Researches in Lactation.

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Although a considerable amount of work has been done and recorded on the subject of milk, lactation in women has received scant attention, and we are in possession of very little accurate knowledge. It was therefore decided to undertake a study of lactation in normal women in the Obstetric Unit of the Royal Free Hospital under the direction of Professor Louise McIlroy.

Investigations were directed to discover the manner in which the milk appeared, and lactation developed during the first l4 days of the puerperium. All the clinical observations were made by as also the collection of samples in the wards. The biochemical work, which was under the charge of S.T.W., was carried out in the Science Laboratories of the London School of Medicine for Women with the co-operation of Miss M. Bond and Mrs. E.I. Taylor. We are also indebted to the Medical Research Council for support and financial assistance.

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The samples of milk were collected by different methods according to the point under investigation, and were taken, immediately after collection, to the laboratories where the appropriate analyses were carried out. In no case was the milk allowed to stand overnight. Sugar, fat, protein, calcium and ash were the constituents chosen for

study. Sixty-three samples were analysed for all these substances, and one hundred and forty for four or less. Total solids were estimated in sixty-five samples.

The mothers selected for observation in this section were all normal in-patients of the Obstetrical Department of the Royal Free Hospital. In a few instances' women admitted to the V.D. wards for gonorrhoea were included, but none with a history of syphilis. A continuous series of observations was made on every woman from the morning after parturition until her discharge from the ward.

At the outset it was found that observations needed to be made according to a method which standardized itself. On the usual clinical chart's observations on the infant's weight, condition of the mother's breasts, quantity and nature of the fluid secreted and the amounts taken by the infant cannot be recorded. These matters were essential to the investigation, and a single chart was therefore constructed to enable such points to be conveniently noted.

