XXXVIII. A STUDY OF THE COMPOSITION OF HUMAN MILK IN THE LATER PERIODS OF LACTATION AND A COMPARISON WITH THAT OF EARLY MILK.

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(Received February 25th, 1930.)

A PREVIOUS investigation gave the variations in the chemical composition of normal human colostrum and early milk [Lowenfeld, Widdows, Bond and Taylor, 1927]. This work has now been extended to a study of the composition of milk secreted after the first month of lactation with the view not only of comparing the relative quantities of the constituents at the different stages of lactation, but also of comparing the relative quantities of these substances at the same stage of lactation. The material for this work has been obtained from the children's department of the Royal Free Hospital, Shoreditch Carnegie Welfare Settlement and from the Mothercraft Training Society, Cromwell House, Highgate.

In the case of the analyses of the later milk it has not been found possible to deal with individual women over the whole course of the observations as was done in the work on early milk, although in some cases it has been managed over two or three months. By studying the composition of the milk of as large a number of women as possible it was thought that to a large extent individual differences would be eliminated and some generalisations indicated.

Altogether 51 women have been studied, and of these 34 paid single, 12 double and 5 triple visits to the Hospital.

The constituents of the milk investigated are sugar, protein, fat, calcium, phosphorus and ash. The methods employed in the estimation of these substances were the same as those for early milk. The number of estimations of each constituent has varied between 50 and 60. The method of extracting the milk was either by means of the breast pump or digital expression. The method of extraction is only of importance in dealing with the percentages of fat, also it was only in the estimation of the fat that separate estimations were made on the milk extracted before and after the baby had fed. The earlier work showed that in the case of the other constituents the differences in the fore and after milk were slight and of no particular significance. Therefore in this work, except in the case of the fat, the estimations were done on the mixed fore and after

milk. The phosphorus, which was not studied in the earlier work, has been estimated by the Martland and Robison [1924] modification, of the Bell-Doisy [1920] and Briggs [1922] method, using Merck's perhydrol as suggested by them and Briggs for the final oxidation of the material for the total phosphorus present.

In considering the results of this work the calcium and phosphorus figures will first be considered separately and then their relationship to the ash and to one another.

The significance of the nitrogen and sugar results will also be dealt with, but the fat results will be left to another communication.

CALCIUM.

The calcium content of the milk of 51 women at all stages in the course of lactation has been determined with a view to ascertaining what variations exist, and whether, if there is variation, the variation follows any general rule. It has been emphasised by us [1927] and also by Hunnaeus [1909] that the individual differences in the calcium content of milk are much greater than the variations from day to day in any one individual. Samples of milk have been analysed, extending from the first to the tenth month of lactation, and the individual variation has again been found to be large, varying from 0.036 to 0.075 g. per 100 cc. of milk.

As our former work showed that the variations in calcium expressed themselves slowly, with the exception of the first month, the averages have been taken over a range of four months. The results of our work are shown on the left of Table I.

Table I.

No. of samples	Average CaO (g. per 100 cc. of milk)	Period of lactation	No. of samples	Average CaO (g. per 100 cc. of milk)	Period of lactation
101	0.0466	1-2 weeks	101	0.0466	1-2 weeks
3	0.0499	3-4 weeks	3	0.0499	3-4 weeks
33	0.0563	2-4 months	15	0.057	2 months
17	0.0499	5-10 months	17	0.0558	3-4 months
			10	0.0521	5-7 months
			8	0.0468	7-10 months

These results show that the calcium content of the milk is lowest for the first two weeks of lactation, rising gradually to a maximum during the first four months, maintaining a fair level after the fourth month, but slowly decreasing. The same result is shown if our numbers are taken over rather shorter intervals of time, as shown on the right of Table I, indicating that the maximum lies between the end of the second and the beginning of the third month.

Among the earlier workers on this subject Hunnaeus [1909], Schloss [1911], Bahrtdt and Edelstein [1910] all agree that the calcium in the milk decreases slowly with the course of lactation.

Bahrddt and Edelstein, while emphasising the high individual differences in the percentage of calcium in milk, concluded that their work showed this gradual decrease. Schloss alone mentions that he found the CaO less during the first week of lactation. The reason why the gradual increase from the first two weeks of lactation to a maximum at the end of the fourth month has not so far been emphasised is probably the fact that in many of the results recorded there are no samples taken until after the first month of lactation.

Table II gives a comparison of our results with those of other workers on milk. The figures of the latter have been averaged over the same periods.

Table II.

