The relationship of single dietary polyunsaturated fatty acids to fatty acid composition of lipids from subcellular particles of liver

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SUMMARY Supplements of purified fatty acid methyl or ethyl esters were fed at levels of 0.8% of dietary calories to each of seven groups of weanling rats for a period of 60 days. The esters were 9,12-octadecadienoate (18:2); 9,12,15-octadecatrienoate (18:3); 10,13-nonadecadienoate (19:2); 5,8,11,14-eicosatetraenoate (20:4); 5,8,11,14,17-eicosapentaenoate (20:5); 4,7,10,13,16,19-docosahexaenoate (22:6); and 12:13-epoxyoctadeca-9-enoate (epoxyoleate). Rats fed a fat-free diet served as control animals. The effects of these dietary supplements on the fatty acid compositions of the nonphospholipids and phospholipids from liver microsomal and mitochondrial particles, and of unfractionated lipids from liver, testes, epididymal, and heart tissues were determined by gas–liquid chromatography.

Epoxyoleate and 19:2, which are structurally related to linoleic acid, did not function as essential fatty acids as judged by the chemical and biological symptoms of fat deficiency. Dietary 20:5 and 22:6, both related to linolenate, were less active than linoleate in preventing the biological symptoms of fat deficiency, but more active in depressing the content of eicosatrienoate. Docosapentaenoic acid of the linoleate family was identified by reductive ozonolysis and shown to increase with an increase in dietary 18:2 or 20:4.

In 1960, Collins (1) found that the most unsaturated of the rat liver lecithins were the most highly labeled when inorganic phosphate-P^{32} was given in vivo, suggesting that the metabolic turnover of polyunsaturated phospholipids is greater than that of less unsaturated ones. Other studies have shown that essential fatty acids (EFA) may satisfy a structural requirement in normal cellular membranes (2–4), and that they may be essential components in the lipid–enzyme complexes of subcellular particles (5). Changes in the composition of dietary fat influence the composition of these subcellular particles and, therefore, may affect the rate of cellular metabolism.

The purpose of the present study was to determine the effects of seven different purified dietary fatty acids on EFA deficiency, and on the composition of rat tissue lipids. The lipids of the enzyme-rich microsomal and mitochondrial fractions of liver were separated into phospholipids and nonphospholipids and examined by gas–liquid chromatography (GLC). Unfractionated lipids of tissues from the same animals, namely epididymal fat, testes, heart, and liver were also examined.

Linoleate (18:2ω6)[1] and arachidonate (20:4ω6) were used as representatives of a family of fatty acids having full EFA activity. Linolenate(18:3ω3), 5,8,11,14,17-eicosapentaenoate (20:5ω3) and 4,7,10,13,16,19-docosahexaneoate (22:6ω3) were selected as representatives of a family of acids having partial activity. The structure of 10,13-nonadecadienoate (19:2ω6) differs by only one carbon atom from that of linoleate (18:2ω6), and 12:13-epoxyoctadeca-9-enoate (epoxyoleate) is structurally related to linoleate by having a polar group in the position occupied by the second double bond of the latter. Epoxyoleate and 19:2ω6 were fed in this experiment in order to study them as possible metabolic substitutes for, or antagonists of, linoleic acid. The availability of a recently described micromethod of

[1] To avoid confusion between isomers and to point out family relationships, acids having the first double bond between the third and fourth carbon atoms from the terminal methyl group are designated ω3 and are related to linolenate. Those related to linoleate are designated ω6 and those related to oleate ω9. All the polyunsaturated acids considered here have the divinylmethane rhythm of double bond spacings.