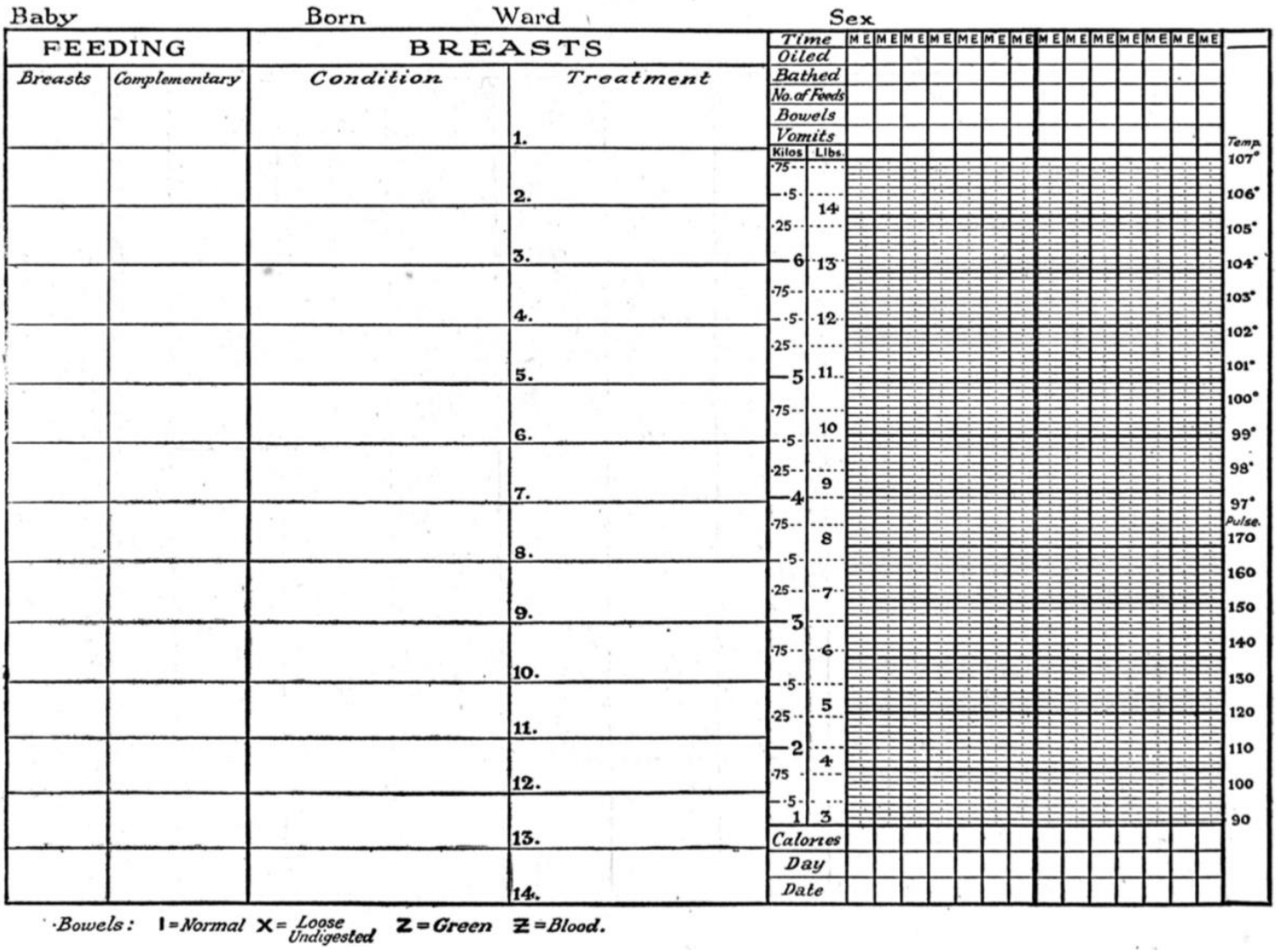
On this chart on the right hand side, the infant's chart, the infant's weight, the nature of its motions, whether it had vomited or not and, if so, the nature of the ejected material and the number of feeds, were entered daily. The number of ounces of milk taken in the day, as ascertained by a test weighing chart, was written on the chart above the daily weight together with the caloric value of the food taken, if desired, calculated from the weighing chart and the biochemical results. Morning and evening temperature and pulse-rate, should circumstances make the record of these desirable, can also be noted in this part of the chart. Owing to the universal use in this country of the ounce measure, records were necessarily made by these weights, but by the provision of an additional table in the metric system, the results can be compared with those of continental workers. The central part of the chart is used for recording the nature and condition of the breasts and milk supply, and also for the prescribing of treatment. The left hand column indicates the feeding methods in use for each particular case. In combination with this chart, the accompanying scheme for test weighing was devised.

Six days charted in this manner can be conveniently grouped together on the same sheet.

With a little practice the nursing staff found this method easy to use. During the observation of each case, the chart remained in the neighbourhood of the weighing scales and was inspected daily by the observer responsible for the ward work and checked and compared with the condition of the infant and mother.

By use of these charts and the biochemical-1 results given by the analyses of samples obtained, the following series of observations was made:—

Variations in Inception of Lactation in Different Women. The course of lactation in 23 individuals was studied, six for 10 to 13 days, five for five to nine days and 12 for one to five days. This forms, except for the work of Woodward[2], 1897, (a study of the milk of six cases from the 1st to the 7th day) and Hammett,3 1917, (a series of five daily samples of milk from eight women from 9.5 cc. From the left side one cc. was expressed before the infant was put to the breast, two drachms taken by the infant, and two cc. by expression after feeding, totalling in all 10.2 cc. The fluid up to this date was very viscid and dense, and of a deep yellow colour at the bottom of the measuring glass. As the fluid left the nipple and streamed down the side of the glass, it was not homogeneous, but was made up of streaks having different colours. This point will be referred to later in discussing the question of colostrum. On the third day the fluid was still very viscid but had become a pale yellow colour and was much more homogeneous in character. A total of three cc. was obtained on this day by expression from one breast, the infant having taken two drachms or 7.2 cc. from the other. On the fourth and fifth days the amount obtained by expression increased to 10 cc. and 25 cc. respectively, and by the eighth day to 73 cc. By this time the fluid had the appearance of rich cow's milk. It was not till the ninth day that the milk reached the usual thin, bluish appearance of normal human milk.



