of pyruvate. When retina is incubated in Ringer phosphate solution the reverse is the case, for in the presence of glucose the rate of oxygen consumption is slightly depressed, although the initial rate is maintained for a longer period; addition of pyruvate causes a small rise in oxygen consumption.

2. Addition of monoiodoacetate at a concentration of mM causes only a small reduction in the amount of oxygen used during incubation in Ringer phosphate solution, although lactic acid production is strongly inhibited.

3. During incubation in Ringer bicarbonate solution containing glucose oxygen consumption is inhibited by 40% in a concentration of 0.1 mM-iodoacetate; an increase in concentration of iodoacetate to 1.0 mM does not bring about a significant further reduction in oxygen uptake.

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The Fatty Acids of Human Milk from Mothers on Diets Taken *ad libitum**

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The unique position of milk as a primary food and as a readily sampled product of active lipid metabolism emphasizes the desirability of obtaining detailed information on human-milk fatty acids. Fatty acids as glycerides comprise the bulk of milk lipids, less than 2% of which are cholesterol and phospholipid (Macy, Kelly & Sloan, 1953). The non-esterified fatty acids of milk, constituting about 2% of total milk lipids, have received slight attention. If analogous to those in serum (Dole, 1956; Gordon & Cherkas, 1956) they might have considerable physiological importance.

The major fatty acids of human milk lipids have been studied in a number of Laboratories (Bosworth, 1934; Hilditch & Meara, 1944; Baldwin & Longenecker, 1944; Brown & Orians, 1946).

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Analyses made by fractional distillation of the methyl esters, followed by crystallization and in some instances by spectroscopy, required sizeable quantities of starting materials; pooled milk specimens were used in all cases. Although studies on individual milks have not been feasible in such detail, Söderhjelm (1953) has defined the di- to hexa-ene contents of individual samples by ultraviolet spectrophotometry after isomerization with alkali. With gas-liquid chromatography of the methyl esters of fatty acids, introduced by James & Martin (1956) and improved by Lovelock & James (1959), precise all-inclusive analyses now can be made on multi-component mixtures totalling 50 µg. or less. Thus it becomes timely to refine the study of lactation by examining individual milks, in order to avoid any imprecisions due to pooling of samples.

The present paper lays a base for further study of physiological variations in human lactation by defining the fatty acid composition of the milk lipids of eleven women on diets taken *ad libitum*. Analyses were made by the combined approach of spectrophotometry and gas-liquid chromatography.

METHODS

Clinical procedure

Milk sampling. Specimens of milk were obtained from six normal women in hospital with their breast-fed newborn infants. These stays in hospital were arranged solely for carrying out biochemical studies on lactation, and the patients were confined to a rooming-in unit in a metabolic ward. Breast feedings were made every 3–4 hr., alternating the breast initially nursed. To make 24 hr. collections, nursing was interrupted and the breasts were emptied at regular nursing times with an electric pump, followed by manual expression as described by Davies (1945). Of the milk collected at each breast 10% was taken for analysis and the remainder fed by bottle to the infant. The individual samples were combined in a pool representing the 24 hr. production of each breast. All milk samples were refrigerated immediately after collection and the final pools were stored at -15° .

In addition, random specimens of milk were obtained from five women living at home. Milk was collected by manual expression from one or both breasts at the end of one or more feedings. The infants of these mothers all received varying amounts of supplementary feedings with milk substitutes or prepared infant foods. In these cases determinations of 24 hr. milk volumes were not made. All samples were refrigerated immediately upon collection and upon delivery to the Laboratory were stored at -15° .

Maternal diets. The patients in hospital were offered a diet of solid foods containing 2200–3000 kcal. with three servings of protein a day. Two of these servings were meat and one was egg, but milk or cheese was provided with each meal bringing the protein intake to 15% of total calories. Fat constituted about 40% of total calories, about half from milk and butter, one-third from meat and eggs and the remainder from vegetables. Precise food consumptions were not determined. The diets of mothers living outside the hospital were not controlled; however, they were not limited by economic factors.

