33) Smith, T., and Little, R. B. The Absorption of Specific Agglutinins in Homologous Serum Fed to Calves during the Early Hours of Life. Jour. Exptl. Med., **36**: 453–468. 1922.

(34) van der Scheer, J., Wyckoff, R. W. G., and Clark, F. H. The Electrophoretic Analysis of Several Hyperimmune Horse Sera. J. Immunol., **39**: 65–71. 1940.

(35) Warner, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., **66**: 1725–1731. 1944.

(36) Wells, H. G., and Osborne, T. B. Anaphylaxis Reactions with Purified Proteins from Milk. J. Infectious Diseases, **29**: 200–216. 1921.

(37) Wyman, J., Jr., Rafferty, J. A., and Ingalls, E. N. Solubility of Adult and Fetal Carbonylhemoglobin of the Cow. J. Biol. Chem., **153**: 275–284. 1944.

THE UTILIZATION OF WHEY: A REVIEW

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The purpose of this review is to make available in one article pertinent information on whey and its constituents and on procedures for the manu-

facture of both food and non-food products from whey. Sufficient details are given so that cheese producers can select processes suited to their individual locations and scales of operation.

THE WHEY DISPOSAL PROBLEM

More than ever before producers of cheese are seeking new methods of utilizing whey. The reasons for this are apparent. Older methods of disposal are becoming less available and several other possible methods are prohibitively expensive to operate. The following procedures either have been employed or have been advocated for disposal of dairy wastes (124):

(a) Running to the sewer. This is practical only when the quantity of whey is very small or the whey can be diluted greatly; otherwise, a nuisance is created or, if the sewage is treated, an inordinate load is placed on the treating plant. Since the biological oxygen demand of whey is high and the quantity usually is large, this method is not feasible in most instances.

(b) Running to a stream. Any appreciable quantity of whey will pollute a small stream sufficiently to kill fish and to produce noxious odors in stagnant areas.

(c) Dumping in abandoned mines or quarries or in holes dug for disposal purposes. The cost of transporting whey to abandoned mines or quarries will be prohibitive in most instances and the odors produced by the decomposing whey will be objectionable unless the place of disposal is at a considerable distance from the cheese plant and from homes.

(d) Dumping in prepared lagoons. Practically the same objections apply to lagooning or spreading on fields as to the preceding method.

(e) Treating in a sewage disposal plant installed for the purpose. The cost of a disposal plant for whey will be unreasonably great because of the high value of the biological oxygen demand of the whey.

(f) Producing fuel gas by anaerobic fermentation. It appears theoretically possible to produce all the heat required in a cheese plant by this procedure, but such a heat source probably would be unreliable. Digestion tanks of about 30 times the daily volume of whey would be required.

(g) Returning the whey to farmers for feeding. This is practical, but only to a limited extent. The large volume of the whey in relation to the quantity of nutrients present and the requirement that whey may not be transported to the farms in the cans used for bringing milk to the plants discourage many farmers from using whey for feeding. Furthermore, the farmers having pigs to feed usually are not the same ones as those delivering milk to the cheese plant.

(h) Evaporating or drying for use as food or feed. Where cheap heat is available and the volume of whey is great enough to justify investment in evaporating or drying equipment, this is a practical means of disposal.

(i) Manufacturing of such products as whey protein, whey cheese, lac-

tose, lactic acid, alcohol, vinegar and food specialties. This and the preceding method are the most desirable from the standpoint of nutritional economy and usually can be operated at the least net cost, and frequently at a profit. The special equipment required is expensive.

QUANTITIES OF WHEY PRODUCED

Approximately 10 billion lb. of whey are produced each year in the United States. About 9 billion lb. are from the manufacture of whole milk cheese and one billion lb. from cottage, pot and bakers' cheese. The 40 million-lb. quantity of whey from the making of casein, though relatively small in amount, is of importance because, until recently, it has been the source of all the lactose produced in this country.

Ten billion pounds of whey contain, in round numbers, 500 million lb. of lactose, 50 million lb. of protein, 40 million lb. of non-protein nitrogenous matter, 30 million lb. of fat, 11 million lb. of phosphorus (P_2O_5), 7 million lb. of calcium (CaO), and 12 thousand lb. of riboflavin. Forty million pounds of ash constituents also are present but are of little or no interest from the standpoint of utilization. The fat can be recovered readily by means of a cream separator for use in making butter and therefore does not contribute to the whey disposal problem.

COMPOSITION AND NUTRITIVE VALUE OF WHEY

A typical cheese whey contains 6.9 per cent total solids, of which percentage 0.6 is ash and 6.3 is organic solids, divided among 0.3 per cent fat, 0.9 per cent nitrogenous compounds (calculated as protein), 4.9 per cent lactose and 0.2 per cent lactic acid. The lactic acid has been formed by fermentation of lactose, and the percentages of these two constituents are somewhat variable, but their sum is consistently close to 5.1 per cent. About five-ninths of the nitrogenous matter is heat-coagulable protein. This protein commonly is called either whey protein or albumin, but the term albumin is an improper one since this material consists of a very small proportion of suspended casein, an "albumin fraction" and a "globulin fraction". These fractions differ in both composition and physical properties and can be fractionated still further.

For the purposes of this review, the term whey protein will include all of the heat-coagulable protein of whey, and its heterogenous composition will be disregarded. The nitrogenous matter that is not coagulable by heat consists of substances precipitable by trichloroacetic acid, which are peptone or proteose in nature, and other simpler substances such as creatin, creatinin, urea, uric acid, amino acids and ammonia. Of the known vitamins, the only one present in appreciable quantities in whey is riboflavin, which occurs to the extent of approximately 1.24 γ per g. of whey, or 0.000124 per cent (25).

The chief individual ash constituents present in whey are: 0.188 per cent

potassium oxide, 0.075 per cent sodium oxide, 0.071 per cent calcium oxide, 0.018 per cent magnesium oxide, 0.001 per cent ferric oxide, 0.110 per cent phosphorus pentoxide, 0.107 per cent chlorine and 0.029 per cent sulfur trioxide. Of these, the calcium and phosphorus are of positive interest because of their nutritional value. The other salt constituents usually have only nuisance value because of the salty flavor that they impart to concentrated whey products and the difficulty of removing them.

Milk is unique as a source of calcium and riboflavin, two nutrients that need to be increased in many American diets. It is unique also in that it contains lactose, a sugar having highly specific nutritive value. These three nutrients largely are left in the whey from the cheesemaking process, together with part of the phosphorus. The whey protein is of excellent quality in that it contains practically all of the essential amino acids.

Lactose brings about increased utilization of calcium, magnesium and phosphorus in young animals (35, 72, 81). This effect may be the real basis for many claims as to the superior assimilability of calcium and phosphorus from whey products. Lactose, unless fed in excessive quantities, is more effective in accelerating growth in young animals than are other common carbohydrates (141). It favors the production of riboflavin and vitamin B_6 in the intestine of the rat (74). The feeding of lactose to rats has caused cataracts, but it has been found that fat, which is necessary for the utilization of dietary lactose, protects against development of cataracts (51, 103). Young rats die when fed lactose in high concentration as the only carbohydrate in the diet (31, 40) unless an unidentified factor associated with casein is present (18). It should be understood that these undesirable effects of lactose have been obtained only in rats and then only on diets that were highly abnormal.

In the poultry industry, dried whey is fed extensively because lactose is effective in preventing coccidiosis, and riboflavin is considered essential to the rapid growth of chicks and to hatchability of eggs and is a preventive of curled toe paralysis.

PROCESSES FOR PRIMARY PRODUCTS

Methods of whey utilization discussed in this review are shown in figure 1. Three primary processes are employed in the preparation of whey for ultimate utilization in feeds, foods, pharmaceuticals, or industrial products. The different primary and final products will be considered here approximately in the order in which they appear in the figure.

If whey is to be processed, the initial processing should be carried out in the plant in which the whey is produced in order that deterioration due to the growth of undesirable organisms will be retarded or prevented. Such treatments may include one or more of the unit operations of pasteurization, concentration or fermentation.