Authors			Holt		Telfer		De Buys and von Meysenburg [1924]		
Period of lacta- tion	No. of samples	Mean value CaO	No. of samples	Mean value CaO	No. of samples	Mean value CaO	Period of lacta- tion	No. of samples	Mean value CaO
First 2 weeks	101	0.0466	5	0.0444	5	0.0398	—	—	—
3-4 weeks	2	0.0499	6	0.0406	4 with rachitics	0.0407	—	—	—
					5	0.0422			
2-4 months (end)	33	0.0563	9	0.0483	9 with rachitics	0.0466	1-3 months	30	0.0435
					18	0.0477			
5-10 months (end)	17	0.0499	8 4-9 months	0.0453	5 with rachitics	0.0472	4-7 months	28	0.0392
					7	0.0474			
10-20 months	—	—	10	0.0388	12-13 months		8-12 months	12	0.0367
					1 with rachitics	0.048			
					4	0.044			
Total no. of samples	152	—	38	—	24 or 39	—	—	70	—

All these results show the same trend to a lower calcium value in the earlier months, gradually rising to about the end of the fourth month and then slowly sinking. Our values differ from those of the other workers in being slightly higher in value, the average value for the interval during 2-4 months being raised by several individual cases with a value 0.06 and there being no very low values. Some of the individual cases of the other workers are high, but their average is lower.

The explanation of the gradual rise in the percentage of calcium in the milk from the first week to the beginning of the third month and the gradual fall in the later months must be related to the physiological needs of the child.

During the first days after birth it must necessarily be trying to accommodate itself to its new environment and it is general that during the first week of life the weight of the child should decrease from that at birth. During this time it will need the minimum amount of calcium. As growth proceeds,

according to the figures of Feldman [1927], the amount of milk taken per day increases from 300 cc. during the first week to 1000 cc. by the end of the sixth month. The increase in intake, however, is not uniform for each month. During the first month the intake rises from 300 cc. to 600 cc. per day, during the second month the average increase on the day's intake is 250 cc., during the third month the intake remains the same, and from the third to the sixth month the average rise is 50 cc. on the day's intake per month.

Table III shows the relationship between the volumes of milk and its calcium oxide content taken per day at the different periods of lactation.

Table III.

Period	1-2 weeks	3-4 weeks	2 months	3 months	4 months	5-7 months	8-10 months
Average CaO (g. per 100 cc.)	0.0466	0.0499	0.0570	0.056	0.055	0.0521	0.0468
Average volume of milk intake per day (cc.)	300	600	850	850	900	950 (5 months) 1000 (6 months)	—
Total intake of CaO per day (g.)	0.1398	0.2994	0.4845	0.476	0.495	0.495 (5 months) 0.521 (6 months)	—

This comparison shows that the gradual rise in percentage of CaO up to its maximum at the end of the second month coincides with the time when the baby is increasing its intake of milk by the largest average daily increase and presumably growing correspondingly fast. During the third month the intake of milk and the percentage of calcium remain practically constant. The gradual decrease in the percentage of CaO after the third month is counterbalanced by the continued increase in the intake of milk, so that the total intake of CaO during the first six months shows a gradually increasing value. The gradual decrease in the percentage of calcium will be balanced in the later months by the supplementary foods that the child will take about the age of six months.

PHOSPHORUS.

The estimations of phosphorus were made on the same samples of milk as were used for the calcium estimations. As no phosphorus estimations were originally made on early milk a separate series of experiments was carried out on the milk from 16 different women for this period. At present the phosphorus content of the milk of one individual has not been estimated over any consecutive length of time, but the cases so far examined on consecutive days or within a short period seem to indicate that as in the case of the calcium probably the individual differences in the phosphorus content are greater than those occurring in the samples of milk of the same individual. The same range of periods has been taken in averaging the percentage of phosphorus as in the case of calcium.

The results are shown on the left of Table IV.

Table IV.

No. of samples	Average P_2O_5 (g. per 100 cc. of milk)	Period of lactation	No. of samples	Average P_2O_5 (g. per 100 cc. of milk)	Period of lactation
14	0.0364	6th-14th day	14	0.0364	6th-14th day
4	0.0387	3-4 weeks	4	0.0387	3-4 weeks
37	0.0412	2-4 months	16	0.423	2 months
18	0.0410	5-10 months	21	0.0404	3-4 months
			9	0.0402	5-7 months
			9	0.0417	8-10 months

The percentages of P_2O_5 in the milk seem to run parallel with those of the calcium oxide. During the first two weeks of lactation the percentage of phosphoric acid is at its lowest. It then rises slowly up to the end of the fourth month when it remains practically constant up to the tenth month with a very slight tendency to decline. The variations in the percentage of phosphorus are not so marked as in the case of calcium.