Chemical methods

Extraction of lipids. The lipids in a sample (1 or 2 ml.) of milk were extracted with 25 vol. of 95% ethanol-ethyl ether (3:1, v/v). Solvent was removed from the filtered extract by low-temperature vacuum-distillation (Craig, Gregory & Hausmann, 1950). Extraction of the residue with light petroleum (b.p. 60–70°) yielded an extract free of non-lipid contaminants. The content of total lipids was determined microgravimetrically as described by Craig, Hausmann, Ahrens & Harfenist (1951). After saponification by refluxing with 5% KOH in absolute methanol for 2 hr., the mixture was acidified and the fatty acids were recovered. Their amount was determined microgravimetrically by the procedure of Ahrens & Craig (1952), in which only the long-chain acids remain to be weighed. All solvents were glass-distilled.

Recovery of fatty acids averaged 92% of the total lipid (theoretical recovery, 95.5% if all lipid were triolein), with losses to be expected during saponification and extraction of the acidified medium. The minimal disagreement between actual and theoretical recoveries indicated that short-chain water-soluble fatty acids (which would have been disregarded by our analytical method) were present only in very small amounts if at all.

The effectiveness of this extraction procedure was evaluated by comparison with three other methods: ethanol-ether extraction of Somogyi's (1930) zinc hydroxide coprecipitate of protein and lipid, chloroform-methanol extraction (Folch, Ascoli, Lees & Meath, 1951) followed by light petroleum rectification, and counter-current distribution (five transfers in a heptane-ether-ethanol-water system) as described by Blankenhorn & Ahrens (1955). Total lipids calculated from gravimetric results were essentially the same by all four methods. However, the small amount of phospholipid present in milk was most effectively extracted by the chloroform-methanol method (84 mg./100 ml. of milk) and less well by direct ethanol-ether extraction with rectification (42 mg.) and by extraction of the zinc-precipitated lipoproteins (12 mg.); the countercurrent-distribution procedure had been designed to avoid this material completely. In view of the small amounts of phospholipid we found in milk, also shown by Macy *et al.* (1953) and by Morrison (1952), our choice of the direct ethanol-ether method was based on merits of technical simplicity. Agreement of the results for total lipid obtained by the four methods indicated that all extracts were free of non-lipid material.

Analysis of fatty acid. The mixed fatty acids were analysed for their content of polyene classes by spectrophotometry and for concentrations of individual acids by gas-liquid chromatography. Alkali isomerization and ultraviolet spectrophotometry were performed on a microscale in duplicate according to Holman & Hayes (1958) and were corrected for preformed conjugation. For gas-liquid chromatography the acids were methylated by refluxing with 5% (v/v) conc. HCl-absolute methanol for 2 hr., but in some experiments the milk glycerides were methylated directly by inter-esterification with HCl-'superdry' methanol as described by Stoffel, Chu & Ahrens (1959). The methyl esters of C_{4-18} acids were chromatographed at 197° on a non-polar stationary phase, Apiezon M, with nitrogen as the mobile phase, with the gas-density balance detector of Martin & James (1956). Methyl esters of C_{19-22} acids were analysed by chromatography at 197° on Orr & Callen's (1959) polar stationary phase, Reoplex 400. The ionization chamber of Lovelock & James (1959) was used as a sensing device. The column efficiency with Apiezon was 3500–4000 theoretical plates, with Reoplex 2100 plates, calculated for methyl stearate according to Desty (1957). Separation factors of methyl oleate and stearate were 1.18 on Apiezon and 1.12 on Reoplex. The complementary data from these two chromatographic analyses were combined by using ratios of single components common to both. Major acids were identified by comparison with the retention volumes of pure standards, all volumes being measured from the air peak. A number of pure polyethenoid acids were identified on the basis of structural studies and chromatographic characteristics carried out in this Laboratory by Stoffel, Insull & Ahrens (1958).

RESULTS

The sources of the milk samples are described in Table 1. The six mothers in the hospital (mean age of 22 years) had been lactating for approximately 12 days. There was considerable variation from patient to patient in volumes of milk secreted in 24 hr. and in concentrations of milk fat; moreover, the simultaneous yields of the two breasts were not similar. However, the values of milk and milk-fat production are all within the normal ranges recorded by Macy *et al.* (1953) and by Morrison (1952). The five out-patients (mean age of 27 years) had been lactating for 35–180 days. The fat concentration in their milk samples was lower than that in the milk of the hospitalized patients.

