

DIET AND FATTY ACID DISTRIBUTION IN SUBCUTANEOUS FAT AND IN THE CHOLESTEROL-TRIGLYCERIDE FRACTION OF SERUM OF YOUNG INFANTS *

By M. J. SWEENEY, J. N. ETTELDORF, L. J. THROOP, D. L. TIMMA, AND
E. L. WRENN

(From the Pediatric Research Laboratory, Division of Pediatrics, College of Medicine, University of Tennessee, and Frank T. Tobey Memorial Children's Hospital, City of Memphis Hospitals, Le Bonheur Children's Hospital, St. Joseph's Hospital, and St. Peter's Orphanage, Memphis, Tenn.; and the Mead Johnson Research Center, Evansville, Ind.)

(Submitted for publication June 19, 1962; accepted September 1, 1962)

The purpose of this paper is to report the fatty acid distribution in subcutaneous fat and in the cholesterol-triglyceride fraction of serum (C-TG) of four groups of infants, each of whom received a different test diet during the first 2 months of life. The major variables in the test diets were: linoleic acid content (approximately 1.2 to 39.2 per cent of dietary fat); percentage of total calories derived from fat (approximately 35 to 50 per cent); main source of dietary protein (cow milk and soybean); and percentage of total calories derived from protein (approximately 9 to 19 per cent). The protein and lipoprotein electrophoretic patterns, total protein, total lipid, and fractional lipid concentrations in the sera of these same groups of infants were subjects of previous reports (1, 2).

SUBJECTS, PROCEDURES, AND METHODS

Subjects. The test subjects were full-term infants born at St. Joseph's Hospital in Memphis, Tennessee, and housed, until adoption or other disposition, in the nursery of St. Peter's Orphanage, Memphis. All infants were placed in the study as soon after birth as possible and in no instance later than the fifth day of life.

Procedures. Four different test diets were employed, and the infants were divided into four groups accordingly. Once assigned to a test diet, each infant remained so until discharged from the study. The diets were fed to the infants in ad lib amounts.

Diet I was derived entirely from animal sources. It included a 20 calories per ounce evaporated milk, water, and lactose formula deriving approximately 15 per cent of its calories from cow milk protein, 35 per cent from butter fat, and 50 per cent from lactose; meats (started at age 6 weeks); and 0.6 cc of supplement¹ containing

vitamin A, 5,000 U, vitamin D, 1,000 U, and ascorbic acid, 50 mg (started at age 4 weeks).

Diet II was derived entirely from nonanimal sources. It included a 20 calories per ounce soybean formula¹ deriving 19 per cent of its calories from soy protein, 35 per cent from a blend of soy and coconut oils, and 46 per cent from a mixture of dextrans, maltose, and sucrose; cereals, fruits, and vegetables (started progressively at age 6 weeks); and the multivitamin supplement.

Diet III was derived from both animal and nonanimal sources. It included a 20 calories per ounce formula¹ deriving 14 per cent of its calories from cow milk protein, 40 per cent from a blend of corn, coconut, and olive oils, and 46 per cent from a mixture of lactose, dextrans, and maltose; meats, cereals, fruits, and vegetables (started progressively at age 6 weeks); and the multivitamin supplement.

Diet IV was derived from both animal and nonanimal sources. It included a 20 calories per ounce formula¹ deriving 9 per cent of its calories from cow milk protein, 50 per cent from a blend of destearinated beef fat, coconut and corn oils, and 41 per cent from lactose; meats, cereals, fruits, and vegetables (started progressively at age 6 weeks); and the multivitamin supplement.

Linoleic acid composed about 1.2 per cent of the fat contained in the formula used in diet I, 39.2 per cent of the fat in the formula used in diet II, 30.1 per cent of the fat in the formula used in diet III, and 21.7 per cent of the fat in the formula used in diet IV. The complete fatty acid spectra of the fat in the various test formulas are shown in Table I. The fatty acid spectra of the fat in the foods started at age 6 weeks was not determined.

Whenever feasible, samples of maternal venous blood and mixed arterial and cord blood were obtained at the time of the infant's birth. Venous blood samples were obtained from the infants at age 5 to 14 days and again during the sixth or seventh week of life. Subcutaneous fat samples were obtained from episiotomy wounds of mothers at the time of parturition and by surgical biopsy from the buttocks of infants during the first or second week of life and again during the sixth or seventh week of life. Most of the infants were discharged from the study within a few days after the second blood and subcutaneous fat samples were obtained. Two infants, how-

* These studies were supported by U. S. Public Health Service Grants HTS-5285 and H-454 and a grant-in-aid from Mead Johnson & Co., Evansville, Ind.

¹ Supplied by Mead Johnson & Co., Evansville, Ind.