The biochemical content of the milk of the two women showed also a wide difference, parallel in nature to the physical appearances. The protein content of the milk of F. never rose above 2.2 per cent. and from the second day to the tenth day showed a curve from 2.2 per cent. to 1.63 per cent. In contrast with this, the protein percentage of H. P. began at 7.65 per cent. on the second day (the first on which an adequate sample could be obtained) and fell on the eighth day to 2.0 per cent., reaching on the eleventh 1.73 per cent. only. The ash content of the milk, as has been recorded already in dealing with the biochemical side of the work, displayed a tendency to be higher in the first type than in the second. The sugar percentage in both types was about the same. As will be shown later, the percentage of fat in the milk was found to depend upon a different set of factors altogether, these factors

remaining constant for either type.

The differentiation between these two types, as might be expected, is not absolute, and cases occurred which could not be placed definitely in either group.

In all cases the appearance of the milk and the biochemical findings corresponded in the manner described above, but it was not invariable that the subjects were respectively multiparae or primiparae. In one case, that of C.B., a multipara, aged 28, in hospital for her second confinement, the milk on the first day after delivery showed the physical appearances described for primiparae, with a protein content of 7.06 per cent. and an ash of .4 per cent. Both percentages, however, fell more rapidly than is usual with primiparae, giving on the sixth day a protein content of 1.58 per cent. with a yield of 22 cc. of milk. Correspondingly, it does occasionally happen that a primipara if young and full-breasted and possessed of a vigorously sucking infant, will show a development approximating to the multiparous type. The caution in this case must be admitted that with the unmarried mother, information regarding these points cannot be altogether relied upon.

From this evidence it is suggested that if milk samples be collected from different women on the same date of the puerperium, they will have no necessary relation in composition to one another. This fact accounts for the remarkable variations found in the bio- chemical milk estimations of many observers. The method of averaging single samples from different women taken at the same date after parturition, is that which has been almost universally employed. But as the composition of these samples will vary widely according to the type of woman chosen this method cannot be asked to give relevant results.

In the second place, the actual amount ingested by the infant in the early days of lactation becomes of less importance. Because of the difference in composition of the fluids secreted, the higher food-values of the one type of milk compensates for the larger available quantity of the other, and a satisfactory gain by the infant can be made on either.

Thirdly, owing to the high food-value of such milk as appears in cases of slow development, delay in appearance of mature milk does not necessarily imply failure in ability to feed.

A further point of interest between these two groups is to be found in the appearance of the early milk of primiparae. If, during the early days of lactation, the nipple of a primipara be carefully inspected while the areola be compressed, different types of fluid will be observed on the surface of the nipple emerging from the different ducts. It has been possible to distinguish four of these kinds. A clear, glairy fluid of very high viscosity, a non-viscid fluid, brown in colour, a thick cheesy—like substance and a thin, limpid, clear fluid resembling a watery solution. All these will be seen flowing from different ducts in the nipple at the same time and joining in a streaky manner on the edge of the collecting-glass, down which they stream to mix at the bottom. In the multiparous type this does not occur, and the milk presents a homogeneous appearance from" the beginning. It is possible that it is due to a lack of distinction between these two types of secretion that the divergences in the descriptions of colostrum have arisen.

Colostrum.

Any study of the early days of lactation must inevitably con- sider the question of colostrum, and review the evidence gained as to possible enlargement of our understanding of the nature of colostrum, of what the usual duration of this stage of milk production can be expected to be, and upon what factors its date of termination depends.