When the above results are set out over shorter intervals of time, as shown on the right of Table IV, it is seen that the maximum percentage for phosphorus falls, as in the case of calcium, between the second and third months of lactation.

The percentage of phosphorus in milk, unlike that of the calcium which slowly decreases, remains almost constant after the fourth month. This may be due to the fact that, although the needs of the child for calcium and phosphorus for bone formation run parallel, it is probable that the demand for phosphorus for the growth of the soft tissues exceeds that for calcium.

The constancy shown by the percentage of calcium during the second and third month of lactation, when the intake of milk by the child remains constant, is not shown in the case of phosphorus. It is found that 0.0386 is the percentage of P_2O_5 for the third month as compared with that of 0.0423 for the second.

This work emphasises a point which Bahrdt and Edelstein [1910] also emphasised, viz. that it is only by comparing over long periods that any general tendency to decrease or increase can be observed.

Schloss [1911] showed, as we have done, a gradual increase in the percentage of phosphorus from the second week up to the third month of lactation, followed by a fall and rise but gradually decreasing to the eleventh month.

Telfer's [1924] results, on the other hand, agree in that they show a gradual rise to a maximum after the first two weeks and then a gradual decrease. His maximum, however, falls later, between the fifth and seventh months, and during the first two weeks his average is higher than at any other time. This may be due to the fact that it is only in the case of country women that the figures for early milk are recorded, and Telfer has pointed out that the percentage of phosphorus in the milk of these women is distinctly higher than that of town dwellers.

Schlossmann [1904] could establish no relationship between the percentage of phosphorus in milk and the duration of lactation, but, although his numbers

vary, they seem to show that the percentage is gradually decreasing with the duration. Although there are exceptions, he thought that a high phosphorus content was associated with a high nitrogen content. So far we have no results to record on the percentage of phosphorus in milk on the first and second days after birth when the nitrogen percentage is very high.

Table V gives a comparison of our averages with those of Telfer and Schloss.

Table V.

Period of lactation	Authors		No. of samples	Telfer		
	No. of samples	P ₂ O ₅ (g. per 100 cc.)		P ₂ O ₅ (g. per 100 cc.)		
5th-14th day inclusive	14	0.0364	5	All cases	Without samples from country women	
3-4 weeks	4	0.0387	5	0.048	—	
2-4 months	37	0.0412	17	0.0384	(3) 0.0363	
5-10 months	18	0.0410	6	0.0395	(13) 0.039	
				0.0421	(5) 0.043	
12-13 months	—	—	4	(6-8 months)		
				0.0377	(4) 0.0377	
		Schloss				
	11-13th day	3-4 wks.	2 mths.	3 mths.	3½ mths.	4½ mths.
P ₂ O ₅ (g. per 100 cc.)	0.0380	0.0393	0.0431	0.0501	0.0342	0.0415
					0.0348	

THE ASH AND ITS RELATIONSHIP TO THE CONTAINED CALCIUM AND PHOSPHORUS.

Our results on the amount of ash show that the ash is high in the early weeks of lactation, the average being 0.289 g. per 100 cc., while towards the end of lactation the average falls to 0.181 per 100 cc. Out of 77 samples during the first two weeks 43 showed an average of 0.3 g. or a little over, 19 of over 0.25 and the rest slightly lower. The above results are in accord with the work of Söldner [1896], who gave as his maximum for early milk 0.3 g. and his minimum 0.18.

The variations in the amount of ash during the course of lactation are given in Table VI.

Table VI.

Period of lactation	1-2 weeks	3-4 weeks	2-4 months	5-7 months	8-10 months
Average ash (g. per 100 cc. of milk)	0.289	0.249	0.243	0.236	0.181
No. of samples	77	4	37	8	8
			2nd month		
			0.247		
			3rd month		
			0.2398		

These figures show a rapid decrease during the first month of lactation, from the end of the first month to the seventh a slow decrease, and then again a rather rapid decrease during the last months, the figure during this last period usually lying between 0.1 and 0.2 g.

In considering the relationship of the calcium in the ash, the percentage of calcium rises from a mean of 15.5 during the first two weeks to 26.1 during the last three months, remaining fairly constant at 23 during the intervening period.

These results are shown in Table VII.

Table VII.

Period of lactation	No. of samples	Average CaO (g. per 100 g. ash)	No. of samples	Average P ₂ O ₅ (g. per 100 g. ash)
1-2 weeks	56	15.5	15	13.56
3-4 weeks	3	18.9	4	15.68
2 months	13	23.8	16	17.3
3 months	13	23.2	13	15.95
4 months	4	23.67	5	17.56
5-6 months	5	23.3	5	18.98
7-10 months	9	26.1	10	21.13

Schloss [1911] reports 11 % CaO in the ash of early milk, rising to 20 % in mature milk. The above higher percentages are probably due to the fact that the average calcium oxide in the samples of milk examined was higher than in those of Schloss.