TABLE I
Percentages of fatty acids in the fat of the formulas used in the four test diets

Fatty acids	Diet I	Diet II	Diet III	Diet IV
Saturated				
Butyric C4:0	6.4			0.5
Caproic C6:0	1.5		Trace	2.3
Caprylic C8:0	1.6	1.3	2.2	2.3
Capric C10:0	3.5	1.3	2.4	1.6
Lauric C12:0	4.0	7.6	13.4	9.3
Myristic C14:0	9.6	2.9	4.8	5.6
Palmitic C16:0	26.5	11.1	8.3	16.5
Stearic C18:0	14.9	4.4	3.1	10.1
Total saturated	68.0	28.6	34.2	48.2
Unsaturated				
Lauroleic C12:1				0.2
Myristoleic C14:1	0.8			0.8
Palmitoleic C16:1	5.0		Trace	0.8
Oleic C18:1	25.9	25.7	35.5	29.0
Linoleic C18:2	1.2	39.2	30.1	21.7
Linolenic C18:3		6.4		
Total unsaturated	32.9	71.3	65.6	51.7
Fat content (g/100 ml)	2.6	2.6	2.8	3.7

ever, one receiving diet I and one receiving diet II, remained in the study until nearly age 1 year. Blood and fat samples were obtained from infants 3 to 6 hours after the last feeding.

As indicated above, solid foods were not introduced into the infants' diet until after day 42 of life. Except for the two infants who remained in the study for a relatively long time, none of the infants received more than one type of solid food (meat for those receiving diet I and prepared cereals for those receiving diets II, III, and IV) before discharge from the study. Relative to the volume of formula received each day, the quantity of solid food offered was small.

Methods. Extracts containing the cholesterol-triglyceride (C-TG) fraction of serum lipid were obtained by the method of Van Handel and Zilversmit (3).

Extracts containing the lipid from subcutaneous fat were obtained as follows: samples of fat were ground with a mortar and pestle in 45 ml of a 1:2 methanol-chloroform mixture, filtered and brought to a 50-ml vol-

ume with the methanol-chloroform mixture. Analyses of the extracts revealed that 98 to 99 per cent of the contained lipid was triglyceride. The gas chromatographic analyses reported below were performed on these extracts and thus are essentially those for the triglyceride component of subcutaneous fat.

The methanol-chloroform extracts of subcutaneous fat and the cholesterol-triglyceride fraction of serum were prepared for gas chromatography in the following manner. The extracts were transferred to 3- \times - $\frac{3}{4}$ -inch ground joint test tubes. The solvents were evaporated in an oil bath at 80° C with the aid of a stream of nitrogen. Five ml of a mixture, prepared by adding 1 ml of concentrated HCl and 4 ml of acetone dimethyl acetal to 20 ml of methanol, was added to each tube. The tubes were fitted with a 4-inch water condenser and a calcium chloride drying tube. After refluxing for one hour, 10 ml of water was added and the methyl esters extracted with three 5 ml portions of petroleum ether. The ether extracts were evaporated, and the methyl esters frozen until chromatographed.

Gas chromatography of the fatty acid methyl esters was carried out in the following manner. The chromatograph used was a Beckman GC-2 equipped with an 8-foot, 20 per cent diethylene glycol succinate on 30-60 mesh firebrick column. The helium flow rate was 100 ml per minute, and the column temperature was 210° C. The methyl esters through C18:2 were eluted in 40 minutes. The subcutaneous fat samples were analyzed by using a thermal conductivity detector, current 250 ma. The serum samples were analyzed with a hydrogen flame detector. The column composition, its temperature, and helium flow rates were identical for both detector systems. The linearity of detector response was established by observing instrument response for various quantities of a synthetic mixture of fatty acids which included the range of fatty acids reported.

The system for identifying the various fatty acids in the remainder of the manuscript is that suggested by Dole and co-workers (4), i.e., the letter C and the first figure denote length of the carbon chain; the second figure denotes the number of double bonds. For example, linoleic acid is designated as C18:2. The values reported below are in terms of saturated fatty acids with 12, 14, 16, and 18 carbon atoms, monounsaturated fatty

TABLE II
Percentage distribution of fatty acids C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2 in the formulas employed in the test diets*

Diet	Adjusted fatty acid distribution in formulas employed in test diets								Fatty acid distribution: saturated, monounsaturated, and diunsaturated acids		
	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	S	US:1	US:2
I	4.6	10.9	0.9	30.1	5.7	16.9	29.5	1.4	62.5	36.1	1.4
II	8.4	3.2	0.0	12.2	0.0	4.8	28.3	43.1	28.6	28.3	43.1
III	14.1	5.0	0.0	8.7	0.0	3.3	37.3	31.6	31.1	37.3	31.6
IV	10.0	6.0	0.2	17.7	0.9	10.8	31.1	23.3	44.5	32.2	23.3

* Values adjusted from those shown in Table I so as to total 100 per cent.

