A Study of the Variations in the Chemical Composition of Normal Human Colostrum and Early Milk

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A very extensive literature has grown up during the past half century, on the subject of the chemistry of normal human milk. So vast has this literature become, that the task of absorbing and correlating its conclusions into a coherent understanding of the subject as a whole has become one of extreme difficulty and laboriousness. On the other hand, some attempt to do so must form the basis of any new work. From a review of the main communications in the literature, a list of which is appended, certain facts stand out and can. we suggest, be made the basis of an attempt to explore the matter in greater detail.

1. We have reliable and accurate knowledge of the general nature of the main constituents of human milk, and adequate methods for their estimation.
2. We have on record a very large number of observations of milk at all stages of lactation and are aware of the changes most usually found.

If, however, we review the aggregate of facts presented by points 1 and 2, an admirable consideration of which appeared in a communication last year, the main feature of the picture is the exceedingly wide range of values shown in normal milk by each constituent. These variations complicate our understanding of the composition of normal milk. and put a serious bar to any study of the conditions of abnormal milk. Furthermore, the great bulk of the work done hitherto on normal milk has not only been done abroad but has also been achieved through the study of wet nurses—that is, of women accustomed to a specialised routine for the production and extraction of milk. As their conditions differ radically from those of ordinary nursing mothers, it cannot safely be assumed that the lactation results will necessarily be the same.

The present paper is the report of an attempt on the basis of work already known to deal with both these positions, to investigate in ordinary normal mothers the factors determining the variations in the chemical composition of their milk.

The period of lactation for investigation was the first 14 days after parturition, the reason for this choice being the accessibility to continuous observation of women in a labour ward, and the scantiness of our knowledge of this period. The mothers selected were all normal women, in-patients of the Obstetric Unit of the Royal Free Hospital. Among these patients were some who had been admitted to the V.D. Section of the Obstetric Unit for gonorrhoea. No mother with a history of syphilis was included.

In the vast majority of previously published communications the milk for analysis was obtained from single samples of the milk of different women. As by this procedure no opportunity if given for the observation of individual differences or their bearing upon general results, our procedure has been instead to make continuous observations of a number of individual women throughout their stay in the maternity wards.

Every sample was collected personally in the wards by one of us (M. F. L.), and taken to the London School of Medicine for Women where the analyses were carried through on the same day by S. T. W., M. B. and E.I.T. In only two instances was the milk kept overnight.

Chemical methods.

The constituents examined were protein, sugar, ash, fat, calcium and solids. Our aim at each point has been, not so much to achieve absolute percentages, as to arrive at some idea of the grounds underlying the differences that we found. The following are the methods of chemical estimation employed.

Protein. The total nitrogen was estimated by the Kjeldahl-Gunning method. 2-5 cc. of milk were usually taken for analysis, and the protein precipitated by saturated tannic acid solution, but where the amounts of milk available for examination were small, 1.5 cc. or occasionally 1 cc. only was used for the determination of N. In all cases where possible, the estimations have been done in duplicate with appropriate blank experiments.

Sugar. The method of Folin and Denis [1918] was employed. Calculation of the sugar content from the figures obtained from titration is according to the following empirical formula given by Folin and Denis:

$$\frac{4.04 \times \text{dilution}}{\text{Titration}}$$

Fat. The method of Gottlieb [1892] has been used, 55 cc. of milk being usually taken; but where the amount of milk available did not allow of this quantity, the maximum amount that could be given was diluted to 5 cc. with water before extraction of fat. In all these estimations the term “fat” is used to denote all the ether-extractable substances.

Solids and ash. 2-5 cc. milk were measured into a weighed platinum basin; the fluid was evaporated on a water-bath for half-an-hour and then transferred in a steam-oven and heated to constant weight. The solid residue was converted into ash by ignition at low temperature, the basin never being allowed to show a red glow. In some cases, a duplicate estimation was made by just charring the solid residue, dissolving out the salts with water and filtering through a. small tared filter. The carbon and filter paper after drying were ignited together, the filtrate added, evaporated to dryness and gently ignited. No difference in result was obtained by these two methods.

Calcium. A modification of the McCrudden [1912] method for small quantities of calcium, as elaborated for the estimation of calcium in blood [Widdows, 1923] was adopted. All the precautions which were taken in the estimation of calcium in blood serum were also observed here. In addition, the milk was diluted (1 in 10) before use for estimation, to render the percentage of calcium comparable with that of serum. The results were calculated to g. of calcium oxide per 100 cc.

Clinical Methods

Very early in the investigation it became quite clear that the method of extraction of the milk had a profound bearing upon the composition of the sample obtained. No uniform technique was therefore adhered to, but the method was varied in each instance according to the problem under consideration. A note of the technique employed, therefore, accompanies each group of figures, the symbol *P* being equivalent to the use of a breast pump, *H* signifying digital expression. For the main part, and unless otherwise stated. the samples were collected between 10 am. and 11 am. and in immediate relation to the 10 am. or 10.30 am. feed. Altogether 164 samples Were investigated—wholly or partly—of 13 women. That is, 5 women through 8—13 days and 8 women through 2-6 days.

