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Studies on Essential Fatty Acid Deficiency

EFFECT OF THE DEFICIENCY ON THE LIPIDS IN VARIOUS RAT TISSUES AND THE INFLUENCE OF DIETARY SUPPLEMENTATION WITH ESSENTIAL FATTY ACIDS ON DEFICIENT RATS

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The composition of the lipids present in a variety of animal tissues has been the subject of numerous reports (Rieckehoff, Holman & Burr, 1949; Gray & Macfarlane, 1958, 1961; Macfarlane, 1961; Getz, Bartley, Stirpe, Notton & Renshaw, 1961; Collins, 1963). The pattern of distribution between the cellular compartments has also been investigated for rat liver (Klein & Johnson, 1954; Macfarlane, Gray & Wheeldon, 1960; Getz & Bartley, 1961; Getz, Bartley, Stirpe, Notton & Renshaw, 1962). Though certain differences exist in the proportions of the principal lipid fractions and of the constituent fatty acids present in different organs and species, a broad similarity of pattern can be recognized. In the tissue phospholipids the incorporation of certain polyunsaturated fatty acids of the linoleic acid series appears to be of special importance for their functional role.

The effect of dietary deficiency of essential fatty acids on the composition of the mixed lipids of a variety of rat tissues was studied by Rieckehoff *et al.* (1949) and by Widmer & Holman (1950). These workers used the alkaline-isomerization technique

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and spectrophotometric characterization of fatty acids used by Holman & Burr (1948). Supplementation of the diet with corn oil or linoleate produced an increase in the dienoic acid and polyenoic acid content of the lipids of heart, liver and kidney when compared with that of deficient animals, together with a decrease of their trienoic acid content. On the other hand, the inclusion of corn oil or linoleate produced little or no effect in the polyenoic acid content of brain, skeletal muscle, skin or depot fat. Supplementation with stearate or oleate was ineffective in raising the tissue content of polyenoic acids. More recently, Mohrhauer & Holman (1963*a, b*), using gas-liquid chromatography, have confirmed these findings. After the administration of graded doses of purified linoleate, linolenate or arachidonate to rats on a diet deficient in essential fatty acids, they recorded a detailed pattern of the constituent fatty acids in the mixed lipids of liver, depot fat and erythrocytes. The present paper extends the investigation of the effect of dietary deficiency of essential fatty acids by fractionating the lipids of a number of tissues into neutral lipids, 'cardiolipin' and other phosphatides, and examining by gas-liquid chromatography the changes in

the fatty acid pattern in each of these groups associated with differences in the fatty acid content of the diet. The influence of dietary supplementation with unsaturated fats on the fatty acid pattern of the tissue lipids of rats deficient in essential fatty acids was also examined.

EXPERIMENTAL

Animals. Wistar strain rats (groups A and B), approx. 60 g. each, were fed on a basal diet containing 20 parts (by wt.) of casein [Genatosan, low in vitamins], extracted twice with 50% (v/v) ethanol, once with 90% (v/v) ethanol and once with diethyl ether, 70 parts (by wt.) of sucrose and 5 parts (by wt.) of mineral salts. The salt mixture was identical with that described by Enser & Bartley (1962). The daily intake of vitamins ($\mu\text{g.}$) was: thiamin (20), riboflavin (40), biotin (0.34), pyridoxine (40), nicotinamide (100), pantothenic acid (240), *p*-aminobenzoic acid (250), choline (2000), inositol (500), calciferol (0.8), vitamin A acetate (40) and tocopherol (530). The last three were given in 0.1 ml. of tributyrin. Rats of group B, which were paired with respect to those of group A, received in addition a daily supplement of 100 mg. of linseed oil. A third group of rats (group S) received a diet of rat cubes (Oxo Ltd., London) given *ad lib*. The daily fatty acid intake of the three groups is shown in Table 1.

After 90–100 days, at a time when rats of group A were showing well-marked signs of deficiency of essential fatty acids, animals from each group were killed for analysis of tissue lipids. The remaining rats of groups A and B thereafter received an additional daily supplement of 0.4 ml. of corn oil (containing 0.2 g. of linoleic acid) for periods of 1–16 days and were then killed.

