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Position of eicosatrienoic acid in the phosphatidylcholine and phosphatidylethanolamine from rats deficient in essential fatty acids

While studying the fatty acid composition of the phospholipids[1] of rats fed diets lacking in essential fatty acids a decrease of arachidonic acid was observed together with an increase of the eicosa-5,8,11-trienoic acid. The latter is synthesized by the rat starting from oleic acid[2]. In order to determine the position occupied by eicosa-5,8,11-trienoic acid in phosphatidylethanolamine and phosphatidylcholine the effect of the phospholipase A (EC 3.1.1.4) of the snake venom (*Crotalus adamantheus*) was studied. This enzyme hydrolyzes the fatty acids bound to the β-carbon as has been demonstrated by TATTRIE[3], HANAHAN, BROKERHOFF AND BARRON[4], SAITO AND HANAHAN[5], ROBERTSON AND LANDS[6] and DE HAAS, DAEMEN AND VAN DEENEN[7].

10 weanling male albino rats from the strain of the Institute were maintained on a fat-free diet[8] for 2 months. After this period the rats were killed and the heart, liver and kidney lipids were extracted by FOLCH-PI, LEES AND SLOANE-STANLEY's method[9]. The phospholipids (62.6 mg) were separated by fixation on activated silicic acid and eluted with methanol[10]. Phosphatidylethanolamine and phosphatidylcholine were separated on Silica Gel G (prepared according to STAHL, E. Merck A.G., Darmstadt, (Germany)) by thin-layer chromatography using chloroform–methanol–water (65:25:4, v/v)[11] as developing solvent. Chromatoplates 200 × 200 mm, 250 μ thickness were used. The spots were made visible with iodine vapor and by carbonization[12]. The spots corresponding to phosphatidylethanolamine and phosphatidylcholine were recognized by running standards and applying ninhydrin and DRAGENDORFF's reaction[13].

The zones corresponding to phosphatidylethanolamine and phosphatidylcholine contained small amounts of plasmalogens. They were separated and eluted with methanol. 27 mg phosphatidylethanolamine and 16 mg phosphatidylcholine were hydrolyzed by snake-venom phospholipase A for 6 h at room temperature. The conditions were the same as those employed by DE HAAS, DAEMEN AND VAN DEENEN[14], incubating ethereal emulsions of 10 mg of phospholipid in 1 ml of borate buffer (pH 7) containing 2.5 mM $CaCl_2$, with 2 mg snake venom (*C. adamantheus*, Ross Allen's Reptile Institute). The reaction was stopped by 1 ml of absolute methanol and the precipitated protein was separated by centrifugation. The supernatant was lyophilized, dissolved in chloroform and fractioned by thin-layer chromatography using the same type of plates and solvents as before. The spots corresponding to the liberated fatty acid and the lysophospholipids formed were detected by means of iodine vapor and recognized with ninhydrin and DRAGENDORFF's reagent. The position of the spots was compared with egg lecithin (R_F 0.32), lysolecithin (R_F 0.08), and fatty acids (R_F 0.82). The liberated fatty acid (R_F 0.82) from phosphatidylethanolamine (R_F 0.54) and phosphatidylcholine (R_F 0.32) gave a much stronger reaction with iodine than the corresponding lysophosphatidylethanolamine (R_F 0.25) and lysophosphatidylcholine (R_F 0.08) which demonstrated that the acids of greater unsaturation were at the β-position. The spots of the fatty acid and lysophospholipids were treated directly with methanol–HCl[15] and the fatty acid methyl esters thus obtained were analysed by gas–liquid chromatography in a Pye apparatus with a polyethylene glycol adipate column.