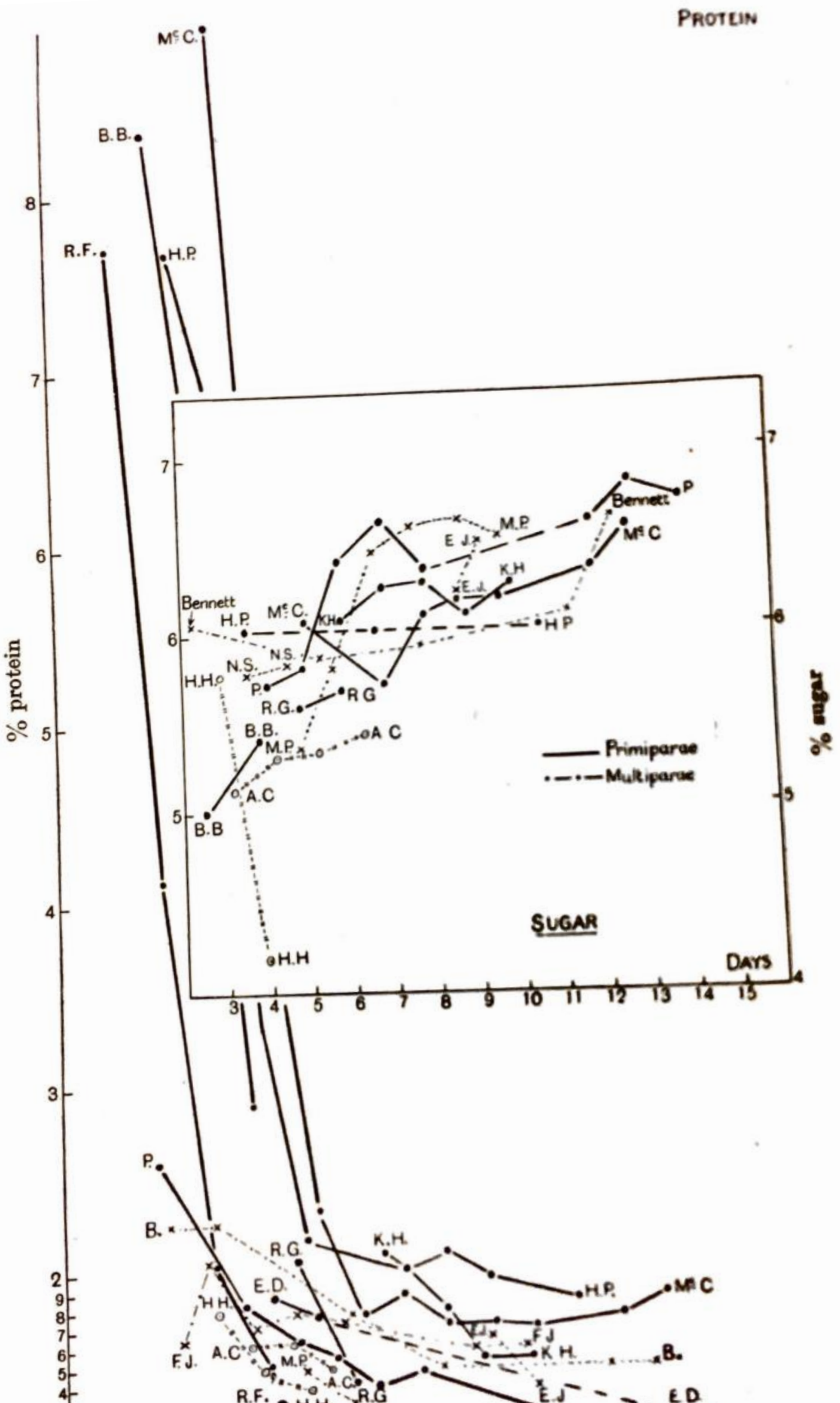

PROTEIN
% protein
1
2
3
4
5
6
7
8
9
DAYS
10
11
12
13
14
15
Mc C.
B.B.
R.F.
H.P.
P.
B.
R.G.
K.H.
E.D.
H.H.
F.J.
A.C.
M.P.
R.F.
H.H.
R.G.
K.H.
E.J.
B.
E.D.
P.
Mc C
SUGAR
% sugar
Bennett
H.P.
Mc C.
KH
N.S.
H.H.
P.
R.G.
B.B.
M.P.
A.C.
E.J.
K.H.
M.P.
Bennett
P
Mc C
H.P
A.C
R.G
H.H
Primiparae
Multiparae

Fig. 1.

RESULTS.

It has been the custom in most papers of this kind to print the values, found for all the constituents of the milks examined, in a single table. By this means, the exact composition of each sample of milk can be seen. How- ever, it has been found that the behaviour of the different constituents in the results to be demonstrated could not be clearly shown unless they were considered separately. The figures in the column headed No. are the laboratory labels of the sample of milk from which the specimen was taken and afford a means of correlation between the various tables. Each of the constituents observed will therefore be examined in order, according to its behaviour during the period under observation and its reaction to circumstances local to the taking of the individual sample of milk.

Protein. In the consideration of the protein content of the milk, 4 women were studied for 11 days, and 8 women for 4—8 days, and Fig. 1 represents the values found. The curves for *primiparae* are shown in an unbroken, and those for *multiparae* in a broken line.

It will be seen that in every case the highest protein percentage is to be found in the first 3 days after parturition, and that the level falls steeply during the first week to reach an approximate average of 1.4% by the end of the 13th day. F.J. is the one exception. It may further be observed that as far as this small number of cases is concerned the initial protein values for *primiparae* appear to be much higher than those for *multiparae*. Moreover, the curves followed by the *multiparae* show a quicker fall than those of the *primiparae* and reach a lower constant level. These observations are borne out by the protein values given by Adriance [1897] and Carter and Richmond [1898] if their tables are arranged in this way.

It is also possible that the quantity of milk available for the sample may be of significance in modifying the percentage of protein in it. The volumes obtained for McC., B. B. and H.P., by expression or when the amount taken by the baby (ascertained by test weighing) is considered in addition, do not exceed 12cc., whereas with F. J. and M.P. the minimum amount obtained was 30 cc. and rose several times to 60 cc, or 90 cc. On the other hand, a percentage of 2.9 has been found on the 5th day in a sample of 105 cc. and 1.74 in a sample of 9.7 cc. It would appear therefore that the total volume of fluid in the breast may correlate with the percentage of protein found in the milk though this consideration, as is shown later, does not seem to influence the sugar or the calcium. It is Worthy of note also that in most cases it is those individuals who show a high protein content in their early milk who have also a high value for total ash.