TABLE III

Mean values for percentage distribution of fatty acids C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2 in the cholesterol-triglyceride fraction of serum of the test subjects*

Group	Mean age		Fatty acid percentage distribution†								Fatty acid distribution: saturated, monounsaturated, and diunsaturated acids		
			C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	S	US:1	US:2
1. Maternal	no.	days	0.9 ±0.1	2.6 ±0.2	0.9 ±0.1	24.7 ±0.5	6.3 ±0.2	4.4 ±0.2	37.7 ±0.6	22.5 ±0.5	32.6	44.9	22.5
2. Cord blood	7	0	2.1 ±0.2	5.4 ±0.8	2.8 ±0.6	26.8 ±1.0	12.2 ±0.6	6.8 ±0.8	31.1 ±1.7	12.8 ±0.8	41.1	46.1	12.8
3. Diet I	6	44	2.3 ±0.7	5.9 ±0.3	1.8 ±0.2	28.5 ±1.3	8.9 ±0.2	6.5 ±0.4	39.2 ±1.7	6.9 ±0.6	43.2	49.9	6.9
4. Diet II	6	48	2.8 ±0.3	4.1 ±0.3	1.9 ±0.2	21.1 ±0.8	6.5 ±0.8	6.0 ±0.7	23.3 ±0.4	34.3 ±1.6	34.0	31.7	34.3
5. Diet III	4	47	3.8 ±0.5	6.6 ±1.0	6.4 ±1.6	18.2 ±1.6	4.3 ±0.9	2.6 ±0.5	18.5 ±2.7	39.6 ±3.4	31.2	29.2	39.6
6. Diet IV	6	48	4.4 ±0.4	4.1 ±0.3	1.4 ±0.2	19.8 ±1.0	5.8 ±0.6	5.2 ±0.5	28.8 ±1.3	30.5 ±1.2	33.5	36.0	30.5

* The sum of the individual values equals 100 per cent.

† Values recorded are means and standard error of the means.

acids with 14, 16, and 18 carbon atoms, and diunsaturated fatty acids with 18 carbon atoms. The percentage distributions of these eight fatty acids were adjusted so that the sum of their individual values equaled 100 per cent. For purposes of comparison, a similar adjustment was made for the fatty acid distribution in the fats provided by the formulas of the four test diets as shown in Table II.

The analytical figures given for dietary fat (Table II), C-TG fraction of serum (Tables III and IV), and subcutaneous fat (Tables V, VI, and VII), i.e., the percentage distribution of the eight fatty acids listed above, refer to the percentage of the total weight of all eight acids represented by the weight of each of the acids; the sum of the percentage values for each of the acids equals 100 per cent. Since these figures are relative rather than absolute, they provide no information concerning 1) the actual quantity (weight) of each fatty acid contained in the test samples, 2) the concentration per unit volume, or the total quantity of the C-TG contained in the serum, 3) the total quantity of depot fat, or 4) the total quantity of any of the eight acids contained in either serum C-TG or depot fat. Relative molar relationships among

the eight fatty acids can be derived from these data but are not included in this paper.

Presentation of the results of this study, therefore, is limited to comparisons of 1) the relative percentages of each of the above eight fatty acids and 2) the over-all patterns characterizing the percentage distribution of all eight acids in the serum C-TG, subcutaneous fat, and dietary fat of the various test groups. Statistical evaluation of the differences among mean values found for each fatty acid in the various groups of test samples was performed. These calculations provided no more meaningful information than that obtained by simple visual inspection of the data and are not included in this report. Attempts to mathematically characterize or statistically evaluate the various over-all fatty acid patterns so as to obtain more information than that provided by visual inspection, likewise, were unproductive.

RESULTS

Cholesterol-triglyceride fraction of serum lipid. Fatty acid percentage distributions in the C-TG

TABLE IV

Mean values for percentage distribution of fatty acids C12:0, C14:0, C14:2, C16:0, C16:1, C18:0, C18:1 and C18:2, in the cholesterol-triglyceride fraction of the sera of the test subjects during the second week of life

Group	Mean age		Fatty acid percentage distribution*							
			C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
Diet I	no.	days	1.8	6.8	2.4	27.1	9.2	6.9	37.8	8.0
Diet II	4	9.0	2.7	3.2	1.4	21.5	7.4	6.7	25.4	31.7
Diet III	6	9.8	5.3	5.9	3.2	19.4	5.4	3.9	27.3	29.6
Diet IV	2	9.5	3.7	3.9	1.8	21.9	7.9	5.9	27.4	27.5

* Values recorded are means.

