CLINICAL AND CHEMICAL STUDIES IN HUMAN LACTATION

BY

F. E. HYTTEN, M.B., B.S.

Midwifery Department, University of Aberdeen

I. COLLECTION OF MILK SAMPLES

The greatest single difficulty in the study of human lactation has always been the collection of milk, and if the study is to be of practical physiological significance the milk collected for analysis must be at least all that the infant would have taken by suckling both in quantity and in composition. Further—and this will be demonstrated in later papers—a true estimate of the quality of lactation at any stage must be based on an analysis of milk representing the whole of every feeding for 24 hours.

To satisfy these conditions the infant must be replaced at each feeding by some mechanism which will remove milk without disturbing the course of lactation. It can probably never be known to what extent this is possible, because the suckling of a baby has, for the mother, emotional overtones beyond the mere local stimulation of the nipple and areola; and the mechanical replacement of this local stimulus, however ingenious, cannot hope to be an entirely effective substitute for the baby.

The shortcomings of artificial milking methods have been controlled in the past, so far as may be, firstly, by the use of methods which are reputed to be effective and to cause little or no discomfort; and, secondly, by the choice of subjects who are interested and willing to take part in the study. The advantages of having co-operative subjects are obvious, but restriction of a study to volunteers tends to select those with higher milk yields, as, for example, those donating a surplus to a milk bank.

Methods of Milk Expression

Most authors in the past have found hand expression more satisfactory than the mechanical pump, and one such manual method, used by Macy in her extensive studies, is described in detail by Davies (1945). It was used in the study by Roderuck *et al.* (1946a, 1946b), and a progressive rise in milk yield during five-day sampling periods found by these authors has been illustrated by Morrison (1952). The level of milk production prior to the sampling periods is not shown, so there is no evidence that the method of sampling revealed the true yield. Morrison interprets the rise as a return towards normality from an initial depression. Deem (1931), who described a similar method, found that an infant put to one breast obtained more milk from it than could be obtained from the other breast by manual expression, but no cross-over experiments were made to eliminate the possibility of unequal breast capacity. She also found manual expression to be unsatisfactory when applied to the "large flat muscular type of breast," and excluded such cases from her study.

Personal experience in the use of both manual expression and the standard types of mechanical breast pumps has shown them to have many drawbacks. Both are uncomfortable, and the pumps often cause oedema and cracking of the nipple. Although skilled manual expression can probably empty a breast, it is time-consuming, and the use of a single operator to maintain a standardized technique throughout a 24-hour period is impracticable. The mechanical pumps are, on the whole, less effective than manual expression in emptying the breast.

The most recent work in man and animals stresses the importance of the milk ejection reflex in assisting milk removal, and it has been shown that injections of pitocin may replace or amplify the mechanism. Newton and Newton (1948), who used a mechanical breast pump, found that more milk was obtained from one breast when the other was simultaneously suckled and even more if pitocin was given first. These results do not suggest that the yield induced by pitocin is necessarily that which most closely represents the normal yield to the infant. It is also probable that the prospect of five intramuscular injections in one day will upset many subjects and thus have the effect of diminishing the milk yield. It would be better, therefore, to try to find a mechanical method which would imitate suckling to the extent of inducing the milk ejection reflex in the normal manner.

In the present studies, a recently devised breast pump, the "humalactor,"* has been used. This apparatus mechanically mimics the mouth action of the baby. It is much more comfortable than manual expression and seems to evoke milk ejection in each case where this has been experienced in suckling the baby. So far as can be judged, it is slightly more effective than expert hand expression in emptying a breast, since it is rarely (and only in some apprehensive subjects) that milk can be forced from the breast by hand when the humalactor has finished, whereas the humalactor can, in many cases, remove a little milk from a breast which has already been stripped by hand.

The humalactor has additional advantages. Firstly, the technique is standardized and there is no dependence on skilled operators; secondly, a great deal of time is saved for the nursing staff; and, thirdly, more patients are willing to undergo a subsequent 24-hour sampling when the initial sampling has been easy and comfortable.

Results

Data by which the two methods can be compared are presented in Tables I and II.

TABLE I.—*Milk Yield*

6th Day	7th Day		6th Day	7th Day	
Yield (ml.)	Yield (ml.)	Fat (g./100 ml.)	Yield (ml.)	Yield (ml.)	Fat (g./100 ml.)
580	695	3·40	720	575	2·95
540	435	1·70	110	105	3·60
305	158	2·85	620	355	2·90
590	370	2·50	80	102	3·45
370	330	2·60	410	130	2·80
360	425	2·50	910	1,110	2·75
350	116	3·80	700	510	2·20
570	325	1·95	270	380	4·75
500	595	3·40	410	490	3·70
170	77	4·60	360	400	3·70
550	680	2·70	380	515	3·05
350	420	2·90	510	506	2·50
160	107	2·90	530	166	1·60
410	510	2·80	410	158	2·50
90	300	2·50	630	545	2·40
210	215	2·75	300	402	3·25
390	355	2·05	340	345	3·00
570	345	4·20	870	860	2·30
230	275	2·75	670	256	3·70
620	530	1·90	390	305	3·70
540	365	3·15	310	275	3·40
1,260	1,250	3·60	440	520	2·00
450	502	2·90	500	380	3·00
520	445	3·70	390	265	1·95
400	270	2·40	580	415	2·20
450	370	2·40	620	490	2·40
650	400	3·50	410	180	2·80
100	90	2·00	310	370	2·25
160	115	5·40	100	100	2·50
210	210	4·05	340	51	3·00
470	335	3·05	240	370	2·65
330	360	2·40	400	150	2·60
500	445	3·90	400	425	2·90
230	76	3·80	580	385	2·80
430	395	3·75	450	540	2·40
570	620	2·80	230	200	2·20
470	512	2·80	390	100	3·00
330	187	4·40	530	765	2·35
530	363	2·70	880	580	2·40
620	370	3·05	340	500	3·05
500	500	2·70	660	620	2·95
270	165	2·60			
		Mean:	441·3	397·6	

*Described in the *British Medical Journal*, 1951, **2**, 234.

TABLE II.—*Milk Yield (The Seventh-day Yield was by the Humalactor)*

6th Day	7th Day		6th Day	7th Day	
Yield (ml.)	Yield (ml.)	Fat (g./100 ml.)	Yield (ml.)	Yield (ml.)	Fat (g./100 ml.)
120	71	5·25	485	690	3·30
370	514	3·10	210	800	2·20
160	258	2·70	540	690	2·60
600	695	1·70	350	380	3·00
430	356	3·40	330	298	3·00
400	352	2·40	450	540	4·10
420	410	3·60	220	250	4·10
130	170	2·70	320	445	1·80
270	150	1·90	400	620	3·70
570	990	4·50	270	375	3·25
660	405	3·70	415	455	3·05
580	380	3·50	505	630	3·80
540	440	4·10	400	420	2·00
600	433	2·10	435	450	3·20
500	220	4·50	170	275	2·10
440	400	2·20	365	375	3·60
520	460	3·20	310	270	2·40
460	330	2·80	350	350	2·70
390	720	2·80	670	510	3·60
75	91	3·40	400	570	3·20
510	385	4·10	360	420	3·40
140	170	3·80	430	450	2·85
390	315	2·70	320	270	3·50
610	470	2·90	410	370	3·50
500	855	2·90	410	670	4·00
640	560	2·80	280	265	4·60
380	350	2·90	680	880	4·35
510	426	1·85	1,180	1,034	3·50
350	265	2·40	350	225	2·20
620	490	3·00	300	260	1·60
460	590	3·80	260	215	2·65
540	590	2·40	390	575	2·25
275	225	5·90	510	545	2·30
410	470	2·50	430	310	2·30
255	555	3·00	490	550	5·00
790	680	3·20	470	330	3·50
395	450	2·40	500	560	3·00
335	275	3·30	500	518	3·65
460	480	4·00	400	610	3·10
415	415	3·75	350	400	2·90
372	310	2·60	340	690	4·10
440	450	2·60	420	440	2·35
220	225	3·00			
		Mean:	419·8	445	

