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Dietary alterations of fatty acids of erythrocytes and mitochondria of brain and liver*

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SUMMARY

The fatty acid composition of erythrocyte and liver mitochondrial lipids was readily and drastically altered by varying the fatty acid content of the diet. Nonessential polyunsaturated fatty acids were found in these tissues when the tissue level of linoleic acid fell below 10% of the total fatty acids. In essential fatty acid deficiency, two isomeric eicosatrienoic acids appeared, except when the diet supplied other more highly unsaturated nonessential fatty acids. Although brain mitochondrial lipids were relatively less affected by dietary manipulations, their fatty acid compositions could be significantly altered by variations of dietary fat; nonessential polyunsaturated fatty acids from cod liver oil were also incorporated into these lipids. It is postulated that such alterations in the polyunsaturated fatty acid content of phospholipids, other lipid complexes, or both, may be of importance in metabolism.

Essential fatty acid deficiency has been described in the rat, mouse, dog, child, and a moth larva (1). Recently it has been pointed out that a high intake of polyunsaturated fatty acids, inadequately protected against autoxidation *in vivo*, facilitates the appearance of encephalomalacia in the chick (2 to 5) and in man (6), nutritional dystrophy in the rat (7), and peroxide hemolysis of human erythrocytes (8, 9, 10). The present report attempts to define in biochemical terms the marginal state of essential fatty acid deficiency and the criteria for determination of this state in individual tissues.

Although it has been shown that the turnover of unsaturated fatty acids in rat liver and carcass is high (11), it has been assumed that the fatty acids of the central nervous system, particularly the brain, turn over far more slowly. However, a previous report (10) has shown that the fatty acid composition of human erythrocytes and chick cerebella were readily altered by variations in dietary fats. In the present investigation the lipids from various tissues of the rat were surveyed to evaluate alterations in fatty acid composition produced by qualitative and quantitative changes in dietary lipids. Tissues containing most of their lipid as triglyceride were not studied, since it has been long recognized (12, 13) that depot fats readily take on the characteristics of the ingested fat. Rather, formed elements of definite structural character, such as brain and liver mitochondria and erythrocyte stroma, were chosen for study. The lipids of these tissues contain most of their fatty acids in the form of phospholipids (see Results), and it was considered adequate for the purposes of this study to analyze these fatty acids without further subfractionation into phospholipid classes. In these studies attention was focused on three effects produced by dietary manipulations: changes in essential fatty acid levels, appearance of nonessential polyunsaturated fatty acids as a criterion of essential fatty acid deficiency, and incorporation of dietary acids (14, 15).

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EXPERIMENTAL

Preparation of Animals and Tissue Lipids. Weanling rats of the Sprague-Dawley strain were raised on synthetic diets (Table 1) containing one of the following fats substituted isocalorically for carbohydrate: 15% coconut oil, 15% corn oil, 7% cod liver oil, and 0.2% corn oil. The latter diet was intended to be a "fat-

TABLE 1. EXPERIMENTAL DIETS

Ingredient	15% Fat by Weight		7% Fat by Weight		0.2% Fat by Weight	
	% of calories	*% by weight*	*% of calories*	*% by weight*	*% of calories*	*% by weight*
Casein (vitamin-test)	21.8	24.8	21.8	22.8	21.8	20.9
Dextrose	26.2	29.8	34.0	35.5	41.5	40.0
Cornstarch	22.7	25.8	28.9.	30.4	36.5	35.1
Coconut oil or corn oil*	29.4	15.0				
Cod liver oil*			15.3	7.0		
Low fat (as corn oil)					0.2	0.2
	g/1,000 cal.	*% by weight*	*g/1,000 cal.*	*% by weight*	*g/1,000 cal.*	*% by weight*
Choline dihydrogen citrate	0.72	0.33	0.72	0.30	0.72	0.27
Vitamin mix†	0.72	0.33	0.72	0.30	0.72	0.27
Salts (USP XIV)	8.7	4.0	8.7	3.7	8.7	3.3

* Major components of dietary oils: Coconut oil 12:0—28%; 14:0—28%; 16:0—17%; 18:0—6%; 18:1—14%; 18:2—4%. Cod liver oil 16:0—8%; 16:1—9%; 18:1—18%; 20:1—13%; 20:4—7%; 20:5—15%; 22:6—12%. Corn oil 16:0—8%; 18:1—30%; 18:2—54%.