The proportions of polyene acids in the milk

fatty acids, determined by alkali-isomerization and spectrophotometry, are shown in Table 2. All polyenes from dienes to hexaenes were present. Analyses for the two groups of patients were very similar in concentrations of total polyenes: 9.9 % of total acids in the hospitalized patients, 10.3 % in the out-patients. The in-patients had a lower mean proportion of dienes and higher mean proportions of trienes, tetraenes, pentaenes and hexaenes, but the differences were not statistically significant. In each patient milks collected simultaneously from the two breasts showed almost identical polyene analyses.

Gas-liquid chromatography of the fatty acid methyl esters revealed the presence of at least thirty-nine different acids (Table 3). Many of these could not be identified on the basis of retention

Table 1. *Description of individual human milk samples collected from eleven normal mothers on diets taken ad libitum*

Clinical data on mothers				Milk samples						
Patient number, initials and age (years)			No. of pregnancy	Study environment	Period post-partum (days)	Volume (ml./24 hr.)		Fat content (g./100 ml. of milk)		
						Right breast	Left breast	Right breast	Left breast	
1	R.Q.	20	1	Metabolic ward	21	67	—	4.68	—	
2*	H.L.	21	1		10	534	413	5.09	5.47	
3*	N.W.	21	1		15	228	338	2.04	2.16	
4	M.T.	23	1		9	239	185	2.27	1.98	
5*	C.M.	23	1		11	243	287	1.37	1.79	
6	E.M.	23	3		10	464	335	3.38	3.07	
7	P.E.	20	2	Home	35	—	—	0.81	—	
8	J.H.	27	1		130	—	—	—	0.63	—
9	L.P.	27	3		60	—	—	—	0.96	—
10	E.J.	29	1		40	—	—	—	2.14	—
11	P.I.	30	1		180	—	—	—	0.66	—

* Negro patient.

Table 2. *Polyene acids as percentages of total fatty acids*

Separate analyses of milks of right and left breasts were averaged in patients 2–6.

Patient number	Dienes	Trienes	Tetraenes	Pentaenes	Hexaenes	Total polyenes	Trihexaenes
In-patients							
1	7.91	1.32	0.72	0.28	0.32	10.55	2.64
2	8.39	1.11	0.68	0.26	0.35	10.79	2.40
3	5.84	1.20	0.73	0.28	0.20	8.25	2.41
4	7.97	1.28	0.89	0.36	0.45	10.95	2.98
5	6.57	1.15	0.73	0.28	0.33	9.06	2.49
6	7.69	1.02	0.69	0.30	0.34	10.04	2.35
Mean	7.40	1.18	0.74	0.29	0.33	9.94	2.55
S.D.	2.19	0.25	0.17	0.08	0.18	2.40	0.53
Out-patients							
7	7.15	0.99	0.60	0.23	0.20	9.17	2.02
8	6.45	1.10	0.42	0.23	0.05	8.25	1.80
9	11.00	1.22	0.45	0.21	0.08	12.96	1.96
10	7.70	1.00	0.48	0.19	0.20	9.57	1.87
11	9.99	0.76	0.40	0.21	0.31	11.67	1.68
Mean	8.46	1.01	0.47	0.21	0.17	10.32	1.87
S.D.	3.89	0.34	0.16	0.03	0.21	3.87	0.27

Table 3. *Fatty acid patterns in milk lipids of eleven mothers on diets taken ad libitum*

Values are expressed as percentages of total fatty acid methyl esters. Current assignment of structure is given under 'Shorthand designation'. Retention volumes on two stationary phases were determined as described by Stoffel, Insull & Ahrens (1958).

