and Harland G. Wood is gratefully acknowledged. Dr. H. S. Isbell, National Bureau of Standards, kindly supplied the 1-C^{14}-glucose.

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ENZYMATIC SYNTHESIS OF PHOSPHORUS-CONTAINING LIPIDES

Sir:

The formation of phosphorus-containing lipid substances from L-α-glycerophosphate (α-GP) and long-chain fatty acids is catalyzed by a partially purified enzyme preparation from rat liver. The reaction has been followed by measuring the incorporation of α-GP labeled with P^{32} into a "phospholipid fraction" (*i.e.*, an ethanol extract of an acid-washed residue precipitated from the incubation mixture by perchloric acid). With enzyme purified 4-fold from homogenates by treatment with calcium phosphate gel or by fractionation with methanol at low temperature, the system requires adenosine triphosphate (ATP), coenzyme A (CoA) and stearic acid (Table I). The latter could be replaced by fatty acids with chain lengths of 12 to 18 carbon atoms (including oleate and linoleate). Labeled inorganic orthophosphate was incorporated only slightly. Addition of choline (0.0025 *M*), phosphorylcholine (0.002 *M*), glycerophosphorylcholine (0.002 *M*) or glycerol (0.05 *M*) did not influence the incorporation of α-GP^{32}.

Two reaction products, tentatively designated as phosphatidic acids, were observed on paper chromatographic analysis of the products resulting from incubation of α-GP^{32} in the presence of stearate-1-C^{14} (Table II). The area occupied by free stearate (Zones 8–10) is widely separated from the bulk (75%) of the P^{32} products (Zones 1–4). Zones 1 and 4 each represent distinct peaks for both C^{14} and P^{32} counts. The ratios of C^{14} to P^{32} counts in the peak zones suggest two compounds, one containing twice as much stearate per mole of phosphate as the other. (The nature of the P^{32} counts in Zones 8–10 is unexplained and may represent an additional component.) Indications of two distinct compounds with C^{14}/P^{32} ratios differing by a factor of 2 were also obtained with acetone and aqueous *n*-butanol as developing solvents. Treatment of the reaction products with 0.2 *N* HCl in ethanol or 0.1 *N* NaOH in ethanol at 75° for one hour resulted in a quantitative conversion of the components in Zones 1–4 to free stearic and glycerophosphoric acids (as judged by paper chromatography with the above-mentioned solvents and also with chloroform).

TABLE I

INCORPORATION OF α-GP^{32} INTO AN ETHANOL FRACTION

The complete incubation mixture (1.0 ml.) contained 0.1 ml. of α-GP^{32} (0.02 *M*, approximately 10^5 counts per minute) 0.2 ml. of ATP (0.03 *M*), 0.05 ml. of CoA (200 units/ml., 50% pure), 0.1 ml. of stearate (0.02 *M*, adjusted to *p*H 9 with NH_4OH), 0.05 ml. of cysteine (0.2 *M*), 0.1 ml. of phosphate buffer (0.5 *M*, *p*H 7.0), 0.2 ml. of water and 0.2 ml. of an enzyme fraction (9 mg. protein/ml.). The latter is prepared from a 0.25 *M* sucrose homogenate (20 mg. of protein/ml.) by collecting a fraction precipitated by methanol (between 7 and 20%) at −5°. Incubation was at 22° for 30 min.

	Ethanol extract (10^3 c.p.m.)
Complete system	10.60
Enzyme heated 2 min. at 50°	0.00
Without ATP	0.24
Without CoA	1.82
Without stearate	2.30
α-GP in place of α-GP^{32}; 10^5 c.p.m. P^{32} as inorganic phosphate	0.02

TABLE II

PAPER CHROMATOGRAM OF THE REACTION PRODUCTS

Incubation conditions were as in Table I except for the inclusion of 0.8 μ curie of C^{14} stearic acid. The ethanol extract (5 ml.) of the acid-washed residue was adjusted to *p*H 4 and concentrated to 0.1 ml. (under a stream of helium at room temperature). An aliquot (0.02 ml.) was chromatographed on Whatman No. 1 filter paper with diisopropyl ether as the solvent. The front advanced 26.5 cm. in two hours. The paper was divided into eleven 2.4-cm. zones and analyzed for C^{14} and P^{32} counts; zone 1 contains the point of origin.

Zone	1	2	3	4	5	6
		10^3 counts per minute				
C^{14}	1.72	0.86	0.79	1.15	0.02	0.06
P^{32}	1.11	.62	.85	1.64	.03	.01
C^{14}/P^{32}	1.6			0.7		

Zone	7	8	9	10	11
	10^3 counts per minute				
C^{14}	0.21	6.47	16.4	5.66	0.00
P^{32}	.02	0.59	0.64	0.11	.00

In view of the participation of ATP and CoA in this system, a mechanism analogous to that proposed for the ATP-CoA activation of acetate (*i.e.*, in choline esterification)[1] may be postulated:

(1) Stearate + CoA $\xrightarrow{\text{ATP}}$ Stearyl-CoA

(2) Stearyl-CoA + α-GP $\longrightarrow$ monostearylphosphatidic acid + CoA

(3) Stearyl-CoA + monostearylphosphatidic acid $\longrightarrow$ distearylphosphatidic acid + CoA

Resolution of the enzyme system into the postulated components and identification of stearyl-CoA are required to validate this mechanism. The possible relation of these findings to the mechanism of esterification of other alcohols (*i.e.*, sterols, glycerol) by long-chain fatty acids is apparent.

It is pertinent to consider the relation of these results to lecithin and cephalin biosynthesis, the nature of which has been obscure. It now appears plausible that phosphatidic acids may serve as precursors, reacting with phosphate esters of the nitrogenous bases. Preliminary observations[2] on the enzymatic incorporation of phosphorylcholine into a phospholipide fraction are consistent with such a scheme.

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(1) For review and primary references see H. A. Barker in "Phosphorus Metabolism," Vol. I, Baltimore, Md., 1951.

(2) A. Kornberg and W. E. Pricer, Jr., *Federation Proc.*, in press.