Sugar. If the sugar values for individual women are plotted in a graph in the same manner as those for protein, a similarly definite relationship between then:

becomes apparent. This, within certain limits, appears to be constant in all women. That is to say, that apart from any other consideration, the day ul lactation upon which a sample of milk is taken will have a marked and definite relationship to the amount of sugar to be found in the milk. The graph (Fig. 1) represents the course taken by the sugar values of 11 cases through 2-11 days. It will be noticed that the lowest values are to be found in the early days of lactation and that these rise irregularly throughout the first 14 days. The direction of the curve taken is, however, opposite to that of protein. No such very low sugars were found by us as by some other observers, our lowest percentage exclusive of case H. H., whose other constituents also showed abnormalities, falling only to 4-5 %. Unfortunately, no sample for sugar' estimation was obtained earlier than the 3rd day after parturition.

As regards comparison between the milk of *multiparae* and *primiparae*, although it would appear possible on the whole that that of *primiparae* shows a somewhat lower sugar value than that of *multiparae*, yet no constant rule is observable for this.

Ash. After as large a quantity of milk had been obtained at a single feed as the circumstances would permit, such estimations of the ash were carried out upon it as the Volume would allow. For the purpose of satisfactory ash analysis the collection of the whole 24 hours' milk is strongly advised by Holt, Courtney and Fales [1915], but, as our interest was in the development of the mineral content in individual Women through a period of days, rather than in the exact constitution of the ash itself, this procedure could not be adopted. The quantity of milk upon which the analysis was done was in most cases 2.5 CC., though in a few cases a somewhat smaller volume had to be taken. Altogether 73 samples of 9 women were investigated representing 2 *multiparae* and *primiparae*. The values obtained are represented in Fig. 2, plotted according to the average results for each day obtained for each individual for the period under observation.

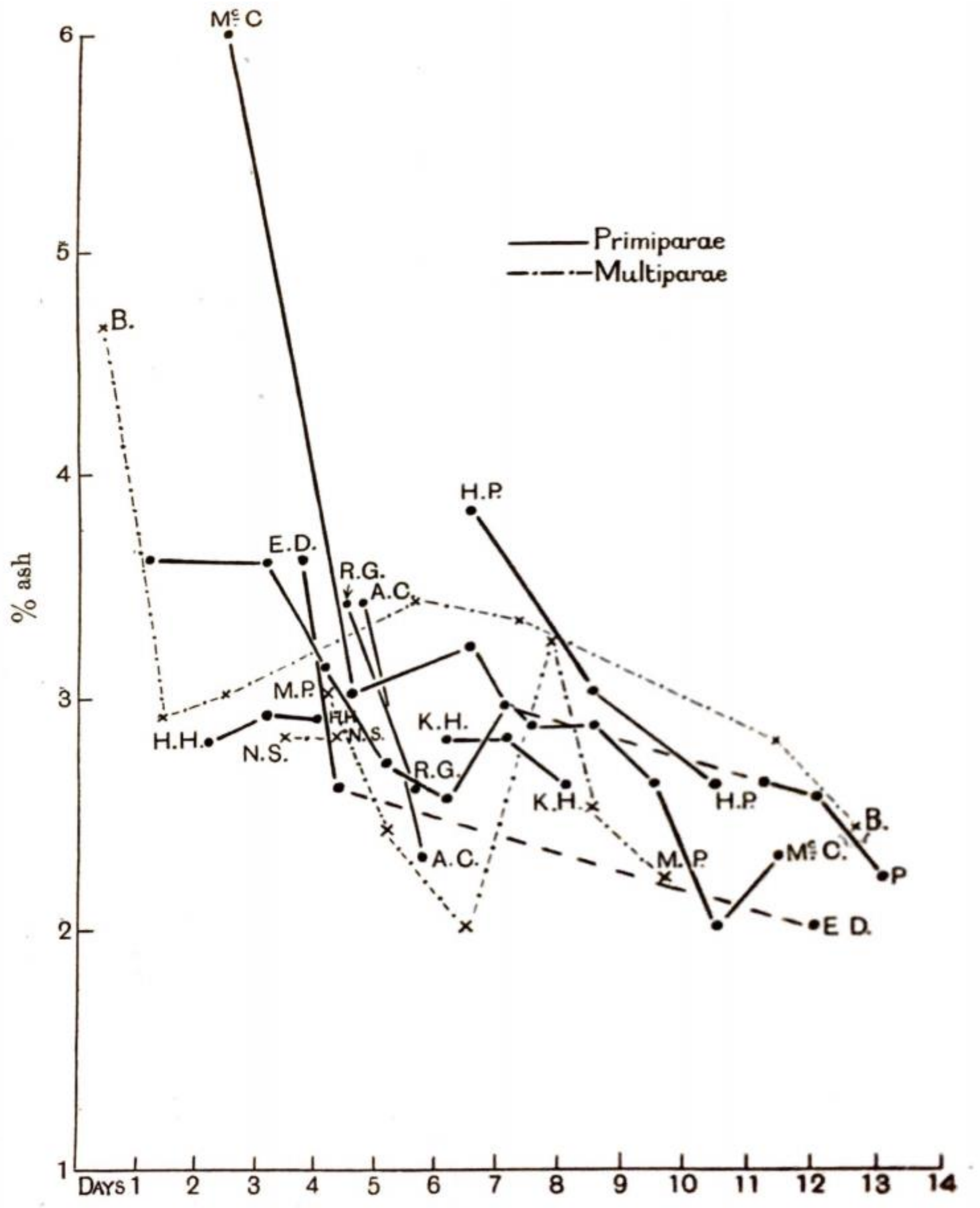


Fig. 2.

It will be seen that the course of the mineral constituents tends to Show the same type of variation as that of protein and sugar, the direction of the curve, however, is analogous with that of the protein. Unfortunately, the number of samples of the milk of *multiparae* is very small and therefore no true comparison can be instituted between the curve of these and the *primiparae*. The tendency, however, seems to be in accordance with that of protein, that is, that the higher percentages appear in the early milk of *primiparae* rather than of *multiparae*. The full is by no means so regular as that of the protein, and there are certain unexplained swings to be noticed in many curves. In the one example of continuous work on early ash found in the literature [Woodward, 1897], the same swinging values are found, although the figures are on the whole lower than our percentages. All the other analyses are unfortunately of single specimens of different women and so not susceptible of comparison.