TABLE V
Mean values for percentage distribution of fatty acids C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2 in the subcutaneous fat of the test subjects*

Group	Mean age		Fatty acid percentage distribution†								Fatty acid distribution: saturated, monounsaturated, and diunsaturated acids		
			C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	S	US:1	US:2
1. Maternal	no. 10	days	0.6 ±0.1	2.9 ±0.2	0.7 ±0.2	23.4 ±1.1	6.7 ±0.5	4.4 ±0.6	50.7 ±1.2	10.6 ±0.9	31.3	58.1	10.6
2. Infant (combined), wk 1	10	5.5	0.3 ±0.1	3.4 ±0.1	0.8 ±0.1	45.1 ±1.0	15.3 ±0.4	3.0 ±0.2	29.8 ±1.3	2.3 ±0.2	51.8	45.9	2.3
3. Diet I	7	43	1.4 ±0.1	7.8 ±0.4	2.1 ±0.1	37.3 ±1.1	13.5 ±0.8	2.8 ±0.5	32.6 ±2.0	2.5 ±0.3	49.3	48.2	2.5
4. Diet II	4	48	3.0 ±0.4	4.0 ±0.3	0.6 ±0.2	26.8 ±1.9	8.7 ±1.0	2.2 ±0.3	28.9 ±0.3	25.8 ±2.0	36.0	38.2	25.8
5. Diet III	5	48	3.8 ±0.4	5.0 ±0.4	1.0 ±0.2	26.9 ±1.5	9.5 ±0.6	1.5 ±0.3	30.1 ±2.4	22.2 ±2.4	37.2	40.6	22.2
6. Diet IV	6	44	4.5 ±0.5	5.1 ±0.1	0.9 ±0.1	27.9 ±1.6	10.3 ±0.9	2.3 ±0.2	32.8 ±0.7	16.2 ±1.3	39.8	44.0	16.2

* The sum of the individual values equal 100 per cent.
† Values recorded are means and standard error of the means.

fraction in maternal venous and mixed cord sera and in the venous sera of the four groups of test infants at age 6 to 8 weeks are shown in Table III. Maternal and cord sera serve as points of reference for the test infants. In addition, maternal sera serves as an interesting point of reference for comparison with cord sera.

The percentage distribution for all fatty acids in the C-TG of neonates differed from that found in maternal C-TG. As shown in Table III and in Figure 1, the C-TG of neonates contained a higher percentage of saturated and a lower percentage of diunsaturated fatty acids. Although the total percentage of monounsaturated fatty acids present in the two groups are similar, neonatal C-TG contained considerably more C16:1 and considerably less C18:1 acids in comparison to maternal C-TG.

Comparison of fatty acid distributions in the C-TG of 6- to 8-week-old infants who received diet

I (predominance of butterfat) with that found in neonates revealed a marked decrease in the percentage of diunsaturated fatty acid (C18:2), an increase in C18:1 acid, a decrease in C16:1 acid, but no differences in C18:0 acid.

The fatty acid distribution in C-TG of 6- to 8-week-old infants receiving diets providing large amounts of linoleic acid (diets II, III, and IV) differs strikingly from that found in neonates and in infants receiving diet I (Table III and Figure 1). Specifically, the percentage of diunsaturated fatty acid (C18:2) is roughly three times that found in neonatal C-TG and five to six times that found in infants receiving diet I. The increased percentage of diunsaturated acid is accompanied by a marked decrease in the percentage of both saturated and monounsaturated acids. With the exception of infants receiving diet III, the percentage of fatty acids derived from C18:0 acid

TABLE VI
Mean values for percentage distribution of fatty acids C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, and C18:2 in the subcutaneous fat of the test subjects during the second week of life

Group	Mean age		Fatty acid percentage distribution*							
			C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
Diet I	no. 9	days 9.8	0.7	4.6	1.3	42.9	14.8	2.7	29.4	3.6
Diet II	7	9.4	0.6	4.3	0.9	42.7	13.7	3.4	30.1	4.3
Diet III	4	11.5	1.1	3.7	1.3	43.0	13.1	3.4	27.5	6.9
Diet IV	7	10.0	0.9	4.1	0.7	40.9	14.6	3.5	30.6	4.7

* Values recorded are means.