† Vitamin mix contains i-inositol, 24.1 mg, para-aminobenzoic acid, 24.1 mg, calcium pantothenate, 13.5 mg, 2-methyl-1, 4-naphthoquinone, 10.9 mg, niacin, 21.8 mg, thiamine HCl, 4.8 mg, pyridoxine-HCl, 4.8 mg, riboflavin, 4.8 mg, folic acid, 0.4 mg, biotin, 98 μg, vitamin B_{12}, 6.5 μg, and starch to 0.72 g. Vitamins A (2,500 I.U.) and D (360 I U.) were given weekly as Oleum Percomorphum drops.

free" diet with sufficient lipid to meet requirements for essential fatty acids. Animals were sacrificed at zero time, 6 weeks, and 21 weeks.

Erythrocytes were lysed and extracted by the procedure of Morris *et al.* (16). Liver and brain were homogenized, and mitochondria isolated by conventional techniques, employing differential centrifugation between 550 and 11,000 $\times$ *g*, as described in the second procedure of Brody and Bain (17). Lipid extracts were obtained as noted above (16). DL-α-tocopherol was added to the extracting solvents and was found to follow the lipid throughout subsequent procedures. All evaporations were performed under nitrogen, and samples were stored in ten times their weight of redistilled petroleum ether under a nitrogen atmosphere at 0°. Methyl esters were obtained by a modification of the method of Stoffel *et al.* (18). Fatty acid esters were determined by the method of Clayton *et al.* (19), and nitrogen by the method of Hiller (20).

Gas-Liquid Chromatography. Fatty acid compositions were determined by gas-liquid chromatography of methyl esters on Celite® columns utilizing Apiezon M, ethylene glycol adipate polyester, ethylene glycol succinate polyester, and silicone grease as liquid phases (Table 2). Most of the reported data were obtained on ethylene glycol succinate polyester columns. Standard mixtures were used to calibrate area measurements. Since ethylene glycol succinate polyester columns do not resolve linolenic acid (18:3) from eicosaenoic acid (20:1) at 170° (21), representative samples were analyzed on ethylene glycol adipate polyester columns. The latter columns clearly resolve these components, but linolenic acid (18:3) was not detected in any of the tissues examined. Structural assignments are based largely on the data of Farquhar *et al.* (22).

The $\Delta^{5,8,11,14,17}$ eicosapentaenoic acid (20:5) and $\Delta^{7,10,13,16,19}$ docosapentaenoic acid (22:5) from cod liver oil (23, 24) fit the data of Farquhar *et al.* (22) better than do the corresponding isomers of these acids found normally in rat tissue lipids. Mead and Slaton (25) have found the major trienoic acid present in an essential fatty acid deficiency to be the $\Delta^{5,8,11}$ eicosatrienoic acid (20:3), while the $\Delta^{7,10,13}$ eicosatrienoic acid (20:3) (26) was found only as a minor constituent. In this paper two peaks on the gas-liquid chromatogram are designated as eicosatrienoic acids (20:3) and their total

TABLE 2. CONDITIONS FOR GAS-LIQUID CHROMATOGRAPHIC ANALYSIS

Liquid Phase	Apiezon M	Ethylene Glycol Adipate Polyester	Ethylene Glycol Succinate Polyester*	Silicone Grease
Percentage of liquid phase by weight	20%	20%	17%	20%
Support	Celite	Celite	Chromosorb W	Celite
Mesh	150–200	140–200	80–100	60–80
Column	glass	glass	glass	stainless steel
Internal diameter (mm)	1	4	6	6
Length (cm)	240	120	240	60
Temperature (°)	190	165	170	240
Carrier gas	argon	argon	argon	helium
Pressure (psi)	90	20	65	20
Flow rate (cc/min)	16	37.5	200	60
Detector	radium-226 strontium-90	radium-226 strontium-90	tritium	thermal conductivity
Detector current (amps)	3×10^{-8} at 1200 v	3×10^{-8} at 1200 v	3×10^{-8} at 900 v	2×10^{-1} at 12 v