TABLE 1 PURITY OF DIETARY SUPPLEMENTS AND THEIR BIOLOGICAL EFFECTS

Dietary Supplements	No. of Animals	Caloric Level	% Major Fatty Acid*	% Contamination by EFA*	% *trans* double bonds†	Food Efficiency g gain/100 g diet	Dermal Score
None	10	—	—	—	—	23.7 ± 0.6	2.8 ± 1.3
Methyl 9,12-octadecadienoate (18:2ω6)	6	0.84	98.3	0.0	0.0	27.8 ± 0.6	0.2 ± 0.2
Methyl 9,12,15-octadecatrienoate (18:3ω3)	6	0.79	99.3	0.0	26.1	26.6 ± 0.5	0.8 ± 0.3
Ethyl 5,8,11,14-eicosatetraenoate (20:4ω6)‡	6	0.87	87.1	0.0	5.1	25.8 ± 0.9	0.2 ± 0.2
Methyl 5,8,11,14,17-eicosapentaenoate (20:5ω3)	6	0.94	81.1	6.7 (20:4ω6)	9.0	25.7 ± 0.8	1.0 ± 0.2
Methyl 4,7,10,13,16,19-docosahexaenoate (22:6ω3)	6	0.82	65.5	9.3 (22:5ω3)	23.6	25.0 ± 0.7	1.9 ± 0.4
Methyl 10,13-nonadecadienoate (19:2ω6)	6	0.89	95.5	3.8 (18:2ω6)	0.0	24.9 ± 2.2	1.1 ± 0.4
Methyl 12:13-epoxyoctadec-9-enoate	6	0.84	100.0	0.0	0.0	22.6 ± 0.6	2.5 ± 0.6

* The fatty acid compositions of the diet supplements were determined by GLC analysis.

† The amounts of *trans* double bonds in the diet supplements were determined by infrared spectral analysis and are expressed as per cent of total double bonds using methyl elaidate as standard.

‡ Provided by Hoffman La Roche.

ozonolysis (6) made it possible to determine the structure of previously unidentified fatty acids encountered during the study.

The conclusions concerning biosynthetic pathways and competitive relationships which appear in this study are drawn from static rather than dynamic analyses and must, therefore, be considered as indicative findings rather than final proof. The authors wish to emphasize, however, that the accumulation of knowledge of the metabolism of fatty acids has, in the past, involved initial static analyses followed by confirmatory dynamic analyses. The present study of steady state concentrations may generate further studies of a dynamic nature.

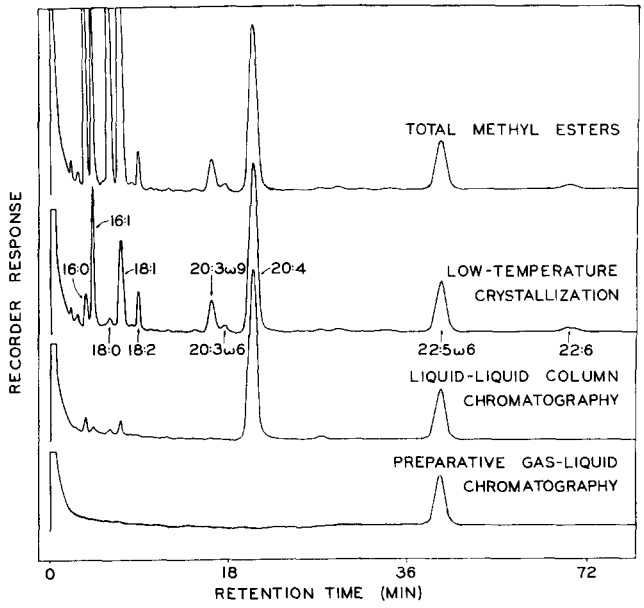

FIG. 1. Purification of methyl 4,7,10,13,16-docosapentaenoate from pooled rat tissues as monitored by analytical GLC. Chromatograms from top to bottom represent: total methyl esters of starting material; mother liquors from crystallization at —77°; the appropriate fraction from liquid–liquid column chromatography; and finally, the 22:5ω6 fraction collected by preparative GLC.

METHODS

Weanling male rats were raised on a fat-free synthetic diet described previously (7), supplemented, where appropriate, at levels of approximately 0.8% of calories with the methyl or ethyl esters of the fatty acids. The major constituents of the basic diet were vitamin-free casein, sucrose, cellulose and required salts and vitamins. The purities of the acids fed are shown in Table 1. The content of *trans* unsaturation, although high in some cases, was not considered to be prohibitive for the present study. Thus, although *trans* isomers have no EFA activity, they are apparently incorporated into higher polyunsaturated compounds; the presence of about one-fourth *trans* double bonds would not prevent the utilization of the all-*cis* isomer present as principal isomer (8). The basic diet was fed ad lib., and the amount consumed daily by each animal was recorded. The supplementary fatty esters (40 μl/day) were administered orally. The caloric intake of supplement was calculated for each animal.

After 59 days the animals were examined and the dermal scores recorded (9). The animals were weighed and the weight gains expressed as food efficiencies (g gain per 100 g diet). These data are given in Table 1. The animals were then sacrificed by ether anesthesia, and the livers were immediately excised and chilled in crushed ice. A 1-g portion of each liver, and also the heart, epididymal fat pad, and testes were stored in saline solution at −20° until analyzed. The remainder of the liver was used for the preparation of cell fractions.

