PHOSPHOLIPID METABOLISM IN ESSENTIAL FATTY ACID DEFICIENT RATS

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Rats and other animals on a fat-free diet develop characteristic symptoms caused by a deficiency of the so-called essential fatty acids. These fatty acids have been shown to have the structure

$$CH_3 - (CH_2)_a - (CH = CH - CH_2)_b - (CH_2)_c - COOH$$

where a is always equal to 4, and all the acids can be converted in vivo to arachidonic acid where b = 4 and c = 2. The double bonds must all have the cis configuration and fatty acids with double bonds in other positions such as linolenic and oleic acids are ineffective in curing the deficiency. This subject has recently been reviewed by Aaes-Jørgensen (1961) and it is clear that research to date does not explain their mode of action. The results of the following experiment may offer such an explanation which can be the subject of further experimentation.

Male weanling rats (Wistar strain) weighing 30 to 40 g. were placed on a diet containing 73% sucrose, 21% fat-free casein (British Drug Houses), 4% salt mixture (U. S. P. XIV, 1950), 2% cellulose and 0.5% vitamin mixture (Nutritional Biochemicals Corp.). After 15 weeks growth stopped and the animals showed the characteristic symptoms of the deficiency. A normal and a deficient rat were each injected intraperitoneally with about 0.4 mc of inorganic P^{32} and after one hour were killed, their livers removed and the lipids extracted. The lecithins and the phosphatidyl ethanolamines were isolated as described by Collins (1960) and the purity of each fraction tested by means of counter-current distribution (see Collins & Shotlander, 1961). A sample of each liver was homogenized in cold 0.8M perchloric acid, the mixtures centrifuged and the inorganic phosphate removed from the supernatant solution by the method of Berenblum and Chain (1938), in order to determine its specific radioactivity.

TABLE 1.

Specific radioactivities of rat liver phospholipids, relative to that of the liver inorganic phosphate

	Normal	Deficient
Lecithins	3.35	6.95
Phosphatidylethanolamines	7.73	13.96
Inorganic phosphate	100	100
(Inorganic phosphate (cts/min/μmole P)	29850	26500)

Four experiments altogether have given results that indicate that after one hour the specific radioactivity of the phospholipids of the deficient animals is greater than that of the normal controls. The fatty acid composition of the deficient animals show the changes that are now recognised as typical. The decrease in linoleic and arachidonic acids is compensated by an increase in palmitoleic and oleic acids and the appearance of an eicosatrienoic acid which has been shown by Fulco and Mead (1959) to be derived from oleic acid.

If it is assumed that each molecule of the lecithins is esterified with no more than one molecule of arachidonic acid, then in the normal liver 57.2% of the lecithins contains arachidonic acid while in the deficient case only 3.8% contains this acid (from Table 2). It is also known (Collins, 1960; Harris, Robinson & Getz, 1960) that the specific radioactivity of the phosphorus of the individual lecithins can vary widely depending on their fatty acid contents while Harris et al. showed it was lower in the fraction high in arachidonic acid.

The above differences can be explained by postulating the existence of two groups of lecithins; those containing arachidonic acid and having a low

TABLE 2

Fatty acid composition of phospholipids

The fatty acids from these fractions were isolated as methyl-esters using the procedure of Stoffel, Chu and Ahrens (1959) and analysed by gas liquid chromatography using an argon ionization detector and a 6 ft. column packed with 12% polyethylene glycol adipate at a temperature of 178°. 3300 theoretical plates were obtained for methyl stearate.

The results are presented as moles per 100 moles.

	Lecithins		Phosphatidylethanolamines	
Fatty acid	Normal	Deficient	Normal	Deficient
14:0	0.4	0.5	0.5	3.3
15:0	0.2	0.0	0.0	0.0
16:0	19.0	21.4	19.2	21.2
16:1	1.3	5.3	1.7	2.5
17:0	0.6	0.0	0.8	0.0
18:0	22.4	22.4	26.7	20.7
18:1	9.6	27.6	8.5	23.4
18:2	15.7	1.4	6.6	1.4
20:3	0.0	19.0	0.0	19.3
20:4	28.6	1.9	32.8	5.8
20 or 22 (?)	0.0	0.4	0.4	1.6
22:6	1.9	0.2	2.7	0.7

specific activity and those without having a high specific activity. The specific radioactivity of lecithins following labelling with tracer amounts of P^{32} is a function of the reaction between cytidine diphosphocholine and various diglycerides during synthesis, and of the subsequent hydrolytic action of a phospholipase. Both the synthetic and hydrolytic reactions are affected by the fatty acids involved and the above results suggest that in the presence of arachidonic acid the rates are slower; however no data are at present available either on the rate of synthesis of lecithins from diglycerides containing arachidonic acid or of the rate of hydrolysis of lecithins with this acid esterified at the β-position.

It is suggested that arachidonic acid acts primarily as a modulator of phospholipid metabolism because molecules containing this acid are more stable. Essential fatty acid deficiency would therefore lead to an increased phospholipid turnover resulting in partial disorganisation of the enzymes and enzyme-chains located on the lipo-protein membranes.

Although the evidence is not as yet conclusive this working hypothesis is put forward as a possible guide to future investigation.

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