TABLE II

THE RELATIVE SPECIFIC RADIOACTIVITY OF EACH PLASMA CHOLESTEROL ESTER FRACTION IN MAN

Plasma free cholesterol of each sample taken as 1.00.

Time (h)	*Whole ester fraction*	*Saturated*	*Δ1*	*Δ2*	*Δ4*
3	0.040	0.037	0.044	0.039	0.04
5	0.087	0.087	0.088	0.085	0.09
7	0.14	0.14	0.14	0.13	0.16
15	0.34	0.35	0.33	0.33	0.36
24	0.50	0.53	0.52	0.48	0.49
72	0.84	0.86	0.86	0.80	0.88

would be expected to result in a more rapid turnover of the poly-unsaturated fatty acid esters of cholesterol.

Very different results were obtained with human and rat plasma cholesterol esters. These findings suggest that plasma cholesterol ester metabolism in man differs significantly from that in the rat. The plasma cholesterol esters of man appear to derive from a pool of esters turning over at the same rate and may comprise a metabolically homogeneous pool.

Further studies of the turnover of individual cholesterol esters in each plasma lipoprotein are currently in progress.

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[1] L. I. GIDEZ AND H. A. EDER, in E. C. HORNING, *Effects of Drugs on Synthesis and Mobilization of Lipids*, Pergamon Press, 1963, p. 67.
[2] A. V. CHOBANIAN, B. A. BURROWS AND W. HOLLANDER, *J. Clin. Invest.*, 41 (1962) 1738.
[3] P. D. KLEIN AND R. A. MARTIN, *J. Biol. Chem.*, 234 (1959) 1685.
[4] M. G. HORNING, E. A. WILLIAMS AND E. C. HORNING, *J. Lipid. Res.*, 1 (1960) 482.
[5] L. J. MORRIS, *J. Lipid Res.*, 4 (1963) 357.
[6] D. S. GOODMAN, D. DEYKIN AND T. SHIRATORI, *J. Biol. Chem.*, in the press.
[7] J. A. GLOMSET, *Biochim. Biophys. Acta*, 65 (1962) 128.

Biochim. Biophys. Acta, 84 (1964) 104–106

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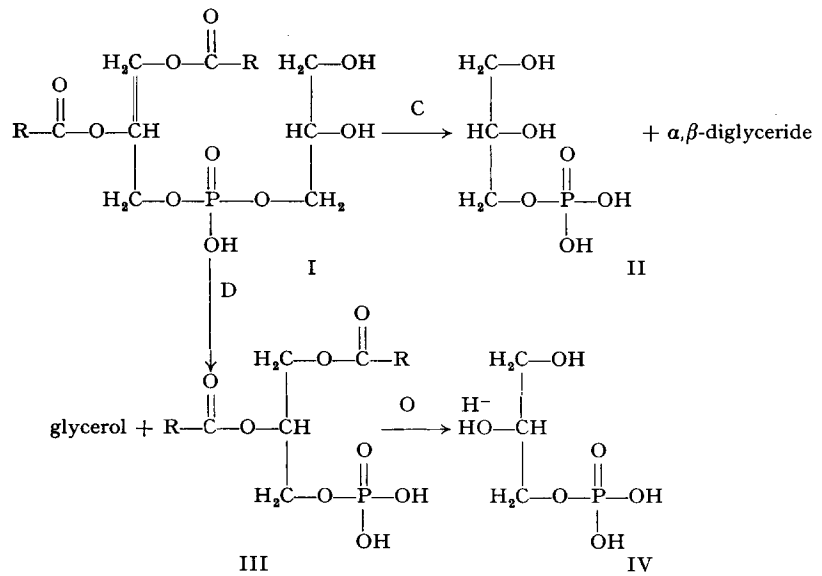
The stereochemical configuration of phosphatidyl glycerol

Phosphatidyl glycerol contains two asymmetric centres and hence can exist theoretically in any of four stereoisomeric forms. BAER AND BUCHNEA[1] synthesized L-α-phosphatidyl-L-glycerol, but BENSON AND MIYANO[2] considered it likely that the

compound present in chloroplasts is identical to L-α-phosphatidyl-D-glycerol. The observations that phosphatidyl glycerol isolated from bacteria and higher plants is susceptible to the action of various types of phospholipases[3,4] made possible an unambiguous determination of its stereochemical configuration.

As outlined in Scheme 1, the use of phospholipase C (EC 3.1.4.3) and phospholipase D (EC 3.1.4.4) respectively enables two α-glycerophosphates differing in the origin of the glycerol moiety to be obtained. The hydrolysis product (II) resulting from the action of phospholipase C contains the unesterified glycerol from I, whereas glycerophosphate (IV) produced by successive action of phospholipase D and alkaline hydrolysis originates from the phosphatidyl part of I. The configuration of II and IV can be established with the aid of L-α-glycerophosphate dehydrogenase (EC 1.1.1.8)



Scheme 1. Production of D-α-glycerophosphate (II) and L-α-glycerophosphate (IV) from phosphatidyl glycerol (I) with the aid of phospholipase C and D.