Table I shows a series of 83 subjects in a study made in 1948–9. Milk yield on the sixth day was computed from test weighing of the infant at each feed, with the addition of any residual milk manually expressed after each feed. Milk yield on the seventh day represents the result of manual expression of the whole of every feeding by an experienced and skilful nurse.

In Table II a series of 85 subjects from a current study, the sixth-day yield is again computed from test weighings and measured strippings, but the seventh-day yield was removed entirely by the humalactor.

In neither table, therefore, are the yields on the sixth and seventh days strictly comparable, since only the seventh-day yields are measured accurately. At this stage of lactation the yield is generally increasing, although many factors affect the day-to-day output, but in two series as large as these some increase in the mean from the sixth to the seventh day would be expected, whatever individual fluctuations there might be.

In the first series the mean seventh-day yield was 379.6 ml. and the sixth-day yield 441.3 ml. The reduction of 61.7 ml. is statistically significant at the 5% level.

In the second series the seventh-day yield averaged 445 ml. and exceeded that of the sixth day, 419.8 ml., by 25.2 ml., a difference which, while not statistically significant, is nevertheless an increase, and in line with the expected trend.

It is well known that a progressive rise in fat content occurs during a feeding, so that milk from completely emptied breasts would be expected to have a higher average fat content than milk from breasts which have been only partially emptied. There is no significant difference between the fat contents of the seventh-day milk in the two series; nor is any difference found if the mean fat content of milks which have decreased from the sixth to the seventh day is compared with that of milks which show an increased yield on the seventh day.

This suggests that both methods of milking can probably empty a given breast at any time in most subjects, but that manual expression, either from its discomfort or because of its lack of natural stimulation, at the time or in anticipation, causes a decreased amount of milk to be secreted.

Summary

The collection of a complete 24-hour milk output involves more than the mere emptying of the breasts at the usual feeding-times. The mechanism for milk removal should be such that not only is all the milk taken from the breast but also normal milk production is undisturbed.

This is probably not possible in all subjects, especially the more excitable, but the use of the humalactor breast pump gives encouragingly better results than manual expression.

I am grateful to Professor Dugald Baird and to the staffs of the Midwifery Department and the Aberdeen Maternity Hospital for invaluable help; to Dr. I. Leitch, of the Commonwealth Bureau of Animal Nutrition, Bucksburn, for criticism and advice; and to Mr. W. Billewicz, of the Social Medicine Research Unit of the Medical Research Council, for statistical assistance.

REFERENCES

Davies, V. (1945). *Amer. J. Dis. Child.*, **70**, 148.
Deem, H. E. (1931). *Arch. Dis. Childh.*, **6**, 53.
Morrison, S. D. (1952). *Human Milk: Yield, Proximate Principles, and Inorganic Constituents.* Commonwealth Bur. Animal Nutrit. Tech Commun No. 18.
Newton, M., and Newton, N. R. (1948). *J. Pediat.*, **33**, 698.
Roderuck, C., Coryell, M. N., Williams, H. H., and Macy, I. G. (1946a) *J. Nutr.*, **32**, 267.
—— Williams, H. H., and Macy, I. G. (1946b). Ibid., **32**, 249.

II. VARIATION IN MAJOR CONSTITUENTS DURING A FEEDING

The change in milk composition during the course of a feeding has been discussed in medical writings since Bartholomeus Metlinger (1473) advised that " . . . the wet nurse should first milk the breast so that the watery part runs from it, and then give the child to suck." During the past 50 years there have been frequent descriptions of the variations, both in man and in animals.

Morrison (1952) has made a comprehensive review of the literature on human milk, and it would be profitless to duplicate this. He quotes few exceptions to the general finding that the fat content increases from the beginning to the end of a feeding. However, Waller (1943) and Newton (1952) found a variable pattern, and Lucignani (1934) observed that the fat increased to a maximum in ten minutes and thereafter declined slightly.

There have been comparatively few observations of the variation of the other major constituents. Novellis di Coarazze (1936) described a diminution in lactose content, followed by a later recovery, in 15 out of 20 cases, but other workers have found no significant variation. A number of investigators describe no significant change in the total nitrogen content, but Macy *et al.* (1931), who examined milk from the first and last halves of the feeding, found a small but highly significant rise. The variation of nitrogen has been studied in more detail by Waisman and Petazze (1947), who found that the casein nitrogen rose and the non-casein nitrogen (including the non-protein nitrogen) fell as the feeding progressed.

Most previous observations have been made on only two or three samples from the feeding. The usual method has been to remove a sample from the breast by hand before the infant is fed and another by stripping the breast when it is finished. One sample, or sometimes two samples, may be removed after interrupting the infant during the course of the feeding. Macy *et al.* (1931) had the entire feeding expressed by hand, but were content to divide it into first and last halves. Thus, in the human, the pattern of change in the constituents of milk throughout a feeding has not been observed in any great detail.

Method

Collection of Samples.—In the present study a number of small samples have been taken during the emptying of a breast by the "humalactor," a machine whose efficiency has been shown in Part I of this paper. The humalactor was applied to the breast in the usual way, using, instead of a collecting-bottle, a test-tube marked to contain 12 ml. When this tube was filled it was replaced by another without stopping the machine. In this way a consecutive series of 12-ml. samples was obtained until the breast was emptied.

Methods of Analysis.—Fat was estimated by the Gerber method, lactose by a modification of the chloramine-T method of Hinton and Macara (1927), and nitrogen by a modification of the micro-Kjeldahl method described by Steel (1946) for plasma protein. Casein was precipitated by acetic acid at 40° C. (Rowland, 1938) and total protein by dialysed iron. When all these constituents were estimated it was necessary to mix adjacent samples to give the necessary volume of milk. This had the effect of halving the number of samples in these cases.