TABLE VII

Fatty acid percentage distribution in the subcutaneous fat of 6- to 8-week-old test infants receiving diets I and II in comparison with those found in the fat of two infants after they had received one of the test diets continuously for 248 and 295 days, respectively

Subjects	Age		Fatty acid percentage distribution								Fatty acid distribution: saturated, monounsaturated, and diunsaturated acids			
			C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	S	US:1	US:2	
Group on diet I	no.	days												
	7	43	1.4	7.8	2.1	37.3	13.5	2.8	32.6	2.5	49.3	48.2	2.5	
Boy Ca on diet I	1	248	1.2	9.3	3.0	28.5	11.0	3.4	40.0	3.8	42.4	54.0	3.8	
Group on diet II	4	48	3.0	4.0	0.6	26.8	8.7	2.2	28.9	25.8	36.0	38.2	25.8	
Girl Sn on diet II	1	295	1.8	2.8	0.9	17.5	6.9	0.9	30.6	38.6	23.0	38.4	38.6	

did not differ from that found in neonates or infants receiving diet I.

By age 6 to 8 weeks, although far from representing an exact copy, the over-all fatty acid pattern in the C-TG of all four groups of test infants

developed considerable resemblance to the pattern present in their respective dietary fats (Tables II and III and Figure 1). Sera of 7- to 14-day old infants subjected to analyses for fatty acid distribution in the C-TG fraction (Table IV) suggested

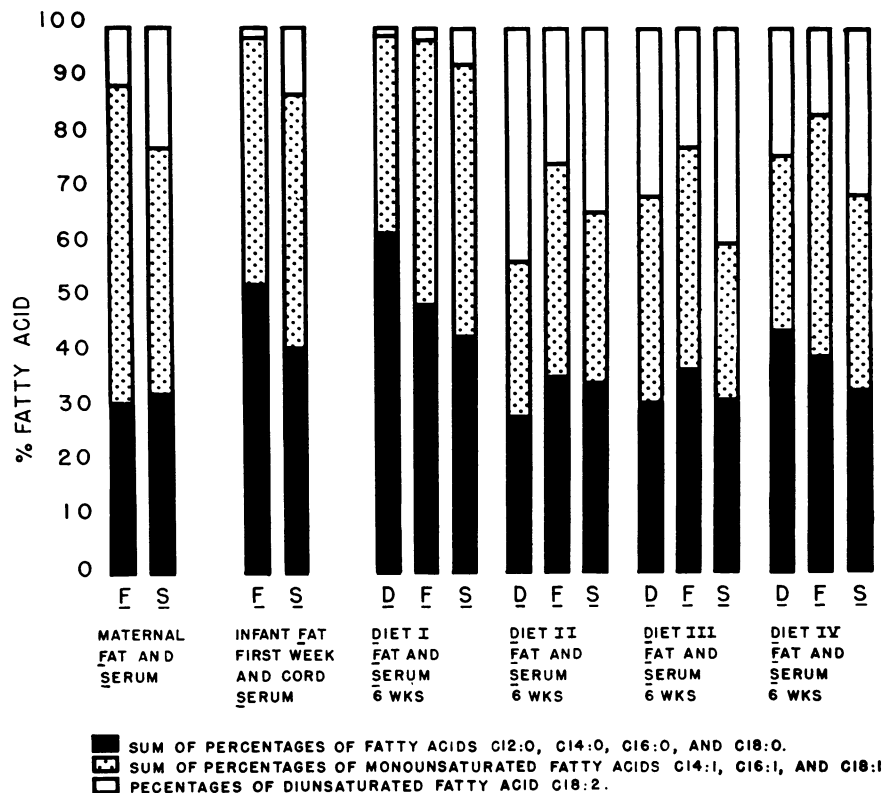


FIG. 1. TOTAL SATURATED, MONOUNSATURATED, AND DIUNSATURATED FATTY ACID PERCENTAGE DISTRIBUTIONS FROM C12:0 THROUGH C18:2 IN THE TEST DIETS, SUBCUTANEOUS FAT OF MOTHERS, AND SUBCUTANEOUS FAT OF TEST INFANTS DURING THE FIRST WEEK OF LIFE AND AT SIX TO EIGHT WEEKS OF AGE, AND IN THE CHOLESTEROL-TRIGLYCERIDE FRACTION OF THE SERA OF MOTHERS, CORD BLOOD, AND SIX- TO EIGHT-WEEK-OLD INFANTS.

that the patterns noted at age 6 to 8 weeks were virtually established by the end of the second week of life.

Subcutaneous fat. Table V shows the fatty acid percentage distribution of subcutaneous fat of mothers and the four groups of test infants at age 6 to 8 weeks. The distribution found in subcutaneous fat of 10 infants during the first week of life also is shown. At the time these latter samples were obtained, four of the infants were receiving diet I, two were on diet II, two on diet III, and two on diet IV. The lack of differences in the fatty acid percentage distribution found in these four groups seemed to justify their combination into one large group which could serve as a point of reference for comparisons between maternal and neonatal fat and between neonatal fat and that of the 6- to 8-week-old infants receiving the different test diets.