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is in reasonable agreement with the triene content of the samples, as determined by alkali isomerization using the extinction coefficients for eicosatrienoic acid (20:3), determined by Holman and Hayes (27). Oxidation experiments are now in progress to determine which peak contains the $\Delta^{5,8,11}$ eicosatrienoic acid (20:3). Assignments of 16:al and 18:al designate saturated aliphatic aldehydes. Data supporting these characterizations will be presented in detail in a future paper. Values for arachidonic acid (20:4) are slightly high, since small amounts of behenic acid (22:0) contaminated the 20:4 peak on ethylene glycol succinate polyester columns.

RESULTS

Fatty Acids of Liver Mitochondrial Lipids. The fatty acids of liver mitochondrial lipids were analyzed as methyl esters by gas-liquid chromatography (Table 3). Five rats were sacrificed for each set of conditions. In weanlings the mitochondrial lipids contained approximately 14% linoleic acid (18:2), 16% arachidonic acid (20:4), and 5% docosahexaenoic acid (22:6). By varying the nature of the dietary lipid, the linoleic acid (18:2) content of liver mitochondrial lipids could be lowered to 5% or raised to 20%, while arachidonic acid (20:4) could be varied from 5% to 24%, and docosahexaenoic acid (22:6) from 2% to 14%. Rat liver mitochondrial lipids contained 93% phospholipid (28), and the fatty acids of this phospholipid might be expected to reflect the composition of the dietary fatty acids.

Direct incorporation of dietary fatty acids was evident in the cod liver oil group in which 9% to 15% of total liver mitochondrial lipids consisted of pentaenoic acids ($\Delta^{5,8,11,14,17}$ 20:5 and $\Delta^{7,10,13,16,19}$ 22:5). An increase in concentration of the eicosatrienoic acids (20:3) was noted in the groups receiving 0.2% corn oil or 15% coconut oil.

Fatty Acids of Erythrocyte Lipids. The stromal lipids of erythrocytes from weanling rats were found to contain 6% linoleic acid (18:2), 11% arachidonic acid (20:4), and 2% docosahexaenoic acid (22:6) (Table 4). Mead and Fillerup (29) found 1% neutral lipid, 30% cholesterol, and 70% phospholipid plus cerebroside in the lipids of rat erythrocytes. Alteration of dietary lipids lowered the linoleic acid (18:2) of erythrocyte lipids to 4%, or raised it to 13%. Similarly, arachidonic acid (20:4) was found to vary from 7% to 23%, and docosahexaenoic acid (22:6) from 1.5% to 6%. Sixteen per cent of the total erythrocyte fatty acids consisted of pentaenoic acids ($\Delta^{5,8,11,14,17}$ 20:5 and $\Delta^{7,10,13,16,19}$ 22:5) in the group receiving cod liver oil. Eicosatrienoic acids (20:3) increased in concentration in the groups receiving 0.2% corn or 15% coconut oil.