TABLE I.—*Fat in g./100 ml.*

Case No.	Post-partum Day	Sample Number									
		1	2	3	4	5	6	7	8	9	01
4	7	0·90	0·90	1·25	1·70	1·80	2·30	2·60	4·15	4·9	
		1·80	1·95	2·05	3·30	3·30	3·45				
5	6	1·35	1·40	1·50	2·40	3·25	3·25	4·65	5·05	5·10	5·95
		3·95	4·45	5·35	5·95						
6	4	1·15	1·20	1·25	1·95	2·45	3·45	3·95			
		1·85	1·35	1·50	1·90	2·25	2·95	2·55			
	6	1·40	1·65	1·40	1·50	2·10	2·50	2·05	3·30	3·20	
		1·70	1·50	2·10	1·95	1·90	2·30	4·05	4·35	4·70	
7	7	1·90	2·60	3·60	4·15	4·80					
8	6	1·80	2·20	2·20	3·10	4·00					
		3·50	3·15	4·20							
10	5	3·60	3·55	4·10	6·60	7·00					
11	6	3·10	3·20	3·90	5·85						
12	5	1·20	1·45	2·15	2·25	2·25	1·90	2·90	4·10		
		1·45	2·05	2·75	3·60	4·20	5·05	4·30	6·10	6·25	
13	7	3·40	3·65	4·35	5·45	7·30					
		2·95	2·55	2·30	2·95	3·55	4·85	4·80			
	9a	3·90	3·40	3·55	5·40	6·60					
		3·90	3·70	4·55	4·80						
	9b	5·40	5·70	6·50	7·00						
		4·40	4·40	5·20	5·80						
	10a	3·95	4·15	5·80	6·85	7·15					
	10b	5·30	6·25	7·20	7·40						
		4·05	4·45	5·20	5·90						
	11	2·35	3·30	4·25	5·75	5·85					
		4·85	5·50	5·00	5·40						
	14	3·50	3·40	4·45	4·85	4·90	5·50	5·65			
		3·35	4·55	6·00	5·95	6·30	7·30	8·90			
	16	1·70	1·65	2·35	2·45	3·50					
		2·30	3·00	5·60	6·35						
	18	3·10	2·90	2·95	3·70						
		0·90	0·55	1·25	1·95	3·70	4·25	4·45			
	23	3·05	4·20	4·95							
		2·10	2·40	3·25	4·40	5·80					
14	5	1·30	4·65	2·30	3·95	3·90					
		1·65	2·10	2·70	3·25						
15	5	1·50	1·50	1·70	2·40	2·45	3·85				
		1·70	1·70	2·20	2·30	2·80	2·35	2·45	1·90	2·50	
16	3	3·85	4·40	3·80	4·40	4·50	6·20	6·30			
		4·90	5·30	5·75	7·95	6·60	10·15				
	7	1·95	2·00	2·05	2·80	2·30	2·45	2·75	4·45	5·50	4·40
		3·00	3·50	3·70	4·30	4·65	4·70				
18	4	0·45	0·70	0·60	1·00	1·05	1·90	2·65	3·10	3·80	4·80*
		0·55	0·70	0·95	2·20	3·70	4·25				
19	3	5·55	6·70	8·15							
		5·40	6·40	8·05							
20	3	1·15	1·60	3·00							
		0·70	1·50	2·45	2·90						
85	6	2·20	4·05	5·95	10·05						
101	8	1·20	1·30	2·50	4·40	7·00					
196	7	2·00	2·10	2·40	2·90	3·40	4·80				
202	7	3·30	3·30	4·95	6·70						
208	8	2·60	3·65	4·90	6·95						

* The amount increased to 5·90 and 6·25 in the eleventh and twelfth samples respectively.

Results

In any one case the breasts seldom produced equal volumes of milk unless both had been emptied completely at the previous feeding. The breast which gave the larger

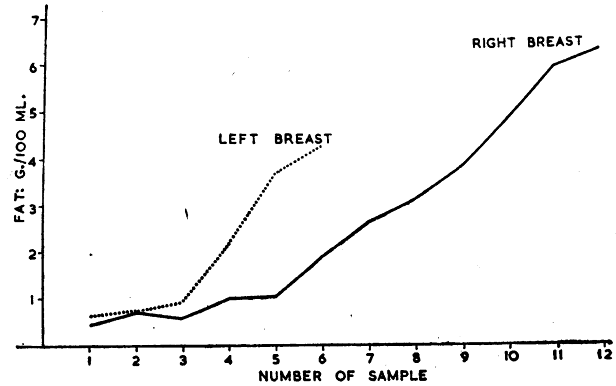

FIG. 1.—Case 18, fourth post-partum day, showing the progressive rise in fat content of the milk during emptying of the breasts.

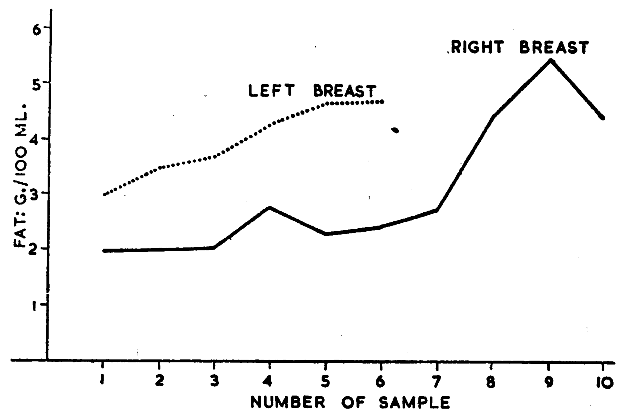

FIG. 2.—Case 16, seventh post-partum day, showing the degree of irregularity which can occur in the usually progressive rise in the fat content of milk during emptying of the breasts.

volume was usually the one at which the infant had suckled last and which had been left with more residual milk than the other.

The changes during a feeding in fat, lactose, and nitrogen found in this investigation are shown in Tables I, II, and III. The experiments are numbered so that when more than one constituent was estimated the values of all constituents can be compared together.

Fat.—A rise in fat concentration always occurred, but there was considerable variation in both the regularity of the rise and its degree (Table I). Fig. 1 demonstrates the most commonly found shape of curve, showing a flat, somewhat irregular start and an increasingly rapid terminal rise. The curve from the

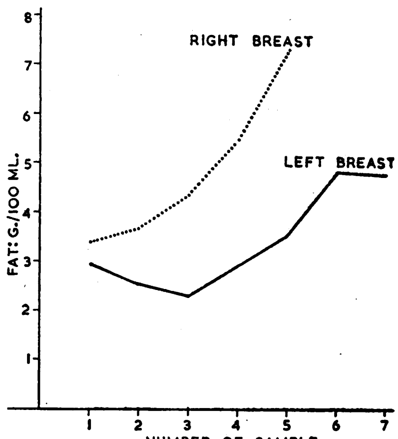

FIG. 3.—Case 13, seventh post-partum day, showing, in the right breast, the smooth and rapidly rising fat content which can occur as the breast is emptied and, in the left breast, the usual degree of irregularity found.

right breast represents the maximum percentage rise found in the series: from 0.45 g. to 6.25 g. per 100 ml. Fig. 2 shows a more irregular rise that was sometimes found. The abrupt changes of direction seen in the curve from the right breast are discussed later. Fig. 3 shows, in the curve from the right breast, the smooth uninterrupted curve which was seen on only three occasions.

Lactose.—The lactose content shows an inverse relationship to the fat, falling from a maximum in the first sample to a minimum at the end of the feed (Table II). This is to be expected, since, in a given quantity of milk, the volume of water is dependent on the volume of fat present, so that a rise in fat will cause, by displacement, a lower volume of water, and therefore less water-soluble constituents. The overall change in lactose was usually small, and the maximum fall, from 6.60 g. to 5.65 g. per 100 ml., was associated with an increase in fat content from 2.20 g. to 10.05 g. per 100 ml.