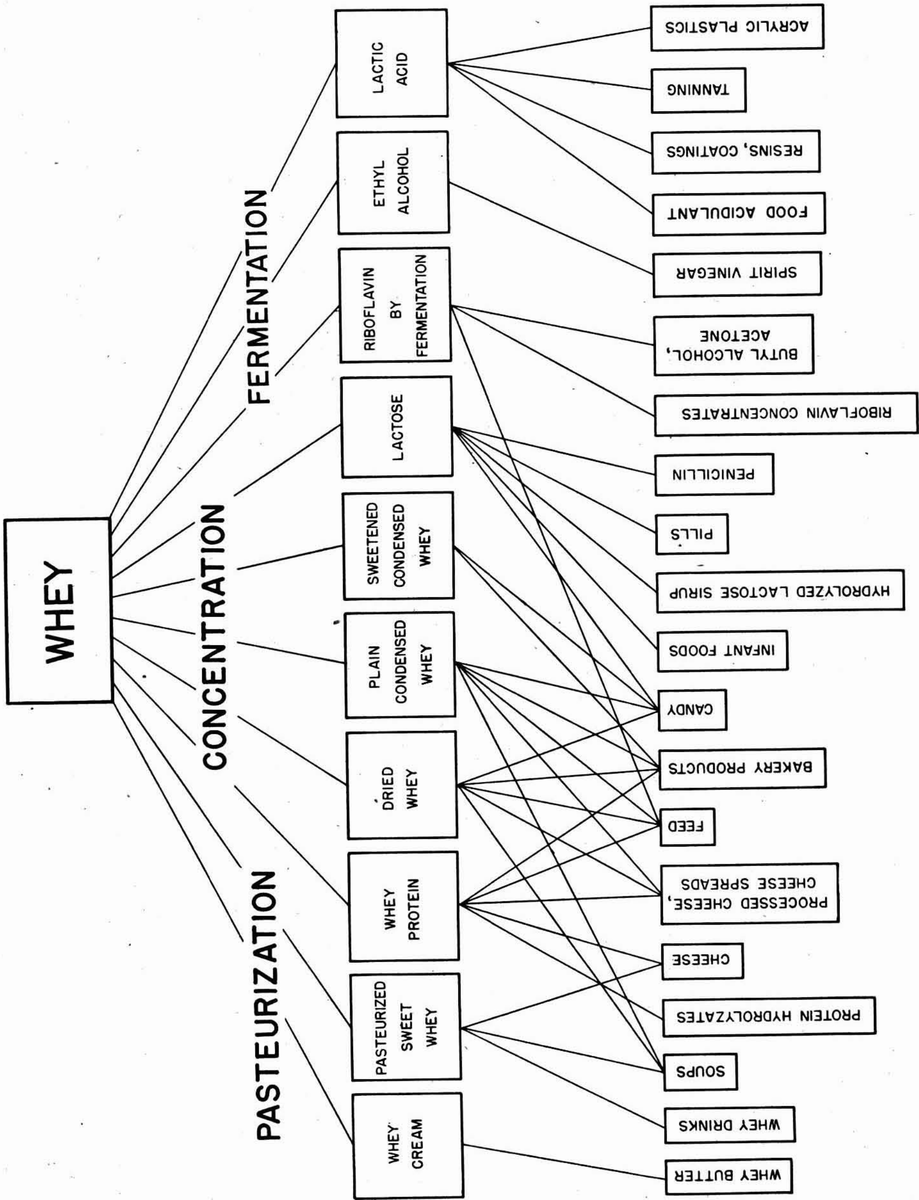

Fig. 1. Flow sheet of products from whey.

Pasteurized Products

Sweet whey. Pasteurization is an essential step in the preservation of sweet whey for further processing and in the production of whey cream. Whey is heated to 145° F. for 30 minutes, or to higher temperatures for shorter times, to retard growth of lactic-acid-producing bacteria which have been active in the cheesemaking process and to destroy pathogenic organisms that may be present if the milk was not pasteurized prior to making the cheese. The whey should be cooled to below 50° F. immediately after pasteurization, unless additional processing is to ensue promptly.

Pasteurized sweet whey is bulky and perishable, but, where there is a convenient source of supply, it can be used successfully in foods. It may be used to advantage in place of water in recipes for beverages, soups, bread and other foods.

Whey cream. It is prepared by putting whey through a cream separator either before or after pasteurization. Products of bacterial growth that produce off flavors may be present in whey that is not properly pasteurized and may be transferred to the cream. When cheese is made in copper equipment, cream made from the whey often has a copper content great enough to catalyze the oxidation of the fat; as a result, off flavors appear in butter or other products made from the cream.

The danger of curdling during the pasteurization of mixtures of whey cream and cream from whole milk can be lessened or avoided by using whey cream of low acidity or by pasteurizing the whey cream separately before it is mixed with the cream from whole milk (123).

Although butter most generally is the product made from whey cream, the cream may be used in foods in which casein coagulates readily. Whey cream contains substantially no casein and will not produce a coagulum when added to cold, acid foods. The coagulum formed during heating is soft and easily dispersed.

Concentrated Products

To remove water from whey, two methods are in use, one employing vacuum evaporators and the other driers. Both require from 40,000 to 100,000 lb. of whey daily for profitable operation. However, if equipment already is available and if the whey cannot be discarded, it may be practicable to concentrate as little as 10,000 lb. of whey daily. The engineering and operating aspects of concentrating equipment have been considered at length by Hunziker (46), Scott (104) and Farrall (32).

Plain condensed whey. This is the simplest of the whey concentrates to manufacture (136). Pasteurized whey is condensed in a vacuum pan to about 68 per cent solids (36.7° Baumé at 115° F.), dropped into cans, barrels or a tank, seeded with lactose or a concentrate from a previous run, and, if possible, cooled with agitation. A multiple-effect evaporator will

condense whey to 40 or 50 per cent solids; a single-effect pan can reduce it to 70 per cent solids. When whey is being condensed to 70 per cent solids, there is a possibility that the concentrated solution suddenly will crystallize in the pan and that the heating surfaces will become badly coated with whey solids (60). To avoid this difficulty, the pan should be clean and free of lactose crystals at the start of each run and the batch should be quickly finished and dropped from the pan (136).

Excessive foaming of whey in the vacuum pan sometimes occurs. The addition of a small quantity of milk fat or other fat to the batch usually will reduce this foaming. Triggs (125) found that if the whey was adjusted to pH 5.5 to 6.0 before it was drawn into the vacuum pan, foaming could be practically eliminated. The acid preferred was phosphoric, and this was neutralized with lime as soon as the concentrated whey was dropped from the pan.

Sweetened condensed whey. This was developed to provide a simple and economical method for the preservation of whey solids for use in food manufacture (92, 137). A quantity of sugar equal in weight to that of the solids in the batch of whey is added to the whey after it has been run through the separator and pasteurized. The mixture is condensed under vacuum to 76 per cent solids (38.4° Baumé at 122° F.). The concentrate is cooled to 95° F., seeded with lactose crystals or with a concentrate from a previous run, stirred for 1 hour and placed in barrels or cans. If a product containing a reduced content of lactose is desired, crystallized lactose may be removed centrifugally before the concentrate is stored. The storage requirements, as well as the manufacturing procedure, are similar to those for sweetened condensed milk. The whey product thickens more slowly and to a lesser extent than does the milk product. Fat-free sweetened condensed whey may be whipped to a dense foam having 200 per cent overrun and a stability of several hours.

Dried whey. When produced by the processes employed in drying milk, dried whey cakes on standing because the anhydrous lactose present gradually absorbs water and crystallizes as a hydrate (108, 110). Many patented processes have been devised principally for the purpose of inducing lactose crystallization prior to the complete drying of the whey. Some of these processes may be applied in either the roller or spray drying procedures.

Processes for controlling the crystallization of lactose during the drying of whey may be divided into four groups: (a) Whey is concentrated to a solids content of 70 per cent or more, the lactose is allowed to crystallize, and then the mass is dried (11, 53, 60, 111). (b) The whey is dried and then allowed to absorb water to force the crystallization of the lactose (20, 30, 87, 130). (c) The whey is concentrated to the point at which it

contains approximately the quantity of water required for hydration of the lactose (6, 58, 59, 85, 88). No water is added and under carefully controlled conditions it is not necessary to remove water after the crystals have formed. In practice, however, often about 2 to 5 per cent of water must be removed after crystallization of lactose is complete. (d) A dried whey in which lactose is largely in the *beta* anhydride form is prepared by seeding a partially concentrated whey with *beta* lactose at a temperature above 200° F. and holding it at this temperature while it crystallizes and dries (20, 59).

Three general drying methods are employed in the manufacture of dried whey: tunnel or shelf drying, drum or roller drying, and spray drying. Since many of the processes and their modifications employed in the drying of whey are patented, the status of the patents in this field should be investigated before manufacturing operations are started. Bosworth (12) concentrated whey under vacuum and then dried it in air at 149° F. for food and pharmaceutical uses.