Mitochondrial and microsomal cell fractions were isolated by differential centrifugation using the method of Hogeboom (10). Liver tissue from each animal was added to ice-cold 0.25 M sucrose to make a 20% suspension (v/v) and homogenized using a Potter-Elvehjem homogenizer. The cell debris and nuclei were discarded after sedimentation at 600 × *g* for 10 min. The mitochondria were next separated at 12,500 × *g* for 10

min, and the microsomes isolated by centrifuging the supernatant fluid for 60 min at 105,000 × *g*. The cell fractions were stored in acetone at the temperature of solid CO_2 until analyzed.

Lipid Extraction and Fractionation

The cell fractions and unfractionated liver tissue from *each* animal, and the epididymal fat, heart, and testes tissues, pooled according to diet group, were homogenized and extracted in a Servall Omni-mixer with chloroform–methanol 2:1 (v/v). The lipids from the subcellular particles were fractionated by silicic acid chromatography to give nonphospholipid and phospholipid fractions. A glass column, 1 cm i.d., packed with 5 g of silicic acid (100–200 mesh), was washed with 100 ml of chloroform prior to being charged with the lipid sample (approx 30 mg). The nonphospholipid fraction was eluted with 50 ml of chloroform at a flow rate of 3.5 ml/min. The column was then stripped of phospholipids by eluting with 75 ml of distilled methanol. The chloroform eluate was monitored by thin-layer chromatography (TLC) to assure complete fractionation. The nonphospholipid fraction consisted of cholesterol, free fatty acids plus diglycerides, triglycerides, and cholesterol esters as determined by TLC.

Methanolysis and GLC

The nonphospholipid and phospholipid fractions from the subcellular particles, and lipid extracts from the unfractionated tissues, were methanolyzed in 5 ml of 5% methanolic HCl, and the methyl esters were analyzed by GLC using the apparatus and conditions described previously (7). The GLC data were calculated as area per cent, a procedure justified in cases such as this where changes in concentration, but not the absolute composition, are to be measured.

Most of the peaks on the GLC chromatograms were identified by reference to fatty acid standards. Three esters remained unidentified by this method. Of these, two with carbon numbers (11) of 21.85 and 22.14 were tentatively identified as 5,8,11-eicosatrienoate (20:3ω9) and 8,11,14-eicosatrienoate (20:3ω6), respectively, by comparison with standards (isolated from brook trout) whose structures had been previously determined by ozonolysis. The third unknown ester (carbon number = 25.22), tentatively identified as 22:4ω3 from retention time data, was isolated from tissue and its structure was determined to be 22:5ω6 by reductive ozonolysis as described below.

Isolation of Unidentified Ester

The lipids from pooled heart, liver, and testes tissues of the rats fed 18:2ω6 and 20:4ω6 were saponified, and the free fatty acids were made up to a 12% solution with acetone and crystallized at −77°. The fatty acids of the filtrate fraction were esterified with diazomethane to yield methyl esters (156 mg). The methyl esters were fractionated by liquid–liquid chromatography using a silicone–Celite (1:1, w/w) column and acetonitrile–water 70:30 (v/v) as eluent (12). The fractions containing the unidentified ester were pooled and subjected to preparative GLC using a Research Specialties instrument equipped with a thermal conductivity detector. Fractions were collected using the apparatus of Schlenk and Sand (13). The column was of aluminum tubing, 10 ft × $^3/_8$ in., packed with 20% β-cyclodextrin acetate (13) on Chromosorb P (30–60 mesh) and eluted with helium at 100 ml/min. The results of the purification of the unidentified ester are shown in Fig. 1. The hydrogenated methyl ester had the same GLC retention time as methyl behenate (22:0).

Identification of Novel Ester

The positions of the double bonds of the C_{22} ester were determined by ozonolysis followed by identification of the aldehyde and ester-aldehyde fragments by GLC analysis, according to Privett and Nickell (6). The results (Fig. 2) show the fragments from the unidentified fatty ester to be the C_4 aldester and the C_6 aldehyde. Assuming a divinylmethane rhythm of unsaturation in the original fatty acid, the fragments must have been derived from 4,7,10,13,16-docosapentaenoate (22:5ω6).