Fatty Acids of Brain Mitochondrial Lipids. In

TABLE 3. FATTY ACID COMPOSITION OF LIVER MITOCHONDRIAL LIPIDS

Fatty Acid	Weanlings at Start of Experiment	Animals on Diets 6 Weeks				Animals on Diets 21 Weeks			
		0.2% Corn Oil	15% Coconut Oil	15% Corn Oil	7% Cod Liver Oil	0.2% Corn Oil	15% Coconut Oil	15% Corn Oil	7% Cod Liver Oil
12:0	0.5* ± 0.3†	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.3	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.2	trace‡
14:0	1.1 ± 0.2	2.6 ± 0.6	2.6 ± 0.6	2.0 ± 0.4	2.1 ± 1.0	0.8 ± 0.2	2.1 ± 0.6	1.2 ± 0.6	0.9 ± 0.2
16:0	18.5 ± 1.2	18.5 ± 2.0	19.8 ± 1.8	17.3 ± 2.6	24.5 ± 3.4	22.1 ± 3.4	17.5 ± 3.0	12.2 ± 2.4	21.6 ± 7.4
16:1	1.5 ± 0.6	7.1 ± 1.0	4.5 ± 0.6	1.7 ± 0.2	5.5 ± 0.6	5.5 ± 1.4	6.5 ± 1.0	2.0 ± 0.6	6.5 ± 1.6
18:0	21.7 ± 1.4	18.3 ± 0.6	19.7 ± 1.2	20.1 ± 3.4	17.5 ± 1.6	16.1 ± 1.2	14.7 ± 2.0	12.1 ± 3.4	12.4 ± 1.2
18:1	14.7 ± 1.4	22.7 ± 1.4	19.5 ± 2.4	12.9 ± 0.8	17.6 ± 1.0	20.3 ± 2.2	18.5 ± 3.0	15.2 ± 2.8	14.3 ± 1.2
18:2	14.1 ± 0.8	6.3 ± 0.4	9.5 ± 1.0	20.3 ± 2.4	5.8 ± 1.2	7.7 ± 1.4	10.1 ± 3.0	20.9 ± 3.6	5.0 ± 1.4
20:0	1.0 ± 0.8	0.4 ± 0.2	0.4 ± 0.4	0.5 ± 0.2	0.4 ± 0.4	0.1 ± 0.0	0.3 ± 0.2	0.3 ± 0.3	0.1 ± 0.1
20:1	0.4 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	1.6 ± 1.0
20:?		0.5 ± 0.0		1.5 ± 0.4		0.1 ± 0.1		0.7 ± 0.3	0.3 ± 0.0
20:3		5.7 ± 1.0	3.9 ± 0.2	trace		3.1 ± 1.4	2.3 ± 0.8		
20:3		1.3 ± 0.2	1.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	1.4 ± 0.6	2.1 ± 0.6	0.6 ± 0.3	0.5 ± 0.2
20:4	16.0 ± 1.6	9.2 ± 1.0	11.4 ± 1.6	17.5 ± 1.4	5.4 ± 0.8	14.8 ± 3.6	17.1 ± 3.0	25.1 ± 4.4	5.7 ± 1.0
20:5	0.7 ± 0.5	0.7 ± 0.2		0.3 ± 0.3		0.6 ± 0.3	1.1 ± 1.0	0.7 ± 0.1	0.6 ± 0.4
20:5§					6.9 ± 0.8	1.0 ± 0.3			12.0 ± 1.6
22:2						0.4 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	0.3 ± 0.3
22:5	4.3 ± 1.6	1.0 ± 0.4	1.1 ± 0.2	0.9 ± 0.6		1.3 ± 0.4	1.3 ± 0.6	1.7 ± 0.8	0.3 ± 0.3
22:5‖					2.2 ± 0.4	0.5 ± 0.2		0.4 ± 0.0	3.5 ± 1.2
22:6	5.2 ± 1.0	1.8 ± 0.2	3.0 ± 1.0	1.6 ± 0.6	7.9 ± 0.4	5.3 ± 1.4	4.1 ± 1.4	3.5 ± 1.6	14.0 ± 3.2
24:0		0.5 ± 0.3	0.8 ± 0.4	1.3 ± 0.6	1.2 ± 0.6	0.5 ± 0.5	0.3 ± 0.2	0.8 ± 0.3	
mg ester/mg nitrogen in mitochondria		0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1

* Percentage of total area of a gas-liquid chromatographic elution diagram.
† Standard deviation (5 samples).
‡ Present, but less than 0.05%.
§ $\Delta^{5,8,11,14,17}$ 20:5 from cod liver oil.
‖ $\Delta^{7,10,13,16,19}$ 22:5 from cod liver oil.

