Fatty Acid Changes in Liver and Plasma Lipid Fractions after Safflower Oil was Fed to Rats Deficient in Essential Fatty Acids

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1. Fatty acid patterns of liver and plasma triglycerides, phospholipids and cholesteryl esters were determined at intervals during 24 hr. after essential fatty acid-deficient rats were given one feeding of linoleate (as safflower oil). 2. Liver triglyceride, phospholipid and cholesteryl ester fatty acid compositions did not change up to 7 hr. after feeding. Between 7 and 10 hr., linoleic acid began to increase in all fractions, but arachidonic acid did not begin to rise in the phospholipid until 14–19 hr. after feeding. 3. Oleic acid and eicosatrienoic acid in liver phospholipid began to decline at about the time that linoleic acid increased, i.e. about 9 hr. before arachidonic acid began to increase. 4. Changes in linoleic acid, arachidonic acid and eicosatrienoic acid in phosphatidylcholine resembled those of the total phospholipid. Phosphatidylethanolamine had a higher percentage content of arachidonic acid before the linoleate was given than did phosphatidylcholine, and after the linoleate was given the fatty acid composition of this fraction was little changed. 5. The behaviour of the plasma lipid fatty acids was similar to that of the liver lipids, with changes in linoleic acid, eicosatrienoic acid and arachidonic acid appearing at the same times as they occurred in the liver. 6. The results indicated that linoleic acid was preferentially incorporated into the liver phospholipid at the expense of eicosatrienoic acid and oleic acid. The decline in these fatty acids apparently resulted from their competition with linoleic acid for available sites in the phospholipids rather than from any direct replacement by arachidonic acid.

Rahm & Holman (1964) have pointed out that significant changes in tissue fatty acid patterns in rats on fat-free diets could be induced by supplementing the diets with linoleate, linolenate or arachidonate for as little as 5 days. Investigations by Brenner & Nervi (1965) of the dynamic aspects of the changes in fatty acids when EFA† were given demonstrated that by 24 hr. dietary linoleate produced changes in eicosatrienoic acid and arachidonic acid in the liver phospholipids of rats fed on a fat-free diet.

Experiments from this Laboratory (Ostwald, Bouchard, Miljanich & Lyman, 1965), comparing the metabolism of EFA in male and female rats, indicated that EFA-deficient rats given one large dose of linoleate increased their liver phospholipid arachidonate more than threefold within the next

24 hr. Concurrent with this increase was an even greater relative rise in phospholipid linoleate, and a fall in 5,8,11-eicosatrienoic acid and oleic acid. The rapidity with which the fatty acid changes took place suggested that a similar procedure might be used to follow the very early changes and the sequence of the displacement and replacement of fatty acids in various lipid classes of liver and plasma of EFA-deficient rats. An experiment was therefore conducted with EFA-deficient rats in which the fatty acid patterns of liver and plasma phospholipid, triglycerides and cholesteryl esters were followed during 24 hr. after a single dose of linoleate was given as part of the diet.

METHODS

Animals and feeding. Forty male weanling rats of the Long-Evans strain were given the EFA-deficient diet for 53 days (Table 1). At the end of the feeding period the average weight of each rat was 217 g. They showed only mild external evidence of EFA deficiency, i.e. dandruff and

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† Abbreviation: EFA, essential fatty acids.

Table 1. *Composition of EFA-deficient diet*

The diet mixed with hydrogenated coconut oil (Plastine; Durkee Famous Foods, Cleveland, Ohio, U.S.A.), had the following (percentage) fatty acid composition (gas-liquid-chromatographic analysis): C₈ acids, 3.0; C₁₀ acids, 4.9; C₁₂ acids, 47.5; C₁₄ acids, 19.8; C₁₆ acids, 10.6; C₁₈ acids, 11.4; C_{18:1} acids, 3.0; C_{18:2} acid, <0.01. The mineral mix provided the following in g./100g. of diet: CaCO₃, 0.725; CaHPO₄, 1.130; Na₂HPO₄, 0.651; KCl, 0.730; MgSO₄, 0.230; MnSO₄·H₂O, 0.0154; CuSO₄, 0.0013; ferric citrate, 0.0151; ZnCO₃, 0.0021; KIO₃, 0.0001. The vitamin B mix supplied the following in mg./100g. of diet: thiamine hydrochloride, 0.5; riboflavin, 0.5; nicotinic acid, 2.5; calcium pantothenate, 2.0; pyridoxine hydrochloride, 0.25; menadione, 0.05; biotin, 0.01; folic acid, 0.02; vitamin B₁₂, 0.002; inositol, 10. Fat-soluble vitamins were supplied in a non-fat form (Nopco Chemical Co., Richmond, Calif. U.S.A.) to provide per 100g. of diet: vitamin A, 1700 i.u.; vitamin D, 100 i.u.; vitamin E (α -tocopherol), 6.66 mg.