Analytical methods. Rats were killed by stunning and decapitation. The brain, heart, liver, kidneys, small intestine and hind-leg skin were immediately excised, gently pressed on filter paper to remove adhering blood and stored on ice until their extraction with lipid solvents (not longer than 1 hr.). Hair and subcutaneous fat were removed from the skin. The intestine was dissected away from attached connective tissue and the intestinal contents were squeezed

out of the intestine, leaving a preparation consisting mainly of smooth muscle. Other tissues were freed as far as possible from attached connective, vascular and adipose tissue. Each tissue was weighed and cut into small pieces. Portions of the chopped tissue were taken for estimation of dry weight and for extraction of lipids.

Extraction of lipids. All the tissues were extracted with a volume of chloroform-methanol (2:1, v/v) equal in millilitres to 20 times the weight (in grams) of the tissue. To ensure maximum extraction, the chopped tissue (except skin) was comminuted in the solvent by vigorously stirring the suspension in an overhead blender for 3 min. Skin was cut into small pieces and ground with coarse sand and a volume of the extracting solvent, and the rest of the solvent was added to this mixture. The suspensions of the tissues were filtered, and the tissue-free extracts were washed with an aqueous NaCl solution as described by Folch, Lees & Sloane-Stanley (1957), any material collecting at the interface of the organic and aqueous layers being discarded, and evaporated to dryness under reduced pressure. The dry lipids were redissolved in chloroform (containing 2% of methanol) and these solutions were used for the estimation of lipid dry weight and analysis. Samples of the lipid extracts were analysed without fractionation by saponification and methylation by the diazomethane method as described by Biran & Bartley (1961).

Silicic acid fractionation. Lipids were fractionated on silicic acid columns ('specially prepared for the chromatography of lipids'; -325 mesh; Bio-Rad Laboratories, Richmond, Calif., U.S.A.). The apparatus and the procedure for the preparation of silicic acid columns were as described by Hirsch & Ahrens (1958), except that diethyl ether was passed through the columns overnight before use. The fractionation was carried out in darkness or in artificial light at 25°. Lipids were transferred to the columns as a suspension in diethyl ether.

Elution of neutral lipid and phospholipid. Neutral lipids were eluted with 1000 ml. of diethyl ether, and the last 100 ml. of the eluate contained no lipid ester. This was followed by elution of the total phospholipids with 1000 ml. of methanol (cf. Hirsch & Ahrens, 1958). The methanolic solution of phospholipids was evaporated to dryness *in vacuo* and the lipids were loaded on to a freshly prepared silicic acid column in chloroform. In some experiments the phospholipids were fractionated on the same column on which they had been separated from the neutral lipids, and the eluents used to fractionate the phospholipids were passed through the column directly. Nitrogenous phospholipids were fractionated in the following sequence. First, 100 ml. of chloroform yielded a chloroform eluate that will not be considered here. This was followed by 100 ml. of chloroform-methanol (19:1, v/v), which eluted the 'cardiolipin' fraction. Finally, 1000 ml. of methanol was passed through the column to yield a fraction designated 'phospholipids other than cardiolipin'.

'Cardiolipin' fraction. Thin-layer chromatograms of the 'cardiolipin' fractions from heart, kidney and liver, obtained as described by Wagner, Hörhammer & Wolff (1961) and treated with ammonium molybdate-perchloric acid, showed one spot with R_f corresponding to that given by these workers for cardiolipin and to that of the cardiolipin fraction of liver mitochondria. On chromatograms treated with bismuth subnitrate (choline-sensitive) or with ninhydrin, no positive reaction was given either by the

Table 1. *Daily fatty acid intake of three experimental groups of rats*

Group A rats were deficient in essential fatty acids, group B rats were given the same deficient diet supplemented with 100 mg. of linseed oil daily, and group S rats were stock-fed animals.