TABLE II.—*Lactose in g./100 ml.*

Case No.	Post-partum Day	Sample Number							
		1	2	3	4	5	6	7	8
11	7a	6·70	6·50						
	7b	6·75	6·64						
		6·81	6·58	6·58	6·64	6·58			
12	5	6·23	6·43	6·40	6·23	6·12	6·10	6·10	6·02
		6·60	6·60	6·50	6·38	6·35	6·40	6·35	6·17
45	4	6·85	6·71	6·57	6·51	6·51			
46	9	6·78	6·94	6·78					
		6·64	6·47						
47	3	6·17	6·60	6·57	6·60	6·55			
	8	6·98	7·08	6·95	6·98	7·08	6·98		
57	5	5·27	5·05	4·95	4·95	4·85			
64	5	6·37	6·44	6·44	6·30	5·96			
78	7	5·20	5·00						
79	5	6·92	6·98	7·04	6·51	6·64	6·57		
85	6	6·60	6·40	5·92	5·65				
86	5	6·15	5·96	5·68	5·48				
101	8	6·84	6·76	6·72	6·55	6·40			

Nitrogen.—The total nitrogen gave a variable pattern, but the changes were in any case usually very small (Table III). In milk of the first three or four days of lactation the total nitrogen falls from beginning to end of the feeding, but after the fifth day there is usually a slight increase.

TABLE III.—*Nitrogen in mg./100 ml.*

Case No.	Post-partum Day	Sample Number						
		1	2	3	4	5	6	7
				Total Nitrogen				
45	4	333	323	318	316	316		
46	9	280	285	282				
		332	330	342				
47	3	425	400	394	372	382		
	8	259	277	270	259	266		
57	5	298	300	307	(Lost)	308		
64	5	283	277	297	297	297		
78	7	356	356	372				
79	5	290	291	297	291	300	302	
85	6	288	292	294	277			
86	5	347	344	342	333			
101	8	238	238	238	232	238		
190	6	295	290	297	307	295	295	
196	7	276	263	267	269	270	269	
197	6	312	321	317	315	304	307	
202	7	312	291	273	276			
203	6	349	335	340	333	336		
208	8	273	276	272	265			
209	6	400	393	399	407	402	399	416
				Casein Nitrogen				
101	8	87	84	83	84	95		
190	6	131	123	133	143	126	128	
196	7	121	113	114	108	117	109	
197	6	126	140	141	139	123	116	
202	7	137	144	134	132			
203	6	104	104	117	112	111		
208	8	112	126	119	121			
209	6	131	162	182	179	177	179	179
				Non-protein Nitrogen				
45	4	56	58	53	52	51		
46	9	70	72	71				
47	3	62	53	50	56	38		
	8	64	67	68	64			
57	5	62	56	62	64	64		
64	5	67	56	62	70	56		
79	5	70	76	79	67	69	78	
85	6	64	53	48	46			
86	5	53	67	67	56			
101	8	47	38	40	34	50		

The inconsistency may be explained if the protein is recognized as two fractions differing in behaviour. Casein, which may be partially adsorbed to the fat, either rises slightly at the end of the feed or remains at an almost constant level, but the soluble protein falls as less water per unit volume is available. The pattern for total nitrogen will depend, therefore, on the relative proportion of its fractions, and in the very early milk the fall is due to the characteristic preponderance of globulin. Non-protein nitrogen showed a haphazard variation in most cases, although changes were small. There was a decrease in a few cases.

Mechanism

A number of theories have been advanced to explain the mechanism of the rise in fat concentration. The belief has been expressed by earlier workers that the fat globules are arrested mechanically by the finer ducts in the breast and are squeezed out finally only by the pressure of milking, but Petersen (1929), who studied the distribution of fat in the bovine udder histologically, denied this. Lowenfeld *et al.* (1927) and von Sydow (1944) have suggested that the magnitude of increase in fat is dependent on the method of extraction, a more forcible process causing a higher final fat content; and Gölz (1940), who attributed the increase to a difference in viscosity between fat and the other solids, believed the increase to become apparent only with inexpert evacuation. Johannson *et al.* (1952), who supported the theory of mechanical arrest of the fat globules, were able to obliterate the rise in fat content, in the cow, by vigorous massage of the udder before milking.

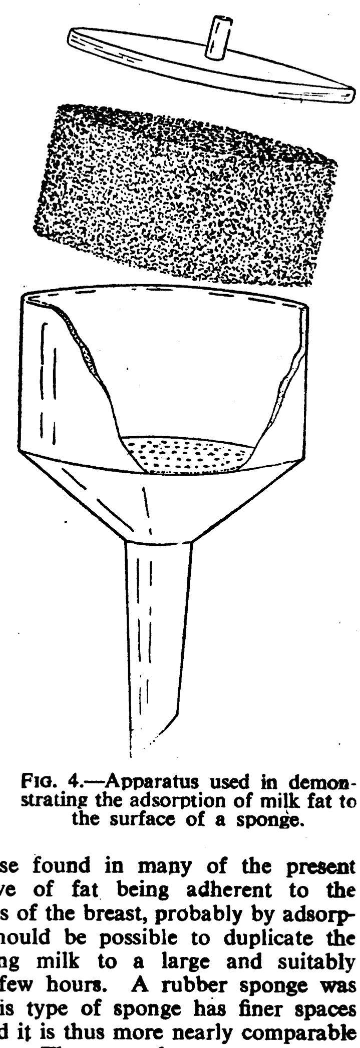

FIG. 4.—Apparatus used in demonstrating the adsorption of milk fat to the surface of a sponge.

The steep terminal rise found in many of the present experiments is suggestive of fat being adherent to the alveolar and duct surfaces of the breast, probably by adsorption; if this is so, it should be possible to duplicate the phenomenon by exposing milk to a large and suitably arranged surface for a few hours. A rubber sponge was chosen to test this. This type of sponge has finer spaces than others available, and it is thus more nearly comparable to the breast in structure. There are, however, no spaces fine enough to hinder the passage of any fat globule. Rubber has no specific affinity for fat in globules. The sponge, cut to fit a porcelain filter funnel (Fig. 4), was filled with milk and allowed to remain immersed in it for four

TABLE IV

Sample	Fat (g./100 ml.)		
	Time of Immersion		
	3 Hours	4 Hours	12 Hours
1	2·30	2·65	0·70
2	2·30	2·85	1·15
3	2·85	3·35	2·00
4	4·00	5·25	4·45
5	6·60	7·75	9·80

hours. At the end of that time it was removed from the milk and the excess not held within it was allowed to drain. It was then placed in the filter funnel and compressed by a plunger until empty. In a number of experiments the sponge was inverted in the filter funnel so that any effect of "creaming" within the spaces could be eliminated. The milk was collected as successive 15-ml. samples, and the fat contents of these samples invariably gave a curve similar to Fig. 5. The steepness of the fat rise varied with the time allowed for adsorption, and Table IV shows the fat contents of samples obtained from a sponge immersed in milk for 3, 4, and 12 hours. This is in accord with the behaviour of the breasts, the gradient for fat being steeper on the side which has been left the longer.