The, next point for general consideration is the question of the possible *variation in the composition of milk during the course of a single feed*. As a means of investigating this point the, following two procedures were adopted. In the first, a sample of milk was taken (either by hand or with a breast pump) before the child was fed. the child was then put to the breast for a varying period of time and finally a sample was taken by hand or pump after it had sucked. By the alternative method the baby was allowed to take his whole feed from the one side, while an equal or larger amount was taken simultaneously or immediately after from the other and analysed in fractional portions. the “first” and “third” of these corresponding to the “before” and "after" of the other method. The composition of the milk of either ample was investigated with regard to the percentage content in it of each constituent in turn. Table I represents these values for protein (i.e N X 6.37) and sugar in percentages.

Table 1. *Variations in single feed. Protein and sugar.*

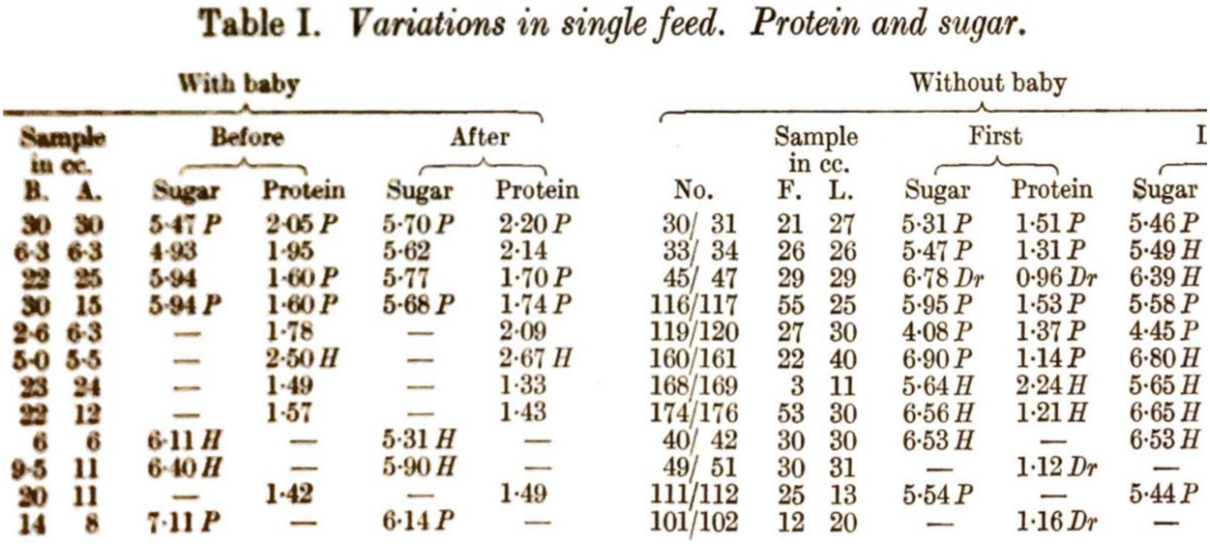


Table I. ***Variations in single feed. Protein and sugar.***

With baby						Without baby					
Sample in cc.		Before		After			Sample in cc.		First		I
B.	A.	Sugar	Protein	Sugar	Protein	No.	F.	L.	Sugar	Protein	Sugar
30	30	5·47 *P*	2·05 *P*	5·70 *P*	2·20 *P*	30/ 31	21	27	5·31 *P*	1·51 *P*	5·46 *P*
6·3	6·3	4·93	1·95	5·62	2·14	33/ 34	26	26	5·47 *P*	1·31 *P*	5·49 *H*
22	25	5·94	1·60 *P*	5·77	1·70 *P*	45/ 47	29	29	6·78 *Dr*	0·96 *Dr*	6·39 *H*
30	15	5·94 *P*	1·60 *P*	5·68 *P*	1·74 *P*	116/117	55	25	5·95 *P*	1·53 *P*	5·58 *P*
2·6	6·3	—	1·78	—	2·09	119/120	27	30	4·08 *P*	1·37 *P*	4·45 *P*
5·0	5·5	—	2·50 *H*	—	2·67 *H*	160/161	22	40	6·90 *P*	1·14 *P*	6·80 *H*
23	24	—	1·49	—	1·33	168/169	3	11	5·64 *H*	2·24 *H*	5·65 *H*
22	12	—	1·57	—	1·43	174/176	53	30	6·56 *H*	1·21 *H*	6·65 *H*
6	6	6·11 *H*	—	5·31 *H*	—	40/ 42	30	30	6·53 *H*	—	6·53 *H*
9·5	11	6·40 *H*	—	5·90 *H*	—	49/ 51	30	31	—	1·12 *Dr*	—
20	11	—	1·42	—	1·49	111/112	25	13	5·54 *P*	—	5·44 *P*
14	8	7·11 *P*	—	6·14 *P*	—	101/102	12	20	—	1·16 *Dr*	—

As regards the protein it will be seen that in the columns representing the values found before and. after a feed there is a larger range of difference than in that of the milk obtained from a complete emptying of one breast, the highest being 0-31%, which represents approximately 14% of the total value found. On the other hand, in the latter case practically all the samples are stationary since differences of 0.01% and 0.02% are, in this case, within the limit of experimental error. If any constant variation therefore can be said to occur in the protein, it is in the direction of a rise in the "after" specimen.

With regard to the *sugar* it will be noticed that in the section “With baby,” 5 show a decrease, 2 an increase, and none is stationary. In the other section, 4 show a decrease, 3 show an increase and 3 are stationary. Both the more frequent and, with one exception, the maximum variations it will be seen are in the downward direction, which is in line with the suggestion already made by Denis and Talbot in this matter [1919], that the sugar content of the milk of the first part of a feed is higher than that of the last. This is to be seen most clearly in the dripped samples (Dr). The method of extraction of the milk appears to have no influence upon the sugar values.