Fatty acid	Fatty acid intake (g./day)		
	Group A	Group B	Group S
Butyric acid	0.07	0.07	0.07
Palmitic acid	—	—	0.07
Palmitoleic acid	—	—	0.004
Stearic acid	—	—	0.006
Oleic acid	—	—	0.12
Linoleic acid	—	0.05	0.16
Linolenic acid	—	0.075	0.01
Arachidonic acid	—	—	0.003
22-6 acid	—	—	0.004
Total	0.07	0.195	0.377

cardiolipin spot or by any other part of the chromatogram. In these three tissues, therefore, chloroform-methanol (19:1, v/v) eluted a non-nitrogenous phospholipid that chromatographically resembled cardiolipin, and that was free from nitrogenous phospholipids as far as could be judged from thin-layer chromatograms. The lipids eluted with chloroform-methanol (19:1, v/v) from the phospholipids of other tissues were analysed only by gas-liquid chromatography. They were classed as cardiolipin on the basis of their fatty acid compositions, which were similar to those of the cardiolipin of liver, heart and kidney, and different from those of the other phospholipids in all the tissues analysed. No attempt was made to elucidate the structure of the cardiolipin from any tissue. In particular, the cardiolipin fractions may have contained an unknown proportion of phosphatidic acid (cf. Hübscher, 1961).

'Phospholipids other than cardiolipin'. The fatty acid composition of the fraction was determined (see above) but it was not further analysed.

Gas-liquid chromatography. Only the common fatty acids

that have been characterized in previous work (see Biran & Bartley, 1961; Mead, 1961) were studied in the experiments on essential-fatty acid deficiency. Acids of chain length less than C_{12} were not estimated. The identity of C_{20} trienoic acid was confirmed by the difference in its relative retention volume on Apiezon L and on polyethylene glycol adipate (LAC-2R-446; Cambridge Industries Co., Cambridge, Mass., U.S.A.) stationary phases and by hydrogenation. Other uncharacterized components were present on all chromatograms, but their proportions were small and could not be correlated with the presence or absence of essential-fatty acid deficiency.

Nomenclature of fatty acids. 'Linoleic acid' refers specifically to $C_{18} \Delta^{9,12}$ acid. Other octadecadienoic acids, or mixtures of octadecadienoic acids including linoleic acid, and other isomers are referred to as '18-2 acid' (notation of Ahrens *et al.* 1959). Similarly, 'arachidonic acid' refers only to $C_{20} \Delta^{5,8,11,14}$ acid. Acids having no trivial names are referred to in terms of the notation of Ahrens *et al.* (1959): thus docosaheptaenoic acid is called 22-6 acid.

Table 2. *Fatty acid composition of neutral-lipid fraction of tissues of rats deficient in essential fatty acids*

The dietary groups of rats were: group A, deficient rats; group B, rats supplemented with 100 mg. of linseed oil daily; group S, stock-fed rats. Results are expressed as percentages of methyl esters in measurable peaks on gas-liquid chromatograms. The contents of fatty acids (μ moles of fatty acid/g. dry wt. of tissue) were as follows. Brain: group A, 14; group B, 9; group S, 27.9. Heart: group A, 69; group B, 58. Kidney: group A, 82; group B, 59. Liver: group A, 555; group B, 390; group S, 83. The values for the fatty acids in the brain of stock-fed animals were taken from Biran & Bartley (1961); those for the fatty acids in the liver of stock-fed animals were taken from Getz *et al.* (1961) and those for the fatty acids in the kidney of stock-fed animals were taken from Veerkamp, Mulder & van Deenen (1962).

Fatty acid	Dietary group	Fatty acid composition (%)					
		Brain	Heart	Kidney	Liver	Intestine	Skin
16-0	A	19.8	21.0	31.0	26.9	23.8	27.6
	B	15.2	29.0	26.5	27.1	32.0	27.2
	S	29.0	25.0	29.5	25.8	.	.
16-1	A	4.1	12.1	10.6	22.8	10.9	12.6
	B	4.6	11.7	10.2	21.9	9.6	13.7
	S	4.5	6.5	9.5	2.9	.	.
18-0	A	16.5	4.7	5.1	1.7	9.2	5.2
	B	14.3	5.8	6.5	1.6	6.2	8.9
	S	26.6	7.6	7.0	1.4	.	.
18-1	A	24.5	46.2	47.7	46.5	43.7	43.2
	B	23.7	46.5	47.6	47.2	40.4	40.5
	S	23.9	31.6	33.0	28.5	.	.
18-2	A	+	+	+	+	+	+
	B	1.5	+	2.7	0.9	3.1	1.3
	S	1.3	18.3	15.5	29.6	.	.
20-3	A	5.6	4.2	1.9	+	1.4	0.6
	B	1.0	0.9	+	+	+	0.5
	S	-	-	-	0.3	.	.
20-4	A	9.0	1.8	+	+	+	+
	B	10.2	1.7	2.0	+	2.4	1.3
	S	9.1	2.3	1.0	1.1	.	.
22-6	A	1.9	+	+	-	+	1.6
	B	2.7	-	-	-	+	2.5
	S	1.4	3.2	-	2.93	.	.
Total unsaturated fatty acids	A	45.1	64.3	60.2	69.3	.	.
	B	43.7	60.8	62.5	70.0	.	.
	S	40.2	61.9	59.0	65.33	.	.
Polyunsaturated fatty acids	A	16.5	6.0	1.9	+	.	.
	B	15.4	2.6	4.7	0.9	.	.
	S	11.8	23.8	16.5	33.93	.	.