Fatty acid			Retention volumes relative to methyl stearate				Means										
No.	Familiar name	Shorthand designation	Apiezon M		Reoplex 400	In-patients					Out-patients				In-patients (6)	Out-patients (5)	Entire group (11)
			1	2		3	4	5	6	7	8	9	10	11			
1	—	<10: 0	—	0.50	0.31	0.22	0.11	0.20	0.15	0.10	0.08	0.18	0.21	0.86	0.26	0.26	
2	Capric (decanoic)	10: 0	0.029	2.80	1.66	2.39	1.05	2.17	0.81	1.42	0.92	1.57	1.98	1.13	1.40	1.63	
3	Undecanoic	11: 0	0.047	—	—	—	—	—	—	—	0.01	—	—	—	0.001	0.001	
4	—	12: hbr?	0.054	—	—	—	—	—	—	—	0.01	—	—	—	0.001	0.001	
5	Lauric	12: 0	0.075	8.46	8.36	9.99	3.82	6.34	5.02	4.89	4.86	7.07	10.75	5.63	6.64	6.84	
6	—	12: br?	0.087	—	—	—	—	—	—	—	0.04	—	—	—	0.004	0.004	
7	Tridecanoic	13: 0	0.112	—	—	0.06	—	—	—	—	—	—	0.09	—	0.006	0.006	
8	Myristic	14: 0	0.174	9.31	10.13	12.11	5.99	8.19	6.84	5.94	8.04	10.14	9.85	7.21	8.24	8.52	
9	Myristoleic	14: 1	0.159	0.30	—	0.17	0.20	0.36	0.35	0.23	0.26	0.26	0.18	0.10	0.21	0.24	
10	—	14: ?	0.147	tr.	—	—	—	—	—	0.05	0.09	—	—	0.08	0.07	0.08	
11	Pentadecanoic	15: 0	0.270	0.49	0.63	0.43	0.50	0.59	0.67	0.58	0.53	0.58	0.43	0.44	0.52	0.53	
12	—	15: br?	0.233	0.37	0.30	0.52	0.53	0.48	0.47	0.30	0.27	0.33	0.28	0.39	0.45	0.39	
13	Palmitic	16: 0	0.419	21.48	19.12	20.62	21.79	19.93	23.30	21.11	18.92	24.58	17.64	21.17	21.04	20.88	
14	Palmitoleic	16: 1	0.366	1.94	1.69	1.74	3.00	1.81	3.17	3.41	3.63	2.45	1.83	2.07	2.23	2.43	
15	Heptadecanoic	17: 0	0.649	0.88	0.86	0.62	0.31	0.48	0.53	0.33	0.39	0.35	0.14	0.70	0.53	0.46	
16	—	17: br?	0.555	0.54	0.69	0.47	0.53	1.12	0.99	0.57	0.87	0.76	0.57	0.87	0.72	0.73	
17	Stearic	18: 0	1.00	6.42	7.50	6.98	6.60	7.88	8.04	6.48	7.74	7.67	6.43	8.95	7.24	7.34	
18	isoOleic	18: 1 iso	0.896	6.84	5.42	9.05	7.83	6.53	5.02	8.10	4.09	3.84	8.64	6.10	6.78	6.50	
19	Oleic	18: 1	0.860	28.36	25.85	25.93	33.26	30.25	29.63	32.41	32.49	24.14	29.37	31.05	28.88	29.34	
20	Linoleic	18: 2	0.801	5.63	7.80	4.27	7.00	6.61	6.98	7.71	8.83	9.76	6.43	7.24	6.35	7.09	
21	—	un	—	1.48-1.50	0.82	0.84	0.29	0.06	0.56	0.69	0.71	0.81	0.99	0.81	0.54	0.67	
22	Linolenic	18: 3	0.801	1.67-1.69	0.55	1.07	0.54	0.98	0.99	0.93	0.90	0.86	0.67	1.02	0.84	0.85	
23	—	20: 0	2.38	1.76-1.78	0.55	1.60	0.95	1.17	1.46	1.60	1.30	1.68	1.05	1.99	1.41	1.31	
24	—	20: 1	1.99	1.97-1.99	0.75	1.55	0.59	1.06	0.80	1.10	0.84	0.99	0.64	0.67	0.84	0.89	
25	—	un	—	2.10-2.14	0.59	0.35	0.24	0.33	0.26	0.33	0.25	0.25	0.18	0.27	0.20	0.30	
26	—	20: 2?	—	2.24-2.26	0.73	0.23	0.16	0.38	0.24	0.25	0.15	0.20	0.12	0.21	0.15	0.25	
27	—	21: 0	—	2.38-2.40	0.46	0.93	0.28	0.68	0.36	0.67	0.38	0.49	0.46	0.21	0.50	0.48	
28	—	20: 3	1.64	2.68-2.70	0.31	0.81	0.19	0.63	0.36	0.49	0.28	0.34	0.59	0.34	0.20	0.41	
29	Arachidonic	20: 4	1.46	2.95-2.98	0.21	1.17	0.34	0.89	0.72	0.69	0.53	0.43	0.52	0.38	0.35	0.45	
30	—	un	—	3.18-3.25	0.05	0.20	0.09	0.15	0.10	0.14	0.12	0.15	0.09	0.08	0.06	0.11	
31	—	22: 1?	—	3.40-3.41	—	tr.	—	—	0.07	0.11	tr.	0.09	0.09	0.06	0.06	0.07	
32	—	un	—	3.56-3.60	0.06	0.08	0.04	0.16	0.10	0.11	0.06	0.79	tr.	0.08	0.09	0.16	
33	—	20: 5	1.46	3.69-3.78	—	0.05	0.04	0.12	0.10	0.08	0.04	0.07	0.23	0.02	0.05	0.08	
34	—	un	—	4.25-4.33	0.02	1.20	0.05	—	tr.	0.06	0.06	0.10	0.05	—	0.09	0.20	
35	—	un	—	5.32-5.39	0.09	0.24	0.09	0.16	0.11	0.09	0.09	0.19	0.10	0.06	0.11	0.12	
36	—	un	—	5.74-5.90	0.10	0.33	0.16	0.10	0.09	0.24	0.17	—	0.23	0.04	0.13	0.13	
37	—	un	—	6.35-6.42	—	tr.	—	tr.	—	0.09	—	—	—	—	0.17	0.13	
38	—	22: 5	3.30	6.75-6.85	0.06	0.15	0.13	0.19	0.14	0.09	0.10	0.17	0.18	0.06	0.13	0.30	
39	—	22: 6	3.00	7.51-7.60	0.17	0.62	0.21	0.50	0.45	0.34	0.30	0.29	0.12	0.13	0.21	0.30	