Ingredient	Composition (% by wt.)
Casein	20
Hydrogenated coconut oil	10
Sucrose	64.4
Mineral mix	3.5
Vitamin B mix	1.0
Fat-soluble vitamin mix	1.0
Choline bitartrate	0.14

some scaliness of feet. Tissue lipid patterns, however, indicated a definite EFA deficiency resembling that obtained in previous experiments of slightly longer duration (Ostwald *et al.* 1965; Lyman, Ostwald, Bouchard & Shannon, 1966).

All animals were starved for 24 hr. before the single feeding by stomach tube. For tube feeding, a 300g. portion of EFA-deficient diet, prepared without hydrogenated coconut oil, was homogenized with 100ml. of water (final volume 275 ml., i.e. about 1g. of diet/ml.). Each animal was then given 2.5 ml. of the homogenate plus 1 ml. of safflower oil (containing 77% of linoleate) per 100g. of body wt. so that each rat received an average of 1500 mg. of linoleate. The feeding apparently caused no discomfort to the animals and there was no diarrhoea. Five rats in each group were killed at 0, 5, 7, 10, 14, 19 and 24 hr. after feeding. A group of rats, force-fed on the regular EFA-deficient diet containing hydrogenated coconut oil, was killed after 24 hr. and served as controls for any effect due to tube feeding.

Tissue preparation. The animals were anaesthetized with pentobarbital sodium. Blood was withdrawn from the heart into heparin-treated syringes and cooled on ice. Livers were removed, weighed and frozen immediately on solid CO₂. Plasma was obtained by centrifugation and equal samples taken for each time-period were pooled and frozen. Liver and plasma were then freeze-dried and stored under vacuum until extracted.

Extraction of liver and plasma lipids. The dehydrated livers were pulverized and extracted twice, at room temperature, for 1 hr. with two 100 ml. portions of CHCl₃-methanol (2:1, v/v) containing 0.1 mg. of quinol. The

CHCl₃-methanol extract was dried in a rotary vacuum evaporator and the lipid was re-extracted into light petroleum (b.p. 30–60°).

Dried plasma was reconstituted with water and held on ice for 20 min. Each pooled sample (about 7–10 ml.) was then extracted with 100 ml. of CHCl₃-methanol (2:1, v/v) and the extract was washed once with an equal volume of water. It was then treated exactly as outlined for the liver extracts.

Lipid analysis. Lipid extracts were fractionated on silicic acid columns into cholesteryl esters, triglycerides, phospholipids and unesterified cholesterol (Lis, Tinoco & Okey, 1961). The total lipid of the original extract and of the triglyceride and phospholipid fractions was determined colorimetrically (Bragdon, 1951). Total and unesterified cholesterol were determined by the method of Sperry & Webb (1950) and phosphorus was determined by the method of Sumner (1944). Recoveries after fractionation of cholesterol, phosphorus and total lipid averaged 93%, 95% and 100% respectively. Fatty acids of the fractions were converted into methyl esters by H₂SO₄-catalysed transesterification in methanol (Tinoco, Lyman & Okey, 1965) before analysis by gas-liquid chromatography.

Phospholipids were separated by thin-layer chromatography (Skipski, Peterson & Barclay, 1964) into phosphatidylcholine, phosphatidylethanolamine, a fraction containing lysophosphatidylcholine and sphingomyelin, and a fraction containing phosphatidylinositol and phosphatidylserine. Phospholipid bands were detected under u.v. light after being sprayed with 2',7'-dichlorofluorescein. The silica gel containing the phospholipid was scraped into flasks and transmethylated directly. Methyl esters were eluted with methanol, then handled as described by Tinoco *et al.* (1965). The aqueous phase was analysed for phosphorus, and the recovery of applied lipid phosphorus averaged 92%. Gas-liquid chromatography was performed on instruments equipped with hydrogen-flame detectors (F and M model 810 and an Aerograph Hy FI, model 600; Wilkens Instrument Co., Walnut Creek, Calif., U.S.A.). Columns were polydiethylene glycol succinate-coated silane-treated Chromasorb W. Each instrument was calibrated daily with a standard containing known proportions of the methyl esters of lauric acid, myristic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid. Standards agreed with each other within $\pm 5\%$ or less of the value of an individual component. An internal standard of heptadecanoic acid (Tinoco, Shannon, Miljanich, Lyman & Okey, 1962) was methylated with each fatty acid sample, and served as an independent means of determining quantitatively the fatty acid recoveries with the total lipid, phosphorus and cholesterol determinations of the respective lipid fractions. Values for triglyceride and phospholipid compared well by both methods. Liver cholesterol esters, however, as obtained from the silicic acid columns, consistently had more fatty acid than could be accounted for by the cholesterol analysis; thus the values in Table 3 may not actually represent entirely cholesteryl ester fatty acids. This discrepancy was not seen with the plasma cholesteryl ester. Positions of double bonds in the eicosatrienoic acid were established by permanganate oxidation, with identification of the dicarboxylic acid by gas-liquid chromatography (Tinoco & Miljanich, 1965). Where pertinent, statistical comparisons between time periods were made by means of the *t* test (Snedecor, 1956).