Different writers have estimated the colostrum stage of lactation as lasting for periods varying from three to fourteen days. Adriance[4] takes it as of two weeks' duration, and his view is supported by Schlossmann.[5] This difference of opinion arises partly from the ambiguity of the term and the difficulty of finding a serviceable criterion for distinguishing colostrum from milk, also, it is suggested, partly from confusion between the two types of lactation development. The crucial difficulty in any estimation of the length of time occupied by cloistral secretion is that of the criterion to be chosen.

By some observers the presence or absence of colostrum corpuscles in the milk is taken as the guide. In the cases examined this was found to vary greatly within wide time limits, and to bear no definite relationship to the other characters of the milk. If instead the biochemical constitution be taken as criterion, and all fluids not giving the approximate seven per cent. of sugar, 3.5 per cent. of fat and 1.3 per cent. of protein of normal human milk, be considered to be colostrum, the duration will vary according to the type of lactation development. That is to say the cloistral period may last from two to eleven days, as shown in the curves in a previous communication. If the physical appearances and the quantity of fluid secreted, be chosen instead, the same variation between the two types will appear. There is, it would appear, no consistent criterion as yet devised that can be used for all cases to distinguish colostrum from, milk. It is therefore difficult to make any dogmatic statement regarding its duration. Since however, by definition colostrum is -the preliminary secretion of the gland before the mature milk appears, it would seem reasonable to expect that the relative rapidity and thoroughness of removal of this early secretion would be correlated with the time of appearance of mature milk. In practice this is found to be the‘ case. In multiparae, that is to say in women whose breasts have already been active, the colostrum stage is usually short and may in rare cases only last for a number of hours. This occurs particularly in those women whose breasts have been secreting during the later months of pregnancy. For its final date of determination it will depend upon the vigour of sucking exhibited by the infant, With a vigorous infant and a mother of this type, the colostrum period as a rule will last one or two days, as in the case of F. J. If, on the other hand, the mother is a primipara from whose breasts no secretion whatever has appeared during pregnancy and if, moreover, the infant be either slightly premature or a very feeble sucker, the colostrum period (considered according to the physical appearance and bio- chemical content of the milk) may extend to thirteen days. This was clearly shown in the case of McG. (*Biochemical journal, 1927, xxi, 1*.)

3. Relation of the Time in the Feeding at which the Sample is taken to the Composition of the Milk resulting. At which the possibility of an association between the time in the feed the sample is taken and the manner of extraction of the milk with the composition of the resulting sample is suggested by the experience both of dairy farmers and clinicians. It has long been known that the fat content of the milk rises at

the end of suckling both in the case of cows and of women. It has also been suggested by Denis and Talbot[6] (1919) Hunnaeus[7] (1909) and Schabad[8] (1911) that the sugar and the calcium content respectively of the milk vary according to the time in the feed when the milk for examination is taken. To elucidate this point the comparative proportions of the different constituents of milk at the beginning, at the middle and at the end of a feed were considered. Samples were taken before and after the infant had fed from the same breast, in some cases and in other cases for comparison, the whole available supply at one feed was taken from one breast, while the infant was fed from the other. This milk was collected into three glasses seriatim, and a full chemical analysis was carried out on each part. When the results obtained were grouped together and studied, the following points emerged :—

The lactose content, in agreement with the results of Denis and Talbot, appeared to be higher at the beginning than at the end of a feed.

The *protein* content of the milk, on the other hand, appeared to rise slightly at the end of a feed. This difference was greater when the samples were taken in relation to an actual feed, than when only the first and last portions of an artificially emptied breast were considered. The differences in percentages found in either constituent are probably not of clinical significance. In the case of the sugar content the variation was the difference between .2 per cent. and .8 per cent- and in that of the protein between .05 per cent. and .6 per cent.

The calcium and the ash percentage did not differ between the beginning and end of the feed.