With regard to the *comparison of samples taken from either breast*, under as nearly as possible similar conditions, out of 12 samples examined for protein. 6 showed no difference, and the difference in the others (0.1% to 0.2%) when taken in association with the fact that the sample from the one breast was of the whole quantity, and the other from the sum of that taken before and after feeding, is probably relative to the variation already considered between the early and late milk of a single feed. With regard to the sugar content, in those instances in which both breasts, at the time at which the sample was taken, were entirely normal, no difference appeared.

As concerns the *ash* with regard to the variations in a single feed and the comparison of the milk of either side, such differences as were found to exist do not follow any rule. The number of our examples here, however, is too small to be of significance.

Fat. It has been seen that protein, sugar and ash show variations in percentage content according to the day of lactation. With fat this factor is not operative. If a list of fat values for a number of cases be written down in sequence, according to the date after parturition, no order is discoverable in their values. In the cases considered some milks showed 1% fat on the 4th day and 7% on the 7th, or 7% on the 2nd and 2% on the 11th. There were *primiparae* who showed a range of 0.6 % to 2% and *multiparae* giving 2.9% to 5.9%. But if, on the other hand, instead of the day of lactation, the variations in a single feed come to be considered, a consistent picture develops.

In representing the amounts taken by the baby, in certain instances. as for example No. 84/85, only the total amount taken from both breasts was known, and as for the purpose of this table only one breast was relevant and the exact amount taken from that side is not discoverable, the quantity, therefore, taken by the baby is represented by the total amount from both sides divided by 2.

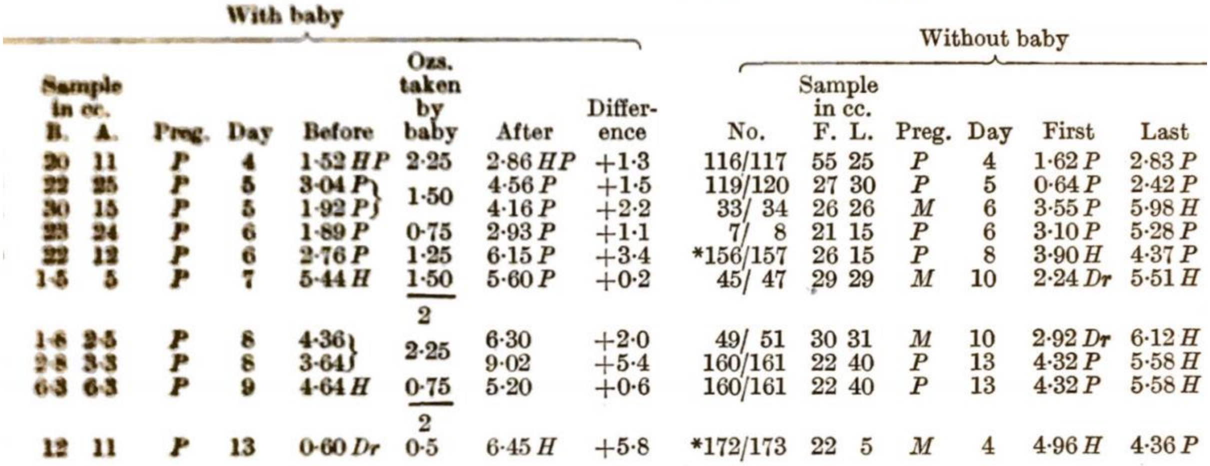
In Table II the following points will be noticed.

1. There is a rise in fat content in all samples but one, and this rise in all cases, other than those specially marked, is more than 1% and can reach as high as 5.8%. If moreover, omitting the cases starred, the average be taken of the differences in the section “With baby” it will be found to be higher than that of the first and lust samples.

2. The greatest difference in both sections appears in those cases (marked Dr and H) where the first sample was of milk that had dripped from one breast while the baby fed from the other, and the last sample was taken by hand.

3. In all cases but four (marked *) the method of procedure was either to take the first sample with a breast pump and to finish by digital expression, or to use the sumo technique for the collection of both samples. In examples 78/79, 88/89, 156/157 and 172/173 the usual procedure was reversed, the first part of the specimen being taken by hand and the last by pump. In the first three of these the result has been to reduce the difference normal for these individuals (1.1%, 5.4% and 1.3% respectively) to 0.2%, 0.6% and 0.4%, whilst in case 172/173 it has fallen to - 0-6.

Table II. *Variations in single feed. Fat* (%).



With baby								Without baby						
Sample in cc. B.	Sample in cc. A.	Preg.	Day	Before	Ozs. taken by baby	After	Difference	No.	Sample in cc. F.	Sample in cc. L.	Preg.	Day	First	Last
20	11	*P*	4	1·52 *HP*	2·25	2·86 *HP*	+1·3	116/117	55	25	*P*	4	1·62 *P*	2·83 *P*
22	25	*P*	5	3·04 *P*	1·50	4·56 *P*	+1·5	119/120	27	30	*P*	5	0·64 *P*	2·42 *P*
30	15	*P*	5	1·92 *P*		4·16 *P*	+2·2	33/ 34	26	26	*M*	6	3·55 *P*	5·98 *H*
23	24	*P*	6	1·89 *P*	0·75	2·93 *P*	+1·1	7/ 8	21	15	*P*	6	3·10 *P*	5·28 *P*
22	12	*P*	6	2·76 *P*	1·25	6·15 *P*	+3·4	*156/157	26	15	*P*	8	3·90 *H*	4·37 *P*
1·5	5	*P*	7	5·44 *H*	$\frac{1·50}{2}$	5·60 *P*	+0·2	45/ 47	29	29	*M*	10	2·24 *Dr*	5·51 *H*
1·6	2·5	*P*	8	4·36	2·25	6·30	+2·0	49/ 51	30	31	*M*	10	2·92 *Dr*	6·12 *H*
2·8	3·3	*P*	8	3·64		9·02	+5·4	160/161	22	40	*P*	13	4·32 *P*	5·58 *H*
6·3	6·3	*P*	9	4·64 *H*	$\frac{0·75}{2}$	5·20	+0·6	160/161	22	40	*P*	13	4·32 *P*	5·58 *H*
12	11	*P*	13	0·60 *Dr*	0·5	6·45 *H*	+5·8	*172/173	22	5	*M*	4	4·96 *H*	4·36 *P*

All these milks were taken after the 3rd day of lactation. When the fat a for these 3 days was examined it was found that in many cases its behaviour was reversed, the fat content being higher in the first sample than in the last. This is shown in Table I II. But if the percentages for these milks are carefully scrutinised it appears that this reversal of values occurs only in the case of *primiparae*.