The marked difference between maternal and neonatal fat is apparent. The fat of the neonate contains twice as much C16:0 acid and 2.5 times as much C16:1 acid, but only 60 per cent as much C18:1 and 20 per cent as much C18:2 acids. Although neither maternal nor neonatal fat contained large amounts of C18:0 acid, a smaller amount was found in neonatal fat.

The percentages of C18:0, C18:1, and C18:2 acids found in subcutaneous fat of 6- to 8-week-old infants receiving diet I (predominance of butterfat) are almost identical with those of the neonates. Modest decreases in C16:0 and C16:1 acids and increases in C12:0, C14:0, and C14:1 acids were found.

The fatty acid percentage distribution in the three groups of 6- to 8-week-old infants receiving diets containing large amounts of linoleic acid (diets II, III, and IV) differed markedly from those found in the infants receiving diet I, the neonates, and the mothers. The most outstanding difference was the marked increase in the percentage of C18:2 acid. The percentage of C18:0 acid decreased somewhat in these three groups of infants in comparison with neonates and those receiving diet I, but the differences were not so striking as those occurring in the percentage of C18:2 acids.

By age 6 to 8 weeks, as with serum C-TG, the over-all distribution pattern of the fatty acids in the subcutaneous fat of all four groups of test in-

fants developed considerable resemblance to those of their respective dietary fats (Tables II and V and Figure 1). In contrast to serum C-TG, analyses of fat samples obtained from several groups of test infants during the second week of life (Table VI) showed only slight changes from those reported in Table V for infants less than age 1 week.

The relative persistence of the values found at age 6 to 8 weeks is illustrated in Table VII. After he had received diet I for 248 days (8 + months), a fat sample was obtained from boy Ca. The resemblance between the fatty acid distribution in his fat and that of the 6- to 8-week-old infants receiving diet I is apparent. Also shown is an analysis of the subcutaneous fat of an infant (Sn) who received diet II for 295 days (9 + months). The fatty acid distribution of her fat continued to generally resemble that of infants receiving diet II during the first 6 to 8 weeks of life.

DISCUSSION

Cholesterol-triglyceride fraction of serum lipid. Calculations based on the triglyceride and cholesterol ester concentrations in the sera of the various test groups indicate that maternal and cord sera contained approximately 1.5 moles of triglyceride fatty acid for each mole of cholesterol ester fatty acid. Similar calculations indicate that sera of the four groups of test infants contained approximately 2 moles of triglyceride fatty acid for each mole of cholesterol ester fatty acid. Since chromatography was performed on extracts which contained fatty acids derived from both triglyceride and cholesterol fractions of serum lipid, the values presented in Tables III and IV provide no specific information concerning the fatty acid percentage distribution in either the triglyceride or cholesterol fractions alone.

No previous reports of the fatty acid distribution in the C-TG fraction of serum lipid of neonates or young infants were found. In acute experiments in human adults, Dole and co-workers (4) found no significant change in the fatty acid distribution of serum C-TG during the course of alimentary lipemia produced with either corn or coconut oil. In these same experiments, the fatty acid patterns of serum FFA, chylomicrons, and phospholipids were also determined. As with the

C-TG, none of these fractions acquired the pattern of dietary fat during the period of alimentary lipemia. The later investigations of Bragdon and Karman (5) and Kuo, Whereat, Bassett, and Staple (6) demonstrated that certain fractions of the chylomicrons do assume a fatty acid pattern similar to dietary fat during alimentary lipemia. However, other than chylomicrons, all three groups of investigators agree that endogenous fatty acid sources and metabolic mechanisms play the dominant role in maintenance of the fatty acid patterns of the various serum lipid fractions.

As shown in Tables II and III and in Figure 1, the fatty acid patterns of serum C-TG in each of the four groups of test infants did develop considerable resemblance to that of their respective dietary fats by age 6 weeks. The work of the above cited investigators suggests that any influence exerted by dietary fat on the fatty acid pattern of serum C-TG was indirect and was mediated by the influence of dietary fat on tissue fat composition. The fact that the fatty acid patterns of serum C-TG seemed to develop a resemblance to dietary fat more rapidly (Table IV) than did those of subcutaneous fat (Tables V and VI) could be explained by the hypothesis suggested by Hirsch and associates (7). These workers suggested that depot fat might consist of a relatively inert large compartment which served as a storage pool for fat calories and a small compartment which was turning over rapidly and which was in close metabolic relation to dietary, serum, and liver lipids. Thus, the relatively rapid change in the fatty acid pattern of serum C-TG noted in the present study might reflect effect of dietary fat on the composition of a small but metabolically active compartment of depot fat which was in close metabolic relation to serum lipids.