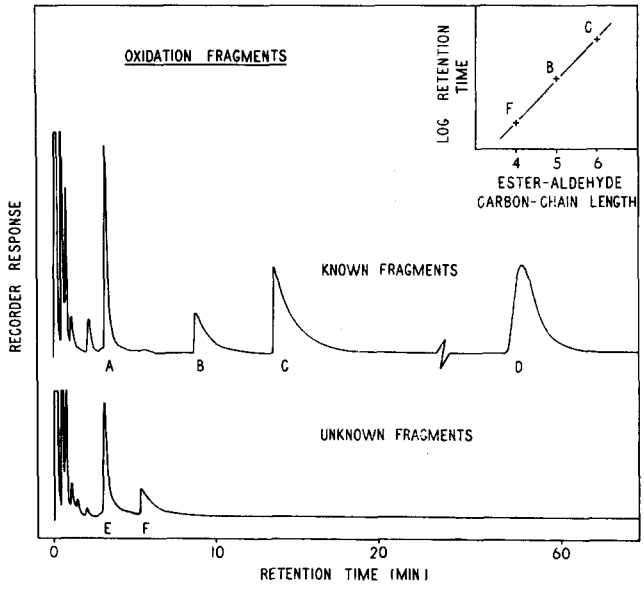

FIG. 2. Structural analysis of methyl 4,7,10,13,16-docosapentaenoate (22:5ω6) by identification of reductive ozonolysis fragments. The fragments were identified by comparison of their GLC retention times with those of standards having known structures. *A*, C_6 aldehyde; *B*, C_5 aldester; *C*, C_6 aldester; *D*, C_{12} aldehyde. Fragment *E* corresponded to the C_6 aldehyde (*A*). The graph in the upper right corner shows that the retention time of fragment *F* corresponded to that of the C_4 aldester.

Methanolysis Products of Epoxyoleate

Epoxy fatty acids undergo molecular alterations during methanolysis procedures. The methanolysis products of epoxyoleate, which have been postulated to be the hydroxymethoxy or isomeric chlorohydrin derivatives of the epoxide group (14, 15), proved to be undetectable by our conventional GLC method of ester analysis. However, they were easily detectable by TLC, in which they migrated together with authentic isomeric chlorohydrins of epoxyoleate, thus providing a qualitative test for epoxyoleate. The products of 2 μg or more of epoxyoleate were detectable under our conditions.

Where feasible, the range of variation of a group of determinations from a mean value was expressed as the standard error of the mean. The significance of the difference between two mean values was calculated by application of the "t" test (16). The limit of significance was set at $P = 0.05$.

RESULTS AND DISCUSSION

Dermal scores and food efficiencies indicated that of the several supplements tested, only linoleate and arachidonate were fully effective as EFA (Table 1). Members of the linolenate family of acids stimulated growth, but failed to prevent the dermatitis of EFA deficiency.

Epoxyoleate when fed could not be detected in subcellular nonphospholipids and phospholipids, or in epididymal unfractionated tissue lipids by TLC. Since epoxyoleate survives the digestion process and is absorbed into the body (17), it presumably was present, but in amounts less than 0.2% of the lipids. The control rats fed a fat-free diet and those fed epoxyoleate supplement had equally low food efficiencies and high dermal scores (see Table 1), indicating that when fed at levels of 0.8% of calories, epoxyoleate does not prevent EFA deficiency. The fact that the EFA-deficiency symptoms were not more severe in the epoxyoleate group suggests that epoxyoleic acid does not function as a metabolic antagonist to linoleic acid.

Nonadecadienoate (19:2ω6) cannot adequately replace 18:2ω6 as an essential fatty acid, although, according to their terminal structures, they are members of the same family of fatty acids. This was indicated by the high dermal scores of the rats fed 19:2ω6. The food efficiency of 19:2-fed rats did not differ significantly from either the fat-free or 18:2-fed group. These results agree with those of Karrer and Koening (18) and of Thomasson (19, 20), who reported 19:2ω6 to have minimal EFA activity on the basis of weight gains.

The fatty acid composition of the lipids from the various body tissues are shown in Tables 2 and 3. Those of the microsomal lipids proved to be nearly identical with those of the mitochondrial lipids for all diet groups and have, therefore, been omitted from the tables. The fatty acid analysis of the epididymal tissue showed only the normal components of chain length less than, and degree of unsaturation less than, that of 18:2, and the data are not presented. Analyses of the lipids of unfractionated tissues from rats fed 18:3ω3 and 20:4ω6 appear in an earlier study (7).