In the case of phosphorus the percentage of P₂O₅ in the ash shows the same general rise from the first week to the tenth month of lactation, as is shown by the calcium oxide. The percentage, however, is lower throughout the course of lactation, rising from 13.56 in the first week to 21.13 during the last (seven to ten) months.

It is to be noticed here that the percentage falls between the second and third months and begins to rise again between the third and fourth months. This runs parallel with the phosphorus estimation, as it has already been shown that the percentage of phosphorus pentoxide in milk rises from the first week to the end of the second month, falls during the third month, rises again during the fourth month, after which a fair level is maintained with a very slow decline in the later months (Table VII).

RELATIONSHIP BETWEEN THE CALCIUM AND PHOSPHORUS.

Among the inorganic constituents of milk the calcium and phosphorus are the most important, and much attention has been focussed on these two elements in the diet in connection with the study of rickets.

It has been established that diets, insufficient in either calcium or phosphorus but adequate in every other respect, result in a retardation of the deposition of calcium phosphate in the bone and, where the deficiency of either element is too great, this cannot be counterbalanced by greatly increasing the antirachitic vitamins in the diet by the addition of cod-liver oil but only by the addition of the deficient calcium or phosphate. This has been established by the work of Sherman and his co-workers [1921, 1925, 1926] and confirmed by the work of McCollum and other workers on rickets.

McCollum *et al.* [1921] were the first to point out and emphasise the fact that the ratio between the calcium and phosphorus in the diet was of infinitely

greater importance than the absolute amounts of the salts themselves. He showed that a deficiency of phosphorus in a diet, insufficiently supplied with "fat-soluble A," gave rise to rickets only when the calcium was present in an amount which gave a calcium-phosphorus ratio considerably above the optimum.

It is suggested that the importance of the ratio between the quantities of calcium and phosphorus in the diet is due to the fact that either excess of calcium or phosphorus, by precipitation of insoluble calcium phosphate in the intestine, would diminish the amount of phosphate absorbed.

Mellanby [1921] showed this to be the case with excessive amounts of phosphorus. The importance of this ratio has also been emphasised by Elliot, Crichton and Orr [1922] for pigs, and by Shohl, Bennett and Weed [1928] for rats.

In the present work this important relationship between the calcium and phosphorus has been studied in human milk. The latter is the natural food of the child during the first six months of life and therefore this ratio in milk should be the best for its nutrition and growth.

From the work already recorded on the variations in the percentages of calcium and phosphorus during the course of lactation a similar rise and fall in the ratio of these elements per 100 cc. of milk over the same period was to be expected. And since the variations in the percentage of P_2O_5 are similar to those of the CaO but are not so great, there should result less variation during the course of lactation in the ratio between these two elements than in the case of the elements alone.

It is found that this ratio, averaging over the same periods as before, does not show a large variation. The ratio rises a little from the first to the fourth month of lactation and then falls during the later months to a value less than that in the early weeks (Table VIII).

Table VIII.

Period of lactation	1-2 weeks	1 month	2-4 months	5-10 months
Average ratio CaO/ P_2O_5	1.35	1.34	1.42	1.25
				4-6 months
				1.3
				7 10 months
				1.23
Ca/P	2.2	2.18	2.3	2.02
No. of samples	11	4	29	17

Although the average ratio over the whole course of lactation does not vary much, the individual variations are greater, varying from 0.81 to 2.

Out of the 61 samples investigated only 9 were between 1.8 and 2, of these 7 came in the period two to four months.

On examining the individual ratios it is found that 9 out of the 60 samples, *i.e.* 15 %, have a percentage of P_2O_5 greater than that of CaO, that in 7 samples the percentages are equal, and in all the other 45 cases the percentage of CaO is higher than that of the P_2O_5 . In many of these cases the ratio is 1.1 or 1.2, but in some higher, the mean at the different periods lying between 1.3 and 1.4. When it has been possible to examine the milk of the same women at different stages of lactation the ratio between the percentage of CaO and P_2O_5 ,

where high, certainly rapidly decreases and in the later months the P_2O_5 tends to become higher than the CaO.

Cases illustrating this are given in Table IX.

Table IX.