The commercial method of concentration and tunnel drying of whey is based on the Simmons patent (111), which only recently has expired. Whey is condensed under vacuum to 70 per cent solids, dropped from the vacuum pan, seeded and held not more than 24 hours to allow the lactose to crystallize. The pasty mass then is dried in a tunnel drier and ground. The finished product is relatively non-hygroscopic and the whey protein is insoluble in water. In the Kraft modification of the Simmons process (53), the whey concentrate containing 70 per cent solids is dried in air by blowing it through a series of conduits and cyclone collectors. The lactose crystallizes during this drying operation. Lavett (60) concentrates whey of 40 per cent solids to 80 per cent on a double drum drier, drops this to cooling and seeding drums, and then to a hot-air drier. Bertram and Lemmerich (11) pass the concentrated whey through a mixing machine in which it is mixed with air and the lactose crystallized. The material then is dried and ground. Peebles and Manning (89) claim that, by heat-coagulating the whey proteins prior to crystallizing the lactose in the concentrate, the protein then will not interfere with crystal formation, and a stable, non-hygroscopic dried whey will be produced.

Drum or roller drying of whey can be carried out by following conventional methods (3), but modifications generally are employed to retard the formation of a sticky, hygroscopic glass on the drums. Lavett (57, 59, 60) uses two double-drum drying units placed one above the other. Whey is concentrated in a vacuum pan to 40 per cent solids and then reduced on the upper drums to 80 per cent solids. The mass is cooled and seeded on the lower drums and finally dried in a rotary drier. When a single pair of rolls is employed, adjustment of the titratable acidity of the whey to be-

tween 0.30 and 0.40 per cent before drying is helpful (61). In one modification, the drying mass is stripped from the drums when it contains from 8 to 15 per cent moisture, the lactose allowed to crystallize, and the drying finished by means of hot air (58).

Drying aids may be added to the whey. Pectic acid is used in the drying of mixtures of whey and fruit juices (143). The dried whey can be scraped off the rollers easier if a finely divided dry material, such as dried whey, is sprinkled on the semi-dried film as the rolls revolve (6). Spellacy (115), Supplee (119), and Jack and Wasson (48) found that whey dried with less difficulty when it was mixed with a material which formed a sheet as it was scraped from the drums. Skim milk, buttermilk and organic, water-insoluble, non-gelatinized substances such as ground and sifted cereals were found to be suitable drying aids. Waite (128) found that cheese whey and hydrochloric acid-casein whey could be drum-dried if they were neutralized with calcium hydroxide, but acetic acid-casein whey could be dried without neutralization.

Spray drying can be accomplished by the processes used for drying milk. However, the hygroscopic nature of spray-dried whey sometimes causes it to cake in the collection system of the drier and obstruct the passages. This tendency is lessened by inducing lactose crystallization during drying by an adaptation of one of the procedures previously described.

The addition of pectic acid to whey (143) and heat-coagulation of the whey protein (89) have been recommended as preliminary treatments for whey that is to be spray-dried.

Casein sometimes is removed from skim milk by treatment with one of several gums (2). The resulting whey is highly viscous; it may be treated with an enzyme that will act on the gum, thus reducing the viscosity of the whey and making it easier to handle in drying equipment (23).

Dried whey often gradually becomes brown subsequent to drying. Doob *et al.* (28) found this objectionable change to be associated with a high content of osmotically held moisture, high titratable acidity and low lactose content.

Lactose. Until recently, lactose was made in this country only from muriatic casein whey. However, the increased demands for lactose in the manufacture of penicillin during World War II, together with the simultaneous decrease in the quantity of casein whey available, made it necessary to use cheese whey as a source of lactose. The difficulties in processing cheese whey for lactose manufacture had been overcome experimentally, and several procedures were available for commercial use. The methods employing casein whey and cheese whey are outlined here.

Whey from casein precipitated by means of sulfuric acid is objectionable

because of the difficulty of removing slightly soluble metal sulfates that impart cloudiness to lactose solutions. Self-sour casein whey is not recommended because so much of the lactose has been converted to lactic acid that yields will be low. The same is true to a less degree of cottage cheese whey. In general, the less the fermentation that has taken place in the whey the greater the yield of lactose that will be obtained.

The whey protein is recovered in an insoluble condition in the process using casein whey and in those processes using cheese whey wherein the whey is clarified by boiling. This insoluble protein is suitable for feed. When a soluble protein suitable for use as food is desired, the whey should not be boiled; under these conditions the lactose obtained will contain a relatively high percentage of protein and ash, usually 1.5 per cent or more of each. Two stages of clarification and boiling are necessary to produce, with one crystallization, lactose containing less than 0.3 per cent protein and 0.3 per cent ash. The recovery of lactose usually is 3.5 to 4 lb. per 100 lb. of whey. A second crystallization is necessary in order to produce USP lactose. For many purposes, however, lactose of crude or technical grade is satisfactory and is less costly to produce.

Muriatic casein whey (78, 117) is heated to boiling in iron tanks with live steam, and lime is added during the heating until the acidity is about 0.5 per cent or the pH value is 6.2. The coagulum is allowed to settle and the clear whey is evaporated in a double-effect evaporator to a concentration of 30 per cent lactose, or 20° Baumé. The hot sirup is filtered in a filter press and is followed by the sludge from the coagulating tank. The clear sirup then is evaporated in a single-effect evaporator to about 40° Baumé, some crystallization or ''graining'' taking place in the evaporating pan. The hot mass is dropped to crystallizing vats, where it is agitated slowly and cooled by water circulating in a jacket. The sugar is freed from mother liquor by spinning in a sugar centrifuge and then washed with cold water. A second crop of crystals can be obtained by concentrating the mother liquor. The wet crude lactose either should be refined or dried promptly to avoid spoilage.

The simplest method of making lactose from cheese whey (9, 10, 138) is to concentrate it in a vacuum evaporator to 55–60 per cent solids content, cool the concentrate with occasional stirring in a vat, separate the lactose in a centrifuge, wash with cold water, and dry in a tunnel drier. The resulting crude sugar will contain approximately 5 per cent impurities (protein and ash) on a dry basis. The whey protein in the mother liquor will be soluble.

Better grades of lactose are obtained when the heat-coagulable fraction is removed from the whey before concentration. During evaporation of the clarified whey further precipitation of insoluble protein and salts occurs.

If the sirup is filtered when its concentration reaches 20° Baumé, or about 30 per cent solids, there will be a further improvement in the finished product. The 20° Baumé sirup also may be decolorized with activated carbon and bone-black to produce a colorless lactose. The resulting technical grade sugar will contain more than 99 per cent lactose and only 0.3 per cent each of protein and ash on the dry basis. A lactose that will not foam in solution may be made by digesting the clarified whey with the enzyme trypsin before concentration.

Several other methods that have not come into commercial use have been described in the literature (142).

For refining (118), crude sugar is dissolved with the aid of steam in sufficient water to give a concentration of 20° Baumé. One pound of decolorizing paste and 0.25 lb. of a filter aid are added for each 100 lb. of sugar present. The solution is heated to boiling and hydrochloric acid is added to give a titratable acidity of 0.09 per cent, expressed as lactic acid. After standing overnight, the batch is heated nearly to boiling and milk of lime added to reduce the acidity to 0.05 per cent, or a pH value of 5.4 to 5.8. The solution then is boiled vigorously for a few minutes and allowed to stand until the insoluble matter has settled. It then is filtered through cloth in a press and again through rag paper supported between perforated copper discs. The acidity of the filtrate is increased slightly by addition of hydrochloric acid, the solution is concentrated to 40° Baumé, and the sugar is crystallized, centrifuged, washed and dried. The product should satisfy the specifications for USP lactose.

Drying lactose solutions by the spray-drying process (8) produces a mixture of the two forms in approximately the equilibrium ratio of 1.65 parts *beta* to 1.00 part *alpha*. The product dissolves much more rapidly than *alpha* lactose but is hygroscopic and has poor wetting properties. The product made by drying lactose solutions on a drum drier will contain as much as 90 per cent of the sugar in the *beta* form under the most favorable drying conditions. Such a product is less hygroscopic than the spray-dried product, has good wetting properties, and is slightly more soluble initially than pure *beta* lactose.

In the process of Supplee and Flanigan (120), a solution of lactose is dried in a thin film at a temperature above the boiling point of water, the film is removed from the source of heat while it contains at least 2 per cent of water, and the heat remaining in the paste completes the drying. The product contains a high proportion of *beta* lactose.

In Sharp's process (106, 107), *alpha* lactose is added to a saturated lactose solution maintained above the critical temperature of 200.4° F. The *alpha* lactose dissolves and reappears as crystalline *beta* lactose, which is separated by filtration in a heated centrifuge.