volumes established to date, and are shown in Table 3 in terms of retention volumes on Reoplex relative to methyl stearate. Since the normal saturated C_{20} acid has a retention volume of $C_{18} \times 1.78$, whereas $n-C_{22} = C_{18} \times 3.17$ and $n-C_{24} = C_{18} \times 5.65$, it is evident that most of the unidentified acids are C_{18-22} unsaturated acids. Eight acids constituted 88.4 % of the total; oleic 29, palmitic 21, myristic 9, linoleic 7, stearic 7, lauric 7, *iso*oleic 7, and palmitoleic acid 2 % of the total. Degradation studies carried out according to James & Webb (1957) showed that a significant part of the *iso*oleic acid is octadeca-11-enoic acid. This acid appeared as a shoulder on the descending limb of the oleate curve on Apiezon, but was poorly resolved from oleic acid on Reoplex. Only 0.5 % of the total acids were C_{10} or shorter. About 2 % of the total acids were odd-numbered, and most of these were C_{15} and C_{17} acids.

About 5 % of total fatty acids were C_{19-22} ; they numbered 17 acids. A number of C_{20} and C_{22} unsaturated acids were recognized which had previously been identified in menhaden body oil by Stoffel *et al.* (1958). These are shown in Table 3 by specific numerical designations. At least eight other unsaturated acids, marked *un* in Table 3, have not been characterized by chain length or double-bond structure.