Case	Period of lactation	Ratio CaO/ P_2O_5
H	6 weeks	1.88
	3 months	1.26
B	2 months	1.9
	3 months	1.3
G	4½ months	1.27
	5 months	0.94
	6 months	1.08
B	3 months	1.8
	6 months	0.91
Ha	9 months	1.01
	10 months	0.81

Although Schabad [1911] says it is not always possible during the first three months of life clinically to diagnose rickets, apparently all the children of the cases examined, with the exception of about two, were healthy children. Therefore our results show that, during the first six months of lactation, a ratio of CaO/ P_2O_5 per 100 cc. of milk greater than 1 and approximating to 1.3 is probably the ratio most suitable for the child provided the vitamin factors are adequate.

That the factor can vary within quite wide limits is also evident and to be expected considering the extraordinary variability and adaptability of the human organism.

Since the ratio during the seventh to tenth months is decreasing (the only high ratio 1.7 being that of a mother with a rickety child, the high ratio being due to a low phosphorus content), it is possible that with milk after the tenth month, which we have not been able to obtain, the average ratio might become 1 or less than 1.

This wide variability of the calcium/phosphorus ratio is in accord with the work on rats which has been done in connection with rickets. Shohl, Bennett and Weed [1928] give the ratio Ca/P used by different workers [Boas, 1924, 1926; Medes, 1926; McCollum, 1922; Osborne and Mendel, 1913] as most suitable for their experimental diets and the ratio varies within the limits 1-2.

Telfer [1924] suggests that in milk a slight excess of phosphoric acid over the calcium equivalent necessary for bone formation is required for the growth of the soft tissue. In the case of his own results, however, out of 27 analyses of the milk of town dwellers, only two show a percentage of P_2O_5 greater than that of CaO. These cases Telfer considers abnormal, as in the analyses of the milk of 12 country women, 7 showed a higher percentage of P_2O_5 than of CaO, 2 an equal percentage, and only 3 a lower percentage.

The results of other workers, Schabad [1911], Schloss [1911], Söldner [1896], Burhaus and Smith [1923], confirm the variability of this ratio and show a higher percentage of calcium oxide in by far the greater number of cases.

Our results are certainly taken from samples of milk of women living in

the poorer districts of London, but, considering the variations in the ratio, that the ratio is nearly always greater than one, that the children are healthy, and that many samples have been analysed, is there not some other physiological explanation for this ratio?

Sherman and Quin [1926], in their work on rats, showed that, during the suckling period, while there was a considerable increase of the soft tissues, where the phosphorus would be in organic combination, the largest gain in calcium and phosphorus was due to the deposition of these elements in the bones, and that, as tricalcium phosphate contains three atoms of calcium to two of phosphorus, the rapid gain of this substance must increase the ratio of the calcium to the phosphorus in the body as a whole. They found that at birth the atomic ratio of calcium to phosphorus in the body was 3 : 5 and that this had become 1 : 1 before the end of the suckling period. They showed that well within this period the percentage of calcium overtakes the percentage of phosphorus in the body.

If the same factors are operating during the suckling period of the child, the fact that the percentage of calcium oxide in the food, the mother's milk, is greater than that of the phosphoric acid has an explanation.

In support of this we have the fact that during the first six months of its life a baby doubles its weight at birth. The ratio $\text{CaO}/\text{P}_2\text{O}_5$ in tricalcium phosphate is about 1.2 and the mean of our ratios falls between 1.3 and 1.4. The gradual decrease in the ratio towards the end of lactation runs parallel with the decrease in the percentage of calcium oxide and phosphorus pentoxide during the later months, the decrease in the calcium being more marked than that of the phosphorus pentoxide.

There may be some differences in the milk of mothers living in the country and in towns as Telfer suggests, but the fact that nature produces a diet richer in calcium than phosphorus and the children bred on it are healthy must have some physiological explanation. The work of Sherman and Quin seems possibly to offer an explanation of the results recorded in this paper¹.

SUGAR.

Fifty-five samples of milk have been examined at different periods of lactation after the first fortnight (Table X). The method of estimation of lactose was that of Folin and Denis [1918].

There are two conclusions to be drawn from the results obtained.

(1) The percentage of sugar in early milk is at its lowest value.

(2) By the end of the first month the percentage has risen to an average which does not materially alter throughout the whole course of lactation up to at any rate the tenth month (no examination having been made of later milk).

¹ (Note added 13th April, 1930.) Parsons (*Brit. Med. J.* 1927, ii, 780) states that "to obtain the maximum absorption of both calcium and phosphorus the diet should contain amounts of these elements sufficient in quantity and properly balanced. The percentage of calcium in the diet should be greater than the percentage of phosphorus."

Table X.