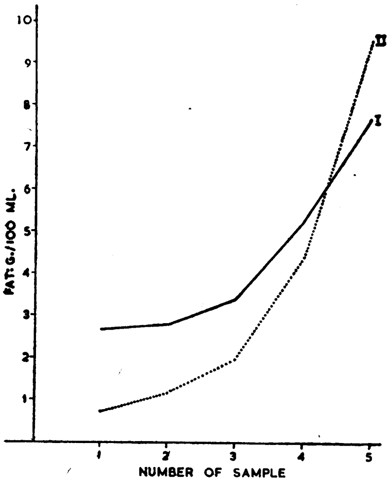

FIG. 5.—The progressive rise in fat content which occurs when a sponge soaked in breast milk is emptied by compression. Curve I is from a sponge after four hours' immersion in milk, and curve II from a sponge after 12 hours' immersion.

In most cases the second breast to be sampled was, as in the usual breast-feeding practice, the breast first suckled at the previous feed. It may therefore have had an hour longer than the other breast for adsorption. This effect has also been demonstrated in the cow, a steeper gradient occurring after the long night interval than after the shorter daytime intervals (Johannson *et al.*, 1952). A breast which has been incompletely emptied at the previous feed will show a higher initial fat content than one which has been stripped completely, since its residual milk, to which the subsequent secretion is added, is of high fat content.

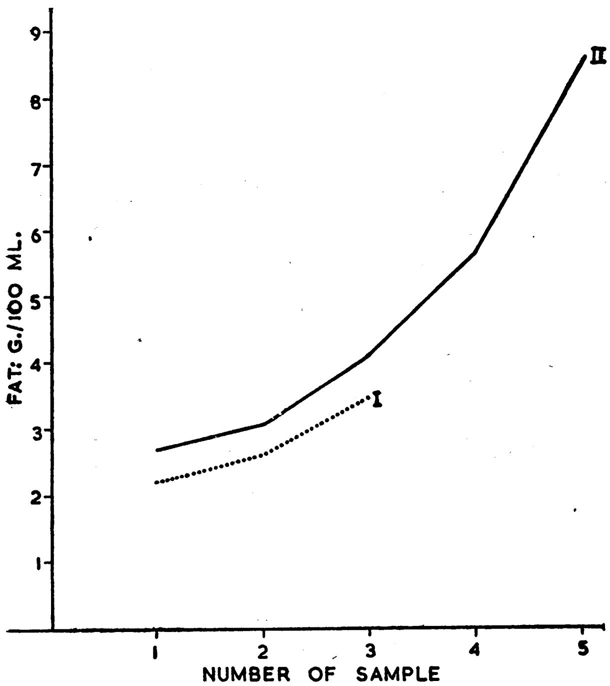

FIG. 6.—The effect of refilling with milk a sponge which has been only partially emptied. Curve I is from a sponge partially emptied after four hours' immersion in milk, and curve II from the same partially emptied sponge after a further four hours' immersion in more of the same milk.

Fig. 6 shows the effect produced when the sponge, after four hours' immersion, is partially emptied of milk and then re-immersed in more of the same milk for a further four hours before it is squeezed empty.

It is not surprising that the breast seldom shows in its output of fat the smooth curve of Fig. 5, since it consists not of a simple unit like the sponge but of up to 20 separately drained lobules. Only if all the lobules act in unison can such a curve be expected. Clinically, this is observed infrequently, and if the collecting-bottle of the breast pump is watched the steady trickles of milk can often be seen to be joined by an additional jet as a new lobule comes into action. In this way the rising fat concentration may be suddenly reduced by the dilute first milk of the new lobule (Fig. 2). This may explain the occasional reverse changes found by some workers, and particularly the terminal drop described by Lucignani. The findings of Lowenfeld *et al.* and of von Sydow also might be explained by the theory of physical adsorption, since the removal of the fat closely adherent to the walls of the ducts would require considerable mechanical force.

The other major constituents behaved in the sponge as they do in the breast. This would confirm that the fall in the water-soluble constituents is due to simple displacement by the fat.

Summary

During the course of a feeding there is a progressive and usually large increase in the concentration of fat, associated with a small decrease in the concentration of lactose. The total nitrogen falls from the begining to the end of a feeding during the first few days of lactation, but as the milk matures there is usually a small increase due to a relative rise in the casein concentration.

It is suggested that the behaviour of the fat is due to the mechanical phenomenon of adsorption of the fat globules to the large secretory and duct surface of the breast, and the changes in the other constituents are secondary to this.

The entire picture has been duplicated by soaking a rubber sponge in breast milk and emptying it by mechanical compression.

I am grateful to Professor Dugald Baird and to the staffs of the Midwifery Department and the Aberdeen Maternity Hospital for invaluable help, and to Dr. I. Leitch, of the Commonwealth Bureau of Animal Nutrition, Bucksburn, for criticism and advice.

REFERENCES

Gölz, H. (1940). *Lait*, 20, 145.
Hinton, C. L., and Macara, T. (1927). *Analyst*, 52, 668.
Johannson, I., Korkman, N., and Nelson, N. J. (1952). *Acta agric. scand.*, 2, 43.
Lowenfeld, M. F., Widdows, S. T., Bond, M., and Taylor, E. I. (1927). *Biochem. J.*, 21, 1.
Lucignani, D. (1934). *Riv. Clin. pediat.*, 32, 412.
Macy, I. G., Nims, B., Brown, M., and Hunscher, H. A. (1931). *Amer. J. Dis. Child.*, 42, 569.
Metlinger, Bartholomeus (1473). *Eyn Vast Nützuck Regiment der Jungen Kinder*, Augsberg. (Quoted by J. Ruhräh, *Amer. J. Dis. Child.*, 1928, 35, 492.)
Morrison, S. D. (1952). *Human Milk: Yield, Proximate Principles, and Inorganic Constituents.* Commonwealth Bur. Animal Nutrit. Tech. Commun. No. 18.
Newton, M. (1952). *Amer. J. Obstet. Gynec.*, 64, 397.
Novellis di Conrazze, C. (1936). *Riv. Clin. pediat.*, 34, 1057.
Petersen, W. E. (1929). *Amer. J. Physiol.*, 90, 592.
Rowland, S. J. (1938). *J. Dairy Res.*, 9, 30.
Steel, A. E. (1946). *Bull. Inst. med. Lab. Tech.*, 12, 1.
von Sydow, G. (1944). *Acta paediat., Stockh.*, 32, 756.
Waisman, S. G., and Petazze, M. L. A. (1947). *Rev. Asoc. argent. Diet.*, 5, 121.
Waller, H. K. (1943). *Lancet*, 1, 69.

III. DIURNAL VARIATION IN MAJOR CONSTITUENTS OF MILK

The more recent literature on human milk has paid particular attention to the diurnal variation of both yield and composition, since most of the earlier work done on milk from a single feeding gave widely divergent results and little indication of the sort of milk which would be available for the whole 24 hours.

Most workers have examined the output of milk at the conventional feeding-times of 6 a.m., 10 a.m., 2 p.m., 6 p.m., and 10 p.m., with an eight-hour interval between 10 p.m. and 6 a.m., and there is general agreement that the volume is maximal at the 6 a.m. feeding and decreases during the rest of the day. Macy *et al.* (1930), however, made an analysis of milk drawn off every four hours during the day and night, including one sample at 2 a.m., and found no such early morning peak. Apart from this unusual sampling method, their results are in any case not strictly comparable with other studies, since their subjects were quite exceptionally large milk producers whose lactation had been artificially maintained and prolonged by manual expression.