In the Sharp and Hand process (109), dry *alpha* lactose is heated in a closed container at 248 to 266° F. Under these conditions, *alpha* lactose loses water of crystallization and changes to the *beta* form. When the conversion has reached completion, or a desired stage short of completion, the water vapor in the container is allowed to escape.

Whey protein. This easily can be recovered as a denatured protein from either concentrated or unconcentrated whey by heat coagulation, but the soluble product is difficult to isolate. Soluble whey protein generally is prepared from a whey concentrate from which the crystallized lactose has been removed. The remaining liquor contains the whey protein, some lactose, and the soluble whey salts, which are difficult to separate from the protein without causing denaturation. Patents were obtained by Dunham in 1902 (29) for precipitating whey albumin from concentrated whey by means of acid, by Weimar in 1921 (139) for preparing a soluble whey protein concentrate from which lactose was partly separated by crystallization and salts by dialysis, and by Meyer in 1931 (71) for removing salts by chemical means.

Bell and Peter (9) and Bell *et al.* (10) improved the Weimar process. Cheese whey is neutralized with sodium hydroxide to pH 7.3, condensed to 62 per cent solids, cooled, and centrifuged to separate the lactose. The mother liquor, which contains soluble whey protein, milk salts and residual lactose, is suitable for use in food preparations. Watson (131) was able to remove most of the salts from the mother liquor of the Bell, Peter and Johnson process by electrodialysis. Perhaps this can be accomplished more readily by application of the more recently developed ion-exchange procedures, first advocated by Lyman (68).

Leviton (62) and Leviton and Leighton (67) extract soluble protein from whey by means of alcohol. Dried whey containing non-crystalline lactose rapidly is mixed with ethanol, and the protein, being insoluble in the alcohol, promptly is separated by filtration and dried. The dried product is soluble in water. Lactose crystallizes from the mother liquor and is recovered by filtration; the alcohol is recovered by distillation, leaving a residue relatively rich in riboflavin. Similarly, Leviton (66) has extracted protein from dried skim milk by means of methanol. The protein complex is redispersed in water and the casein is precipitated by acid or rennet, leaving soluble whey protein in solution.

Another method for the separation of water-soluble protein from whey was devised by Gordon (38). The protein is precipitated at pH 3 by addition of a soluble metaphosphate, separated by filtration, and washed and treated with excess calcium hydroxide at pH 9 to precipitate calcium phosphate. The mixure then is adjusted to pH 7 and centrifuged. The filtrate contains the whey protein and is evaporated under vacuum to yield the protein in a dry undenatured state.

Water-insoluble whey protein may be separated from whey by heat coagulation. Investigators have determined quantitatively the effect of reaction and temperature on the heat coagulation of protein in cheese whey (47, 76, 80). In general, 50 to 60 per cent of the nitrogen in whey is recovered as coagulated protein when the whey at a reaction between pH 4.5 and 5.0 is boiled. Several practical processes have been developed (15, 34, 130). According to Burkey and Walter (16), sweet whey (pH 6.3) is heated to 200° F. and acidified to pH 5.0 with any suitable acid or with sour whey. The sour whey used should contain 2 per cent lactic acid and is added in an amount equal to 10 per cent of the whey being treated. During acidification the whey is stirred; then it is held hot and without stirring for about 15 minutes. The clear liquid may be siphoned or drained off, or the flocculated protein dipped into cheese cloths and drained and washed. Curd that has been drained and washed contains more than 74 per cent moisture and may be preserved by drying or freezing. The clarified whey remaining after removal of the protein may be used for lactose manufacture or for animal feed.

Centrifugal separation and washing of heat-coagulated whey protein have been accomplished by means of specially built, high-speed centrifuges. The composition of the protein suspension produced may vary widely. One procedure produces a concentrate containing about 2 per cent whey protein and very small quantities of lactose and salts. Some of these suspensions resemble skim milk in appearance and can be used in food manufacture either directly or after concentration by vacuum evaporation or by drying.

Fermentation Products

The substances that can be produced by the fermentation of the lactose of whey can be produced by fermentation of cane, beet or corn sugar. Whether it is practical to utilize whey in making fermentation products depends in general on whether a suitable organism is available to convert lactose into the desired product and whether whey is a less costly source of fermentable sugar than is molasses or corn sugar.

Of the many substances that can be produced by fermentation of lactose, the only ones being produced in this country are lactic acid, ethyl alcohol and riboflavin. Lactose is used in penicillin production because its slow rate of acid production under the required conditions favors increased formation of penicillin, but it is not essential to the fermentation. Since lactose, rather than whey, is used in this fermentation, the process is not described here.

Lactic acid. It is produced commercially from whey by means of a mixed culture of a lactobacillus and a mycoderm, American Type Culture Collection no. 9223 (17, 49, 140, 142). The efficiency of conversion is greater than 90 per cent; the acid is the inactive mixture of the dextro and levo forms, and no objectionable by-products are formed.

A starter culture is built up by successive inoculations and incubations of batches of whey of increasing size. Five hundred gallons of starter are added to 5,000 gallons of raw whey maintained at 43° C. (110° F.). Every 6 hours, or whenever the reaction approaches pH 5.0, a slurry of slaked lime is added in quantity sufficient to bring the reaction to pH 6.0 but not higher. When chemical tests show that practically all the sugar has been fermented, or when the quantity of lime consumed indicates that the conversion is complete, the whey is neutralized to pH 6.5 to 7.5 with lime slurry and heated to the boiling point. After 10 minutes at the boiling point, the coagulum is allowed to settle, the clear liquid is run to a filter press and is followed by the sludge. The hot filtrate is treated with a small percentage of decolorizing carbon, stirred, and brought to a pH value of 10.0 by addition of lime slurry. As soon as a test on a sample shows that sedimentation will be rapid, the precipitate is allowed to settle and the batch again is filtered. The filtrate is neutralized with lactic acid and concentrated in a vacuum pan at 15° Baumé. The concentrate is run to jacketed crystallizers, and, by circulating cold water in the jacket, it is cooled to 10–15° C. (50–60° F.). After 12 hours, the crystalline mass is spun in a basket centrifuge until no more filtrate is obtained and the crystals are washed lightly with cold water. The mother liquor and washings are concentrated to 13.5° Baumé to obtain a second crop of crystals. The calcium lactate obtained may be recrystallized to produce USP calcium lactate or it may be treated with sulfuric acid to convert it to lactic acid either before or after crystallization.

Lactic acid comes on the market principally as 22 and 44 per cent crude, 50 per cent edible, and 65 per cent USP acid.

Ethyl alcohol. The production of ethyl alcohol from the lactose of whey (50, 77, 97) requires a lactose-fermenting yeast. *Torula cremoris,* American Type Culture Collection no. 2512, is the most efficient yeast found for the purpose; it produces 84 to 90 per cent of the theoretical yield.

The whey is heated to boiling, acidified to pH 5.0 with sour whey or acid, and the precipitated protein removed by filtering. After the filtrate has cooled to 33–34° C. (93° F.), 1 lb. of the yeast is added for each 120 gallons of whey, and the fermentation continued at constant temperature until it is complete, usually for about 50 hours. The yeast is removed and the alcohol recovered by distillation. The protein, spent yeast and residues from the still are suitable for feed. The alcohol is of sufficiently good quality to be used for the production of spirit vinegar, as described later.

Riboflavin. Dried whey is fed to chickens not alone for its protein and lactose contents but also for its riboflavin content. During World War II when drying equipment was being used extensively in drying whole and skim milk, the difficulty of producing enough dried whey to satisfy the

needs for riboflavin in feeds was overcome by increasing the riboflavin content of whey before drying by means of a fermentation process (4, 65, 69, 73, 96, 144).

The reaction of raw whey is adjusted to between pH 6.0 and 7.0, and its iron content adjusted to between 1 and 2 parts per million. Five pounds of corn meal and 2 lb. of calcium carbonate are added for each 1,000 lb. of whey and the mixture sterilized by heating under pressure at 250° F. for 15 to 20 minutes. After the whey has been cooled to 100° F., 50 lb. of an active starter of *Clostridium acetobutylicum* (American Type Culture Collection no. 824 or other suitable strain) is added for each 1,000 lb. of whey and fermentation allowed to continue at 86 to 98° F. for 48 hours, or until riboflavin concentration no longer increases. A yield of at least 30 γ of riboflavin per g. of whey can be expected. About 30 per cent of the lactose of the whey is converted during the fermentation to alcohols and acetone. Of these compounds, two-thirds is butyl alcohol, which is of sufficient value to warrant recovery by distillation. It usually is not feasible to recover the small quantities of ethyl alcohol and acetone present.