Epoxyoleate, 19:2ω6 and Fat-Free Diets

The similarity in fatty acid composition of the lipids from the animals fed epoxyoleate, 19:2ω6, and fat-free diets indicates that general fatty acid metabolism was not altered by ingested epoxyoleate or 19:2ω6. In this experiment the only fatty acid to be found in equal amounts in the subcellular nonphospholipid and phospholipid fractions was 19:2ω6. Therefore, the mechanism that controls the unequal distribution of normal amounts of dietary polyunsaturated fatty acids between the nonphospholipid and phospholipid fractions is not functional in distributing this unnatural fatty acid. Moreover, feeding equal amounts of 18:2ω6 and 19:2ω6 resulted in equal levels of these fatty acids in the subcellular phospholipid fractions, indicating that the mechanisms incorporating linoleate into phospholipids were unable to distinquish the one-carbon difference between these two fatty acids. The concentration of 19:2ω6 itself in the various tissues ranged from traces in the lipids from epididymal tissues to 6.7% of total fatty acids in the heart lipids, whereas possible higher metabolites of 19:2ω6, such as 21:2, 21:3, or 21:4, could not be detected. Thus it is apparent that the mechanisms involved in the *metabolism* of 18:2ω6 can discern a one-carbon structural difference at the carboxyl end of the molecule, and 19:2 is not lengthened and dehydrogenated as is linoleate. A specific search for 21:4ω6 made by Schlenk and Sen (21) did not detect any trace of this most likely metabolite of 19:2ω6.

Linoleate and Arachidonate

Previous studies (4, 22–24) have shown that the fatty acid composition of subcellular particles can be altered by ingested fat. For the study of EFA, it is of further advantage to separate phospholipids and nonphospholipids, for the liver mitochondrial phospholipids reflect magnified differences in polyunsaturated fatty acid compositions in response to dietary change. 8,11,14-Eicosatrienoic acid (20:3ω6) appeared in the liver lipids in measurable amounts only in tissues of the 18:2-fed animals. This acid has been reported to be a rapidly metabolized intermediate in the conversion of 18:2ω6 to 20:4ω6 (25), and Stoffel (26) found that the dehydrogenating enzyme system, which is active in this conversion, is located in the microsomal fraction of the cell. However, 20:3ω6 was found in the lipids of both the