The fat content, however, was found to be profoundly affected by this and by the next factor to be considered and the key to its variations lies in a combination of the two factors.

4. The relation of the manner of extraction of the sample upon the resulting composition of the milk obtained. It is obviously impossible to obtain for analysis at any time an exact sample of the milk that would have been actually ingested by an infant. No procedure has yet been devised which accurately reproduces the sucking

action of the infant. But by varying the method of extraction, a series of samples was obtained which can be considered as approximating to that probably obtained by the infant.

As has already been described, milk was taken both with a breast pump and by digital expression. A difference was made and noted between a vigorous and gentle use of either method. The biochemical results obtained were grouped in such a way that the effect of all four degrees, i.e., very gentle use of pump (e. g., suction only) firm use of pump, gentle expression (e. g. pressure only) and vigorous expression could be compared, and associated with the effect of the beginning or end of the feed. All constituents considered were examined in this way, but the most definite results were found in regard to the fat. Although it has long been agreed in clinical practice that the fat content of the milk is higher at the end of a feed than at the beginning, the figures of investigators have not always shown this rise. Indeed, it has been the common impression of most milk investigators that no rule could be laid down which would entirely govern the percentage of fat in milk. It has, in addition, been assumed clinically that the milk obtained in similar circumstances by different infants from the same mother would be identical in composition.

In order to investigate this point a series of samples of milk was taken, first before the feed by use of a pump and by digital expression after the infant had fed. The procedure was then reversed, digital expression being used for the sample before the feed, and the breast pump for that obtained after. To compare with these, a second series of samples was then obtained from the first and last parts of the whole available content of a single breast of the same individual, in the same manner. In all cases the quantity of the fluid obtained was noted. These two groups of figures were tabulated in series and studied. The resulting Tables, an example of which is given below, showed that the high percentages of fat in the milk occurred in the relatively small amounts of fluid, while the low percentages of fat in the milk were found in the large amounts of fluid. Further, the higher percentages occurred in samples obtained with maximum pressure (e. g., vigorous digital expression) and the lower percentages in samples extracted with minimal pressure (e. g. gentle use of a pump.). On consideration, this would appear a reasonable result since fat in milk is present in the form of an emulsion. All fats tend to emulsify more successfully under greater

pressure, and percentage emulsification of any fat is increased as the proportion of suspensory fluid decreases. To illustrate this point, the fat percentages of H. P. are given herewith.

Table 1. Percentage of Fat in milk in relation to feed.

TABLE I.

CASE H.P. Percentage of Fat in milk in relation to feed.

	Before Feed.	After Feed.	Difference.
PROCEDURE I.	Extraction by Pump Minimal Pressure	Digital Expression Maximal Pressure	——
	4.36 3.64 3.78	6.30 9.02 7.16	+1.94 +5.38 +3.38
PROCEDURE II.	Digital Expression Maximal Pressure	Extraction by Pump Minimal Pressure	——
	5.44 4.64	5.60 5.20	+0.16 +0.56

In another case it was possible to obliterate the usual difference and to obtain a minus figure.

It would appear therefore that the percentage of fat in milk depends upon two factors, the one directly and the other inversely. It will depend inversely upon the amount of fluid available in the breast at the time the sample is taken, and directly upon the degree of pressure put upon the breast in the process of extraction. The final percentage will depend upon the balance of these two factors. These results are paralleled by the experience of dairy farmers, who find that an excess supply of fluid

to the cows diminishes the fat content of the milk, and that variation in efficiency of milking results in different percentages of fat from the same cow under otherwise identical conditions.

Clinically, these facts suggest certain conclusions. The importance of the fat element in milk in relation to the nutritional disturbances of childhood, has long been appreciated. Attempts are very generally made to arrive at some estimate of the fat content of the milk of unsatisfactory cases. If the above deductions be true, this is not a factor which has any existence apart from .the individual infant obtaining the milk. That is to say, unless the action of the infant to be watched and note taken of the vigour of its sucking, it is unlikely that a sample obtained artificially for examination as to the fat content, will resemble at all in this)articular, the milk ingested by it. In other words, it is the degree of pressure of the jaws of the infant upon the areola and nipple, as well as the length of the feed, which determines the amount of fat obtained in the milk.