The amounts of the main fatty acids found are presented in Table I, which shows that saturated acids mainly appear in lysophospholipids whereas polyunsaturated fatty acids and more than a half of palmitoleic and oleic acids are hydrolyzed by snake venom. The eicosa-5,8,11-trienoic and arachidonic acids are hydrolyzed in a similar way by phospholipase A and therefore they both occupy the β-position in phosphatidylethanolamine and phosphatidylcholine. Small amounts of both acids are nevertheless found in lysophosphatidylethanolamine and lysophosphatidylcholine. It might be inferred, then, that these small amounts would be found in the α-position, but these acids might remain in the lysophospholipids if, as MARINETTI, ERBLAND, TEMPLE AND STOTZ[16] and BENNET AND TATTRIE[17] think, the phospholipase A possesses in addition to the preferential action on the β-position, a certain amount of activity in the α-position. DE HAAS, DAEMEN AND VAN DEENEN[7], on the contrary, show conclusively that the phospholipase A from *C. adamantheus* is specifically a β-esterase and the small amounts of polyunsaturated fatty acids found in phospholipids according to our investigations might be attributed to a weak non-specific hydrolysis produced independently from the phospholipase A.

TABLE I

FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE

*Fatty acids**	*Phosphatidylethanolamine*			*Phosphatidylcholine*	
	Untreated (mole %)	*Lyso derivative (mole %)*	*Liberated fatty acids (mole %)*	*Lyso derivative (mole %)*	*Liberated fatty acids (mole %)*
16	11.9	33.1	8.2	29.8	13.2
16:1**	2.2	2.8	4.4	2.8	4.5
18	20.6	30.8	5.8	23.4	2.9
18:1	23.3	21.1	26.6	17.9	38.1
20:3	7.8	1.9	12.6	1.8	14.0
20:4	19.1	4.2	31.9	2.5	13.4

* Minor components contribute the remaining percentage.
** 16 = number of carbon atoms; 1 = number of double bonds.

In any case, it is confirmed that the eicosa-5,8,11-trienoic and arachidonic acids are both esterified only or at least mainly in the β-position and that the eicosa-5,8,11-trienoic acid replaces the arachidonic acid in the phosphatidylethanolamine and phosphatidylcholine of rats deficient in essential fatty acid.

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On the nature of dialyzable phosphate associated with the di- and tri-phosphoinositides of brain phospholipids

While preparing a concentrate of the phosphoinositides from calf brain, FOLCH[1] noted the presence of large amounts of P_I. He reported, however, that removal of the latter by dialysis interfered with the purification of the "diphosphoinositide", which was accomplished by multiple reprecipitation of the methanol-insoluble phospholipids (FF-I) from chloroform. More recently this product has been shown to contain TPI and DPI (see refs. 2, 3) as well as MPI (see ref. 4). The possibility that P_I might be attached to the PP-I was suggested by a number of observations made during the preparation of TPI in this laboratory.

Although P_I may be removed by prolonged dialysis against water, very little is released by a short period of shaking with water or dilute trichloroacetic acid. It is released within 3 min by 0.1 N H_2SO_4 at 100°, and within a few seconds by alkaline methanolysis (0.3 N methanolic KOH at 37°), and either of these procedures may be used for its quantitative determination.

The PP-I secured by counter-current distribution of FF-I between light petroleum and 96% methanol[4] was found to contain P in excess of that required for TPI. The amount of P_I released by acid hydrolysis or by alkaline methanolysis was nearly identical, and approximated 2 moles of P_I per 3 moles of inositol-bound P, or 40% of the total P. Similar results were obtained when FF-I was distributed between 96% methanol and a 1:1 mixture of CCl_4 and light petroleum[5]. The distribution of P_I and P_O was such that P_I represented 40% of the total P in the non-polar phase, whereas in the starting material P_I was 30–32% of the total P.

PP-I in the form of its magnesium or calcium salt shows strongly hydrophobic properties which are reversed once the metal ions and P_I are removed. Thus the ratio of P concentration in the two phases of COLE's solvent system[5] (non-polar–polar) was found to be 24 after FF-I had been equilibrated 15 times with the polar phase. In the system light petroleum–96% methanol[4] it was 31. This affinity for the non-

Abbreviations: P_O, organic phosphate; FF-I, FOLCH Fractions I and II (see ref. 1); TPI, DPI and MPI, tri-, di-, and monophosphoinositides; PP-I, mixed TPI and DPI.