Now since it is known that the fat in milk is present in the form of an emulsion and is therefore liable to respond, as to its degree of emulsification, to changes in physical conditions, it would appear reasonable that these conditions should play a large part in the determination of the resultant fat percentage in the milk. A consideration of the above figures suggests that the percentage of fat in milk depends upon two factors, the one inversely and the other directly. The first of these is the quantity of liquid available as a suspensory fluid. In a very full breast just before a feed, when stimulation of the one Hide produces dripping from the other, the fluid will be at a maximum, and therefore. according to this point of View, the fat should be at a minimum. This is exactly what in fact occurs—see cases 102/103, 45/47 and 49/51 (Table II). On the other hand. at the end of a feed when the breast is near exhaustion. the fluid will be at a minimum, and one would expect, therefore, a rise in fat content. and. as will be seen by the above tables; this is in every instance the ease. The second factor in operation is the question of direct force applied to the breast itself. This is absent in the case of a milk that drips. at its least in the gentle use of a breast pump, and at its maximum in Vigorous digital expression or the action of the jaws of a strongly sucking baby. The influence of this factor is direct upon the proportion of fat in the resulting milk. that is to say, in an opposite direction to the action of the fluid factor. The percentage of fat resulting in the milk depends, therefore, upon a balance of these two forces, the fat rising at the end of a feed if all other conditions are equal. But if greater pressure be applied at the beginning than at the end this rise can be very much modified (78/79, 88/89, 156/157) or even altogether obliterated (172/173).

As a further experiment in confirmation of these contentions, in one case a first sample was taken by vigorous finger expression and set aside. A second sample was then taken by very gentle use of the pump, and finally the remaining milk was withdrawn by hand. The values of the fat found were, first sample 3.88% (*H*), second 4.08% (*P*), last 5.34% (*H*). That is to say. that although the sample with 5.34% of fat followed immediately upon the sample with 4.08 % and was not very much in excess in quantity, yet the fat difference was 1.26%. To illustrate both factors at one time, in two other samples a “before” and "'after” specimen was taken by pump and hand on the 4th day from the left breast 6 hours after the last feed on that side, and the estimated fat content was 1.62% and 2.86 % respectively, and the total amount secreted 3 ¼ ozs. (2 ¼ ozs. sucked and 1 oz. expressed). The whole available quantity of the right breast, where the baby had fed 3 hours before, expressed by a pump, reached a total of 113 ozs. with a fat content of 2.9%. That is to say, that on the one side the factor of digital expression plus that of the end of the feed operating in a large quantity of fluid resulted in a fat percentage just equal to that appearing in a very much smaller quantity obtained by the gentler method of expression from the other side. As the procedure hitherto adopted for the study of the fat content of milk by most observers has been either to mix the fore milk and strippings [Denis and Talbot, 1919], or to take the middle milk [Adriance, 1897; Woodward, 1897], it was thought to be of interest to investigate in a series of cases the fat content of these relative to each other. Table IV represents the figures obtained. the second column being the fat percentage in a sample composed of equal parts of first and last milk and the third that of the middle milk of the same sample.

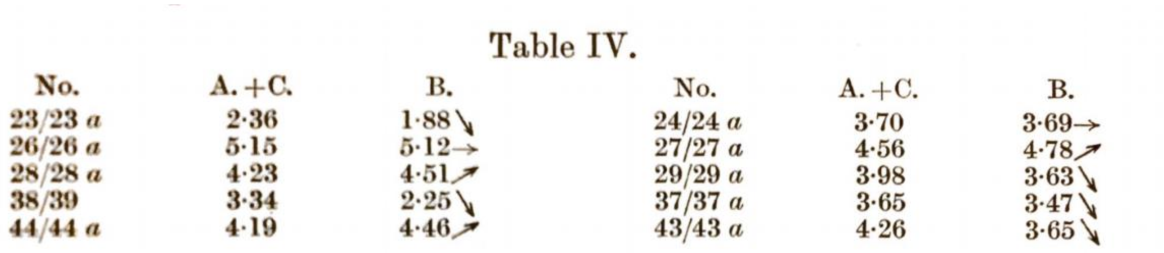


Table IV.