Phosphatidyl glycerol was isolated from spinach leaves by a minor modification of the procedure previously reported[4]. This preparation (phosphorus:glycerol = 1:2.08) exhibited an optical rotation different from the value reported by BAER AND BUCHNEA[1]. With a view to the high content of poly-unsaturated acids, a partial conversion of these fatty acids constituents during the elaborate isolation procedure cannot be precluded. For this reason no definite conclusions can be made with respect to any difference in the configuration between the synthetic and natural product. An amount of 2 mg of I was emulsified in 0.5 ml of Tris buffer (pH 7.1) together with a crude preparation of phospholipase C obtained from *Bacillus cereus*[5]. After addition of 1 ml of ether the mixture was incubated 2 h at 37°. As demonstrated by thin-layer chromatography a complete hydrolysis of I was obtained, and the diglyceride formed, extracted by ether, turned out to be identical to an α,β-diglyceride[6]. The water layer was shown by paper-chromatography[3] to contain α-glycerophosphate only. Quantitative assays of phosphorus[7] and vicinal hydroxyl

groups by periodate oxidation[8] revealed confirmatory evidence. Determinations with L-α-glycerophosphate dehydrogenase[9] showed that less than 1% of the α-glycerophosphate was present as the L-α-isomer. Thus it can be concluded that II is identical to D-α-glycerophosphate or glycerol-1-phosphate[10].

Degradation of I with phospholipase D was carried out by incubation of 3 mg of phospholipid with an equal amount of crude enzyme (C. F. Boehringer und Söhne, Mannheim) in 2 ml of acetate buffer (pH 5.6; 0.02 M $CaCl_2$), to which 2 ml of ether were added. The mixture was vigourously shaken for 4 h at 30°. Thin-layer chromatograms[3] revealed a complete breakdown of I into phosphatidic acid (III) and glycerol. After extraction of the lipids by repeated ether treatment, a quantitative determination[8] carried out on the water-layer showed that one equivalent of glycerol had been formed. The lipid fraction was hydrolysed with 1 N KOH in methanol for 30 min at 55°. After dilution with two volumes of water the pH was adjusted to 8 and the lipid-soluble material was removed by extraction with chloroform. The yield of α-glycerophosphate (IV) turned out to be 72% calculated on I. Determination with L-α-glycerophosphate dehydrogenase showed that IV is completely present in the form of the L-α-isomer, thus indicating that according to expectation the phosphatidyl moiety of I belongs to the L-α-series. Hence, it can be concluded that phosphatidyl glycerol from spinach leaves is identical to L-α-phosphatidyl-D-glycerol or 1,2-diacyl-glyceryl-3-phosphoryl-1'-glycerol[10]. Determination of the nature of α-glycerophosphate (II) obtained by the action of phospholipase C on phosphatidyl glycerol isolated from *Bacillus cereus* also demonstrated that the glycerol present as end group had the D-configuration. Though in this case no investigations of the configuration of the phosphatidyl moiety have yet been made indirect evidence suggests that the given configuration is valid also for phosphatidyl glycerol from this origin.

The results of this investigation are in good agreement with the recent ascertainment of the biosynthesis of phosphatidyl glycerol in animal tissues by KENNEDY *et al.*[11] and therefore this pathway may be operating in higher plants and bacteria as well. This study was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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[1] E. BAER AND D. BUCHNEA, *J. Biol. Chem.*, 232 (1958) 895.
[2] A. A. BENSON AND M. MIYANO, *Biochem. J.*, 81 (1961) 31P.
[3] F. HAVERKATE, U. M. T. HOUTSMULLER AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 63 (1962) 547.
[4] F. HAVERKATE AND L. L. M. VAN DEENEN, *Biochem. J.*, 88 (1963) 42P.
[5] H. P. CHU, *J. Gen. Microbiol.*, 2 (1949) 255.
[6] O. S. PRIVETT, M. L. BLANK AND W. O. LUNDBERG, *J. Am. Oil Chem. Soc.*, 38 (1961) 312.
[7] G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466.
[8] R. M. BURTON, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, p. 246.
[9] H. J. HOHORST, in H. U. BERGMEYER, *Methods of Enzymatic Analysis*, Academic Press, New York, 1963, p. 215.
[10] H. HIRSCHMANN, *J. Biol. Chem.*, 235 (1960) 2762.
[11] J. Y. KIYASU, R. A. PIERINGER, H. PAULUS AND E. P. KENNEDY, *J. Biol. Chem.*, 238 (1963) 2293.

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