RESULTS AND DISCUSSION

The total quantity of lipid in the tissue did not change except in the liver, where the content of neutral lipid increased some fourfold.

Neutral lipids. The results of gas-liquid chromatographic analysis of methyl esters from the hydrolysis of neutral lipids from the tissues of rats deficient in essential fatty acids (group A) and from those of rats receiving daily supplements of 0.1 ml. of linseed oil (group B) are given in Table 2.

In all tissues studied (except brain) the major non-essential acids were palmitic acid (15–30 %), palmitoleic acid (10–23 %), stearic acid (2–9 %) and oleic acid (33–50 %). Myristic acid (1–4 %) was found in all neutral-lipid fractions. Brain neutral lipids presented some special features. In both groups A and B oleic acid constituted only 25 % of the fatty acids, compared with 30–50 % in other tissues, whereas the percentages of stearic acid was

higher (14–16 %). The general pattern of non-essential fatty acids in the deficient group was not dissimilar to that of rats receiving linseed oil, or of stock-fed animals.

In the unsaturated fatty acid constituents of neutral lipids the amount of 18–2 acids in the tissues of rats receiving linoleic acid was only marginally higher than in the deficient group, and was in marked contrast with the high amounts seen in stock-fed rats. The amounts of 20–4 acid in the brain, heart and liver was similar in both groups A and B and were of a similar order to that of stock-fed animals. Since neutral-lipid esters in brain constitute only 2–3 % of the total lipid, any contamination with hydrolysis products of phospholipids would have a large influence on their composition, and the values for 20–4 acid in this tissue must be accepted with some caution. In kidney and intestine the contents of 20–4 acid was lower in the deficient animals than in those receiving linseed oil.

Table 3. *Fatty acid composition of phospholipid fraction of tissues of rats deficient in essential fatty acids*

The dietary groups of rats were: group A, deficient rats; group B, rats supplemented with 100 mg. of linseed oil daily; group S, stock-fed rats. Results are expressed as percentages of methyl esters in measurable peaks on gas-liquid chromatograms. The contents of fatty acids (μ moles of fatty acid/g. dry wt. of tissue) were as follows. Brain: group A, 465; group B, 419; group S, 610. Heart: group A, 329; group B, 313. Kidney: group A, 215; group B, 285. Liver: group A, 238; group B, 241. The values for the fatty acids in the brain and kidney of stock-fed animals were taken from Veerkamp *et al.* (1962).

Fatty acid	Dietary group	Fatty acid composition (%)					
		Brain	Heart	Kidney	Liver	Intestine	Skin
16-0	A	17.3	10.3	17.6	16.1	6.3	15.1
	B	17.6	13.0	20.0	26.9	7.6	22.5
	S	—	15.0	24.5	24.5	—	—
16-1	A	1.4	5.2	6.8	7.9	3.4	14.6
	B	1.7	4.3	4.3	2.9	3.1	7.8
	S	—	1.5	3.0	3.0	.	.
18-0	A	18.1	23.0	17.3	18.3	23.4	6.9
	B	16.4	18.9	18.6	18.1	19.7	11.4
	S	—	19.1	20.5	28.0	.	.
18-1	A	27.3	27.4	27.9	26.6	17.3	38.2
	B	27.9	19.6	19.4	14.5	16.7	28.4
	S	—	10.2	18.5	14.0	.	.
18-2	A	+	+	0.7	2.8	+	+
	B	+	12.9	7.1	3.3	3.6	3.7
	S	—	24.0	12.5	14.0	.	.
20-3	A	4.1	25.4	15.9	22.3	23.4	12.6
	B	0.5	3.8	2.4	2.0	2.1	1.6
	S	—	—	0.5	0.3	.	.
20-4	A	6.2	7.6	10.1	4.8	5.2	3.2
	B	7.9	13.9	19.5	6.0	13.6	8.1
	S	—	15.5	20.0	16.0	.	.
22-6	A	10.2	—	+	—	—	+
	B	13.5	7.4	—	14.3	6.1	+
	S	—	9.5	—	—	.	.
Total unsaturated fatty acids	A	49.2	65.6	61.4	64.4	.	.
	B	51.5	61.9	52.7	43.0	.	.
	S	—	60.7	54.5	47.3	.	.
Polyunsaturated fatty acids	A	20.5	33.0	26.7	29.9	.	.
	B	21.9	38.0	29.0	25.6	.	.
	S	—	49.0	33.0	30.3	.	.