RESULTS

Table 2 shows that giving linoleate to the EFA-deficient animals had little effect on the total amounts of liver phospholipid or cholesterol during the 24 hr. experimental period. The liver triglyceride increase apparently resulted from the feeding conditions rather than from the linoleate, since the tube-fed controls exhibited a similar increase. Concentrations of plasma cholesterol and phospholipid also were not significantly affected by the feeding with linoleate during the time-intervals covered by the experiment.

The major fatty acids in the liver lipids after feeding with linoleate are shown in Table 3. Values have been expressed as mg. of fatty acid in the lipid fraction, so that real rather than relative changes in the individual fatty acid may be shown.

Phospholipid fatty acids made up the largest part of the lipid fatty acids and were characterized by high concentrations of stearic acid and palmitic acid, which remained nearly constant during the post-feeding period. No consistent change occurred in the phospholipid linoleic acid until 7-10 hr. after feeding. By 24 hr. it had risen to about three times its initial concentration. Arachidonic acid, on the other hand, did not show any increase until 14-19 hr. Although oleic acid in the phospholipid had somewhat decreased by 24 hr., the largest quantitative decrease occurred in the eicosatrienoic acid. Its depletion started shortly after linoleate was given and continued throughout the post-feeding period, apparently unrelated to the changes in arachidonic acid.

Triglyceride fatty acids constituted the second largest source of liver lipid fatty acids and were the only liver lipids to increase appreciably during the feeding conditions. Almost all of the increase in

this lipid was in linoleic acid and oleic acid and, to a smaller degree, palmitic acid. Though relatively small amounts of linoleic acid were present initially in the triglyceride, the linoleate content had risen by 7-10 hr. post-feeding, and the rise continued until about 19 hr. This lipid fraction also exhibited a delay interval, similar to that in the phospholipids, between the time linoleate was given and its appearance in the liver lipids. The amount of eicosatrienoic acid and arachidonic acid in this fraction were very low and are not shown.

Cholesteryl ester fatty acids made up the smallest portion of the liver lipid fatty acids and contained initially small amounts of linoleic acid and arachidonic acid. As mentioned above, the fatty acids presented may not be entirely those of the cholesteryl ester fraction. Nevertheless, their composition appears characteristic for this lipid fraction, and the changes in linoleic acid were similar to those occurring in the phospholipid and the triglyceride.

Liver phospholipids were separated by thin-layer chromatography into phosphatidylcholine and phosphatidylethanolamine fractions and into two combined fractions, one mainly phosphatidylinositol with small amounts of phosphatidylserine, and the other a mixture of sphingomyelin and lysophosphatidylcholine. Phosphatidylcholine and phosphatidylethanolamine made up the major part of the phospholipid phosphorus (50% and 19% respectively) with the phosphatidylinositol and phosphatidylserine accounting for about 14% and the sphingomyelin and lysophosphatidylcholine for about 6.5%. The remaining 10% of phosphorus was distributed about equally between the origin and the solvent front. The proportions of the phospholipid fractions were not influenced by the feeding with linoleate. Table 4 shows the major

Table 2. *Liver and plasma lipids of EFA-deficient rats during feeding with linoleate*

Rats were fed by stomach tube on a fat-free diet supplemented with safflower oil at zero time and killed at intervals thereafter. Tube-fed controls were given the EFA-deficient diet and killed after 24 hr. Liver lipid means $\pm$ S.E.M. are from five animals per time-period. Plasma lipids were determined on pooled samples from five animals.