Points in management suggested by these results :—

The advisability, if fat dyspepsia is suspected, of putting-the infant, as a routine, to both breasts rather than one. In this way the fore milk of both breasts, which is poor in fat, is obtained, and only little of the rich after milk.

The advisability, if the fat still remains too high, of giving water immediately before the feed, as a dilution of the fat content.

The possibility with an infant of feeble jaw action of starting the flow of milk with the fingers or pump before putting the child to the breast. By this means the available energy in the infant can be conserved to obtain the highest fat content possible. In some cases it may even be wise to draw off and discard the fore milk from either breast so as to give the infant the chance of obtaining the later and richer part only of the milk from both breasts.

The importance of diagnosis in all cases of digestive disturbance in breast-fed infants and of observing the type of action of the infant in sucking.

Table 2

Case.	Sample in cc. Before Feed	Sample in cc. After Feed	Parity.	Day.	Grams of Fat Per 100 cc. Before Feed	Grams of Fat Per 100 cc. After Feed	Diff.	Amount taken by Infant
H.P.	1.0	2.0	P.	2	7.14	5.02R	− 2.12	7.5 cc.
	0.7	1.6	P.	2	6.92	5.74L	− 1 18	7.5 cc.
	1.5	5.0	P.	2	9.98	8.66L*	− 1.32	45.0 cc.
	1.3	8.5	P.	7	5.44	5.6R*	+ 0.16	45.0 cc.
	1.8	2.5	P.	8	4.36	6.3L	+ 1.94	67.5 cc.
	2.8	3.3	P.	8	3.64	9.02R	+ 5.38	67.5 cc.
R.P.	1.7	1.5	P.	1	0.95	0.53R	− 0.42	Nil.
	1.7	2.3	P.	2	1.66	1.26L	− 0.39	0.25 cc.
	3.3	5.5	P.	3	2.73	3.35L	+ 0.62	1.25 ce.
	20.0	11.0	P.	4	1.52	2.86L	+ 1.34	2.5 cc.

*These samples were taken at the same feed from right and left breasts respectively. They Show the appearance of homogeneous milk later in the one breast than in the other. It will be noted that by the next day the usual rise occurs.

As a corollary to these general observations, a curious fact was observed during the first days after parturition. Here, until the fluid became homogeneous, the fat content at the beginning of a feed was higher than that at the end. This is of physiological rather than of clinical interest and would appear to be caused by the initial breakdown of the cells lining the ducts and potential ducts of the glands. Table 2 illustrates this point.

The Influence upon. the Composition of the Milk of Morbid States of the Breast. The range of experiences of this nature embraced by the work was small, since it was in the main an endeavour to study the development of normal lactation. But in one or two instances in the cases under observation the breasts at one time or another showed mild morbid states. In connexion with this point the composition of the milk taken simultaneously from either breast was considered. 80 long as the condition of the breasts was normal the variations in composition between the milk from either breast were. slight. When, however, any morbid condition appeared in either breast.

a disturbance was observed in the sugar values. Table III gives the comparative sugar values of either breast in a number of different women. Numbers 1-8 are in cases in which the breasts were normal, and 9-13 in which some disturbance was evident. It will be noted that from 1-8 the variation shown ranges from .02 per cent. to .09 per cent. with an average of .046 per cent. whereas from 9-13 the differences shown range from .19 per cent. to .46 per cent. with an average of .286 per cent.

Table 3

SUGAR: COMPARISON BETWEEN BREASTS.