A higher amount of 20-3 acid in the neutral-lipid fraction of the deficient group was apparent in brain, heart, kidney and intestine, though this was much less conspicuous than in the phospholipid fraction. The rats of group B had substantial amounts of linolenic acid in the diet, but there was no indication in the neutral lipids that the quantity of 22-6 acid in the tissues was affected. The total of unsaturated fatty acids in the tissues was similar in all three groups.

Nitrogenous phospholipids. The gas-liquid-chromatographic analysis of fatty acids derived from the nitrogenous phospholipids of the tissues of rats of groups A and B, and the corresponding values for brain, heart, kidney and liver for stock-fed animals based on the present work and on the data of Veerkamp *et al.* (1962), are given in Table 3. No very marked differences in the pattern of saturated fatty acids of the tissue phospholipids in groups A and B and in stock-fed animals was evident in any of the tissues examined except brain. In the latter tissue the proportions of palmitic acid and stearic acid, though similar for groups A and B, were markedly lower than the values reported by Veerkamp *et al.* (1962) for stock-fed animals.

The proportions of the unsaturated fatty acid constituents of the phospholipids assumed a characteristic pattern in the deficient animals of group A. In heart, kidney, liver and skin there was an elevation in the proportions of palmitoleic acid and oleic acid and a striking increase of that of 20-3

acid as compared with those of group B or stock-fed animals. In these tissues there was a parallel fall in the amounts of 18-2 acid and 20-4 acid in the deficient group as compared with those of groups B and S. In the brain, where the normal concentration of linoleic acid is low, little change was observed in the deficient group in the amount of this acid or of 20-4 acid. The brain phospholipids appear to be more resistant to change in their fatty acid composition than those of other tissues.

The values recorded for group B receiving linseed oil are intermediate between those of the deficient group and stock-fed animals, though approximating more closely to those of the former. The dietary intake of linoleic acid of the stock-fed animals was, however, some three times as great as that of the animals supplemented with linseed oil. Nevertheless, the animals of group B remained entirely free from clinical signs of deficiency and their growth rate was superior to that of the deficient group. Veerkamp *et al.* (1962) give no values for 22-6 in their stock-fed rats, but the analysis of Getz *et al.* (1961) suggests a value close to that found for the animals on diet B. Mohrhauser & Holman (1963*a*, *b*) have indicated that, where the dietary intake of linoleic acid falls below 1 % of the total calories, the trienoic acid:tetraenoic acid ratio rises above the value, 0.4, that is associated with a normal essential fatty acid status. In these circumstances the synthesis of arachidonic acid from linoleic acid does not proceed sufficiently rapidly, and the synthesis of

Table 4. *Fatty acid composition of cardiolipin fraction of tissues of rats deficient in essential fatty acids*

The dietary groups of rats were: group A, deficient rats; group B, rats supplemented with 100 mg. of linseed oil daily. Results are expressed as percentages of methyl esters in measurable peaks on gas-liquid chromatograms.