Time after feeding (hr.)	Liver (mg./liver)				Plasma (mg./100ml.)	
	Triglycerides	Phospholipid	Cholesteryl ester	Total cholesterol	Phospholipid	Total cholesterol
0	52 $\pm$ 17	235 $\pm$ 24	3.8 $\pm$ 0.8	17.7 $\pm$ 1.9	90.0	40.1
5	41 $\pm$ 6	231 $\pm$ 17	3.4 $\pm$ 1.0	15.9 $\pm$ 1.5	85.8	43.6
7	40 $\pm$ 6	249 $\pm$ 9	2.8 $\pm$ 0.9	16.4 $\pm$ 0.6	92.0	45.6
10	66 $\pm$ 4	245 $\pm$ 9	2.3 $\pm$ 0.8	15.6 $\pm$ 0.8	110.0	44.4
14	85 $\pm$ 24	231 $\pm$ 24	3.2 $\pm$ 0.7	15.3 $\pm$ 1.7	103.0	42.3
19	116 $\pm$ 26	272 $\pm$ 6	4.9 $\pm$ 0.6	19.0 $\pm$ 0.7	91.8	41.1
24	77 $\pm$ 13	265 $\pm$ 13	3.5 $\pm$ 0.4	17.7 $\pm$ 0.7	86.0	40.4
Controls (tube-fed)	123 $\pm$ 16	263 $\pm$ 6	7.2 $\pm$ 1.1	21.2 $\pm$ 1.5	80.2	42.6

Table 3. *Changes in fatty acids of liver cholesteryl ester, triglyceride and phospholipid in EFA-deficient rats after feeding with linoleate*

Rats were fed by stomach tube on a fat-free diet supplemented with safflower oil at zero time and killed at intervals thereafter. Tube-fed controls were given the EFA-deficient diet and killed at 24 hr. Values are means of five animals per time-period. Fatty acids below C₁₆ and beyond C_{20:4} have been omitted for convenience of presentation. Standard errors are given only for those fatty acids in which significant changes are emphasized.

	Time after feeding (hr.)	Liver fatty acids (mg./liver)						
		C _{16:0} acid	C _{18:0} acid	C _{16:1} acid	C _{18:1} acid	C _{18:2} acid	C _{20:3} acids*	C _{20:4} acid
Cholesteryl esters								
	0	1.00	0.20	0.31	1.79	0.05	0.08	0.07
	5	0.96	0.23	0.25	0.69	0.09	0.07	0.08
	7	0.77	0.16	0.20	0.48	0.06	0.06	0.07
	10	0.68	0.12	0.15	0.37	0.18	0.04	0.04
	14	0.82	0.16	0.21	0.57	0.24	0.05	0.06
	19	0.93	0.21	0.27	1.08	0.72	0.05	0.08
	24	0.89	0.16	0.19	0.53	0.46	0.03	0.12
Controls (tube-fed)	24	1.62	0.32	0.64	1.88	0.10	0.10	0.12
Triglyceride								
	0	16.2	2.0	4.2	23.3	0.8	—	—
	5	13.0	2.2	2.2	18.1	1.1	—	—
	7	13.6	2.0	2.4	18.2	0.6	—	—
	10	19.2	2.5	3.1	22.9	11.0	—	—
	14	27.0	2.4	5.8	35.7	21.6	—	—
	19	28.3	2.6	4.6	35.0	32.9	—	—
	24	20.1	1.9	3.6	20.2	23.4	—	—
Controls (tube-fed)	24	41.0	4.0	9.3	57.2	0.8	—	—
Phospholipid								
	0	26.5	43.2	—	25.8	8.2 ± 1.6	27.4 ± 2.0	25.5 ± 4.2
	5	30.4	40.4	—	24.4	12.0 ± 0.9	19.1 ± 2.4	26.2 ± 2.1
	7	33.3	45.3	—	29.6	8.3 ± 0.7	24.0 ± 1.6	23.7 ± 4.2
	10	28.5	42.6	—	25.1	17.6 ± 3.7	20.6 ± 3.7	25.1 ± 1.9
	14	27.2	39.3	—	23.8	19.8 ± 3.6	16.9 ± 2.4	25.1 ± 2.9
	19	27.8	43.4	—	20.9	29.7 ± 1.1	15.6 ± 1.2	34.4 ± 2.1
	24	32.4	49.0	—	17.2	25.7 ± 1.3	10.3 ± 1.2	40.2 ± 1.7
Controls (tube-fed)	24	37.1	45.1	—	35.8	9.5 ± 0.8	23.7 ± 2.4	22.1 ± 1.8

* Sum of both isomers.

fatty acids in the phosphatidylcholine, phosphatidylethanolamine and the combined phosphatidylinositol and phosphatidylserine fractions during the post-feeding period.