Deem (1931) studied the output of a single breast in 27 cases and was able to demonstrate a mean yield dropping from 150 ml. at 6 a.m. to a level of approximately 75 ml. for all other feeds. That the yield following an eight-hour interval was double the yield after a four-hour interval strongly suggests a constant hourly secretion rate for the whole 24 hours, but no other studies have been able to confirm this, and Morrison (1952), who reviewed this literature in some detail, believes there is insufficient evidence that the yield varies only with the interval between milkings.

There is more general agreement about the very considerable variation in the fat content, the agreed pattern showing a minimum value at 6 a.m., rising steeply to a maximum at 10 a.m., and falling off during the following three feeds.

TABLE I.—*Yield in Millilitres*

Case	Day	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
28	3	116	61	118	115	51
29	3	14	12	13	37	45
31	3	14	10	28	31	41
42	3	235	125	84	155	100
172	3	67	33	68	61	94
21	4	152	142	67	143	62
27	4	74	78	57	68	48
43	4	71	136	116	88	48
172	4	69	33	77	142	95
23	5	82	85	57	46	72
26	5	87	38	39	49	74
30	5	81	82	14	41	32
34	5	109	87	51	21	36
172	5	212	56	92	26	45
22	6	163	155	150	116	97
33	6	175	137	162	133	131
37	6	80	85	72	66	69
40	6	103	97	102	77	39
41	6	210	107	59	76	50
172	6	64	150	85	170	55
25	7	224	126	97	128	116
36	7	149	122	76	89	78
39	7	93	133	127	145	24
172	7	133	90	120	140	100
181	7	100	32	76	133	90
200	7	76	54	114	42	70
163	7	166	74	95	109	78
38	8	50	61	24	61	23
172	8	210	135	100	120	105
Mean	.. ..	116·5	87·4	80·7	90·6	67·9
35	21	290	192	143	157	172
159	22	100	58	36	53	52
162	32	178	86	100	133	118
35	37	281	174	153	138	189
198	38	222	120	114	126	99
138	40	95	120	72	108	80
119	41	60	81	68	54	45
151	47	192	130	129	147	145
116	48	135	88	128	95	84
161	48	158	137	50	74	62
163	48	238	205	164	173	177
115	49	66	63	56	51	66
74	50	114	113	117	99	117
102	50	143	88	149	56	87
139	50	229	227	206	146	146
110	53	120	75	89	130	97
177	53	148	125	93	130	137
121	62	228	180	133	92	142
174	62	172	79	48	59	85
75	135	198	198	135	118	113
Mean	.. ..	168·3	126·9	109·1	106·8	110·6

No systematic change in any of the nitrogen fractions or in lactose has been described, although for both there may be substantial differences from feed to feed in the same individual.

The collection of the entire milk output for 24 hours presents considerable difficulties, particularly without the convenience of a hospital, and it would be much more practicable to collect merely the total output of one feeding. The following study was partly undertaken, therefore, to discover to what extent any single feed is representative of the whole day.

TABLE II.—*Fat in g./100 ml.*

Case	Day	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
28	3	2·55	2·45	2·30	3·00	2·25
29	3	2·20	2·60	2·25	2·25	2·20
31	3	1·55	2·15	1·65	1·90	1·95
42	3	1·95	3·45	2·70	2·25	2·05
172	3	2·40	4·40	3·50	3·30	4·00
21	4	2·10	3·35	3·50	2·90	3·40
27	4	3·90	3·95	4·25	3·50	3·50
43	4	1·70	2·65	3·75	4·35	3·30
172	4	2·70	2·80	2·50	2·20	3·60
23	5	1·90	3·05	2·80	2·15	2·00
26	5	2·95	3·05	1·95	1·70	1·65
30	5	1·00	2·30	3·05	2·20	2·60
34	5	1·20	3·50	5·60	2·75	4·05
172	5	3·40	3·90	4·05	3·20	2·80
22	6	2·60	3·40	3·35	4·50	4·00
33	6	2·90	4·05	4·65	2·70	3·90
37	6	1·65	2·30	4·20	2·85	3·35
40	6	1·30	1·95	2·35	2·90	2·45
41	6	3·95	6·70	5·85	4·40	3·80
172	6	1·90	3·20	3·60	2·75	2·95
25	7	3·35	4·70	4·30	3·10	3·95
36	7	2·70	2·75	3·30	3·60	3·30
39	7	2·00	2·90	2·40	3·15	2·90
163	7	3·20	4·70	3·50	4·10	3·40
172	7	1·90	3·00	3·10	2·70	3·10
181	7	2·20	1·80	2·70	4·00	3·00
200	7	2·50	4·00	4·10	3·70	3·00
38	8	1·85	1·40	1·35	1·30	1·35
172	8	2·40	3·90	3·80	2·90	2·60
Mean	.. ..	2·34	3·25	3·32	2·98	2·98
35	21	2·95	4·15	4·65	3·65	3·30
159	22	3·30	5·70	5·00	4·30	3·80
162	32	2·60	5·80	3·20	3·40	3·80
35	37	2·15	5·30	3·35	3·35	2·75
198	38	3·10	4·20	4·60	4·00	3·00
138	40	3·20	3·75	3·80	3·00	3·40
119	41	2·30	3·80	4·00	3·90	3·40
151	47	2·80	5·20	3·20	4·95	5·25
116	48	1·90	3·10	2·80	3·30	2·20
161	48	1·50	2·70	2·10	2·60	1·40
163	48	3·20	4·60	4·50	4·30	4·50
115	49	1·40	2·60	1·60	0·70	0·95
74	50	1·80	2·50	2·70	3·10	2·90
102	50	2·60	3·00	3·80	4·15	3·00
139	50	2·70	4·30	4·00	4·20	4·00
110	53	3·00	4·00	3·00	2·80	2·10
177	53	2·55	3·20	4·00	3·70	3·10
121	62	3·90	4·70	5·90	5·50	4·10
174	62	2·90	5·00	5·40	3·15	2·20
75	135	3·00	5·30	4·50	3·60	3·20
Mean	.. ..	2·64	4·15	3·81	3·58	3·12

Method

Altogether, 49 24-hour samples were collected from 42 subjects. The 24-hour sample comprised the whole output of both breasts collected by the "humalactor" at the usual feeding-times of 6 a.m., 10 a.m., 2 p.m., 6 p.m., and 10 p.m. To ensure that no residual milk from the previous 24 hours was included, the breasts were completely emptied by the humalactor after the last feed prior to the sampling period. Samples from the first week of lactation were obtained from patients in the Aberdeen Maternity Hospital, and the more mature samples from patients in the mother and baby unit of the Royal Aberdeen Hospital for Sick Children and from unmarried mothers in the Aberdeen Mother and Baby Home. The methods of chemical analysis used have been outlined in Part II of this paper.

Results

All estimations of yield and of fat, lactose, and nitrogen content are shown in Tables I–V. When any relationship has been tested statistically the level of significance is shown in parentheses.