TABLE 2 FATTY ACID COMPOSITION OF LIPIDS OF LIVER MITOCHONDRIA

Diet and Lipid Fraction	14:0 (14.00)*	16:0 (16.00)	16:1 (16.78)	18:0 (18.00)	18:1 (18.67)	18:2ω6 (19.47)	18:3ω3 (20.61)	19:2ω6 (20.64)	20:3ω9 (21.85)	20:3ω6 (22.14)	20:4ω6 (22.72)	20:5ω3 (23.80)	22:5ω6 (25.22)	22:5ω3 (25.70)	22:6ω3 (26.32)
	% of total fatty acids†														
Fat-free															
Phospholipids	0.54±0.17	21.1±0.9	6.62±0.41	20.0 ±0.7	25.4±0.7	1.32±0.09	—‡	—	16.5 ±0.9	—	6.05±0.38	—	1.08±0.08	—	1.23±0.09
Nonphospholipids	3.61±0.40	31.6±1.4	11.8 ±0.9	7.96±0.40	39.0±1.7	0.96±0.16	—	—	2.92±0.29	—	1.14±0.14	—	0.23±0.02	—	0.37±0.05
18:2ω6															
Phospholipids	0.29±0.01	20.9±0.6	4.98±0.42	20.1 ±0.7	20.1±0.6	4.29±0.19	—	—	6.11±0.43	1.64±0.09	17.1 ±0.8	—	3.18±0.13	—	0.74±0.04
Nonphospholipids	3.42±0.31	32.1±0.4	12.8 ±0.7	8.61±0.33	34.7±0.9	2.00±0.19	—	—	1.47±0.20	0.27±0.05	3.47±0.50	—	0.29±0.07	—	0.49±0.10
18:3ω3															
Phospholipids	0.32±0.05	25.7±0.7	6.50±0.57	19.2 ±1.0	21.3±0.6	0.91±0.13	0.35±0.05	—	3.82±0.46	—	2.36±0.21	5.08±0.41	—	1.53±0.19	13.6 ±1.0
Nonphospholipids	3.04±0.48	32.9±0.8	11.8 ±0.7	7.77±0.24	35.0±1.8	0.78±0.15	0.79±0.12	—	1.14±0.14	—	0.80±0.12	2.28±0.33	—	0.53±0.14	3.17±0.43
20:4ω6															
Phospholipids	0.44±0.08	23.0±0.7	5.61±0.53	19.6 ±1.2	18.1±0.8	0.62±0.05	—	—	1.48±0.31	—	25.0 ±1.0	—	5.29±0.36	—	0.71±0.07
Nonphospholipids	3.37±0.45	33.0±0.7	12.4 ±1.0	7.99±0.24	34.1±1.1	0.99±0.19	—	—	0.61±0.14	—	6.37±0.78	—	0.75±0.11	—	0.46±0.23
20:5ω3															
Phospholipids	0.42±0.03	26.1±0.9	6.97±0.42	15.5 ±1.1	18.2±0.6	0.90±0.06	—	—	1.31±0.21	—	4.95±0.20	7.66±0.26	—	3.68±0.35	14.3 ±0.7
Nonphospholipids	3.06±0.41	32.5±1.5	12.3 ±0.9	7.05±0.95	33.6±2.2	0.88±0.12	—	—	0.55±0.12	—	1.48±0.33	4.27±1.04	—	1.24±0.40	2.72±0.72
22:6ω3															
Phospholipids	0.26±0.05	27.8±0.7	6.18±0.26	18.1 ±1.6	21.2±0.6	1.09±0.20	—	—	1.95±0.23	—	2.59±0.26	2.84±0.38	—	1.07±0.13	17.0 ±1.3
Nonphospholipids	2.53±0.18	34.4±0.4	11.1 ±0.5	7.17±0.44	36.0±0.7	1.51±0.30	—	—	0.70±0.07	—	0.82±0.11	1.99±0.29	—	0.37±0.05	3.46±0.24
19:2ω6															
Phospholipids	0.45±0.05	22.7±0.7	6.09±0.25	18.7 ±0.5	23.1±0.5	1.20±0.08	—	4.51±0.56	11.93±0.74	—	7.38±0.62	—	2.02±0.24	—	1.97±0.17
Nonphospholipids	3.49±0.19	31.4±0.9	12.6 ±0.8	7.77±0.29	35.5±0.7	0.64±0.04	—	4.04±0.35	2.39±0.33	—	1.28±0.15	—	0.40±0.10	—	0.42±0.06
Epoxyoleate															
Phospholipids	0.31±0.03	22.4±0.7	7.02±0.69	19.8 ±1.0	24.8±0.4	1.42±0.09	—	—	16.13±0.32	—	6.13±0.36	—	1.02±0.09	—	1.04±0.04
Nonphospholipids	3.91±1.00	34.2±2.1	11.4 ±0.6	8.29±0.91	37.2±3.6	1.75±0.41	—	—	2.08±0.48	—	0.70±0.15	—	0.23±0.05	—	0.25±0.06

* Calculated carbon number.

† Areas of GLC peaks determined by triangulation and reported as percentage of total area.

‡ Unreported values indicated by — imply that the substance was below the limit of sensitivity of the analytical method.