The phosphatidylcholine fraction showed the largest composition changes. The linoleic acid had increased significantly above its 7 hr. value by 10 hr. and remained significantly higher throughout the rest of the experiment ($P < 0.01$). Eicosatrienoic acid began to decrease at about the same time and was significantly below its 7 hr. value by 14, 19 and 24 hr. ($P < 0.01$). Arachidonic acid, however, showed no significant rise above its 7 hr. value until 24 hr. after the feeding ($P < 0.01$). It therefore appeared that the decline in eicosatrienoic acid in the phosphatidylcholine was not dependent on a reciprocal increase in arachidonic acid. The amount of the normal intermediate (8,11,14-eicosatrienoic

acid) remained constant at about 1.5 mg./liver during the entire period.

The phosphatidylethanolamine fraction, though showing a significant increase in linoleic acid by 10 hr. after feeding, showed very little change in either eicosatrienoic acid or arachidonic acid. The resistance of the phosphatidylethanolamine fraction to the dietary supplementation is evidenced also by the small and insignificant changes in oleic acid. Palmitic acid and stearic acid in both the phosphatidylcholine and phosphatidylethanolamine fractions remained unaffected by the linoleate supplement.

The phosphatidylinositol and phosphatidylserine fraction contained mainly stearic acid, eicosatrienoic acid and arachidonic acid. Feeding with linoleate had almost no effect on the amounts of fatty acid in these phospholipids. As the total

Fig. 1. Changes in the major unsaturated fatty acids of plasma cholesteryl ester (a), phospholipid (b) and triglyceride (c) in EFA-deficient rats given linoleate. Rats were fed by stomach tube on a fat-free diet supplemented with safflower oil at zero time and killed at intervals thereafter. Points on the graph are values from the extract of a pool of plasma from five rats. $\circ$, $C_{18:1}$ acid; $\bullet$, $C_{18:2}$ acid; $\square$, $C_{20:3}$ acids; $\blacksquare$, $C_{20:4}$ acid.

content of polyunsaturated fatty acid in the lysophosphatidylcholine and sphingomyelin fraction was only about 1mg. or less, no values are presented.

Changes induced by feeding with linoleate in the major polyunsaturated fatty acids of plasma cholesteryl esters, phospholipids and triglycerides are shown in Fig. 1. As the linoleate did not appreciably affect the plasma concentration of the cholesteryl esters or phospholipids, the percentages of fatty acids are proportional to their masses. The rise in linoleic acid and arachidonic acid and the fall in eicosatrienoic acid follow similar patterns

and time-sequences to those of the liver lipids. The progressive decrease in eicosatrienoic acid in both plasma cholesteryl esters and phospholipids apparently is not associated with the rise in arachidonic acid. No quantitative estimation of plasma triglyceride was made, but qualitatively the major change in this fraction was in the linoleic acid. Only traces of arachidonic acid and eicosatrienoic acid were detectable, and their proportions remained constant during the experiment.

DISCUSSION

The results show that EFA-deficient rats can rapidly utilize linoleic acid, initially for incorporation into liver and plasma lipids, and later for conversion into arachidonic acid. Although a relatively large amount of fat was given (about 25% of the diet), it did not influence the concentrations of liver or plasma cholesteryl ester and phospholipid (Table 2). Liver triglyceride concentration, however, was increased markedly, but apparently because of the feeding technique rather than because of the linoleate content of the diet.

The delay in the appearance of linoleic acid in the lipid fractions until 7–10 hr. after linoleate had been given was unexpected. That the fat from the diet was probably absorbed is indicated by the experiment of Ostwald, Bouchard, Shannon, Miljanich & Lyman (1966), who showed that 6 hr. after EFA-deficient rats had been given, by stomach tube, a comparable amount of safflower oil mixed with [^{14}C]linoleate, over 40% of the linoleic acid had been absorbed, and 50% of that absorbed was present in the carcass fat and 25% in the liver. A similar degree of linoleate absorption (54%) was obtained by Reinius & Turpeinen (1954) at 6 hr. after feeding EFA-deficient rats, and in this experiment also the major portion of the linoleic acid appeared in the carcass triglyceride. It is possible that linoleic acid is absorbed differently from linoleate in triglycerides. However, if only 25% of the oil given in the present experiment had been absorbed by 7 hr., nearly 400 mg. of linoleate would have entered the animal. Since linoleic acid did not appear in the liver lipids at this time, it may have been utilized initially to restore muscle and adipose-tissue stores of linoleic acid and have become available only later for transport to the liver lipids. This possibility bears further investigation.