UTILIZATION OF PRIMARY PRODUCTS

Feed Uses

Fluid, concentrated and dried whey. The use of whey in feeding animals has been mentioned briefly at the beginning of this review. It should be emphasized that condensed and dried whey are the forms most practical to use in feeding pigs and chickens (105, 126), especially those that are being raised on farms distant from cheese factories. Dried whey is considered especially useful in feeding chickens because of the effects of lactose in preventing coccidiosis and the effects of riboflavin on growth of chicks, hatchability of eggs, and prevention of curled toe paralysis. The riboflavin content of whey that is to be dried for chicken feed can be increased advantageously prior to drying by the fermentation procedure outlined in the preceding section.

The concentrated and dried forms of whey usually are fed in mixtures with other feed materials, or the mixing may be done prior to concentration (75, 83). Sweet or soured whey, preferably somewhat concentrated, is recommended as an addition in the making of silage, especially grass silage to which it furnishes nutrients and lactic acid as a preservative (1, 45, 116).

Food Uses

An early record of the benefits to health to be gained through the consumption of whey as a food is found in Hoffmann's "Treatise on the Virtues and Uses of Whey", published in 1761 (44). But, although whey long has been offered as a cure for many illnesses, it is only recently that serious efforts have been made to incorporate it in foods.

The use of whey products in food manufacture affords a much more efficient means for utilization of whey from the standpoint of human nutrition than does feeding to animals followed by consuming the animals as food. The food value of the solids of whey is high and under favorable conditions these solids can effectively transfer milk flavor to foods. It is highly advantageous that whey protein will not coagulate at low temperatures in the presence of fruit acids and that it forms a soft, easily dispersible curd during the heat treatments used in cooking and canning (132).

Foams and emulsifiers. The foaming properties of whey protein have been studied and compared with those of egg albumin by Peter and Bell (90). They found that the stability of foams made from concentrated whey from which part of the lactose has been removed may be increased by neutralization or by the addition of small quantities of tannic acid, saponin, or bisulfites. Beeching and Severn (7) report the preparation of a heat-coagulable foaming agent by the neutralization and filtration of the mother liquor from lactose manufacture. Whey protein foams may be used in many food preparations. However, whey protein cannot be used in place of egg white in certain cakes and custards in which air must be incorporated by whipping and a firm structure set up by heat coagulation. A whey protein whip will not support other ingredients when coagulated by heat.

Sweetened condensed whey can be whipped in 4 minutes to a foam having 200 per cent overrun and a stability of 15 hours (92). This whip, which resembles marshmallow in appearance, is useful for toppings, icings, fruit whips and similar products.

A foaming material for use in non-alcoholic beverages has been prepared by dissolving whey protein in sufficient sodium hydroxide so that the solution has a pH value of 7.0, and then adding an edible acid, such as citric, to bring the reaction to pH 4.0–5.0 (33).

A mixture of 10 lb. of lipoid-free dried whey and 80 lb. of whole egg was found by Clickner (21) to have as good emulsifying properties as pure egg yolk and to be suitable for use in mayonnaise.

Whey drinks. These are made by adding highly flavored fruit or vegetable juices to whey. Whey adds to the nutritive value of a beverage, but it generally does not improve its flavor. A tomato-whey drink is made by mixing 65 per cent tomato juice, 0.4 per cent salt, and 34.6 per cent whey, including enough whey cream to give 2 per cent fat in the finished beverage (133). The reaction should be pH 4.4. The mixture is heated to 140° F., homogenized at 2,500 lb. pressure, canned, and sterilized by heating at 200° F. for 25 minutes. Other vegetable juices or fruit juices may be substituted for tomato juice. These beverages, when freshly mixed and promptly used, have flavor, color, and body superior to those of the canned and sterilized product.

A buttermilk type of beverage may be made by segregating the whey protein in a part of the whey (113). The whey from which the fat has been separated is boiled to coagulate the whey protein, the clear portion (five-sixths of the total) drawn off and discarded, and the remainder homogenized to redisperse the protein. The product contains about 4.1 per cent whey protein, 4.8 per cent lactose, and 0.5 per cent each of ash and fat. It should be possible to prepare a product of approximately this composition by means of high-speed centrifugal separation of boiled cheese whey, as discussed in the section on whey protein.

Soups. For immediate consumption soups can be prepared by using fresh whey in place of water. Soups that are to be canned and sterilized are made more easily with whey than with milk solids. A tomato soup containing whey solids retains the natural tomato acidity and does not contain clots or lumps of protein after heating. A formula for cream-style tomato soup follows (132):

> Whey solids, 4 per cent; milk fat, 4 per cent; flour or starch binder, 2.8 per cent; salt, 1 per cent; sugar, 1 per cent; fresh tomato juice, 70 per cent; added water, 17.2 per cent. Warm the mixture to 110° F., homogenize it at 2,500 lb., heat to 176° F., can, and sterilize by heating at 240° F. for 60 minutes without agitation.

Cheese and cheese foods. These may be divided into three classes: (a) whey cheese, (b) whey protein cheese, and (c) process cheese foods.

Whey cheese, known as mysost or primost, has been made for centuries in northern Europe, but the quantity produced in the United States is small. It is made by boiling the whey, generally in an open iron pan 8 to 10 feet in diameter. When it has the consistency of mortar, the hot, pasty mass is placed in tubs in which it is cooled and stirred in order to cause the lactose to form small crystals (27, 102, 122). Primost is packed into greased, wooden, cubical molds to cool and harden.

Albumin cheese was described in 1895 by Babcock (5). Ricotta or Ziger is produced from protein that has been removed from whey by one of the methods described earlier. Sammis (101) states that 5 to 10 per cent of skim or whole milk may be added to the whey before it is heated. The curd is placed in metal hoops, allowed to settle overnight, bandaged and pressed. The cheese may be salted and sold in fresh condition or dried at 110° F.

Whey protein curd recently has been converted into a Roquefort-type cheese (94). Four pounds of curd are recovered from 100 lb. of separated whey and, when pressed, the curd contains about 77 per cent moisture, 16.5 per cent protein, and 2.5 per cent fat. It is probable that whey protein curd can be converted into soft cheeses of other types or into a suitable base for cheese spreads.

Process cheese foods provide one of the largest uses for whey solids. The whey is added to the emulsified cheese mixture in the form of plain condensed or dried whey. Such mixtures contain at least 51 per cent cheese, less than 3 per cent emulsifying salts, organic acids to adjust the reaction to not less than pH 4.5, and a seasoning agent (24). A process cheese food may be made by mixing 93.5 per cent natural cheese, 5 per cent condensed whey containing 65 per cent solids, and 1.5 per cent emulsifying salts, heating with stirring at 165° F., and packaging hot (114).

Bakery products. Products containing whey solids are superior to those containing no milk products, but the products containing whey solids usually are considered inferior to those containing whole or skim milk concentrates. However, Davies (26) has stated that in England ". . . the use of dried whey in bread-making has proved time and again that the size of loaf, texture, taste, and general appeal of the bread are quite equal to that of milk bread." Any inferiority for this use of whey in comparison with milk is due principally to the relatively high salt and low protein of the whey solids, though the condition of the protein evidently is a factor. Concentrated whey products sometimes impart a salty, acid, or even a bitter taste to bread or cake. There is current interest in new types of whey concentrates developed especially for use in bakery products.

Since Greenbank *et al.* (39) showed that high heat treatment of milk contributed to improvement of bread containing it, whey protein has been suspected of playing an important role in bakery products containing milk. Studies on nitrogen distribution have shown that more than 90 per cent of the whey protein is coagulated when milk is heated above 200° F. for a few minutes (42, 70, 98, 99). This relationship between heat treatment and denaturation of the whey protein has prompted the suggestion that the determination of soluble or undenatured whey protein might be used as a test for the baking quality of dried skim milk (37, 41). In any case, heat treatment of whey destined for use in baked products appears highly desirable.

Processes have been patented for conditioning whey protein (86) and for separating it (54) for use in bread. A dry, comminuted, siftable, water-dispersible shortening composed of particles of fat coated with whey solids has been produced for use in prepared dry mixes and for other bakery purposes (19, 52).

Funder (36), in an extensive study, compared the volumes of water, whey and skim milk breads. For each 100 kg. of flour, he found volume increases over water bread of 6.2 kg. for fluid whey, 13.3 kg. for whey concentrated in the ratio of 2 to 1, and 9.5 kg. for plain skim milk. Several other workers have investigated the use of whey in bread (43, 56, 91, 121).