TABLE III.—*Lactose in g./100 ml.*

Case	Day	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
28	3	6·67	6·53	6·60	6·53	6·78
42	3	6·23	6·16	6·08	6·16	6·13
172	3	5·72	6·09	5·89	5·75	5·20
21	4	6·12	6·17	6·00	6·30	6·18
27	4	6·12	6·19	6·67	6·74	6·85
43	4	6·45	6·02	5·68	6·02	6·44
172	4	5·48	5·96	5·82	6·20	6·30
34	5	4·80	4·64	4·80	4·88	5·41
172	5	6·37	6·23	6·85	6·02	6·37
33	6	6·71	6·50	6·64	6·67	6·67
37	6	6·71	6·54	6·51	6·47	6·54
40	6	6·44	6·27	6·09	6·36	6·30
41	6	5·96	5·73	6·00	6·16	6·33
172	6	6·16	6·57	6·54	6·51	6·33
36	7	6·46	6·47	6·47	6·44	6·37
39	7	6·57	6·54	6·40	6·16	6·37
172	7	6·64	6·44	6·51	6·33	6·37
38	8	6·68	6·40	6·37	6·66	6·68
Mean	.. ..	6·24	6·19	6·22	6·24	6·31
35	21	6·98	6·78	6·85	6·81	6·85
35	37	7·27	7·10	7·07	7·07	6·85
138	40	6·33	6·68	6·60	6·47	6·37
151	47	6·78	6·78	7·15	6·82	6·95
163	48	7·33	6·85	6·78	7·05	6·92
115	49	6·64	7·08	7·15	6·92	6·92
74	50	7·12	7·12	7·29	7·10	7·22
102	50	7·15	7·26	6·85	6·92	6·92
139	50	6·85	6·85	6·92	6·88	6·81
75	135	7·19	7·05	7·05	7·05	6·78
Mean	.. ..	6·96	6·96	6·97	6·91	6·86

TABLE IV.—*Total Nitrogen in mg./100 ml.*

Case	Day	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
28	3	360	392	379	368	355
42	3	288	277	266	283	274
172	3	483	420	363	321	309
27	4	435	441	398	378	372
43	4	353	361	364	367	353
172	4	301	287	314	290	281
34	5	525	497	470	575	481
172	5	295	293	301	265	294
33	6	255	260	238	253	257
37	6	294	308	316	288	277
40	6	286	305	295	321	316
41	6	388	414	405	402	360
172	6	287	284	281	281	284
39	7	336	312	327	319	343
172	7	290	273	276	265	267
38	8	260	260	256	250	245
Mean	.. ..	339·7	336·5	328·0	326·6	316·7
35	21	268	254	275	266	270
138	40	231	221	207	225	223
151	47	213	203	214	218	213
163	48	209	203	218	214	200
115	49	182	216	207	190	186
74	50	194	183	178	179	189
102	50	181	192	206	193	178
139	50	200	195	195	189	193
75	135	147	141	144	147	141
Mean	.. ..	202·8	200·8	204·8	202·3	199·2

TABLE V.—*Non-protein Nitrogen in mg./100 ml.*

Case	Day	6 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
28	3	74	68	75	62	74
42	3	48	50	49	53	52
43	4	81	60	66	71	71
172	4	68	68	64	65	52
34	5	61	61	63	62	62
172	5	63	60	63	57	65
33	6	63	55	72	73	70
40	6	69	73	67	70	70
41	6	91	83	80	69	69
172	6	54	63	55	68	54
39	7	80	65	71	63	65
172	7	60	57	71	65	65
38	8	65	52	84	78	70
Mean	.. ..	67·5	62·7	67·7	65·8	64·5
35	21	69	68	72	62	63
35	37	47	43	50	54	44
138	40	60	59	52	60	52
151	47	70	62	81	68	76
163	48	55	52	63	52	53
115	49	59	42	62	53	62
74	50	60	62	60	62	65
102	50	45	53	56	48	34
139	50	39	62	48	55	62
75	135	41	32	39	45	40
Mean	.. ..	54·5	53·5	58·3	55·9	55·1

Yield.—The mean diurnal variation in yield is shown graphically in Fig. 1; milk from subjects on the 21st post-partum day or later is shown separately from the earlier milk. In both series the yield at 6 a.m. was significantly greater (1%) than at the other feeds, which were not significantly different from each other, except for the 10 p.m. feed in the earlier milk (Table I). This feed had a significantly (1%) lower volume than the other feeds.

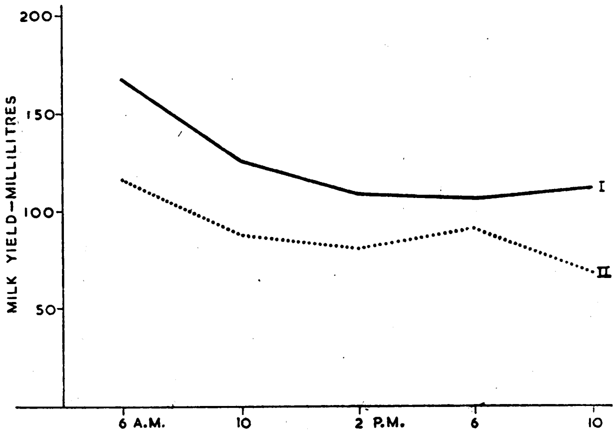

FIG. 1.—Mean diurnal variation in the yield of milk. Curve I represents milk from the 21st day of lactation or later, and curve II earlier milk.

Fat.—Fig. 2 shows mean variations throughout the 24 hours in the fat content of both early and more mature milk. Apart from the overall rise in fat content which occurs as the milk matures, the flat "peak" occurring in

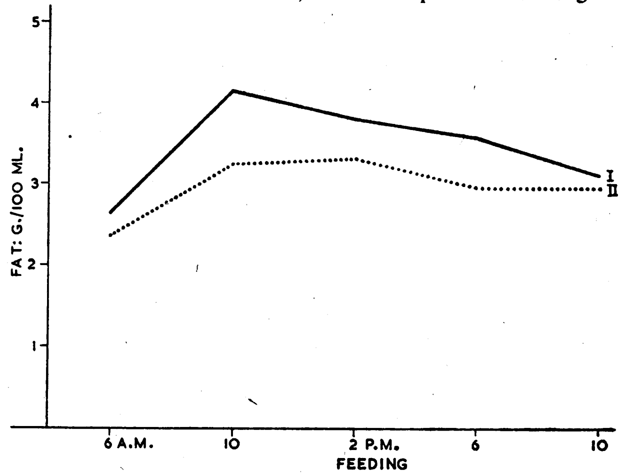

FIG. 2.—Mean diurnal variation in the fat content of milk. Curve I represents milk from the 21st day of lactation or later, and curve II earlier milk.

the middle of the day for early milk changes to the high 10 a.m. peak, which is generally described. There is a considerable spread of results about the means, as can be seen from Table II, but the variation at 6 a.m. in both series is significantly (5%) less than at any other feed. The early milk is notably more irregular in pattern than the more mature milk, but even in established lactation the rise from 6 a.m. to 10 a.m. is the only invariable characteristic.

Lactose.—There was no systematic change in the lactose content throughout the day, although differences of the order of 10% occurred in some cases between one feed and another (Table III). As with the fat, this haphazard irregularity was more noticeable in the early milk, and is clearly illustrated by Case 172. In this subject every feeding was analysed from the third to the seventh days, and the maximum value for lactose for each of these five days occurred at 10 a.m., 10 p.m., 2 p.m., 10 a.m., and 6 a.m.