At about the time (14–19 hr.) that linoleic acid had reached a maximum level in the liver phospholipid (Table 3), arachidonic acid in this fraction had begun to rise and by 24 hr. was still increasing. Brenner & Nervi (1965) reported that when 110 mg. of linoleate was given daily to fat-deficient rats, arachidonic acid in the phosphatidylcholine did not

begin to increase until after 24 hr. In their experiment, however, phospholipid linoleate rose to only about 4–5% of the fatty acid present. Expression of the phosphatidylcholine-linoleate in Table 4 as a percentage of the total fatty acid shows that it increased to a maximum of about 20% before beginning to decrease. This is much higher than the 5–6% of linoleic acid reported for this phospholipid fraction in control animals (Brenner & Nervi, 1965; Lyman *et al.* 1967; Tischer & Glenn, 1965). Thus our feeding procedure, though essentially saturating the phosphatidylcholine with linoleate, did not increase the rate of appearance of arachidonic acid much more than when phosphatidylcholine linoleate proportions remained low (Brenner & Nervi, 1965). The delay in arachidonic acid synthesis therefore cannot have resulted from any limitation in the availability of linoleic acid. If any of the intermediate steps in desaturation or elongation were rate-limiting in the conversion, an accumulation of an intermediate might be expected. As indicated above, 8,11,14-eicosatrienoic acid, a normal intermediate in arachidonic acid synthesis, was demonstrated at each time-period, but the amount was low compared with the amount of 5,8,11-eicosatrienoic acid, and remained constant throughout. Also, the amount of the more highly unsaturated fatty acids (beyond $C_{20,4}$) remained nearly constant and there was no evidence of any other intermediates accumulating.

Dietary studies with EFA-deficient rats by Holman & Mohrhauer (1963) and Mohrhauer & Holman (1963), and more recent investigations *in vitro* with deficient rat liver microsomes (Brenner & Peluffo, 1966), have indicated that linoleate inhibits the formation of 5,8,11-eicosatrienoic acid from oleic acid by competing for the enzymes involved in the formation of the polyunsaturated fatty acids. Feeding with arachidonic acid, however, also depressed the percentages of 5,8,11-eicosatrienoic but had no effect on linoleic acid (Brenner & Nervi, 1965; Mohrhauer & Holman, 1963). Since arachidonic acid would not be expected to affect intermediate enzyme reactions, it was proposed that it competed with the eicosatrienoic acid for the β -position of the phospholipid, thus causing the trienoic acid content to decrease (Brenner & Nervi, 1965; Brenner & Jose, 1965). In the present experiment, the decline in eicosatrienoic acid occurred before the increase in arachidonic acid and was quantitatively similar to the increase in linoleic acid (Tables 3 and 4). It is difficult to see how competition by the linoleate for the enzymes involved in the formation of eicosatrienoic acid could have been a factor in those changes, inasmuch as no increase in arachidonic acid occurred until 14–19 hr. after the feeding. Arachidonic acid therefore could not be decreasing the

trienoic acid concentration through competition for its place on the phospholipid molecule. As there were no consistent changes in the amount of liver phospholipid during the 24 hr. post-feeding period (Table 2), the influx of linoleic acid into the phospholipids must have occurred either through an exchange of the linoleate for the fatty acids already present on the phospholipids (β -acyltransferase reaction described by Lands & Merkl, 1963) or by synthesis of new phospholipid at the expense of that already present. Our data are insufficient to indicate which possibility may have predominated, but it seems reasonable to assume that the decrease in eicosatrienoic acid occurred because it was unable to compete with the relatively large amount of linoleic acid for the enzymes and sites involved in its incorporation into newly synthesized phospholipid. This interpretation would be consistent with that of Brenner & Nervi (1965), who calculated that at least 20–30%, and probably more, of the phosphatidylcholine and phosphatidylethanolamine present after feeding with linoleate arose from new synthesis.

The changes in fatty acid composition induced by feeding with linoleate in phosphatidylcholine were greater than those in phosphatidylethanolamine and in the phosphatidylinositol and phosphatidylserine fraction (Table 4). This suggests that the turnover of polyunsaturated fatty acids in phosphatidylethanolamine is slower than in phosphatidylcholine. Stitt & Johnson (1966), on the other hand, have reported that the incorporation *in vitro* of labelled linoleate into phosphatidylethanolamine and phosphatidylcholine by liver homogenates from EFA-deficient rats was about the same, and when liver microsomes only were incubated the incorporation into phosphatidylethanolamine was 3 times that into phosphatidylcholine. Studies *in vivo* by Johnson & Ito (1965) and Tischer & Glenn (1965), however, have shown that, even in severely EFA-deficient rats, significantly higher proportions of arachidonic acid are maintained in phosphatidylethanolamine than in phosphatidylcholine. Thus, in the intact animal, the depletion and, apparently, the repletion of phosphatidylethanolamine with EFA is a slower process than it is for phosphatidylcholine. The greater stability of the fatty acids on this phospholipid suggests that its function in the cell membrane may differ from that of the phosphatidylcholine.