TABLE 4. FATTY ACID COMPOSITION OF ERYTHROCYTE LIPIDS. ANIMALS ON DIETS 21 WEEKS

Fatty Acid	Weanlings at Start of Experiment	0.2% Corn Oil	15% Coconut Oil	15% Corn Oil	7% Cod Liver Oil
12:0	0.3* ± 0.2†		0.3 ± 0.2		
14:0	2.3 ± 1.3	0.9 ± 0.4	1.7 ± 0.2	0.9 ± 0.3	0.7 ± 0.2
16:al‡	2.2 ± 0.6	3.0 ± 1.0	3.0 ± 0.4	2.6 ± 0.2	3.3 ± 0.2
16:0	16.7 ± 2.7	20.9 ± 4.4	15.1 ± 4.4	21.6 ± 3.8	22.9 ± 4.6
16:1	6.4 ± 2.3	2.1 ± 0.6	1.3 ± 0.4	0.6 ± 0.3	2.4 ± 0.6
18:al‡	1.0 ± 0.5	2.4 ± 0.6	2.6 ± 0.8	2.2 ± 0.2	2.8 ± 0.2
18:0	11.9 ± 1.7	16.4 ± 2.0	15.5 ± 2.2	16.5 ± 1.2	13.9 ± 1.0
18:1	12.1 ± 0.5	14.8 ± 1.2	13.7 ± 1.4	11.4 ± 1.4	15.7 ± 1.0
18:2	5.7 ± 1.3	5.3 ± 1.0	8.1 ± 0.6	13.3 ± 1.4	3.9 ± 0.6
20:0	0.7 ± 0.3				
20:1	1.6 ± 1.0	0.6 ± 0.1	0.8 ± 0.5	0.4 ± 0.2	1.7 ± 0.6
20:?	0.7 ± 0.6			0.5 ± 0.2	
20:3	1.0 ± 0.4	2.4 ± 1.0	1.3 ± 0.4		
20:3		0.7 ± 0.2	1.2 ± 0.6	0.4 ± 0.3	0.4 ± 0.2
20:4	11.4 ± 2.1	22.9 ± 3.0	22.6 ± 2.0	23.3 ± 5.2	7.1 ± 0.8
20:5	1.4 ± 0.8	0.7 ± 0.5	1.3 ± 0.8	1.3 ± 0.6	
20:5§	2.1 ± 1.5				13.0 ± 2.4
22:2		0.5 ± 0.2	0.4 ± 0.2	0.8 ± 0.6	0.4 ± 0.2
22:5	2.4 ± 0.9	1.5 ± 1.2	1.9 ± 0.6	0.8 ± 0.2	1.1 ± 0.2
22:5‖	2.3 ± 0.9				3.6 ± 0.8
22:6	1.7 ± 0.2	1.5 ± 0.2	3.6 ± 2.4	0.8 ± 0.4	5.7 ± 1.2
24:0	3.1 ± 0.5	1.7 ± 0.8	2.8 ± 1.8	2.7 ± 0.4	0.5 ± 0.2

* Percentage of total area of gas-liquid chromatographic elution diagram.
† Standard deviation (5 samples; last column, 4 samples).
‡ Saturated aliphatic aldehydes.
§ $\Delta^{5,8,11,14,17}$ 20:5 from cod liver oil.
‖ $\Delta^{7,10,13,16,19}$ 22:5 from cod liver oil.

this laboratory the approximate composition of brain mitochondrial lipids has been found to be 22% cholesterol, 7% cholesterol ester, and 71% phospholipid. The lipids of brain mitochondria from weanling rats contained 1.7% linoleic acid (18:2), 11% arachidonic acid (20:4), and 9% docosahexaenoic acid (22:6) (Table 5). By varying the fatty acid composition of the dietary lipid, it was possible to lower the linoleic acid (18:2) content of the brain mitochondrial lipids to 0.5%. The arachidonic acid (20:4) levels could be varied from 8% to 13%, and docosahexaenoic acid (22:6) from 9% to 17%. Incorporation of pentaenoic acid isomers ($\Delta^{5,8,11,14,17}$ 20:5 and $\Delta^{7,10,13,16,19}$ 22:5) from cod liver oil into brain mitochondrial lipids was noted. Eicosatrienoic acids (20:3) did not appear to increase in concentration in the groups receiving 0.2% corn oil or 15% coconut oil.