TABLE 3 FATTY ACID COMPOSITION OF LIPIDS OF UNFRACTIONATED TISSUES

Diet	14:0	16:0	16:1	18:0	18:1	18:2ω6	19:2ω6	20:3ω9	20:3ω6	20:4ω6	20:5ω3	22:5ω6	22:5ω3	22:6ω3
							% of total fatty acids							
Fat-Free														
Liver	1.09±0.14	24.7±1.4	10.9 ±0.7	10.6 ±0.9	36.9±1.6	0.83±0.13	—*	8.03±1.21	—	3.66±0.45	—	0.59±0.08	—	0.62±0.10
Testes	1.20	37.3	8.87	4.60	32.6	0.57	—	3.58	—	3.88	1.00	5.42	0.47	0.41
Heart	0.85	16.4	6.67	17.4	33.1	3.22	—	12.6	—	8.28	—	0.56	—	0.62
18:2ω6														
Liver	0.79±0.05	24.3±0.4	8.56±0.49	12.4±0.5	31.4±0.5	3.47±0.15	—	4.36±0.44	1.16±0.20	11.0±0.7	—	2.03±0.11	—	0.37±0.03
Testes	1.04	37.1	7.41	4.38	25.0	1.60	—	0.85	0.54	8.43	0.33	13.0	0.26	—
Heart	0.59	15.8	4.23	17.9	25.0	12.1	—	3.12	—	18.4	—	2.17	—	0.57
20:5ω3														
Liver	0.85±0.07	25.4±1.0	10.1 ±0.4	10.7 ±0.8	31.8±1.5	0.72±0.10	—	0.89±0.23	—	3.18±0.36	5.73±0.63	—	2.20±0.44	7.95±0.72
Testes	1.50	36.6	11.3	4.23	34.0	0.68	—	0.93	0.30	3.84	0.93	3.06	0.48	2.14
Heart	0.52	16.7	5.56	19.6	26.5	2.68	—	1.04	—	11.1	4.87	—	4.41	6.86
22:6ω3														
Liver	0.61±0.03	26.2±0.5	8.70±0.48	12.4±0.5	33.0±0.5	0.72±0.13	—	1.41±0.16	—	1.96±0.13	3.19±0.27	—	0.48±0.09	11.3 ±0.8
Testes	1.12	39.6	9.41	3.11	32.1	0.68	—	2.20	0.61	4.01	0.82	3.11	0.35	2.85
Heart	0.64	17.7	5.82	19.9	29.0	4.00	—	2.24	—	6.94	2.37	—	0.58	10.8
19:2ω6														
Liver	0.88±0.09	24.9±0.9	9.84±0.51	11.0 ±0.9	36.0±1.6	1.08±0.27	3.19±0.55	6.74±0.34	—	4.22±0.59	—	1.16±0.18	—	1.00±0.13
Testes	1.10	41.0	7.17	4.82	28.0	0.39	2.36	3.02	—	3.98	1.18	6.00	0.62	0.52
Heart	0.45	14.0	4.57	20.4	26.5	2.88	6.66	10.7	—	11.5		0.93	—	1.33
Epoxyoleate														
Liver	0.93±0.19	23.7±0.3	12.1 ±1.4	9.50±0.84	39.8±1.7	0.67±0.08	—	8.24±0.86	—	3.48±0.43	—	0.63±0.11	—	0.63±0.12
Testes	1.00	38.7	7.13	5.45	29.5	0.49	—	4.55	—	4.19	1.25	6.29	0.76	0.59
Heart	0.53	16.0	4.83	19.1	29.9	3.88	—	16.2	—	8.31	—	0.66	—	0.65

* Content less than the limit of sensitivity of the analytical method.

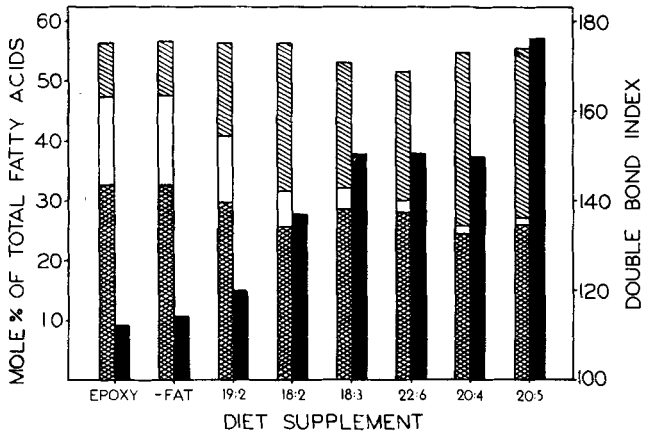

FIG. 3. Effects of dietary supplements on the content of unsaturated fatty acids and on double bond indices of mitochondrial phospholipids of liver. Graph designations are as follows: crosshatch = polyenes; white = 20:3ω9; double crosshatch = monoenes; black = double bond index.

microsomes and mitochondria, suggesting that it may be functional other than as a metabolic intermediate.

The isolation and identification of 4,7,10,13,16-docosapentaenoic acid (22:5ω6) has confirmed and extended our previous studies (27) and those of Mead's group (25, 28–30) on the in vivo conversions of linoleic acid. The increase of 22:5ω6 only in the lipids of the rats fed linoleate (18:2ω6) and arachidonate (20:4ω6) and the similarity of the structures of these three acids strongly suggest that the 22:5ω6 is derived from linoleate via arachidonate.

