drocarbons before reaching the layer of cobalt trifluoride. The amount of hydrocarbon introduced during each cycle necessarily was somewhat less than the theoretical amount which could be fluorinated completely, based on the amount of cobalt trifluoride present, its oxidizing value, and the particular hydrocarbon used. The spent cobalt difluoride then was reoxidized with chlorine trifluoride, after the system had been flushed thoroughly with nitrogen. In this cycle the valve leading to trap 1 was kept closed while the valve to trap 3 was opened. Passing the chlorine trifluoride over the cobalt difluoride for only five minutes resulted in a conversion to the cobalt III fluoride, with an oxidizing value greater than 90% of theoretical.

It might be thought possible to mix the vapors of chlorine trifluoride and hydrocarbon and to pass the mixture over cobalt trifluoride to effect fluorination. However, it was found that if the chlorine trifluoride were mixed directly with the hydrocarbon, either as vapor in the reactor tube or as liquid in the copper traps at Dry Ice temperature, only tarry and carbonaceous material resulted. The repetitive batch process of fluorination with CoF_3 must therefore be used.

DEPARTMENT OF CHEMISTRY
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RECEIVED SEPTEMBER 12, 1951

COMMUNICATIONS TO THE EDITOR

THE ORIGINS OF GLUCURONIC ACID

Sir:

The observations that three-carbon compounds, such as lactate and pyruvate, stimulate glucuronic acid synthesis *in vitro* to a greater extent than six-carbon compounds, such as glucose, suggests that the former are either precursors of glucuronic acid or that their oxidation supplies energy for the reactions by which conjugated glucuronides are synthesized from other sources.[1]

In an attempt to clarify this problem, the synthesis of menthol glucuronic acid by liver slices of fasted guinea pigs was studied using, as substrates, glucose, and lactate, in each of which a single carbon was labeled with C^{14} as indicated in the table below. In each experiment the slices were incubated in the same modified Ringer's solution containing glucose, lactate, menthol and a sodium bicarbonate:carbon dioxide buffer system. Only one substrate was labeled in each experiment. The menthol glucuronic acid synthesized by such a system was isolated, purified to constant radioactivity and oxidized, either with periodate (which gave carbon 1, carbons 2-3-4, and carbons 5-6 as three separate fractions), or with 12% hydrochloric acid (which gave carbon 6 as a separate fraction).[2] Each fraction was isolated, specifically converted to carbon dioxide, and plated and counted as barium carbonate. The results are summarized as

Substrate	Labeled position of substrate	C-1	C-2,3,4	C-5,6
	Counts per minute per mM. carbon			
Glucose-1-C^{14}	53.8×10^5	56,500	3,200	1,320
Lactate-3-C^{14}	11.7×10^5	4,150	2,060	6,540
	8.8×10^5	830	470	1,270

It is apparent from the data with glucose-1-C^{14} that this compound enters the glucuronic acid molecule with no major redistribution of C^{14} from the 1-position of the glucose molecule. This might be interpreted as evidence for the direct conversion of glucose to glucuronic acid. However, if glucose were the sole source of glucuronic acid, it would be expected, in accordance with current concepts of glycogenesis, that symmetrical labeling of the glucuronic acid would occur when lactate-3-C^{14} was the labeled substrate. Contrary to this expectation, the average specific activity of carbons 5 and 6 of the glucuronic acid is higher than that of carbon 1, indicating that this substrate is converted to the distal portion (carbons 4, 5, 6) of the glucuronic acid molecule to a greater extent than to the proximal portion. Decarboxylation of the menthol glucuronic acid obtained with lactate-3-C^{14}, resulting in the isolation of carbon 6 as a discrete fraction, shows that practically all the radioactivity in the C-5,6 fraction resides in C-6, the specific activity of the latter being approximately 2.5 times than of C-1. It would appear from these results that the entire glucose molecule is not the sole source of the glucuronic acid. Since lactate is rapidly metabolized by pathways other than anabolic reactions, it is to be expected that considerable dilution and redistribution of the radioactivity of the original substrate will occur and will be apparent in the labeling of the menthol glucuronic acid even though lactate were a precursor.

These data, therefore, do not exclude the possibility that, in the biosynthesis of conjugated glucuronic acid, a triose, produced from lactate, condenses with another triose formed from glucose and that, in this manner, carbons 1, 2 and 3 of the glucuronic acid originate predominantly from carbons 1, 2 and 3 of glucose while carbons 4, 5 and 6 originate from a three-carbon compound. Further work, designed to test these possibilities, is in progress.

This work was done while the author was a Postdoctoral Fellow of the National Institutes of Health, U.S.P.H.S., and, subsequently, with the aid of a grant from the Nutrition Foundation, Inc. The continued guidance and assistance of Dr. Ernest Bueding and the interest of Drs. Warwick Sakami

(1) W. L. Lipschitz and E. Bueding, *J. Biol. Chem.*, **129**, 333 (1939).
(2) C. F. Huebner, R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *ibid.*, **159**, 503 (1945).

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

DEPARTMENT OF PHARMACOLOGY
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RECEIVED JANUARY 2, 1952

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

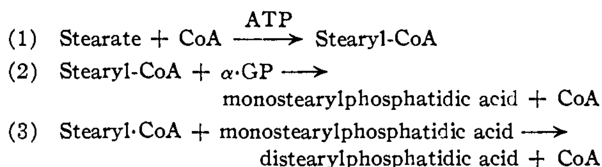
PAPER CHROMATOGRAM OF THE REACTION PRODUCTS

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:



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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RECEIVED FEBRUARY 20, 1952

(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.