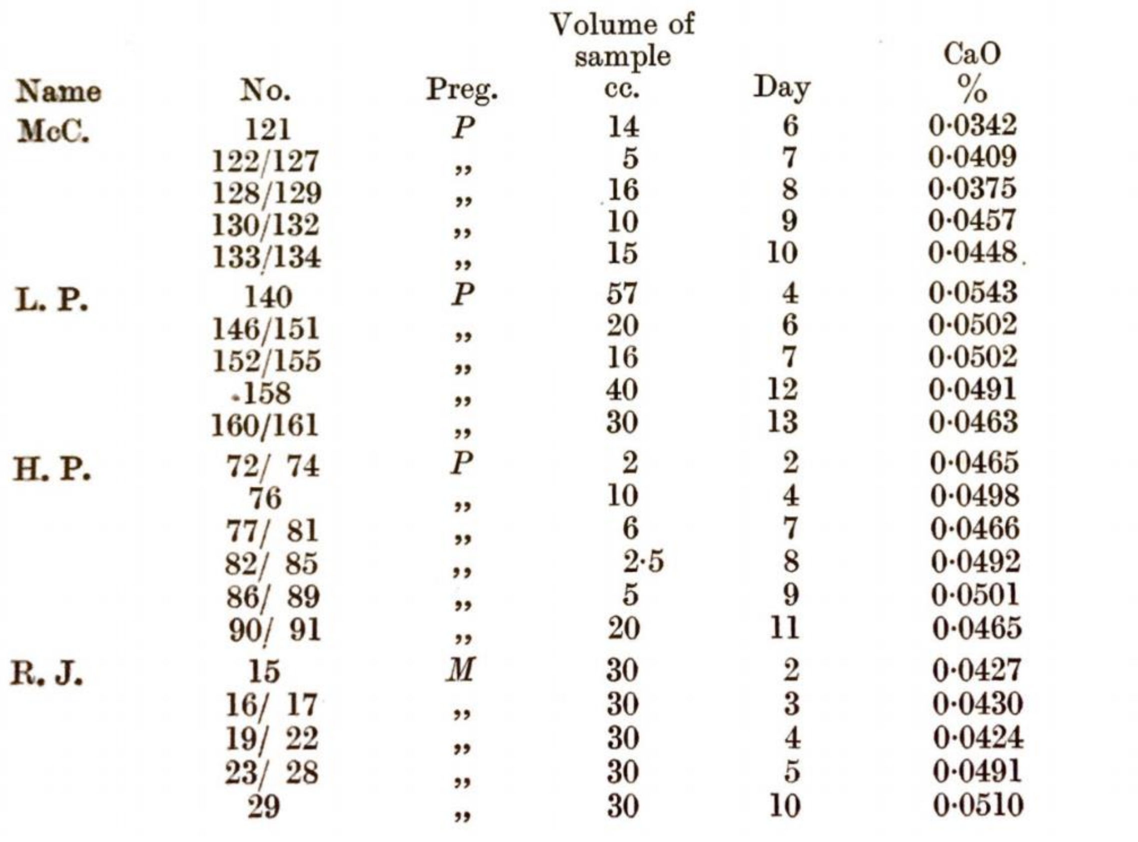
No.	A. + C.	B.	No.	A. + C.	B.
23/23 *a*	2·36	1·88 ↘	24/24 *a*	3·70	3·69 →
26/26 *a*	5·15	5·12 →	27/27 *a*	4·56	4·78 ↗
28/28 *a*	4·23	4·51 ↗	29/29 *a*	3·98	3·63 ↘
38/39	3·34	2·25 ↘	37/37 *a*	3·65	3·47 ↘
44/44 *a*	4·19	4·46 ↗	43/43 *a*	4·26	3·65 ↘

The arrows at the side of the columns indicate by their direction the relative values of the fats of these two columns. It will be seen that the relationship between them is fortuitous.

It is postulated. therefore, that, whereas the other constituents of the milk so far considered vary in percentage according to the general influences of the development of lactation, the percentage of fat depends alone upon factors local to the breast and the manner of extraction of the sample.

Calcium. The calcium content of the milk of 18 women was estimated over a period of 2—15 days and according to the same schema as with the other constituents. The following table shows the results obtained in 5 typical cases ranged according to days after parturition.

Table V. Values for calcium in the milk of 4 women.



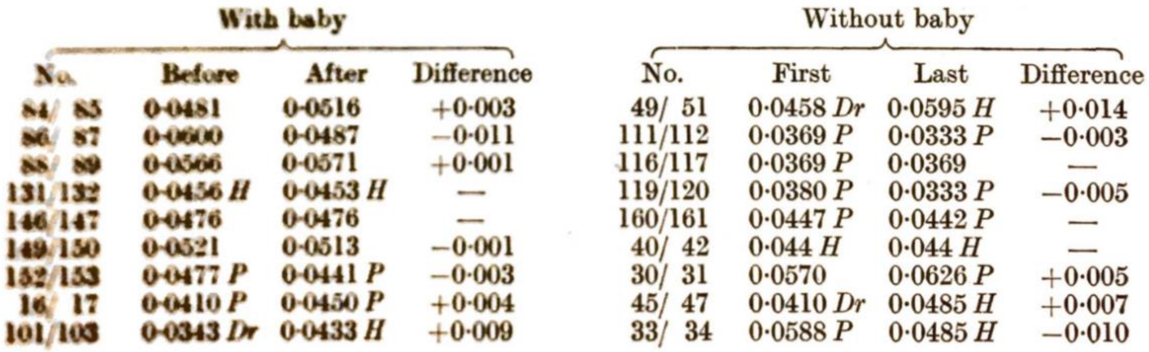
Name	No.	Preg.	Volume of sample cc.	Day	CaO %
McC.	121	*P*	14	6	0·0342
	122/127	,,	5	7	0·0409
	128/129	,,	16	8	0·0375
	130/132	,,	10	9	0·0457
	133/134	,,	15	10	0·0448
L. P.	140	*P*	57	4	0·0543
	146/151	,,	20	6	0·0502
	152/155	,,	16	7	0·0502
	158	,,	40	12	0·0491
	160/161	,,	30	13	0·0463
H. P.	72/ 74	*P*	2	2	0·0465
	76	,,	10	4	0·0498
	77/ 81	,,	6	7	0·0466
	82/ 85	,,	2·5	8	0·0492
	86/ 89	,,	5	9	0·0501
	90/ 91	,,	20	11	0·0465
R. J.	15	*M*	30	2	0·0427
	16/ 17	,,	30	3	0·0430
	19/ 22	,,	30	4	0·0424
	23/ 28	,,	30	5	0·0491
	29	,,	30	10	0·0510

NOTE. The figures for calcium given above represent the average figure for all samples estimated on the given day.

It will be seen that no variation is perceptible according to the day of lactation as far as the period 2-15 days is concerned. This naturally does not in any way prejudice the possibility of a later rise and fall with the later development of lactation, as has been suggested by Schabad [1911] and Hunaeus [1909]. It will be seen also that there is a definite regularity in content of the milk of the individual women themselves. This has been the case with all but one of the women investigated, the maximum range of variation among all the samples taken of any one woman being from 0.009% to 0.0017%. In one case, however. a greater variation occurred, the lowest figure reaching 0.0399% and the highest 0.628%, this over a range of 5 days and 20 samples. This can be accounted for either by the hypothesis of Schabad (1911), that such a degree of variation clues occurs in a certain proportion of women, or by the possible inaccuracies introduced into the work by the case of a breast pump. It would therefore appear that the difference in calcium values shown between the milks of individual women during the period under observation is far greater mater than that occurring among the samples of milk of any single individual. This fact was also found by Schabad for the milk of the later periods of lactation.