			RIGHT			LEFT		
No.	Preg.	Day.	Notes Sample.	Sugar gms. per 100 c.c.	Notes Mother.	Notes Sample.	Sugar gms. per 100 c.c.	Notes Mother.
			Total			Total		
1.	P.	4	1 oz.	5.10	Normal	1 oz.	5.12	Normal
2.	P.	4	Samedy4.30	5.14	Normal	as other	5.05	Normal
3.	P.	6	Whole	5.65	Normal	Bef. Aft.	5.67	Normal
4.	P.	7	Bef. Aft.	5.75	Normal	Bef. Aft.	5.71	Normal
5.	P.	7	Whole	6.61	Normal	Bef. Aft.	6.62	Normal
6.	P.	8	Bef. Aft.	6.13	Normal	Bef. Aft.	6.09	Normal
7.	P.	10	Bef. Aft.	6.21	Normal	Bef. Aft.	6.15	Normal
8.	P.	9	Bef. Aft.	6.11	Normal	Bef. Aft.	6.20	Normal
9.	P.	6	Bef.	6.19	V. Full	Bef.	6.00	V. Full
10.	P.	7	Bef. Aft.	6.19	Engorged	Bef. Aft.	6.30	Engorged
11.	P.	5	Mainly Aft.	4.64	V. Hard	Mainly Aft.	4.32	V. Hard
12.	M.	5	Bef. Aft.	5.69	Engorged	Whole	5.92	Engorged
13.	H.H.	3	1, 3.	5.49	Flushed	1, 3.	6.03	Flushed

In this table " Bef. and Aft." means that equal volumes of milk were taken before and after feed, mixed and analysed. " I and 3 " means that equal volumes of the first and last milk without the baby feeding were mixed and analysed. The sugar percentage is given in gram per 100 cc. milk.

In connexion with this point the case of is of interest. Although cited as an example of normality in the physical appearances and development of her milk, she gave a very peculiar curve in the development of her sugar values.

Table IV

Day.	Time.	Relation to Feed.	Percentage of Sugar.	Breast.
2	10 a.m.	Before After	5 47 5,70	
4	10 a.m.	Before and After	6 20	
4	1 p.m.	Before After	8 90 10 00	Right
4	4 p.m.	Before and After	10.40	Left
5	1 p.m.	Before and After	7.50	Left
5	4 p.m.	Before and After	9.60	Right
6	10 a.m.	Before and After	5.95	Left
6	4 p.m.	Before and After	5.97	
7	10 p.m.	Before and After	5.72	

After the seventh day the sugar percentage became normal. On the fifth day lactose, identified by a lactosazone, was identified in the urine, but unfortunately no further specimens were obtained. There was here no clinical disturbance in infant or mother. Reference to curve in the Biochemical Journal will show one individual, H. H., whose curve for sugar from the second day to the fourth day showed an inversion of the usual order. In all other cases, both of our own and of the series of other observers, the course of development of the sugar value in milk is a steady rise from a value lying between five per cent. and six per cent. on the second day to between 6.8 per cent. and seven per cent. between the tenth and fourteenth days. But with H. H. the initial value of 5.25 per cent. on the second day was not maintained but showed a steep decline to 3.67 per cent. on the fourth day. This patient had been admitted to the Venereal Disease Ward for gonorrhoea. and the investigation had to be discontinued owing to the development of acute mastitis in the mother, and in the child of ophthalmia neonatorum.

It would appear therefore that disturbances in the normal function of the breast are reflected by an alteration in the sugar content of the milk, but we have no evidence at present to suggest that any other constituents in the milk are affected.