DISCUSSION

From the experiments described above it is quite evident that the fatty acid composition of liver mitochondrial lipids can be altered by dietary fatty acids. Definite changes in the essential fatty acid content of mitochondrial lipids were observed, and direct incorporation of dietary fatty acids could be seen in the group receiving cod liver oil. On diets marginally deficient in essential fatty acids, eicosatrienoic acids (20:3) were found to increase in concentration.

Whereas it was originally thought that the stroma of the erythrocyte would represent a fatty acid composition intermediate in stability between liver mitochondria and brain mitochondria, this was not found to be the case in young animals. It was apparent that the lipids of the erythrocyte respond just as positively to alterations of dietary fatty acids as do liver mitochondria.

Brain mitochondrial lipids were more resistant to change than were the lipids of either of the other tissues studied, but even this fatty acid composition could be significantly altered by dietary fat. Direct incorporation of pentaenoic acids ($\Delta^{5,8,11,14,17}$ 20:5 and $\Delta^{7,10,13,16,19}$ 22:5) could be demonstrated when the diet contained cod liver oil. The essential fatty acid requirements of brain mitochondria were apparently met, as judged by their content of nonessential polyunsaturated fatty acids, even when other tissues in the

TABLE 5. FATTY ACID COMPOSITION OF BRAIN MITOCHONDRIAL LIPIDS

Fatty Acid	Weanlings at Start of Experiment	Animals on Diets 6 Weeks				Animals on Diets 21 Weeks			
		0.2% Corn Oil	15% Coconut Oil	15% Corn Oil	7% Cod Liver Oil	0.2% Corn Oil	15% Coconut Oil	15% Corn Oil	7% Cod Liver Oil
12:40	0.3* ± 0.1†	0.3 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
14:0	0.7 ± 0.1	0.6 ± 0.3	1.2 ± 0.6	0.6 ± 0.3	0.6 ± 0.3	0.8 ± 0.5	0.8 ± 0.4	0.5 ± 0.3	0.7 ± 0.4
16:al‡	3.2 ± 0.4	2.5 ± 0.2	2.0 ± 1.2	2.6 ± 0.6	2.7 ± 1.0	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.2	2.2 ± 0.6
16:0	19.6 ± 2.8	18.1 ± 1.8	17.1 ± 2.6	16.8 ± 1.2	16.6 ± 1.4	15.1 ± 2.2	14.8 ± 1.0	18.5 ± 1.2	14.3 ± 0.8
16:1	1.5 ± 0.6	1.6 ± 0.8	1.7 ± 0.6	0.8 ± 0.1	1.2 ± 0.2	0.8 ± 0.4	0.9 ± 0.4	0.5 ± 0.2	0.9 ± 0.6
18:al‡	2.8 ± 1.0	4.6 ± 2.6	2.7 ± 0.4	3.0 ± 1.0	3.3 ± 1.4	3.3 ± 0.8	3.7 ± 0.6	3.8 ± 0.8	3.4 ± 0.6
18:0	20.9 ± 0.8	19.4 ± 1.2	20.0 ± 1.8	17.6 ± 1.4	19.0 ± 0.8	20.7 ± 2.0	20.3 ± 2.2	21.2 ± 2.0	18.7 ± 2.0
18:1	20.8 ± 1.0	24.2 ± 3.0	23.3 ± 5.6	19.5 ± 3.2	21.9 ± 2.8	24.1 ± 2.6	21.7 ± 1.6	22.1 ± 2.0	22.8 ± 1.4
18:2	1.7 ± 0.2	0.6 ± 0.4	0.5 ± 0.2	1.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.7	0.9 ± 0.2	1.3 ± 0.2	0.8 ± 0.4
20:0	1.3 ± 0.6	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
20:1		1.7 ± 0.6	1.8 ± 0.6	1.6 ± 0.2	1.6 ± 0.6	2.9 ± 0.6	3.0 ± 0.6	3.0 ± 0.4	2.8 ± 0.4
20:?						0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.2	
20:3		0.5 ± 0.2	0.3 ± 0.3			0.5 ± 0.2	0.1 ± 0.1		0.1 ± 0.1
20:3	0.6 ± 0.5		0.5 ± 0.4	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.3
20:4	11.4 ± 1.2	9.5 ± 1.4	9.2 ± 3.2	12.8 ± 0.4	8.4 ± 0.8	9.3 ± 1.8	10.7 ± 0.8	10.2 ± 1.4	8.0 ± 1.0
20:5						0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	
20:5§									0.7 ± 0.2
22:2						0.4 ± 0.3	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.3
22:5	1.8 ± 1.0	1.6 ± 0.4	2.8 ± 1.2	2.6 ± 0.6		2.6 ± 1.0	2.4 ± 0.8	2.1 ± 0.6	1.3 ± 0.8
22:5‖					0.8 ± 0.3				1.0 ± 0.1
22:6	9.1 ± 1.8	10.9 ± 3.0	9.7 ± 2.6	14.9 ± 1.8	18.4 ± 2.6	9.6 ± 2.6	10.9 ± 1.8	8.7 ± 2.2	16.6 ± 3.8
24:0	2.9 ± 0.4	2.9 ± 1.4	3.3 ± 0.4	3.9 ± 0.4	2.2 ± 1.0	3.2 ± 1.2	3.6 ± 1.0	3.4 ± 0.6	1.3 ± 0.8
mg ester/mg nitrogen in mitochondria		1.4 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.2 ± 0.2		1.4 ± 0.5	1.3 ± 0.1	1.2 ± 0.2