Recent studies have shown that linolenic acid (18:3-ω3) has an influence on the concentration of metabolites of 18:2ω6 in tissue lipids (7, 23, 31–35). In our present study, a reduction in concentration of 20:4ω6 in lipid fatty acids by dietary supplementation with 18:3ω3 was most noticeable in the subcellular phospholipid fractions (Table 2). The levels of 20:4ω6 in the subcellular phospholipids of the 22:6ω3-supplemented animals were significantly lower than in the control (fat-free) animals, indicating that inhibition of 18:2ω6 metabolism is not limited to 18:3ω3. The decreased concentration of 22:5ω6 in the lipids of animals fed the ω3 family of fatty acids, compared with those of the control animals, was even more striking.

The 22:5ω6 is the longest chain, most unsaturated member of the linoleate family thus far detected. If 22:5ω6 is the terminal member of the metabolic sequence, its EFA activity could be expected to be greater than that of 20:4ω6, since the activity of 20:4ω6 is greater than that of its precursor 18:2ω6. That 22:5ω6, as terminal metabolite, may be the unique functional essential fatty acid is not borne out by the data available. We have found no positive correlation in previous work (7), or in this report, between the content of 22:5ω6 in the liver and the prevention of the dermatitis or inhibition of growth caused by EFA deficiency. Thus, polyunsaturates other than 22:5ω6 must have contributed to the EFA functions in these two studies.

Unsaturated/Saturated Ratio

The total mole percentage of unsaturated fatty acids from the subcellular lipids remained fairly constant in all diet groups despite wide differences in the supplements (Fig. 3). Through the synthesis of 16:1, 18:1, and 20:3ω9, a constant ratio of saturated to unsaturated fatty acids in the liver lipids was maintained even without an exogenous source of unsaturated fatty acids. Evidence of a regulated synthesis and distribution of fatty acids, as described above, has been reported in studies of unfractioned tissue (35–38) and has been suggested to be a mechanism for maintaining a constant physical state of body lipids (39, 40). The double bond indices[2] of the mitochondrial phospholipids were calculated, because the physical state of a lipid may be more directly dependent upon the total number of double bonds present than upon the percentage of unsaturated fatty acids. Although the rats were able to maintain a constant concentration of unsaturated fatty acids, they were unable to maintain a constant number of total double bonds in the phospholipids (Fig. 3). The constancy of the ratio of saturated to unsaturated fatty acids does not preclude, therefore, a changing physical state of tissue lipids, which may contribute to the morphological symptoms of deficiency.

Penta- and Hexaenoates

The rats of the 20:5ω3 and 22:6ω3 diet groups had higher dermal scores and lower food efficiencies than the 18:2-fed rats, indicating EFA deficiency (Table 1). However, they also had much lower levels of tissue 20:3ω9 than the 18:2-fed rats. Low levels of tissue 20:3ω9 are generally associated with adequate levels of dietary EFA. The noncorrelation of dermal scores and food efficiencies with the levels of 20:3ω9 in tissue lipids indicates that these parameters are not directly related. One can depress the levels of 20:3ω9 in tissue lipids and yet have significant biological symptoms of EFA deficiency in rats fed purified fatty acids of the linolenate family in the absence of acids of the linoleate family.

Fatty Acids of Different Tissues

The compositions of the polyunsaturated fatty acids in the tissue lipids were different in the various body organs analyzed (Table 3). The levels of 18:2ω6 and 20:4ω6 were much higher in the heart lipids than in the lipids of

[2] The double bond index equals the sum of the products of the mole fraction and the number of double bonds for each fatty acid.

either the liver or testes. The major fatty acid of the ω6 family in the heart and liver lipids of the 18:2ω6-supplemented animals was 20:4ω6, whereas 22:5ω6 was the major fatty acid of this family found in the lipids of the testes. The levels of 22:6ω3 in the liver and heart lipids of the 22:6-fed rats were about four times higher than that in the testes lipids of these animals. The lipids of the testes appear to be unique because of their low levels of linolenate type fatty acids and because 22:5ω6, rather than 20:4ω6, was the major unsaturated fatty acid in the linoleate-supplemented animals.

The authors are indebted to Hans Mohrhauer for many helpful discussions and suggestions. We wish to thank Hermann Schlenk and Donald Sand for their practical advice on the fatty acid separations, and Nirmal Sen for preparing the 10,13-nonadecadienoate used in this experiment.

These studies were supported in part by PHS Grant AM 04524 from the National Institutes of Health, U.S. Public Health Service; the Life Insurance Medical Research Fund; and the National Dairy Council.

The work reported here is abstracted from the doctoral dissertation of Joseph J. Rahm, University of Minnesota, June 1963.

Manuscript received August 2, 1963; accepted October 20, 1963.

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