Subcutaneous fat. The fatty acid percentage distributions found in this study in subcutaneous fat of mothers and neonates were similar to those reported by Hirsch and associates (7) for three post partum women and three full term infants. Presumably, the findings in subcutaneous fat were representative of all depot fat in the body (8). The cause, or causes, of the marked differences between fatty acid distributions of maternal and neonatal fat are not known. Hirsch and associates (7) hypothesized that *a*) late in pregnancy, calories in excess of those needed for growth become

available to the fetus and that lipogenesis from carbohydrate may predominate, a situation which animal studies suggest is associated with decreased C18:2 and increased C16:0 and C16:1 fatty acid synthesis, or alternatively, *b*) the enzymatic apparatus responsible for conversion of carbohydrate to fat in fetal adipose tissue matures late in pregnancy, giving rise to a sudden burst of C16:0 and C16:1 acid synthesis. Since the test diets used in the present study provided 40 to 50 per cent of dietary calories as carbohydrate, the changes that occurred in the percentages of C16:0, C16:1, and C18:2 acids in the subcutaneous fat of the four groups of infants at age 6 to 8 weeks are of interest. Infants receiving diet I (predominance of butterfat and approximately 50 per cent of dietary calories as carbohydrate) showed slight decreases in C16:0 and C16:1 acid percentages compared with those found in neonates, but these decreases were not nearly so great as those found in infants receiving diets II, III, and IV (containing large amounts of linoleic acid and 41 to 46 per cent of calories as carbohydrate). The percentages of C18:2 acids in neonates and those receiving diet I did not differ, but the striking increase in C18:2 acid percentages in infants receiving diets II, III, and IV is apparent. The present study provides no explanation concerning the mechanisms responsible for these changes. Presumably, they represent the net effect of a number of endogenous and exogenous forces among which are the activity of the enzymatic apparatus responsible for conversion of carbohydrate to fat and the composition of dietary fat.

In comparison with adults in whom a high intake of linoleic acid for 6 months or longer was required before significant changes occurred in the fatty acid composition of depot fat (7), changes occurred in the depot fat of these groups of infants in a rather short period of time. As in the adults, these changes were in a direction which brought about considerable resemblance between the composition of subcutaneous and dietary fats.

Although the reasons depot fat composition came to resemble dietary fat more rapidly in the infants are not known and none of the data obtained in the present study throw any light on this matter, certain speculations seem of interest. One potential factor is related to the rapid accumulation of new tissue mass occurring in all growing

infants (infants in this study grew normally). The raw material required for synthesis of this new tissue must come from the diet. If the infants 1) absorb dietary fat well and without any radical change in its fatty acid pattern, 2) accumulate new fat mass as rapidly as other types of tissue, and 3) accumulate new fat tissue with a fatty acid pattern grossly similar to dietary fat, changes in subcutaneous fat composition such as found in this study would occur more rapidly than in nongrowing adults.

A second factor which potentially could exert an influence concerns differences in the quantity of fat ingested by infants and adults when such are compared in terms of body weight and per cent of body weight represented by body fat. The total caloric intakes of the average infant and adult, when expressed in terms of body surface area, are roughly comparable, that is, approximately 1,500 calories per m² per day. In terms of body weight, however, this represents a total caloric intake of about 100 to 130 calories per kg in the infant and 40 calories per kg in the adult. In addition, the body of the average adult contains roughly 150 to 200 g fat per kg (15 to 20 per cent of the body weight), and that of the infant contains about 120 g fat per kg (12 per cent of the body weight) (9). Thus, for diets with similar caloric distributions, the infant may be consuming three to five times or more fat per gram of depot fat than the adult. If infants absorb dietary fat as well as adults and if the composition and quantity of depot fat laid down bears any relationship to the composition and the quantity of fat ingested per unit of body weight, the composition of the infant's depot fat should come to resemble that of dietary fat more rapidly than this occurs in adults.

A third factor that potentially could play a role in this regard concerns the rates of metabolic expenditure per unit of body weight in the infant and the adult. Studies of oxygen consumption (10) and basal metabolism (11, 12) in neonates suggest their energy expenditure is two to three times that of the adult when this is expressed in terms of body weight. If the rate at which depot fat contributes calories for such expenditure bears any relationship to over-all energy expenditure, a gram of depot fat in the neonate will be utilized more rapidly than a gram of depot fat in the

adult. This, particularly in combination with accretion of new fat tissue during growth and a greater intake of fat per unit of body weight, also would favor a more rapid effect of dietary fat on depot fat.