In all reports of work upon lactation the use of the phrase " to empty the breast" is customary, but in practice this is illusory. As a result of many experiments it has become a matter of considerable doubt in the minds of the authors if this can ever be accomplished. The regulation of milk supply in the breast is under the control of factors as yet only partially understood. Cases in this series have been observed in which, in spite of obvious engorgement of the breasts, it has been impossible by any procedure to obtain more than a very small quantity of milk, cases in which the infant is able to obtain one or two ounces of fluid after all attempts at digital expression or pump extraction had reported an "empty" breast, and very occasional examples of the reverse occurrence. This experience will be borne out by all workers in this field. It is paralleled by the phenomenon known to every farmer of the difference in yield obtained from the same cow by different milkers, and by the occasional apparent refusal of the cow to " give down " her milk to any, though the udders may be full.

Formerly in the Obstetrical 'Unit of the Royal Free Hospital Caesarean section, when necessary, was performed in the majority of cases prior to the onset of labour. For several days following the operation supplementary feeds had to be given to the infant owing to the scarcity of milk in the breasts. During the last two years it has been the practice of Professor McIlroy to operate after labour has begun, and it is now found that lactation becomes established earlier than formerly. This suggests that labour in itself is a preparation for lactation. Further investigations are being continued on this point.

Summary.

1. Our knowledge of the phenomena of early lactation is very scanty, and the need for further special study of it is imperative.
2. The development of lactation in different women varies considerably both in the rate of appearance of the fluid and the composition of the milk secreted.

3. Two main types of development can be distinguished. In the first, the breast shows a tendency to activity during the later weeks of pregnancy, the milk comes in early, is not viscid, rarely deeper in colour than primrose, homogeneous, early obtainable in definite amounts, the protein content is low and probably the ash also. The calcium content remains unchanged. In the second, the breast is inactive during pregnancy, does not produce an appreciable secretion till after the first twenty-four hours after delivery, is viscid, variable in colour, not homogeneous and obtainable in very small quantities. The protein and ash contents are high. The line between these two types ‘is not absolute, and cases were found of multiparae and of primiparae the development of which approximated rather more to the other group than their own.
4. The duration of the colostrum depends upon a balance of factors between mother and infant, i.e.-, upon the type of development present and upon the vigour of sucking of the infant.
5. Small quantities of early milk have a food value approximating to larger quantities of mature milk.
6. The percentages of sugar and protein vary slightly at the beginning and end of a feed, but the differences are without clinical significance.
7. The time in the feed when the sample is taken and the method of extraction of the milk do not affect the percentage of calcium and ash in the milk resulting.
8. The percentage of fat in milk is dependent inversely upon the amount of fluid present in the breast at the time of extraction, and directly upon the amount of pressure exerted upon the areola.
9. Morbid states of the breast appear to be correlated with disturbances in the sugar content of the milk. Cases can, how- ever, occur with profound modification of the sugar content, without any corresponding morbid state in the mother or in the infant. Our thanks are due to Professor Winifred Cullis for help and advice during the investigation.

References

1. Lowenfeld, M. F. and S. T. Widdows. “A study in the variations in the chemical composition of normal human colostrum and early milk.” Biochemical Mum, 1927, xxi, I.
2. Woodward, G. “Chemistry of colostrum milk : a study of six cases.” form-z, Expert. Med., 1897, ii, 217.
3. Hammett, F. S. “Variations in the composition of human milk during the first eleven days after parturition.” *Journ. Biochem*.,1917, xxiv, 2.
4. Adriance, V. “A clinical report on the chemical examination of two hundred cases of human breast milk.” Arch, Pediatr, 1897, xiv, 22, 85.
5. Schlossmanm, A. “Zur frage der naturlichen Sauglingernfihrung.” Arch. f. I., 1900, XXX, 288, 1902, xxxiii, 338.
6. Dennis, M. and F. B. Talbot. “A study of the lactose, fat and protein content of human milk.” Amer. Journ. Dis. Ch., 1919, xviii, 93.
7. Hunnaeus. “Uber den Kalkgehalt der Frauenmilch.” Biochem. Zeit., XXii, 442..
8. Schabad, J. A. “Der Kalkgehalt der Frauenmilch.” Jahrb. f. Kinderh, 1911, N.F., lxxiv, 511.