The changes induced in the composition of the plasma lipids (Fig. 1) closely paralleled those in the liver. The decreased proportions of arachidonic acid in the plasma phospholipid (normal rat, about 30–33%) (Tinoco, Shannon & Lyman, 1964; Sugano & Portman, 1965) were reflected in similarly low proportions of cholesteryl arachidonate (normal rat, about 55–65%) (Swell, Law &

Table 4. *Changes in fatty acids of some liver phospholipids of EFA-deficient rats after feeding with linoleate*

Rats were fed by stomach tube on a fat-free diet supplemented with safflower oil at zero time and killed at intervals thereafter. Tube-fed controls were given the EFA-deficient diet and killed after 24 hr. Values are means $\pm$ s.e.m. from five animals per time period. Fatty acids below C₁₆ have been omitted for convenience of presentation.

Time after feeding (hr.)	Fatty acids (mg./phospholipid/liver)							
	C _{16:0} acid	C _{16:1} acid	C _{18:0} acid	C _{18:1} acid	C _{18:2} acid	C _{20:3} acids*	C _{20:4} acid	> C _{20:4} acids
Phosphatidylcholine								
0	15.6 $\pm$ 2.2	0.8	19.7 $\pm$ 3.3	12.7 $\pm$ 0.8	4.0 $\pm$ 1.0	14.5 $\pm$ 0.9	7.9 $\pm$ 1.7	4.5
5	16.3 $\pm$ 2.3	2.2	18.2 $\pm$ 1.3	14.0 $\pm$ 1.7	7.1 $\pm$ 0.5	12.3 $\pm$ 2.1	9.4 $\pm$ 1.1	2.6
7	17.1 $\pm$ 1.5	1.5	19.9 $\pm$ 1.9	19.3 $\pm$ 2.5	5.2 $\pm$ 0.4	15.6 $\pm$ 0.7	9.1 $\pm$ 0.9	4.2
10	16.5 $\pm$ 1.8	1.0	20.5 $\pm$ 1.9	15.2 $\pm$ 2.3	11.6 $\pm$ 2.6	11.1 $\pm$ 1.9	9.4 $\pm$ 1.4	3.8
14	18.2 $\pm$ 0.6	1.5	19.3 $\pm$ 1.7	12.6 $\pm$ 1.8	14.9 $\pm$ 1.2	9.1 $\pm$ 1.3	10.5 $\pm$ 1.7	2.5
19	17.0 $\pm$ 1.2	0.3	23.0 $\pm$ 1.2	9.9 $\pm$ 0.7	17.6 $\pm$ 0.8	8.4 $\pm$ 1.1	12.8 $\pm$ 1.6	2.1
24	16.2 $\pm$ 0.6	3.8	22.7 $\pm$ 1.1	7.3 $\pm$ 0.8	14.5 $\pm$ 0.8	5.3 $\pm$ 0.9	22.3 $\pm$ 2.1	4.5
Controls (tube-fed)	17.6 $\pm$ 3.0	2.7	20.8 $\pm$ 2.9	21.8 $\pm$ 0.9	5.2 $\pm$ 0.4	15.7 $\pm$ 1.9	8.2 $\pm$ 0.7	1.9
Phosphatidylethanolamine								
0	3.9 $\pm$ 0.3	0.2	7.2 $\pm$ 0.6	3.2 $\pm$ 0.6	0.4 $\pm$ 0.7	4.1 $\pm$ 0.1	6.7 $\pm$ 1.7	1.2
5	4.1 $\pm$ 0.5	0.2	7.2 $\pm$ 0.7	2.9 $\pm$ 0.4	0.7 $\pm$ 0.1	2.9 $\pm$ 0.7	7.6 $\pm$ 0.6	2.8
7	3.4 $\pm$ 0.5	0.1	6.7 $\pm$ 1.1	3.1 $\pm$ 0.3	0.4 $\pm$ 0.1	3.7 $\pm$ 0.1	7.7 $\pm$ 1.0	2.9
10	3.9 $\pm$ 0.4	0.2	7.3 $\pm$ 0.6	3.0 $\pm$ 0.6	1.5 $\pm$ 0.4	3.8 $\pm$ 0.9	8.6 $\pm$ 1.2	3.4
14	5.0 $\pm$ 0.3	Trace	9.9 $\pm$ 0.6	3.8 $\pm$ 0.5	2.7 $\pm$ 0.6	3.6 $\pm$ 0.3	11.1 $\pm$ 0.9	6.3
19	4.9 $\pm$ 0.6	0.2	10.7 $\pm$ 1.4	4.3 $\pm$ 0.4	3.2 $\pm$ 0.6	4.0 $\pm$ 0.1	9.2 $\pm$ 1.0	3.1
24	5.0 $\pm$ 0.4	0.1	9.8 $\pm$ 0.6	3.5 $\pm$ 0.3	3.5 $\pm$ 0.5	3.1 $\pm$ 0.4	11.6 $\pm$ 1.0	5.7
Controls (tube-fed)	4.9 $\pm$ 0.7	0.5	7.8 $\pm$ 0.9	4.7 $\pm$ 0.5	0.8 $\pm$ 0.1	4.6 $\pm$ 0.7	8.9 $\pm$ 0.5	3.1
Phosphatidylinositol + phosphatidylserine								
0	2.1 $\pm$ 0.9	0.1	8.8 $\pm$ 2.0	1.4 $\pm$ 0.6	0.2 $\pm$ 0.1	5.1 $\pm$ 0.7	3.6 $\pm$ 1.3	2.0
5	2.8 $\pm$ 0.3	0.1	8.0 $\pm$ 0.7	2.1 $\pm$ 0.3	0.9 $\pm$ 0.3	3.6 $\pm$ 0.2	4.7 $\pm$ 0.7	1.5
7	2.6 $\pm$ 0.2	0.1	8.0 $\pm$ 0.4	6.0 $\pm$ 0.4	0.3 $\pm$ 0.1	6.7 $\pm$ 1.0	4.6 $\pm$ 0.5	1.4
10	2.4 $\pm$ 0.3	0.2	8.6 $\pm$ 0.6	1.3 $\pm$ 0.1	0.7 $\pm$ 0.3	4.2 $\pm$ 0.3	3.6 $\pm$ 0.3	1.1
14	2.7 $\pm$ 0.5	0.1	9.1 $\pm$ 1.3	1.6 $\pm$ —	0.9 $\pm$ —	3.8 $\pm$ 0.4	3.5 $\pm$ 0.4	0.8
19	2.5 $\pm$ 0.5	—	8.7 $\pm$ 1.8	2.0 $\pm$ 0.4	1.5 $\pm$ 0.4	3.2 $\pm$ 0.7	3.6 $\pm$ 0.8	0.8
24	1.7 $\pm$ 0.3	—	7.7 $\pm$ 0.8	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2	3.3 $\pm$ 0.2	5.2 $\pm$ 0.5	1.3
Controls (tube-fed)	3.4 $\pm$ 0.5	0.3	8.9 $\pm$ 0.9	2.4 $\pm$ 0.4	0.4 $\pm$ —	5.3 $\pm$ 0.2	4.1 $\pm$ 0.5	1.2