At any rate, this study does demonstrate the relative rapidity with which the composition of depot fat of infants comes to resemble that of their dietary fat. In addition, it also illustrates development of a resemblance between the fatty acid patterns of serum C-TG and dietary fat and suggests that this occurs somewhat more rapidly than does development of the resemblance between depot fat and dietary fat. However, the study does not define the mechanism, or mechanisms, that bring these changes about, nor does it provide any information concerning the clinical significance or usefulness of these findings.

SUMMARY AND CONCLUSIONS

The fatty acid percentage distribution from C12:0 through C18:2 in subcutaneous fat and in the cholesterol-triglyceride fraction of serum lipid was determined in four groups of 6- to 8-week-old infants, each of whom received a different test diet. Major variables in the test diets included: percentage of total calories derived from fat (35 to 50 per cent), major source of dietary protein (cow milk and soybean), and percentage of total calories derived from protein (9 to 19 per cent). Linoleic acid content of dietary fat in diet I was approximately 1.2 per cent, in diet II 39.2 per cent, in diet III 30.1 per cent, and in diet IV 21.7 per cent.

Fatty acid distributions in serum C-TG and subcutaneous fat of neonates differed markedly from those of maternal serum and subcutaneous fat. By age 6 to 8 weeks, fatty acid distributions found in serum C-TG and subcutaneous fat of each of the test groups bore considerable resemblance to that of their respective dietary fats. Changes in the serum lipid fraction appeared to occur more rapidly than those in subcutaneous fat. Analyses of depot fat of two infants who remained on test diet I and test diet II for 8 and 9 months, respectively, indicated apparent persistence of the effect of dietary fat on composition of depot fat.

Similar studies in adults by others indicate that

6 or more months are required before the fatty acid composition of dietary fat produces a demonstrable effect on the fatty acid composition of depot fat. The reasons such changes occurred more rapidly in our infants are not known. The clinical usefulness and significance of the findings of the present study must await further investigation.

REFERENCES

1. Sweeney, M. J., Etteldorf, J. N., Dobbins, W. T., Somervill, B., Fischer, R., and Ferrell, C. Dietary fat and concentrations of lipid in the serum during the first six to eight weeks of life. *Pediatrics* 1961, **27**, 765.
2. Sweeney, M. J., Etteldorf, J. N., Orban, P. M., and Fischer, R. Effect of diet during the first six to eight weeks of life on total concentrations of serum protein and on electrophoretic patterns of serum protein and lipoprotein. *Pediatrics* 1962, **29**, 82.
3. Van Handel, E., and Zilversmit, D. B. Micromethod for the direct determination of serum triglycerides. *J. Lab. clin. Med.* 1957, **50**, 152.
4. Dole, V. P., James, A. T., Webb, J. B. W., Rizack, M. A., and Sturman, M. F. The fatty acid patterns of plasma lipids during alimentary lipemia. *J. clin. Invest.* 1959, **38**, 1544.
5. Bradgon, J. H., and Karman, A. The fatty acid composition of chylomicrons of chyle and serum following the ingestion of different oils. *J. Lipid Res.* 1960, **1**, 167.
6. Kuo, P. T., Whereat, A. F., Bassett, D. R., and Staple, E. Study of the mechanism of hyperlipemia. Serum chylomicron fatty acid patterns of hyperlipemic patients before and after the ingestion of different food fats. *Circulation* 1961, **24**, 213.
7. Hirsch, J., Farquhar, J. W., Ahrens, E. H., Jr., Peterson, M. L., and Stoffel, W. Studies of adipose tissue in man; a microtechnic for sampling and analysis. *Amer. J. clin. Nutr.* 1960, **8**, 499.
8. Hegsted, D. N., Jack, C. W., and Stare, F. J. The composition of human adipose tissue from several parts of the world. *Amer. J. clin. Nutr.* 1962, **10**, 11.
9. Forbes, G. B. Methods for determining composition of the human body with a note on the effect of diet on body composition. *Pediatrics* 1962, **29**, 477.
10. Cook, C. D., Cherry, R. B., O'Brien, D., Karlberg, P., and Smith, C. A. Studies of respiratory physiology in the newborn infant. I. Observations on normal premature and full-term infants. *J. clin. Invest.* 1955, **34**, 975.
11. Marsh, M. E., and Murlin, J. R. Energy metabolism of premature and undersized infants. *Amer. J. Dis. Child.* 1925, **30**, 310.
12. DuBois, E. F. *Basal Metabolism in Health and Disease*, 3rd ed. Philadelphia, Lea & Febiger, 1936.

SPECIAL NOTICE TO SUBSCRIBERS

Post Offices will no longer forward the Journal when you move.

Please notify The Journal of Clinical Investigation, Business Office, 10 Stoughton Street, Boston 18, Mass., at once when you have a change of address, and do not omit the zone number if there is one.