Fatty acid	Dietary group	Fatty acid composition (%)				
		Brain	Heart	Kidney	Liver	Intestine
16-0	A	9.5	4.9	5.6	3.3	5.8
	B	6.0	5.1	5.7	4.4	7.9
16-1	A	6.3	21.4	27.2	33.5	16.8
	B	3.2	11.8	13.7	12.7	7.1
18-0	A	10.2	3.7	3.4	8.6	3.1
	B	10.5	4.9	3.5	8.9	4.8
18-1	A	42.7	39.9	46.2	34.5	42.2
	B	38.3	21.2	31.7	29.7	21.5
18-2	A	—	7.1	5.0	5.8	3.5
	B	1.0	40.7	28.3	22.9	25.1
20-3	A	2.2	5.1	2.2	4.8	4.4
	B	+	2.3	+	1.5	—
20-4	A	5.5	3.9	3.3	4.2	2.1
	B	7.0	1.8	4.6	0.9	2.5
22-6	A	11.1	—	—	+	—
	B	18.6	—	—	10.7	+
Total unsaturated fatty acids	A	67.8	77.4	83.9	82.8	.
	B	68.1	77.8	78.3	78.4	.
Polyunsaturated fatty acids	A	18.8	16.1	10.5	14.8	.
	B	26.6	44.8	32.9	36.0	.

Table 5. *Fatty acid composition of neutral-lipid, nitrogenous-phospholipid and cardiolipin fractions of tissues of rats initially deficient in essential fatty acids and of rats receiving linseed oil, before and after supplementation with corn oil*

The dietary groups of rats were: group A, deficient rats; group B, rats supplemented with 100 mg. of linseed oil daily. After 90–100 days, a daily supplement of 0.4 ml. of corn oil (equivalent to 200 mg. of linoleic acid) was given for 16 days. Results are expressed as percentages of methyl esters in measurable peaks on gas–liquid chromatograms.

Fatty acid composition (%). Initial										
Fatty acid	16-1		18-1		18-2		20-3		20-4	
	A	B	A	B	A	B	A	B	A	B
Neutral lipid										
Heart	8.1	12.3	35.2	37.6	2.4	1.9	4.1	1.3	+	1.3
Kidney	10.7	16.8	39.7	45.3	+	+	4.0	0.7	1.4	0.7
Liver	13.0	13.7	50.8	45.4	–	+	–	–	–	–
Nitrogenous phospholipid										
Heart	2.3	3.6	20.8	.	+	.	30.9	.	9.9	.
Kidney	5.6	4.7	23.4	19.3	1.8	4.2	21.8	2.5	10.6	13.0
Liver	7.6	7.1	24.4	17.6	+	2.3	21.4	3.3	7.5	4.9
Cardiolipin										
Heart	16.4	9.1	46.3	.	10.0	.	7.0	.	6.0	.
Kidney	21.2	9.9	55.1	29.5	4.7	31.7	3.7	–	4.2	–
Liver	23.0	24.4	40.8	40.1	9.8	14.7	2.3	–	3.1	–
Fatty acid composition (%). After corn-oil supplementation for 16 days										
Fatty acid	16-1		18-1		18-2		20-3		20-4	
	A	B	A	B	A	B	A	B	A	B
Neutral lipid										
Heart	9.4	15.1	29.9	44.3	33.2	5.8	+	–	0.4	–
Kidney	12.5	13.6	45.6	45.2	5.3	4.3	+	–	1.4	–
Liver	15.6	11.8	42.8	47.8	2.5	4.7	–	–	0.8	–
Nitrogenous phospholipid										
Heart	1.6	.	15.4	.	11.7	.	5.5	.	21.2	.
Kidney	17.7	2.0	12.9	14.9	4.6	9.3	2.4	0.6	41.4	25.1
Liver	3.8	1.8	7.4	10.6	4.8	7.5	2.9	1.5	26.8	16.0
Cardiolipin										
Heart	7.5	.	11.8	.	51.2	.	–	.	–	.
Kidney	7.6	4.9	22.7	18.1	48.5	57.4	+	1.1	3.8	3.5
Liver	13.0	9.6	22.8	19.0	24.8	52.2	+	1.2	12.8	2.1

trienoic acid from palmitoleic acid and oleic acid becomes predominant. This appears to be a characteristic feature of essential fatty acid deficiency.