* Percentage of total area of gas-liquid chromatographic elution diagram.
† Standard deviation (5 samples).
‡ Saturated aliphatic aldehydes.
§ $\Delta^{5,8,11,14,17}$ 20:5 from cod liver oil.
‖ $\Delta^{7,10,13,16,19}$ 22:5 from cod liver oil.

same animal showed a marginal deficiency by this criterion.

The fatty acid compositions of tissues including brain have been found to depend upon the composition of the dietary fat. A logical question follows: What concentrations of various polyunsaturated acids in different tissues suffice to prevent an essential fatty acid deficiency, and how may a marginal deficiency be recognized?

Polyunsaturated fatty acids fall into three groups depending on whether the first double bond, numbered from the terminal methyl group, is in the 3-, 6-, or 9-position. Compounds having full essential fatty acid activity have the first double bond in the 6-position (30). Linolenic acid (18:3) does not fulfill all the functions of an essential fatty acid (31). Appearance in the tissue of fatty acids belonging to the other groups, particularly eicosatrienoic acid (20:3), has been considered as a symptom of essential fatty acid deficiency (15) in which the body desaturates nonessential fatty acids in a futile attempt to meet its requirements.

When the linoleic acid (18:2) content of liver mitochondrial lipids fell below approximately 10% in animals on the 0.2% corn oil or 15% coconut oil diets, nonessential polyunsaturated fatty acids (20:3) accumulated. This occurred despite the presence of 9% to 17% arachidonic acid (20:4) in the same tissue. These nonessential polyunsaturated fatty acids were not elevated in the liver mitochondrial lipids of the weanlings or the corn oil groups (14% and 20% to 21% linoleic acid [18:2], respectively). In the groups receiving cod liver oil, the linoleic acid (18:2) concentration in the fatty acids of the liver mitochondrial lipids fell to 5% to 6% without production of eicosatrienoic acids (20:3), possibly as a result of the presence of 9% to 15% of nonessential pentaenoic acids ($\Delta^{5,8,11,14,17}$ 20:5 and $\Delta^{7,10,13,16,19}$ 22:5) derived from the diet. Arachidonic acid (20:4) was also very low (5% to 6%) in this group. The elevation of docosahexaenoic acid (22:6) concentration probably arises largely from incorporation of a dietary fatty acid present in cod liver oil with the same retention time as the docosahexaenoic acid (22:6) usually present in mammalian tissue. Since this fatty acid from cod liver oil probably has the 4, 7, 10, 13, 16, 19 double bond sequence (24), and is therefore a member of the linolenic acid (18:3) family, full essential fatty acid activity is precluded (30).