Nitrogen.—There was no significant variation in the total nitrogen from feed to feed in the more mature milk, but in milk from the first week of lactation the decreasing nitrogen content characteristic of this period is so rapid that it is obvious even from feed to feed, and a progressive drop throughout the day can be seen (Table IV). In this small series the drop is not, however, statistically significant. Non-protein nitrogen, in general, remains almost constant through the day, although there are quite large variations in some cases (Table V). The differences between feeds are not statistically significant, and the slightly lower mean value at 10 a.m. is due to a few cases in which the value was much lower than the average. This may have occurred because the 10 a.m. specimen was usually analysed within one hour of its removal from the breast, whereas the other specimens were stored in a refrigerator for periods up to 20 hours, and some protein breakdown may have occurred.

Apart from the milk volume, which can be estimated with reasonable accuracy by test-weighing the infant, fat is the only variable of any practical consequence, and the results were examined to determine whether any particular feed had a fat content similar to the overall fat content of the whole 24-hour output. The fat contents were considered to be similar if they were within 0.1 g. per 100 ml. of each other. In 26 cases there was no one feed similar to the average for the 24 hours, and in 23 cases one feed was similar—the 6 a.m. in four cases, the 10 a.m. in five cases, the 2 p.m. in three cases, the 6 p.m. in five cases, and the 10 p.m. in six cases.

Discussion

The difficulties of obtaining milk samples which represent the normal yield have been stressed in Part I of this paper, and for this reason the average values, by ironing out such sampling irregularities as occur in the individual case, may have more real meaning. With the exception of the low 10 p.m. volume found in the earlier milk, the graphs in Fig. 1 show a levelling off during the day similar to those published by Deem; but although the volumes of the 10 a.m., 2 p.m., 6 p.m., and 10 p.m. feeds are almost constant, the volumes at 6 a.m. do not support her evidence of a constant hourly secretion rate in either the early or the more mature milk. There are, in fact, only three cases out of the 49 in which the 6 a.m. yield is double the yields of the other feeds. This does not, in itself, nullify the hypothesis, since the breast must have a limited capacity for milk storage, so that although most breasts can probably store four hours' full secretion, only a few may be able to accommodate twice that volume. A more detailed discussion of breast size and capacity will be presented in a later publication, but a striking illustration of this limiting effect is shown by Case 74. This woman had particularly small breasts, and her output at the five feedings of 114, 113, 117, 99, and 117 ml., strongly suggests a ceiling of milk storage.

The low fat content at 6 a.m. rising steeply to a maximum value at 10 a.m. and falling off during the rest of the day confirms previous results, although there have been no published descriptions of the variation in early milk and of the changing pattern as the milk matures. There is no evidence to support Ruzicic's (1936) belief that the variation is merely a reflection of meal-times, since all the subjects supplying early milk, and many of those giving mature samples, showed widely different patterns while living on an institutional diet which was uniform in composition and eaten at strictly regulated times. Nor does it seem to be an individual characteristic, since the same subject was able to produce different patterns on different days. For example, Case 35 on the twenty-first day had fat contents of 2.95, 4.15, 4.65, 3.65, and 3.30 g. per 100 ml., but on the thirty-seventh day produced 2.15, 5.30, 3.35, 3.35, and 2.75 g. per 100 ml. at the five feeding times. The pattern of variation was apparently unaffected by the level of total daily fat secretion, and two extreme cases—No. 41 with an overall fat content of 4.80 g./100 ml., and No. 115 with 1.45 g./100 ml.—both produced the characteristic changes throughout the day.

Bartlett (1929) found that the fat content of morning milk was particularly low in cows with small udders. He believed that the increased tension caused resorption of milk, but this would imply that the fat was selectively resorbed, which seems unlikely. In these series the low fat content at 6 a.m. occurred even when the breast was in no way distended or even apparently full.

Kon and Mawson (1950) found that the fat content tended to be high in samples taken within four hours of the last feed and low in samples taken after six hours. This result could be explained if the breasts had not been fully emptied, as is possible with manual expression, since the first milk of a breast left longer will contain less fat than that from a breast left only a short time (Part II of this paper).

It might seem at first sight that there is some interdependence of fat concentration and milk volume, so that the rising fat and the falling milk yield would combine to produce a constant amount of fat for each feed. Fig. 3

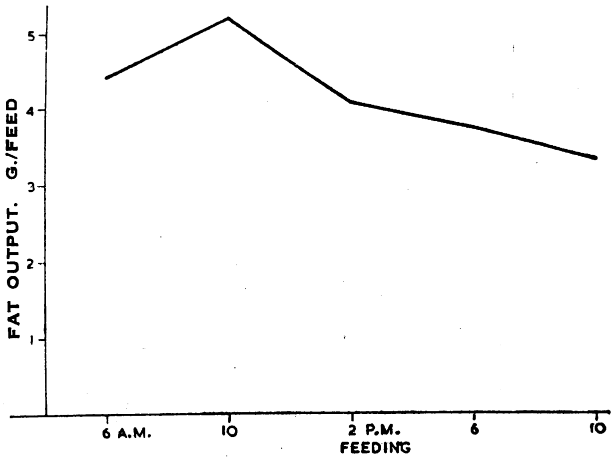

FIG. 3.—Mean diurnal variation in the output of fat in milk from the 21st day of lactation or later.

shows the mean quantities of fat at each feeding, in mature milk, and it can be seen that almost half the daily fat output is available in the first two feeds. There is no obvious physiological advantage to the infant in this arrangement.

It is clear from the above data that the total daily output of fat cannot be assessed accurately from the analysis of any single feed.

Summary

There is a very marked diurnal variation in both the volume and the fat content of human milk, but the other major constituents show no systematic change. The variation, particularly in fat content, is very irregular in milk from the first week of lactation, but it tends to conform to a pattern in the more mature milk.

The volume of milk obtained at a feeding may be due to a combination of two factors—the length of time since the last milking and the storage capacity of the breast—but no clear conclusion can be reached from the data presented.

The reason for the very substantial variation in the fat content remains obscure.

I am grateful to Professor Dugald Baird and to the staffs of the Midwifery Department, the Aberdeen Maternity Hospital, and the Royal Aberdeen Hospital for Sick Children for invaluable help; to Dr. I. Leitch, of the Commonwealth Bureau of Animal Nutrition, Bucksburn, for criticism and advice; and to Mr. W. Billewicz for statistical assistance.

REFERENCES

Bartlett, S. (1929). *J. agric. Sci.*, 19, 36.
Deem, H. E. (1931). *Arch. Dis. Childh.*, 6, 53.
Kon, S. K., and Mawson, E. H. (1950). *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 269.
Macy, I. G., Hunscher, H. A., Donelsen, E., and Nims, B. (1930). *Amer. J. Dis. Child.*, 39, 1186.
Morrison, S. D. (1952). *Human Milk: Yield, Proximate Principles and Inorganic Constituents.* Commonwealth Bur. Animal Nutrit. Tech. Commun. No. 18.
Ruzicic, U. S. (1936). *Mschr. Kinderheilk.*, 67, 415.