Period of lactation	1st-14th day	3-4 weeks	2-4 months	5-7 months	8-10 months
Average lactose (g. per 100 cc. milk)	5.845	7.08	7.099 2 months 7.18 3-4 months 6.97	6.93	7.09
Range of average	4.41-6.9	—	6.72-7.38 1 sample 8.4	5.83-7.35	6.12-7.77
No. of samples	115	2	29	12	12

To compare the percentage of lactose in early milk, that is up to the end of the first fortnight, with that found by other investigators, it has been found convenient to tabulate the results in days and these are given in Table XI.

No other observations have been recorded over the whole consecutive fourteen days for comparison with our results.

Although the numbers fluctuate a little there is a steady rise in the proportion of sugar during this period up to an average of 6.89 g., culminating in a value over 7 by the end of the first month.

Bracketed numbers refer to number of samples analysed.

Table XI. *Lactose (g. per 100 cc. milk).*

Days	Authors	Hammett [1917]	Bell	Camerer and Söldner [1896, 1898]	Söldner [1896]
1	4.74 (1)	—	—	—	—
2	5.56 (3)	—	—	—	—
3	5.33 (14)	5.43 (8)	—	4.782 (2)	—
4	5.47 (11)	—	—	—	—
5	5.42 (16)	6.08 (8)	6.42 (88)	5.6 (2)	—
6	5.78 (13)	—	—	—	—
7	6.17 (10)	6.21 (8)	—	—	—
8	6.34 (10)	—	—	—	—
9	6.24 (9)	6.32 (8)	6.73 (88)	6.73 (1)	6.75 (2)
10	6.42 (9)	—	—	—	6.62 (2)
11	—	6.42 (7)	—	6.35 (1)	—
12	6.12 (11)	—	—	—	—
13	6.69 (5)	—	—	—	—
14	6.89 (2)	—	—	—	—

Schlossmann [1900] recorded a value of 6.92 g. for the ninth to tenth day and Holt, Courtney and Fales [1915] gave an average of 6.5 for the first twelve days of lactation.

There is general agreement that during the first fortnight the average, although gradually rising, never rises to the figure 7 which is characteristic of mature milk.

From our results and those of Camerer and Söldner, it is probable that the percentage of sugar on the first day lies between 4 and 5, but too few samples have been examined on this day to establish this point.

The comparison of our numbers for the percentage of sugar in the later months with those of other workers is shown in Table XII.

Table XII.

Period of lactation	Authors	Schabad [1911]	Schlossmann [1902, 1904]		Holt, Courtney and Fales [1915]	Söldner [1896]	Camerer and Söldner [1898]
3-4 weeks	7.08 (2)	—	—	6.81 (65) from 11th day	7.81 12th-30th day 5.54 (1)	7.15 (4)	6.52 (15) 6.36 (4) 6.36 (7)
2 months	7.18 (14)	4.7 (2)	7.01 (30)	7.05 (56)	—	—	—
3-4 months	6.97 (15)	4.72 (4)	7.35 (56)	6.87 (53)	7.98 (10)	7.42 (3)	6.81 (14) 6.66 (5)
5-7 months	6.93 (12)	4.36 (2)	7.11 (90)	6.89 (64)	8.15 (3)	—	6.87 (4)
8-10 months	7.09 (12)	5.48 (2)	—	7.33 (17)	7.66 (3)	7.28 (1)	6.78 (10) (5½-11 months)
10-20 months	—	—	—	—	7.41	—	—

Although our figures differ to a certain extent from those of the other workers, their general significance is the same. They all seem to indicate that there is an average percentage of sugar in milk which is well maintained throughout the course of lactation.

A different level of average percentages is to be expected where, as in all this work, the average is taken from a study of a large number of different individuals, as individual variations certainly exist. Schlossmann, for instance, finds in the case of some women a percentage of lactose as high as 10.75, while out of all our 55 samples only one was as high as 8. Considering also the variation in the methods of analysis of the sugar the differences in the averages are no greater than would be expected.

Hammett [1917] commented on the fact that the sugar estimations of the American workers were higher than those of others and certainly the results of Holt, Courtney and Fales [1915] are distinctly above those of the other workers recorded. Schabad's [1911] numbers are the only ones which are markedly different from all the others, the average being between 4 and 5 g. per 100 cc., the proportion associated by us with milk of the first few days of lactation. He also gets a very noticeable rise during the last few months. The small number of Schabad's samples may account for his figures.

In those cases in which we have been able to examine the milk of the same women at different stages in the lactation, there seems to be little variation in the percentage of the sugar except in two cases not included in the table.

This is evident from the cases quoted in Table XIII.