In the lipids of the erythrocyte, concentrations of 6% linoleic acid (18:2) and 11% arachidonic acid (20:4) appeared to meet adequately the essential fatty acid requirements of the weanling, while concentrations

of 6% to 8% linoleic acid (18:2) and 23% arachidonic acid (20:4) did not quite adequately meet the requirements for the 24-week-old animals, as judged by the appearance of nonessential polyunsaturated fatty acids. The erythrocytes from the 15% corn oil group contained 13% linoleic acid (18:2), and 23% arachidonic acid (20:4). The feeding of the nonessential polyunsaturated fatty acids of cod liver oil produced in erythrocytes the same fatty acid patterns as those described above for liver mitochondrial lipids.

Brain mitochondrial lipids contain very little linoleic acid (18:2). However, eicosatrienoic acids (20:3) did not accumulate when marginal essential fatty acid deficiency was evidenced by other tissues. The data suggest that linoleic acid (18:2) and arachidonic acid (20:4) should probably account for 1% to 2% and 10% to 12%, respectively, of the brain mitochondrial fatty acids of rats on an adequate diet, and that levels lower than these may be considered representative of a deficiency. In weanlings, the brain mitochondrial lipids contained little linoleic acid (18:2); however, when 0.2% corn oil or 15% coconut oil diets were fed, there were no appreciable accumulations of eicosatrienoic acids (20:3). When the cod liver oil diet was fed, arachidonic acid (20:4) was considerably decreased, and in all groups docosahexaenoic acid (22:6) constituted the major polyenoic acid.

The data presented above suggest that the relative content of essential fatty acids in a given tissue may be an index of the nutritional status of the animal. Normal requirements for these acids in the tissues studied are higher than anticipated. These needs cannot be judged in terms of their collective total, but rather in terms of requirements for the individual fatty acids for a given tissue (2, 3). For example, the erythrocyte and the mitochondria of the brain and liver contain approximately 25% to 35% of essential fatty acids. In the case of the erythrocyte, arachidonic acid (20:4) is most prevalent; in the brain mitochondria, docosahexaenoic acid (22:6) is a principal constituent; and in the liver, arachidonic acid (20:4) and linoleic acid (18:2) are present in similar high concentrations. It should be noted that while the first measurable indication of a marginal deficiency is the accumulation of nonessential polyunsaturated fatty acids, particularly the trienoic acids (20:3), this test fails if the diet supplies even more highly unsaturated nonessential fatty acids such as those in cod liver oil. The trienoic acids (20:3) do not appear in this case.

Although the appearance of a trienoic acid ($\Delta^{5,18,11}$ 20:3) has been recognized as the first sign of impending deficiency (14, 15), the existence of two isomeric trienoic acids (20:3), appears to have been overlooked in studies utilizing alkali isomerization as the sole analytical method. This represents a serious drawback to the use of the alkali isomerization technique in studies of essential fatty acid deficiency. In the liver mitochondrial lipids, where deficiency was noted when linoleic acid (18:2) constituted less than approximately 10% of the fatty acids, the user of the alkali isomerization technique would measure approximately 6% dienoic acid, and base his estimate of nonessential polyunsaturated fatty acids on the detection of 7% trienoic acids (20:3) without realizing two fatty acids were making up the observed triene content.

In recent years there has been growing awareness of the biochemical importance of the fatty acid composition of the phospholipids. This has been especially pertinent with respect to the unsaturated fatty acids in the diglycerides, which are the preferred substrates for phosphatidic acid synthesis (32, 33). Furthermore, where phosphate exchange in phosphatidic acids has been studied in relation to cholinergic agents, the fatty acid composition of the phosphatidic acid may be a determinant in the rate of active transport across a membrane (34). Collins (35) has shown that rat liver lecithins which contained the most highly unsaturated fatty acids were most highly labeled when P^{32} inorganic phosphate was given *in vivo*. A large difference in specific activity was noted between the most highly unsaturated lecithins.

When essential fatty acids are replaced by nonessential polyunsaturated fatty acids, well-known symptoms of essential fatty acid deficiency become apparent. Conversely, if there is a high intake of polyunsaturated fatty acids, inadequately protected against *in vivo* autoxidation, other pathological manifestations may appear (2, 36).

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