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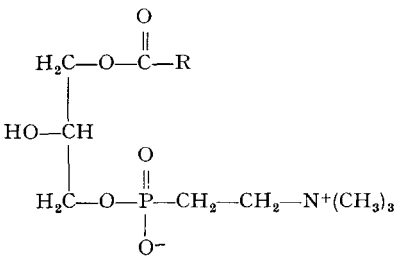
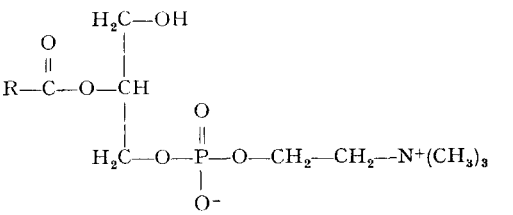
The formation of isomeric lysolecithins

Although the pancreatic phospholipase A (EC 3.1.1.4) has been shown to act exclusively on the 2-ester position of phosphoglycerides[1], the mode of action of phosphatido monoacyl hydrolases involved in the intracellular metabolism remains to be established. The independent turnover of saturated and unsaturated fatty acids located preferentially at the 1 and 2 positions respectively of phosphoglycerides was established by HANAHAN[2]. Recently LANDS[3] showed that the microsomal fraction of animal tissues is capable of acylating lysolecithins; with the aid of structural isomeric lysolecithins (I and II) the enzyme was shown preferentially to esterify unsaturated fatty acids at the 2 position and saturated acids at the 1 position so as to form lecithin[4]. The enzymic reacylation of lyso-derivatives has been confirmed for a diversity of phospholipid classes[5–7], and this activity was detected in mitochondrial[8] and microsomal fractions as well as in red-cell membranes[9,10]. The physiological significance of these reactions may be endorsed by the proof that the cell organelles are able to produce two structurally isomeric lyso-phosphoglycerides. Recently TATTRIE AND CYR[11] reported that the fatty acid composition of lysolecithins isolated from egg, bovine lung, human plasma and yeast was consistent with the simultaneous occurrence of both isomers. On the other hand a more direct proof of the structure of lysolecithin from yeast, involving a chemical reacylation and hydrolysis of the formed lecithin by phospholipase A, enabled LETTERS AND SNELL[12] to conclude that only 1-acylglycerol-3-phosphorylcholine (I) was present. LLOVERAS *et al.*[13] observed that the nature of the liberated fatty acids and the fatty acid constituents of lysolecithin produced from egg lecithin by the action of an extract of spleen was compatible only with the presence of a phospholipase attacking both ester positions. An able approach of ROBERTSON AND LANDS[14] with lecithins containing labeled fatty acids in either the 1 or 2 position, however, failed since no accumulation of lysolecithin was obtained.



I



II

In this study tissue homogenates have been incubated with [^{32}P]lecithin containing in 2-position a [^{14}C]linoleic acid in the presence of deoxycholate, an agent well known to inhibit lysophospholipase (EC 3.1.1.5) activity. Under these conditions a fair amount of the added lecithin was hydrolyzed, while the breakdown of the formed lysolecithin into water-soluble products was limited (Table I).

For the preparation of the double-labeled substrate 400 mg of rat-liver slices were incubated in 1 ml of Krebs–Ringer solution containing 2 mC of [^{32}P]P_i. After

TABLE I

HYDROLYSIS OF LECITHIN BY RAT-TISSUE HOMOGENATES

The incubation mixture consisted of 100 mg tissue (wet weight) in 1.0 ml of Krebs–Ringer solution, containing an emulsion of 3 mg of sodium deoxycholate and a tracer amount of labeled lecithin. After 4 h incubation at 37°, the lipids were separated on paper chromatograms and scanned for ^{32}P activity. Each value is the mean ± S.D. of the mean of 7 experiments.

Tissue	*Distribution of radio-activity*		
	Deacylated water-soluble products (%)	*Lysolecithin (%)*	*Lecithin (%)*
Liver	4 ± 1.5	21 ± 5	75 ± 6
Lung	5 ± 1.9	26 ± 6	69 ± 6
Spleen	8 ± 0.6	39 ± 3	53 ± 3

extraction of the lipids[15], lecithin was isolated by chromatography on silicic acid according to HANAHAN's procedure. For the introduction of the labeled fatty acid, the biosynthetic procedure of LANDS[3] was utilized: 100 mg rat liver was incubated for 2 h in the presence of 4 μC of [1-^{14}C]linoleic acid, ATP, CoA and 1-acylglycerol-3-phosphorylcholine, the latter being obtained by snake-venom degradation of the liver lecithin. The ratio between ^{32}P and ^{14}C activity in the substrate molecule was determined with a Packard Tricarb spectrometer, and three preparations with different isotopic proportions have been used. By virtue of the positional specificity of phospholipase A from *Crotalus adamanteus*, 95 ± 3% of the [1-^{14}C]linoleic acid was found to be located at the 2 position in the lecithin preparations.