Whey may be used in a standard bread formula by adding either 3 to 4 lb. of whey solids (as fluid, plain condensed, or dried whey) or 7 to 10 lb. of sweetened condensed whey per 100 lb. of flour. The sugar and water in the formula should be adjusted to compensate for the addition of these ingredients in the whey.

Whey helps in producing a cake-like texture in sweet goods, and it may be added to conventional formulas for cakes and cookies (82, 136). A canned pudding has been developed in which as much as 22 per cent of the solids are whey solids (13).

Candies. Such types of candies as fudge, caramel and taffy can be made with a whey solids content of 14 to 40 per cent (55, 134, 135). Plain condensed, sweetened condensed, or dried whey may be used as a source of whey solids. Sweet rennet-type whey is preferred to neutralized acid whey because of its superior flavor. Whey is especially useful in fudge; the lactose on crystallizing contributes to the desired grainy texture. Whey caramels should be fortified with casein-containing milk solids in order to produce the characteristic chewy body. Whipped sweetened condensed whey may be used to incorporate air in special types of candy. A formula for whey fudge follows (134):

> Sweetened condensed whey, 43 per cent; sugar, 11 per cent; corn sirup, 9 per cent; invert sirup, 3 per cent; butterfat, 2.5 per cent; chocolate, 6 per cent; fondant, 20 per cent; powdered lactose, 0.1 per cent; nuts (optional), 5.4 per cent; vanilla to flavor. Cook (with stirring) the condensed whey, sugar, invert sirup, and half the corn sirup. The butterfat is added as cream or butter after the sirup has been partly boiled down. Cook to 248° F. Cool 25 or 30° or transfer to smaller pouring kettles, add the remaining corn sirup, the fondant and chocolate, and stir well for several minutes. Add the powdered lactose, flavoring and nuts. Stir. Pour into wooden forms.

Spirit vinegar. It may be made from the alcohol produced by fermentation of whey by the procedure described earlier. A simple distillation of the fermented whey will yield a dilute alcohol suitable for conversion to vinegar. The dilute alcohol is allowed to trickle over beech shavings or birch twigs impregnated with the acetic-acid-producing organism. A current of air passing upward through the vinegar converter accelerates the fermentation.

Food acidulant. Colorless 50 per cent lactic acid is used as a food acidulant in sherbets and bottled beverages and as a preservative and firming agent for pickles. The production of edible lactic acid from crude acid is a highly technical chemical process (17).

Whey butter. This is made from whey cream in accordance with usual buttermaking procedures. The low ash and the absence of casein make

possible rapid churning. Hence, a butter of good body and texture can be produced by churning at a lower temperature than customarily is employed in churning cream from whole milk. The churning time of cream from whole milk can be shortened somewhat by adding whey cream.

Pharmaceutical Uses

Whey furnishes raw material for the preparation of several important pharmaceuticals, such as protein hydrolyzates and penicillin. Certain other products derived wholly or in part from whey and sold over the pharmaceutical counter in drug stores might properly be classed as foods, such as lactose and infant foods. The use of lactose as a basis for pills definitely is a pharmaceutical use.

Lactose. This often is added to infant foods based on cows' milk for the purpose of making the composition of the food more nearly like that of human milk. High grade technical or USP lactose is added to modified cows' milk in sufficient quantity so that it has a concentration of carbohydrate that may be as great as 52 per cent of the solids of the milk. Such infant foods generally are either dried or canned and sterilized. Lactose also is sold for use in feeding formulas prescribed for preparation in the home. Because the common or *alpha* lactose is only slowly soluble, it has been found more convenient to use the more rapidly soluble *beta* form of this sugar. The resulting solution is the equilibrium mixture of the two forms, whether *alpha* or *beta* lactose is used in making the solution.

Riboflavin concentrates. Riboflavin and other materials can be purified and concentrated by adsorption on lactose (63, 64, 84). By suitable choice of degree of super-saturation of the lactose, concentration of riboflavin, and working temperatures, adsorbates containing approximately 300 γ of riboflavin per g. of lactose can be produced. It is possible to obtain even greater concentrations if excessive time is allowed for the crystallization of the sugar. The mother liquor of lactose manufacture may be used for the preparation of riboflavin adsorbate. After the removal of crude lactose that has crystallized at 140° F., the filtrate is cooled to between 40 and 50° F. and seeded with lactose crystals. A second crop of lactose crystallizes slowly over a period of 24 hours and contains about 100 γ of riboflavin per g. of sugar. By recrystallization of this crude adsorbate, the concentration of riboflavin can be increased to about 300 γ per g. of lactose.

Penicillin. Since 1943, several million lb. of lactose have been used each year as a component of the nutrient mixture in which penicillin is produced. Lactose acts to ensure higher yields than are obtained when it is not used, but it is not essential in the process. The possibilities of substitution

of other carbohydrates for lactose, of synthetic production of penicillin, and of the discovery of other antibiotics that will supersede penicillin make it seem unlikely that lactose long will be used for this purpose.

Hydrolyzed lactose. Lactose hydrolysis, which produces a mixture of glucose and galactose, offers the possibility of preparing a sweeter, more soluble form of carbohydrate from lactose for food use. Impure lactose, or the lactose in whey, when hydrolyzed by acid, yields a product which has a disagreeable taste and is contaminated with whey salts, humin and other products of protein hydrolysis.

Ramsdell and Webb (93) in hydrolyzing pure lactose found that there was a gradual destruction of glucose as it was formed. They found the optimal procedure to be as follows:

> Heat 2,100 g. of pure lactose, 49 g. of N hydrochloric acid and 4,851 g. of water to 297° F. in a pressure kettle. Allow 60 minutes to reach 297° and hold 5 minutes. Add carbon black, filter, concentrate to 60 per cent solids, and adjust the reaction to pH 5.0.

Glucose and galactose to the amount of 93 per cent of the theoretical are obtained. The solubility of a mixture of equal parts of both is 42 per cent. The maximal concentration of a mixture of the two hexoses soluble at 77° F. is 58.3 per cent and consists of 49.8 per cent glucose and 8.5 per cent galactose. A process for making hydrolyzed lactose caramel has been described (79).

Enzymic hydrolysis of lactose in whey and other dairy products would be the simplest method of reducing the concentration of lactose and increasing the sweetness of the product. The use of an enzyme obtained from kefir grains to treat milk products to be used in ice cream has been suggested by Turnbow (127). However, no adequate source of a lactase has been available. Browne and Webb (14) investigated a number of possible sources, but were unable to obtain uniformly active lactase preparations suitable for commercial use. Current work in several laboratories, however, seems likely to result in the preparation of an enzyme of adequate strength in commercial quantities.

Hydrolyzed protein. Protein hydrolyzates usually are prepared by pharmaceutical companies, although several of the large dairy organizations have manufactured the hydrolyzate from whey protein. The flavor of the hydrolyzate derived from whey protein is said to be less objectionable than that derived from casein. Protein may be hydrolyzed by acid, by alkali or by enzymes. The reaction is complete when all the peptide linkages are broken. The rate of hydrolysis is that of a second-order reaction.

Sahyun (100) has reviewed the factors concerned with protein hydrolysis. Acid hydrolysis may be conducted with any of several acids, although

sulfuric generally is preferred. Tryptophane is destroyed during acid hydrolysis. The following directions for hydrolysis of protein by acid and by enzyme were given by Sahyun (100):

> Two kilograms of protein is mixed with 14 l. of 5 N (25 per cent) sulfuric acid, the mixture autoclaved at 248° F. for 16 hours, limed to pH 10, filtered, and the residual calcium and sulfate ions removed. The amino acid preparation then is concentrated, and sterilized or dried.

There are numerous proteolytic enzymes capable of hydrolyzing proteins. Some of the amino acids are liberated sooner than others, but the degradation follows the general pattern: Protein → proteoses → peptones → peptides → amino acids.

> To hydrolyze 100 g. of protein, it is mixed with 700 cc. of water, 10 cc. chloroform, 0.5 g. pancreas extract (trypsin), and sufficient 5 N (20 per cent) sodium hydroxide to produce a reaction of pH 8. The mixture is incubated under controlled conditions for 8 to 12 days, heated, filtered, concentrated and dried. (100)

When proteins are hydrolyzed by alkali, no humin is formed, as is the case in acid hydrolysis. However, the amino acids, with the exception of glycine, are racemized, which is objectionable, since not all of the racemic amino acids are utilized in animal metabolism.