DISCUSSION

Analysis of the polyethenoid fatty acids of milk by spectrophotometry and gas-liquid chromatography offers an unique opportunity for rigorous comparison of these two techniques. Results on a variety

of human milks obtained under experimental conditions (W. Insull & E. H. Ahrens, unpublished work) are presented in Table 4. Since linoleate and linolenate overlap when gas-liquid chromatography is carried out on Apiezon M, the sum of these two esters was compared by the two methods of analysis; agreement in results is seen to be excellent. By contrast, when chromatography is carried out on Reoplex 400, the linoleate-linolenate overlap is easily resolved. In addition, the entire range of polyenes can be evaluated by the two methods, the results in Tables 2 and 3 being used. This comparison (Table 5) is made with the full realization that many of the C_{19-22} polyenes remain unidentified and that the extinction coefficients used for calculation of these polyenes have not been determined. Nevertheless the two methods of analysis give results on the proportions of trienes, tetraenes, pentaenes and hexaenes which are reassuringly similar in order of magnitude.

Our analyses of individual milk specimens by gas-liquid chromatography agree with previous studies of pooled milk samples in the proportions of major components (Table 6). Furthermore, analyses on the milks of the two breasts collected simultaneously were nearly identical in every instance. The data assembled from the literature indicate that eight acids characteristically constitute about 90 % of the total acids in human milk. The total of these eight acids was only 89 % in the present study, the lowest yet reported, perhaps because our analyses demonstrated a number of acids previously overlooked. C_{8-9} acids were present only in traces, and there was indirect evidence that acids shorter than C_8 , if present,

Table 4. Comparison between gas-liquid chromatographic and spectrophotometric estimations of dienes and trienes in human milk

Results are expressed as percentages of total acids. All differences (last column) were statistically insignificant.

Source of fat in maternal diet	Number of analyses	Spectrophotometric			Chromatographic (Linoleic + linolenic)	Difference between methods
		Dienes	Trienes	Di- + tri-enes		
Mixed diet	11	8.41	1.05	9.46	8.96	-0.50
Maize oil	12	41.62	1.15	42.77	42.88	+0.11
Linseed oil	6	8.09	36.58	44.67	42.95	-1.72

Table 5. Comparison between gas-liquid chromatographic and spectrophotometric estimations of all polyenes in human milks from eleven mothers on diets taken ad libitum

Results are expressed as percentages of total acids.

Method	Dienes	Trienes	Tetraenes	Pentaenes	Hexaenes
Spectrophotometric (Table 2)	7.88	1.10	0.62	0.25	0.26
Gas-liquid chromatography (Table 3)*	7.34	1.26	0.57	0.21	0.30

* Totals from Table 3 are: dienes (acids 20 + 26); trienes (acids 22 + 28); tetraenes (acid 29); pentaenes (acids 33 + 38); hexaenes (acid 39).

occurred only in very small proportions. Monoenoic C_{10-18} acids were found regularly. Odd-numbered acids C_{9-17} were always present, as noted by James, Webb, Stapleton & McDonald (1958). Long-chain branched acids, isolated and positively identified by Shorland (1955) in butter and mutton fat, were recognized by James and co-workers (James *et al.* 1958) in their gas-liquid chromatographic analyses of goat milk and human sebum (James & Wheatley, 1956); our results suggest their presence in human milk in small amounts. We have noted a wide array of C_{19-23} unsaturated acids and the regular occurrence of one or more oleic acid isomers.

The lactation of eleven in- and out-patients was fully established, as judged by Morrison's evaluation of daily milk-production figures. Since samples from the in-patients were obtained after 10 days of lactation whereas the out-patients had been lactating 35-180 days, some interesting comparisons can be made between the two groups. Despite wide differences in fat content of the milk samples of the two groups, the fatty acid compositions differed only to a minor degree. The question can be raised whether the random samples obtained from the out-patients group can properly be compared with the truly representative samples of 24 hr. output of the in-patients. A partial answer to this query is found in our preliminary comparisons of fatty acid patterns of milks obtained at the onset and at the end of a nursing period: no differences were seen. Nor have we been able to demonstrate any diurnal variation in milk-fatty acid patterns of mothers fed with experimental diets containing 40 % of calories as fat, although some variation was seen when mothers ingested fat-free diets.

The present analyses of the proportions of major and minor components in human milk of mothers fed *ad libitum* form a basis for comparison with milks produced under experimental conditions.