Homogenates of liver, lung and spleen tissue of the rat were incubated in Krebs–Ringer solution containing a tracer amount of the labeled substrate for 4 h at 37°.

TABLE II

ISOTOPIC DISTRIBUTION IN LECITHIN AND LYSOLECITHIN AFTER INCUBATION

The experimental conditions were as in Table I. In the separate experimental series the $^{32}P/^{14}C$ ratios of the lecithin substrate, and 1-acylglyceryl-3-phosphorylcholine (I) prepared by phospholipase A (*C. adamanteus*) degradation, were respectively: a: 2.31, 38.5; b: 0.140, 0.80; c: 0.313, 10.7. Each value is the mean of two experiments. The percentage of 2-acyl isomer (II) in lysolecithin was calculated taking into account the amount of ^{14}C-labeled fatty acid present at the 1-position of the lecithin substrate. After this correction the known $^{32}P/^{14}C$ ratios for both individual lysolecithins enabled to derive their proportion in the enzymic hydrolysate.

Tissue	*Experimental series*	*$^{32}P/^{14}C$ ratio lecithin*	*Lysolecithin*	*% II in lysolecithin (calc.)*
Liver	a	2.32	4.82	44
	b	0.140	0.221	64
	c	0.320	0.443	71
Lung	a	2.32	9.9	19
	b	0.137	0.329	35
	c	0.300	1.00	30
Spleen	b	0.128	0.370	29
	c	0.301	1.44	20

The extracted lipids were separated on silica-impregnated paper[16]. After being stained with rhodamine 6G, the spots of lecithin and lysolecithin were cut out and counted for ^{32}P and ^{14}C activity with a liquid scintillation spectrometer. The ratio of both isotopes in the lecithin fraction was identical to that of the control lecithin. The values obtained on the lysolecithin fraction, after correction for the amount of ^{14}C-labeled fatty acid present at the 1 position of the glyceride molecule, indicated that both structural isomers I and II were formed in the tissue homogenates (Table II). The proportions of the two lysolecithins formed differed between the various tissues, although the results obtained in the separate experiments with liver tissue showed a notable scattering. Since the processes are complex and may involve more than one enzyme, further work is necessary before final conclusions can be made about the exact ratios of both lysolecithin isomers. Theoretically, the conversions might be effected either by one enzyme attacking both ester positions, or two distinct phospholipases each being responsible for the hydrolysis of one defined ester position, or by the combined action of one phospholipase and a lysolecithin acylmutase[17] (EC 5.4.1.1). The results obtained so far point to the two first-mentioned possibilities, but do not rule out a simultaneous occurrence of the last process. Studies with the aid of synthetic lecithins[18], containing at given positions two differently-labeled fatty acids, may help to elucidate some of the problems remaining, particularly when combined with direct structural investigation of the lysolecithins formed.

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[1] L. L. M. VAN DEENEN, G. H. DE HAAS AND C. H. TH. HEEMSKERK, *Biochim. Biophys. Acta*, 67 (1963) 295.
[2] D. J. HANAHAN, *Lipid Chemistry*, New York and London, 1960.
[3] W. E. M. LANDS, *J. Biol. Chem.*, 235 (1960) 2233.
[4] W. E. M. LANDS AND I. MERKL, *J. Biol. Chem.*, 238 (1963) 898.
[5] I. MERKL AND W. E. M. LANDS, *J. Biol. Chem.*, 238 (1963) 905.
[6] R. A. PIERINGER AND L. E. HOKIN, *J. Biol. Chem.*, 237 (1962) 659.
[7] R. W. KEENAN AND L. E. HOKIN, *Biochim. Biophys. Acta*, 60 (1962) 428.
[8] G. R. WEBSTER, *Biochim. Biophys. Acta*, 64 (1962) 573.
[9] M. M. OLIVEIRA AND M. VAUGHAN, *Federation Proc.*, 21 (1962) 296.
[10] L. L. M. VAN DEENEN, J. DE GIER, U. M. T. HOUTSMULLER, A. MONTFOORT AND E. MULDER, in A. C. FRAZER, *Biochemical Problems of Lipids*, Elsevier, Amsterdam, 1963, p. 404.
[11] N. H. TATTRIE AND C. CYR, *Biochim. Biophys. Acta*, 70 (1963) 693.
[12] R. LETTERS AND B. K. SNELL, *J. Chem. Soc.*, (1963) 5127.
[13] J. LLOVERAS, L. DOUSTE-BLAZY AND P. VALDIGUIÉ, *Compt. Rend.*, 256 (1963) 1861.
[14] A. F. ROBERTSON AND W. E. M. LANDS, *Biochemistry*, 1 (1962) 804.
[15] E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
[16] G. V. MARINETTI, *J. Lipid Res.*, 3 (1962) 20.
[17] M. UZIEL AND D. J. HANAHAN, *J. Biol. Chem.*, 226 (1957) 789.
[18] G. H. DE HAAS AND L. L. M. VAN DEENEN, *Tetrahedron Letters*, 22 (1960) 7.

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