Therapeutic products. Products consisting chiefly of the protein and mineral constituents of whey have been prepared by patented processes. One method is to precipitate the protein-mineral complex with alkali, separate it, wash or reprecipitate it to free it from any objectionable protein decomposition products, and finally dry it (95, 129). Another method is to precipitate electrolytically, filter, and wash and dry the precipitate. A product thus is obtained containing approximately 18 per cent calcium, 6 per cent phosphorus and 20 per cent protein (22). Another therapeutic product consists of whey, an edestin-calcium solution derived from hempseed, and magnesium sulfate (112).

Chemical Uses

Lactic acid. This is used industrially in leather manufacture (142). The highly colored, crude grades, marketed in 22 or 44 per cent concentration, are used in diluted condition to neutralize the lime in limed hides. The requirements for this purpose of a weak acid forming a soluble calcium salt are satisfied by lactic acid. Sodium lactate solutions resemble glycerol in consistency and are used as substitutes for it in textile printing and in paper-making. Ethyl, butyl and other lactate esters have use as solvents and plasticizers. Lactic esters can be used as starting materials for the production of the industrially important acrylates.

Butyl alcohol. This is a by-product of the fermentation producing riboflavin, and its esters are useful as solvents.

REFERENCES

(1) ALLEN, L. A., AND WATSON, S. J. Lait, **14**: 889–895. 1934.
(2) AMBROSE, A. S. U. S. Patent 1,991,189, Feb. 12, 1935.
(3) ANONYMOUS. Food, **14**(171): 319–322. 1945.
(4) ARZBERGER, C. F. U. S. Patent 2,326,425, Aug. 10, 1943.
(5) BABCOCK, S. M. Wis. Agr. Expt. Sta. Rept., **12**: 134. 1895.
(6) BEARDSLEE, A. C. U. S. Patent 2,336,461, Dec. 14, 1943.
(7) BEECHING, E. I., AND SEVERN, G. W. C. British Patent 560,840, July 19, 1943.
(8) BELL, R. W. Ind. Eng. Chem., **22**: 51–55. 1930.
(9) BELL, R. W., AND PETER, P. N. Ind. Eng. Chem., **20**: 510–512. 1928.
(10) BELL, R. W., PETER, P. N., AND JOHNSON, W. T., JR. J. Dairy Sci., **11**: 163–174. 1928.
(11) BERTRAM, K., AND LEMMERICH, E. U. S. Patent 2,335,380, Nov. 30, 1943.
(12) BOSWORTH, A. W. U. S. Patent 1,246,858, Nov. 20, 1917.
(13) BROWNE, H. H., AND WEBB, B. H. Food Inds., **13**: 36–38, 96. Nov., 1941.
(14) BROWNE, H. H., AND WEBB, B. H. Unpublished results. 1942.
(15) BURKEY, L. A., AND WALTER, H. E. J. Dairy Sci., **29**: 503. 1946.
(16) BURKEY, L. A., AND WALTER, H. E. BDIM-Inf-46, Bur. Dairy Inds., U. S. Dept. Agr. 1947.
(17) BURTON, L. V. Food Inds., **9**: 571–575. 1937.
(18) CARY, C. A., AND HARTMAN, A. M. Unpublished work.
(19) CHAPIN, E. K. U. S. Patent 2,392,833, Jan. 15, 1946.
(20) CHUCK, F. Y. U. S. Patent 2,016,592, Oct. 8, 1935.
(21) CLICKNER, F. H. U. S. Patent 2,030,964, Feb. 18, 1936.
(22) CLICKNER, F. H. U. S. Patent 2,076,400, April 6, 1937.
(23) CLICKNER, F. H. U. S. Patent 2,091,629, Aug. 31, 1937.
(24) COLLINS, M. Federal Register, **12**: 1203–1204. Feb. 21, 1947.
(25) DANIEL, L., AND NORRIS, L. C. Food Research, **9**: 313–318. 1943.
(26) DAVIES, W. L. Dairy Inds., **2**: 213–215. June, 1937.
(27) DOAN, C. F., AND MATHESON, K. J. U. S. Dept. Agr. Bull. 608. 1932.
(28) DOOB, H., JR., WILLMANN, A., AND SHARP, P. F. Ind. Eng. Chem., **34**: 1460–1468. 1942.
(29) DUNHAM, H. V. U. S. Patent 709,003, Sept. 16, 1902.
(30) ELDREDGE, E. E. U. S. Patent 1,923,427, Aug. 22, 1933.
(31) ERSHOFF, B. H., AND DEUEL, H. J., JR. J. Nutrition, **28**: 225–234. 1944.
(32) FARRALL, A. W. Dairy Engineering. John Wiley & Sons, New York, N. Y. 1942.
(33) FAULKNER, J. E. U. S. Patent 2,391,559, Dec. 25, 1945.
(34) FLANIGAN, G. E., AND SUPPLEE, G. C. U. S. Patent 2,023,014, Dec. 3, 1935.
(35) FRENCH, R. B., AND COWGILL, G. R. J. Nutrition, **14**: 383–394. 1937.
(36) FUNDER, L. Myse Og Skummet Melk Til Brodbakning. Statens Meieriforsok, Beretning Nr. 15. 1922.
(37) GEDDES, W. F., AND JENNESS, R. Dairy Products Inst., Univ. Minn., Abs. Pp. 158–159. Sept., 1947.
(38) GORDON, W. G. U. S. Patent 2,377,624, June 5, 1945.
(39) GREENBANK, G. R., STEINBARGER, M. C., DEYSHER, E. F., AND HOLM, G. E. J. Dairy Sci., **10**: 335–342. 1927.
(40) HANDLER, P. J. J. Nutrition, **33**: 221–234. 1947.
(41) HARLAND, H. A., AND ASHWORTH, U. S. Food Research, **12**: 247–251. 1941.

(42) HARLAND, H. A., AND ASHWORTH, U. S. J. Dairy Sci., 28: 879–886. 1945.
(43) HENRY, K. M., HOUSTON, J., KON, S. K., POWELL, J., CARTER, R. H., AND HALTON, P. J. Dairy Research, 12: 184–212. 1941.
(44) HOFFMANN, F. Treatise on the Virtues and Uses of Whey. L. Davis and C. Reymers. London, 1761.
(45) HUIZENGA, S. Centr. Zuckerind., 43(51): 1054–1056. 1935.
(46) HUNZIKER, O. F. Condensed Milk and Milk Powder. 6th Ed. Published by the author at La Grange, Ill. 1946.
(47) IRVINE, O. R., AND SPROULE, W. H. Can. Dairy Ice Cream J., 19(3): 62–64. 1940.
(48) JACK, E. L., AND WASSON, A. J. J. Dairy Sci., 24: 85–92. 1941.
(49) JOHNSON, A. H., WEISBERG, S. M., JOHNSON, J. J., AND PARKER, M. E. U. S. Patent 2,071,346, Feb. 23, 1937.
(50) KAUFFMANN, W., AND VAN DER LEE, P. J. U. S. Patent 2,183,141, Dec. 12, 1939.
(51) KON, S. K. J. Dairy Research, 9: 248–249. 1938.
(52) KRAFT, G. H. U. S. Patent 2,035,899, March 31, 1936.
(53) KRAFT, G. H. U. S. Patent 2,118,252, May 24, 1938.
(54) KREMERS, K. U. S. Patent 2,349,969, May 30, 1944.
(55) LANGWILL, K. E. Mfg. Confectioner, 21(8): 15–16. 1941.
(56) LARSEN, L. Tids. Norske Landbruk., 40: 18–25. 1933.
(57) LAVETT, C. O. U. S. Patent 2,143,019, Jan. 10, 1939.
(58) LAVETT, C. O. U. S. Patent 2,172,393, Sept. 12, 1939.
(59) LAVETT, C. O. U. S. Patent 2,188,907, Feb. 6, 1940.
(60) LAVETT, C. O. U. S. Patent 2,197,804, April 23, 1940.
(61) LAVETT, C. O. U. S. Patent 2,232,248, Feb. 18, 1941.
(62) LEVITON, A. U. S. Patent 2,129,222, Sept. 6, 1938.
(63) LEVITON, A. Ind. Eng. Chem., 35: 589–593. 1943.
(64) LEVITON, A. Ind. Eng. Chem., 36: 744–745. 1944.
(65) LEVITON, A. J. Am. Chem. Soc., 68: 835–840. 1946.
(66) LEVITON, A. In press.
(67) LEVITON, A., AND LEIGHTON, A. Ind. Eng. Chem., 30: 1305–1311. 1938.
(68) LYMAN, J. F. U. S. Patent 1,954,769, April 10, 1934.
(69) MEADE, R. E., POLLARD, H. L., AND RODGERS, N. E. U. S. Patent 2,369,680, Feb. 20, 1945.
(70) MENEFEE, S. G., OVERMAN, O. R., AND TRACY, P. H. J. Dairy Sci., 24: 953–968. 1941.
(71) MEYER, E. M. U. S. Patent 1,787,754, Jan. 6, 1931.
(72) MILLS, R., BREITER, H., KEMPSTER, E., MCKEY, B., PICKENS, M., AND OUTHOUSE, JULIA. J. Nutrition, 20: 467–476. 1940.
(73) MINER, C. S. U. S. Patent 2,202,161, May 25, 1940.
(74) MORGAN, A. F., COOK, B. B., AND DAVISON, H. G. J. Nutrition, 15: 27–43. 1938.
(75) MÜLLER, W. Molkerei-Ztg. (Hildesheim), 53: 442. 1939.
(76) MUNIN, F. Fette u. Seifen, 48: 144. 1941.
(77) MYERS, R. P., AND WEISBERG, S. M. U. S. Patent 2,128,845, Aug. 30, 1938.
(78) NABENHAUER, F. B. Ind. Eng. Chem., 22: 54–56. 1930.
(79) N. V. Lyemph Leeuwarder Ijs Melkproductenfabrick. Dutch Patent 57,374, April 15, 1946.
(80) OKUDA, Y., AND ZOLLER, H. F. J. Ind. Eng. Chem., 13: 515–519. 1921.
(81) OUTHOUSE, JULIA. Ill. Agr. Expt. Sta., Ann. Rept., 1934–1935: 293–294. 1936.
(82) PEARCE, J. A. Can. Dairy Ice Cream J., 26(1): 64, 66. 1947.
(83) PEEBLES, D. D. U. S. Patent 1,976,246, Sept. 10, 1934.
(84) PEEBLES, D. D. U. S. Patent 2,069,388, Feb. 2, 1937.