* Sum of both isomers.

Treadwell, 1964; Sugano & Portman, 1965; Tinoco *et al.* 1964). This relationship between plasma cholesteryl esters and phospholipids has previously been observed in EFA-deficient rats (Lyman *et al.* 1966; Swell *et al.* 1964) and is consistent with the concept of a plasma transesterification of fatty acid from the β -position of phosphatidylcholine to the cholesterol. Recent evidence suggests that such a reaction on the high-density lipoproteins may contribute appreciably to the fatty acid composition of rat plasma cholesteryl esters (Glomset, Janssen, Kennedy & Dobbins, 1966; Gidez, Roheim & Eder, 1967). Nonetheless, it is apparent that linoleic acid was preferentially incorporated into the plasma cholesteryl esters, displacing principally oleic acid as well as some of the eicosatrienoic acid, before appreciable amounts of arachidonic acid appeared in the phospholipid. As the content of arachidonic

acid in the phospholipid increased, it, in turn, displaced the linoleic acid.

Under the conditions of this experiment, the early changes in the unsaturated fatty acids of the liver and plasma lipids that were induced when linoleate was given to EFA-deficient rats seem to have arisen from a competition for sites of esterification rather than from a direct competition for desaturation and chain-elongating enzymes. The avidity with which linoleic acid is incorporated into the phospholipid and plasma cholesteryl esters of the EFA-deficient rats indicates that this acid is preferred to the non-essential unsaturated fatty acids, and temporarily substitutes for arachidonic acid when the latter is in short supply. However, as soon as synthesis of arachidonic acid begins, it quickly replaces linoleic acid. The mechanism of the conversion of linoleic acid into arachidonic

acid must involve an initial deacylation since, according to Brenner & Peluffo (1966), the linoleic acid is desaturated and probably elongated as the CoA derivative, then esterified to an appropriate glycerophosphatide.

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