Table XIII.

Case	Month	Lactose (g. per 100 cc.)	Case	Month	Lactose (g. per 100 cc.)
A	5	6.8	E	5½	7.35
	8	7.05		6½	7.35
B	3	7.02	F	4½	7.03
	6	7.00		7½	7.18
C	9	7.31	G	1½	6.97
	10	7.18		2½	7.34
D	1½	6.94			
	3	6.90			

We have no explanation to offer of the two exceptions, nor has Schlossmann for similar variations that he records.

From the few cases at present examined, however, a pathological condition of the breast seems to influence the sugar percentage.

In one case, five days after birth, the percentage of sugar was 4.27 and in another case, fifteen days after birth, the percentage was only 3.97 and 3.65.

In all these cases the breast was very engorged and the percentage of sugar was found to be much below the average for the period of lactation.

Myers [1927] also gives a case of abscess of the breast, where at four and a half months the percentage of lactose is 2.16.

The low values for the sugar of early milk are of interest in comparison with the very general practice in the artificial feeding of young infants of the use of a highly sweetened food.

PROTEIN.

In the case of early milk it was shown that the highest percentage of protein occurred in the first three days after parturition and that the level fell steeply during the first week, to reach an approximate average of 1.4 % at the end of the thirteenth day. The present work on later milk shows that although after the first month the level is fairly constant for some months, yet a very slow continuous decrease is taking place from 1.4 at the end of the first fortnight to 0.9337 during the last month of lactation.

This gradual decrease is shown in Table XIV.

Table XIV.

Period of lactation	1st-3rd day	4th-7th day	8th-13th day	3-4 wks.	2 mths.	3 and 4 mths.	5 and 6 mths.	9-10 mths.
Average protein (g. per 100 co. milk) (g. N $\times$ 6.37)	4.357	1.702	1.449	1.175	1.174	1.111	0.9855	0.9337
No. of samples	30	52	26	2	16	19	7	12
Range of percentages	8.47 -1.19	2.586 1.18	1.984 0.98	—	1.407 -0.882	1.367 -0.891	1.032 -0.961	1.24 -0.803
			1 only under 1	—	2 only under 1 and 2 only over 1.25	3 under 1 4 above 1.158	4 under 1	9 under 1 2, 1.035

There are several points of interest arising out of the above results.

(1) The percentage of protein during the first three days after parturition in milk rises in some cases to 6, 7, or 8, the highest value recorded being 8.4.

Other workers have noted high protein in early milk. Söldner [1896] records one between 5 and 6 % for the second day, Holt, Courtney and Fales [1915] 2.06 for the third day, and Hammett [1917] 5.79 for a third day, but no numbers are recorded as high as ours.

Among our estimations of the protein in milk during the first three days of lactation 6 are made on milk taken the first day after parturition, giving an average of 7.48 %, and 8 on the second day, giving an average of 4.58 %. This accounts for our high early values compared with those of other workers, for,

with one or two exceptions, their earliest estimations are made on the third day of lactation.

It was thought possible, and suggested in our previous paper, that the high percentage of protein in the colostrum period might be connected with the small quantity of milk available at this period. Our earlier experiments [1927] support this explanation.

(2) It was also noticed in the cases examined that the initial protein values for the primiparae were in all cases much higher than those of the multiparae. The figures for the latter fell to a constant level much more rapidly than did those for the former. Since no distinctions of this kind have been recorded by other workers, comparison on this point is not possible.

In comparing values of the percentage of protein in milk it must be borne in mind that the several workers in this field have used different factors for converting the protein nitrogen obtained from the precipitated protein into protein itself. The factor used in this work is 6.37. Söldner, Schlossmann, Holt, Courtney and Fales use 6.25, Hammett and Bell 6.38.

In comparing the figures of these workers with ours, Söldner's numbers have been corrected to the 6.37 factor, but those of Schlossmann, Holt, Courtney and Fales are their recorded values.

(3) From the first day of lactation to the end of the first fortnight there is a rapid decrease in the average percentage of protein, the number falling from an average of 4.357 to 1.449.

(4) During the first and second months the level of the protein remains fairly constant. As lactation progresses, however, there is undoubtedly a gradual and continuous decrease to an average of about 0.9337 in the tenth month. This gradual decrease is also found by other observers.

(5) It is of interest that our range of numbers after the first fortnight are in accord with those of Söldner, the values in both series being considerably below those of Schlossmann and lower than those of Holt, Courtney and Fales. In the case of the latter workers the final average approaches ours, but not until much later in the course of lactation. Between five and six months our average is 0.9855 g. protein per 100 cc., while theirs becomes 0.972 after the tenth month.