(85) PEEBLES, D. D. U. S. Patent 2,126,807, Aug. 16, 1938.
(86) PEEBLES, D. D. U. S. Patent 2,336,634, Dec. 14, 1943.
(87) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 1,928,135, Sept. 26, 1933.
(88) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 2,088,606, Aug. 3, 1937.
(89) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 2,181,146, Nov. 28, 1939.
(90) PETER, P. N., AND BELL, R. W. Ind. Eng. Chem., 22: 1124–1128. 1930.
(91) PROCOPIO, M. Chim. ind. agr. biol., 19: 64–66. 1943.
(92) RAMSDELL, G. A., AND WEBB, B. H. J. Dairy Sci., 21: 305–314. 1938.
(93) RAMSDELL, G. A., AND WEBB, B. H. J. Dairy Sci., 28: 677–686. 1945.
(94) REED, O. E. Rept. of the Chief, Bur. Dairy Ind., U. S. Dept. Agr. 1945.
(95) RIGGS, L. K., AND CLICKNER, F. H. U. S. Patent 2,123,203, July 12, 1938.
(96) ROGOSA, M. J. Bact., 45: 459–460. 1943.
(97) ROGOSA, M., BROWNE, H. H., AND WHITTIER, E. O. J. Dairy Sci., 30: 263–269. 1947.
(98) ROWLAND, S. J. J. Dairy Research, 5: 46–53. 1933.
(99) ROWLAND, S. J. J. Dairy Research, 9: 42–46. 1938.
(100) SAHYUN, M. Outline of the Amino Acids and Proteins. Chap. 4. Reinhold Pub. Corp., New York, N. Y. 1944.
(101) SAMMIS, J. L. Cheese Making. 11th Ed. The Cheese Maker Book Co., Madison, Wis. Pp. 304–305. 1946.
(102) SAMMIS, J. L. Cheese Making. 11th Ed. The Cheese Maker Book Co., Madison, Wis. Pp. 308–310. 1946.
(103) SCHANTZ, E. J., ELVEHJEM, C. A., AND HART, E. B. J. Biol. Chem., 122: 381–390. 1938.
(104) SCOTT, A. W. Hannah Dairy Research Institute, Bull. 4. 1932.
(105) SCOTT, M. J., AND GRAHAM, R. W. New Zealand J. Agr., 38: 112–114. 1929.
(106) SHARP, P. F. U. S. Patent 1,810,682, June 16, 1931.
(107) SHARP, P. F. U. S. Patent 1,956,811, May 1, 1934.
(108) SHARP, P. F., AND DOOB, H., JR. J. Dairy Sci., 24: 679–690. 1941.
(109) SHARP, P. F., AND HAND, D. B. U. S. Patents 2,182,618 and 2,182,619, Dec. 5, 1939.
(110) SHARP, P. F., AND TROY, H. C. J. Dairy Sci., 13: 140–157. 1930.
(111) SIMMONS, N. L. U. S. Patent 1,763,633, June 10, 1933.
(112) SIREK, J. U. S. Patent 2,023,359, Dec. 3, 1935.
(113) SMILLIE, D. M., AND WIGHT, J. British Patent 548,836, Oct. 27, 1941.
(114) SOMMER, H. H., AND TEMPLETON, H. L. Wis. Agr. Expt. Sta. Research Bull. 137. 1939.
(115) SPELLACY, J. R. U. S. Patent 2,163,331, June 20, 1939.
(116) STEFANIAK, J. J., RUPEL, I. W., BOHSTEDT, G., AND PETERSON, W. H. J. Dairy Sci., 30: 103–114. 1947.
(117) STRINGER, W. E. Food Inds., 11: 72–74. 1939.
(118) STRINGER, W. E. Food Inds., 11: 262–263, 290. 1939.
(119) SUPPLEE, G. C. U. S. Patent 2,173,922, Sept. 26, 1939.
(120) SUPPLEE, G. C., AND FLANIGAN, G. E. U. S. Patent 1,954,602, April 10, 1934.
(121) SVENSON, J. Mehl u. Brot, 39: 357–360. 1939.
(122) THOM, C., AND FISK, W. W. The Book of Cheese. MacMillan Co., New York, N. Y. 295. 1938.
(123) THOMSEN, L. C. J. Dairy Sci., 19: 503. 1936.
(124) TREBLER, H. A., AND HARDING, H. G. Chem. Eng. Progress, 43: 255–266. 1947.
(125) TRIGGS, W. W. British Patent 434,058, Oct. 27, 1934.
(126) TUFFT, E. Natl. Butter Cheese J., 32(8): 11. 1941.
(127) TURNBOW, G. D. U. S. Patent 1,737,101, Nov. 26, 1929.

(128) WAITE, R. J. Dairy Research, 12: 71–77. 1941.
(129) WANSHENK, O. B. U. S. Patent 2,123,218, July 12, 1938.
(130) WASHBURN, R. M. U. S. Patent 1,870,270, Aug. 9, 1932.
(131) WATSON, P. D. Ind. Eng. Chem., 26: 640–645. 1934.
(132) WEBB, B. H. Food Research, 3: 233–238. 1938.
(133) WEBB, B. H. BDIM 847, Bur. Dairy Ind., U. S. Dept. Agr. 1939.
(134) WEBB, B. H. Mfg. Confectioner, 21(3): 14–16. 1941.
(135) WEBB, B. H., AND HUFNAGEL, C. F. Food Research, 5: 185–195. 1940.
(136) WEBB, B. H., AND HUFNAGEL, C. F. Natl. Butter Cheese J., 37(12): 34, 35, 68, 70, 72. 1946.
(137) WEBB, B. H., AND RAMSDELL, G. A. U. S. Patent 2,119,614, June 7, 1938.
(138) WEBB, B. H., AND RAMSDELL, G. A. Natl. Butter Cheese J., 35(7): 18–24; 35(8): 18. 1944.
(139) WEIMAR, A. C. U. S. Patent 1,381,605, June 14, 1921.
(140) WEISBERG, S. M., CHAPPELL, F. L., STRINGER, W. E., AND STEVENS, S. U. S. Patent 2,071,368, Feb. 23, 1937.
(141) WHITTIER, E. O., CARY, C. A., AND ELLIS, N. R. J. Nutrition, 9: 521–532. 1935.
(142) WHITTIER, E. O. J. Dairy Sci., 27: 505–537. 1944.
(143) WILSON, C. W. U. S. Patent 1,975,998, Oct. 9, 1934.
(144) YAMASAKI, I. U. S. Patent 2,297,671, Sept. 25, 1942.