As regards the question of the influence upon the calcium content of the place of the ample in a feed, it has been suggested by Schabad [1911] and Hunacus [1909] that the value of the calcium falls at the end of a feed. In order to study this point, 22 samples were investigated of the first and last milk of a feed and of that taken before and after a feed. Three specimens also analysed *fractionally* from breasts that had dripped. The results are given. below:

Table VI. Variations in single feed. CaO (%).



With baby				Without baby			
No.	Before	After	Difference	No.	First	Last	Difference
84/ 85	0·0481	0·0516	+0·003	49/ 51	0·0458 *Dr*	0·0595 *H*	+0·014
86/ 87	0·0600	0·0487	−0·011	111/112	0·0369 *P*	0·0333 *P*	−0·003
88/ 89	0·0566	0·0571	+0·001	116/117	0·0369 *P*	0·0369	—
131/132	0·0456 *H*	0·0453 *H*	—	119/120	0·0380 *P*	0·0333 *P*	−0·005
146/147	0·0476	0·0476	—	160/161	0·0447 *P*	0·0442 *P*	—
149/150	0·0521	0·0513	−0·001	40/ 42	0·044 *H*	0·044 *H*	—
152/153	0·0477 *P*	0·0441 *P*	−0·003	30/ 31	0·0570	0·0626 *P*	+0·005
16/ 17	0·0410 *P*	0·0450 *P*	+0·004	45/ 47	0·0410 *Dr*	0·0485 *H*	+0·007
101/103	0·0343 *Dr*	0·0433 *H*	+0·009	33/ 34	0·0588 *P*	0·0485 *H*	−0·010

If the range of experimental error be taken to be approximately 0.003% it will be seen that 11 samples show no variation while 5 show a rise and 2 a fall. If, on the other hand, the figures be taken as they stand, 5 are stationary, 7 show a rise and 6 a fall. It would appear therefore, that such differences as occur, are either fortuitous in direction or negligible in amount. We would suggest that, since Schabad gives only 3 samples, and Hunaeus 6, a selection of such as, for instance. 119/120, 86/87 and 33/34 would account for their findings.

When the calcium content of the milk of either breast was compared, the variations found were as follows: in 8 out of 17 cases there was a variation of 0.003% or under, in 6 out of 17 cases a variation of 0.004 % to 0.008%, in 3 out of 17 cases variations respectively of 0.014 %, 0.013% and 0.023%; for this latter instance no reason is apparent.

In comparing the values for calcium with those for ash, it is worthy of note that the high ash values found at the beginning of lactation are not represented in the calcium. The excess of mineral in the cloistral and early milk must therefore be due to some component other than calcium, possibly magnesium, which, according to Holt, Courtney and Fales [1915], is variable throughout lactation and highest in the colostrum period (considered as 1-12‘ days) as also are potassium and sodium.

Solids. The total solids were estimated in 67 samples from 16 women, and show a variation of from 7.6 % to 18.9%. But if one case showing 7.6% and a single example of 18.9% be omitted, the range of the remaining samples is 9.76% to 14.98%. The height of the figures for solids is found consistently throughout all samples to vary in general relationship to the fat and to give an average mean value of 12.68%.

It is to be noted that the estimated total solids have not, in a considerable number of cases, reached the same total as an arithmetic addition of the results for sugar, fat, protein and ash would give for the same milks, the difference between the two varying from plus 0.046% to plus 2%, with a mean of 1.18%. This is in agreement with the work of Camerer and Söldner [1896] and earlier observers, who found in the case of colostrum and early milk a similar discrepancy. In working with later milk Söldner did not find this discrepancy to occur, and suggested, therefore, that its appearance in the early milk could not be due entirely to experimental error, as had hitherto been suggested, but must represent actual unanalysed substances in the milk. In the analyses recorded in this paper only the precipitable protein nitrogen was estimated. The soluble bodies containing nitrogen therefore, which are known to exist in milk, would necessarily form part of the discrepancy found. Furthermore, the extractives containing no nitrogen which are known to be present in milk, and citric acid, have not been considered.

To sum up, it is suggested that the usual view of milk, as a secretion homogeneous in its response to stimuli, cannot be maintained, and indeed lies at the root of a number of the contradictions and obscurities found in the results of milk analyses. On the contrary, in agreement with the view first put forward by Forster [1881], it is contended that milk is composed of elements opposite in behaviour and very varied m' then response to identical stimuli.

CONCLUSIONS.

Our conclusions with regard to the chemical composition of milk of the first 14 days of lactation and the factors influencing its variation may be summarised as follows.

1. There appears to be a difference between the milk of *primiparae* and *multiparae* With regard to the behaviour, in the early days of lactation, of the protein and fat.

2. The protein and ash content of the milk vary regularly according to the day of lactation, being very high in *primiparae*, less so in *multiparae* on the 2nd and 3rd days, and falling steeply during the first week to reach an approximately normal level of from 0.98% to 1.3% for the protein, and 0.20% to 0.25% for the ash.

3. The sugar content varies also according to the day of lactation, but inversely as the protein, and less regularly, being at its lowest in the early days and rising irregularly to a normal level of approximately 6.5% by the end of the second week.

4. The fat content of the milk varies only according to the physical conditions of the breast and the actual extraction, and not in relation to the day after parturition, but in *primiparae* the general behaviour is reversed in the first days of lactation.

5. The calcium content of the milk' varies up to the 15th day between individual women, but not according to any factors concerned with individual lactation and is uninfluenced by factors local to the breast.

6. There is some possibility that the protein content of the milk rises slightly at the end of a single feed, the difference being greater when the samples are taken in relation to an actual feed than when only the first and last portions of an artificially emptied gland are considered.
7. The lactose content of the milk appears to be higher at the beginning than at the end of a feed.

8. The total solids vary roughly as the fat and show a range from 9.6% to 14.98%.

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