A comparison of our results with those of other workers can be seen in the collected results in Table XV.

The average percentage of protein in mature human milk is recorded as 1.5 by Feldman [1927] and 1.6 by Sherman.

Our results suggest that after the first month of lactation a distinctly lower percentage of protein than 1.5-1.6 is present in milk, and that no single value can be taken as representative of the percentage over the whole course of ten months.

The fact that it has fallen to 0.934 by the end of the tenth month does not mean that the child is requiring less protein, as any slight lowering of the percentage of protein during the late months is counterbalanced by the larger volume of milk consumed.

Table XV. *Average protein (g. per 100 cc.).*

	1-3 days	4-7 days	8-13 days	3-4 wks.	2 mths.	3-4 mths.	5-6 mths.	7-10 mths.	11-20 mths.
Authors g. N $\times$ 6.37 per 100 cc.	4.35 (30)	1.702 (52)	1.449 (26)	1.175 (2)	1.174 (16)	1.111 (19)	0.9855 (7)	0.934 (12)	—
Camerer and Söld- ner [1896, 1898] g. N $\times$ 6.37 per 100 g.	2.05 (1)	1.77 (3)	1.66 (10)	1.22 (15)	—	1.019 (14)	0.873 (10)	—	—
	1.624 (1)	—	—	—	—	3-4½ mths.	5½ and over	—	—
Schlossmann [1900] g. N $\times$ 6.25 per 100 g.	—	—	1.81 (5)	1.94 (41)	1.99 (30)	1.54 (60)	1.50 (60)	1.57 (30)	—
Holt, Courtney and Fales [1915] g. N $\times$ 6.25 per 100 cc.	—	2.206 (3)	2.19 (1)	1.17 (1)	1.05 (2)	1.141 (8)	1.13 (3)	1.27 (3)	0.972 (10)
Hammett [1917] g. N $\times$ 6.38 per 100 g.	3.52 (8)	1.64 (16)	1.57 (15)	—	—	—	—	—	—
	3rd day	5 and 7 days	9 and 11 days	—	—	—	—	—	—
Bell [1928] g. N $\times$ 6.38 per 100 g.	—	2.0 (88)	1.73 (88)	1.37 (88)	1.30 (88)	—	—	—	—
		5th day	9th day		5-6 wks. 1.21 (88) 6-8 wks.				

Since human milk is the natural food of the child during its first six months of life these results should be of interest from the point of view of the preparation of artificial foods for infants.

SUMMARY.

1. The composition of human milk has been studied in the later stages of lactation and the calcium, phosphorus, ash, sugar and protein constituents compared with their proportions in early milk.

2. The percentages of calcium and phosphorus follow parallel curves throughout the course of lactation, though the variations in phosphorus are less than those in calcium. The percentage of both is less in the early weeks, rising to a maximum at the end of the second month, then slowly declining to the end of the tenth month. The relationship between the volume of milk assimilated and the percentage of calcium and phosphorus is noted.

3. The percentage of ash is shown to be highest during the first two weeks and then falls continually throughout lactation, the steepest fall being during the last month.

4. The percentage of both the calcium oxide and phosphorus pentoxide gradually rises in the ash to reach a maximum during the last months. That of the calcium oxide rises from 15.5 in the early weeks to 26.1 in the later months, while that of the phosphorus pentoxide rises from 13.56 to 21.13.

5. The ratio of the percentage of calcium oxide to that of phosphorus pentoxide in the milk has been studied at the different periods. It is shown to have a rise and fall following the corresponding rises and falls of the calcium

and phosphorus, but the variations are not great. The average rises from 1.35 to 1.42 and falls to 1.25 during the last months.

6. The fact that in the larger number of samples the percentage of the CaO is greater than that of the P_2O_5 is discussed and an explanation suggested.

7. The percentage value of the protein decreases rapidly from a very high value immediately after parturition to the end of the first month, and then gradually but continuously till it reaches an average of about 0.9337 by the end of the 10th month.

8. The figures for the average percentage of protein from the end of the first month till the tenth suggest that this is not a fixed value and that it has a lower average than 1.5 which is usually taken.

9. The percentage of sugar is at its lowest value in early milk. After the first month it varies little throughout lactation.

10. In those cases examined in which there was a pathological condition of the breast, the percentage of sugar is affected and the value lowered.

We wish here to thank Dr Chodak Gregory, Dr Olivier Richards and Miss Liddiard for affording us the facilities for obtaining the material for this study and Prof. Winifred Cullis for help and advice throughout the work. We also wish to thank the Medical Research Council for their help in the present investigation.

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