Studies by Insull, Hirsch, James & Ahrens (1959) have shown that wide variations in human-milk-fat composition can be produced by alterations of caloric intakes as well as by exchange of dietary fats. Under rigidly controlled experimental conditions it was demonstrated that dietary fatty acids are rapidly transferred into the milk lipids. Thus within 2 or 3 days the character of the breast-milk fat can be radically changed to mimic the dietary fat. Moreover, when too few calories are fed to maintain the mother's body weight, adipose tissue fatty acids are rapidly transferred into the milk lipids. However, when excess of calories of a fat-free diet are fed, synthesis of fat from carbohydrate occurs in the breast, and milk of a very distinctive type is produced, poor in all unsaturated acids and rich in C_{12} , C_{14} and C_{16} saturated acids.

With these facts in mind it may seem remarkable that the milk fatty acids of mothers on diets taken *ad libitum* are so similar, whether in the early or late post-partum period. In part, the resemblances between the data presented by four Laboratories (Table 6) might be due to qualitative similarities in the dietary fats of the mothers who furnished the milk for these studies. Excluding the unusual circumstance of a nursing mother gaining weight on a very low fat diet, it is reasonable to assume that under ordinary conditions the major determinant of milk-fatty acid composition is the nature and quantity of the maternal dietary-fat intake. However, from one meal to the next there must be marked qualitative differences in maternal dietary fats. Nevertheless, the full effect of these alterations is not manifested in the breast milk within hours, but only in days. Thus the irregularities due to varying intakes are evened out by the time the dietary fatty acids are transported into the milk. It seems necessary to postulate that the dietary fatty acids are first transported into some intermediate pool of considerable size and later into the

Table 6. *Major fatty acids in human milk*

Results are expressed as percentages (by wt.) of total fatty acid methyl esters.

Acid	Pooled milk			Present results (averages of 11 individual samples)
	Baldwin & Longenecker (1944) (mature milk)	Hilditch & Meara (1944) (late milk)	Brown & Orians (1946) (sample I)	
Lauric	5.5	7.0	5.4	7.0
Myristic	8.5	13.9	6.7	8.8
Palmitic	23.2	24.1	21.0	21.0
Palmitoleic	3.0	2.8	2.7	2.2
Stearic	6.9	9.6	6.8	7.2
Oleic	36.5	30.2	39.1	35.8*
Linoleic	7.8	5.5	10.3	6.4
Total	91.4	93.1	92.0	88.4

* Total of acids numbered 18 and 19 in Table 3.

milk. Whether this pool is the mammary gland itself or some other depot fat cannot be answered at present. However, in such a pool the qualitative differences in the fatty acids eaten at one or another time would be averaged. Thus the milk lipids, formed predominantly by transference from this pool, must represent the last stage of a process which is not unlike that of a 'moving average' in statistics. The advantage of this seemingly homeostatic mechanism to the child is obvious.

SUMMARY

1. The fatty acid compositions of human milk obtained on individual samples from eleven mothers on diets taken *ad libitum* were determined by gas-liquid chromatography and by ultraviolet spectrophotometry after isomerization of polyenoic acids with alkali.

2. The patterns of acids from the two breasts were nearly identical. A major proportion (88.4 %, calculated as the methyl esters) was made up of eight components: oleic 29 %, palmitic 21 %, myristic 9 %, linoleic 7 %, stearic 7 %, lauric 7 %, isooleic 7 %, and palmitoleic 2 % of the total. However, at least 30 other acids were seen in minor amounts, odd-numbered and branched-chain acids as well as a wide variety of C_{18-29} unsaturated acids. The two complementary methods of analysis produced results in reasonable agreement.

3. The fatty acid composition of milks of mothers in the early and late post-partum periods were very similar, although the fat concentrations of the samples differed greatly.

4. It is suggested that the similarities in milk-fatty acid patterns of the in- and out-patients were due in part to similarities in the quality of dietary fat ingested by the two groups. However, in addition, the existence of a pool intermediate between dietary fat and milk fat is postulated, which acts to buffer radical changes in milk-fatty acid composition under ordinary circumstances.

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