Cardiolipin. This fraction comprised 10–15 % of the total phospholipid fatty acids in heart, muscle and liver in both the supplemented and the deficient group. In spleen, testis and brain the cardiolipin fatty acids contributed 3–5 % of the phospholipid fatty acids. Cardiolipin with its content of 70–80 % of linoleic acid in all mammalian tissues studied except brain has a fatty acid distribution different from that of any other lipid (Gray & Macfarlane, 1961; Getz *et al.* 1962). The effect of deficiency on the pattern of the cardiolipin fatty acids is in some respects similar to that of the nitrogenous-phospholipid fraction (Table 4). There was a conspicuous rise in the amounts of 16–1 acid, 18–1 acid and 20–3 acid, and a corresponding fall in that of 18–2 acid, in the deficient group; this is particularly evident in brain, heart, kidney, liver

and intestine. With the 20–4 acid, however, there was little or no change (brain, kidney and intestine) or an increase of doubtful significance (heart and liver) in the deficient group. The group supplemented with linseed oil had substantial amounts of 22–6 acid in the cardiolipin.

Comparison of the data for the neutral-lipid, nitrogenous-phospholipid and cardiolipin fractions indicates that dietary deficiency of essential fatty acids results in a pattern characterized by a fall in the proportion of linoleic acid and an elevation in those of oleic acid, palmitoleic acid and eicosatrienoic acid. Though there is also some decline in the amount of arachidonic acid in all tissues studied, the change resulting from deficiency is less marked, particularly in the cardiolipin fraction, than is the case for linoleic acid.

Effect of supplementation with corn oil. After a period of 90–100 days on the experimental diet, at a time when rats of group A were showing well-

marked signs of deficiency, some of the animals received daily supplements of 0.4 ml. of corn oil (equivalent to 200 mg. of linoleic acid) for periods of 1-16 days, after which they were killed for fatty acid analysis of the neutral-lipid, nitrogenous-phospholipid and cardiolipin fractions of heart, kidney and liver. Animals of group B receiving linseed oil similarly received additional supplements of corn oil for 1-16 days. Table 5 records the distribution of 16-1, 18-1, 18-2, 20-3 and 20-4 acids of the neutral-lipid fraction for those tissues at day zero and after supplementation with corn oil for 16 days. In group A there was an elevation of the proportion of linoleic acid particularly in heart and a fall in that of 20-3 acid in all tissues, but little rise in the content of arachidonic acid. A similar trend was observed in animals of group B. Table 5 also records the distribution of the fatty acids of the nitrogenous-phospholipid and cardiolipin fractions for heart, kidney and liver of animals of group A and corresponding values for kidney and liver of animals of group B. In the nitrogenous phospholipids, supplementation with corn oil over a period of 16 days resulted in a restoration of the characteristic normal pattern of phospholipid fatty acids. In all three tissues there was a fall in the amounts of 18-1 acid and 20-3 acid and elevation in the amount of 18-2 acid. In contrast with the neutral-lipid fraction there was also a marked rise in the content of 20-4 acid in kidney and liver. These changes were evident in those animals of group B that had received a supplement of linseed oil throughout the entire experimental period as well as in the deficient animals of group A. The changes in the fatty acid distribution in the cardiolipin fractions of kidney and liver were similar to those of the nitrogenous phospholipids except that the rise in the amount of 20-4 acid was less marked.

SUMMARY

1. Deficiency of essential fatty acids in the diet of the rat resulted in marked changes in constituent fatty acids of the nitrogenous-phospholipid and cardiolipin fractions. In the former there is a replacement of arachidonic acid and linoleic acids by palmitoleic acid, oleic acid and eicosatrienoic acids. In the cardiolipin fraction, where linoleic acid is normally the predominant unsaturated fatty acid, dietary deficiency results in replacement of this acid and to a less extent of arachidonic acid by palmitoleic acid, oleic acid and eicosatrienoic acid.

2. Dietary deficiency of essential fatty acids resulted in a fall in the proportion of linoleic acid and a rise in those of palmitoleic acid, oleic acid and eicosatrienoic acid in the neutral-lipid fractions of heart, kidney and liver when comparison is made with the corresponding values for stock-fed animals. The proportion of arachidonic acid, which is low in the neutral lipids of most normal tissues except brain, are little changed as a result of dietary deficiency.

3. Dietary supplementation of animals with corn oil over a period of 16 days reversed the changes induced by deficiency in the fatty acid constituents of the neutral-lipid, nitrogenous-phospholipid and cardiolipin fractions of heart, kidney and liver.

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