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
HARVARD UNIVERSITY
CAMBRIDGE, MASS. 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
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO THOMAS G. BIDDER

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:

(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.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH ARTHUR KORNBERG
BETHESDA, MARYLAND W. E. PRICER, JR.

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.

A NEW SOLVENT EXTRACTION METHOD FOR THE SEPARATION OF NIOBIUM AND TANTALUM

Sir:

We wish to make a preliminary report of the separation of niobium and tantalum by a new solvent extraction technique. It has been found that niobium may be extracted essentially quantitatively from strong hydrochloric acid with a solution of methyldioctylamine in xylene. Under these conditions the extraction of tantalum appears to be negligible. The niobium may then be "stripped" from the organic phase with nitric acid, sulfuric acid or dilute hydrochloric acid.

Methyldioctylamine, a water-insoluble tertiary amine, is known[1] to form the corresponding amine acid salts which are also water-insoluble, in general, and preferentially extract into organic solvents. The work to date suggests that approximately 8 *M* hydrochloric acid concentration is satisfactory for the separation.

In a typical experiment an aqueous phase containing Nb^{95} tracer or Ta^{182} tracer was extracted for five minutes with an equal volume of a 5% solution of methyldioctylamine in xylene. Each phase was checked for Nb^{95} γ or Ta^{182} γ radioactivity by use of a scintillation counter. The results of the initial investigation of the effect of hydrochloric acid concentration on the extraction of niobium and tantalum are given in Tables I and II, respectively.

TABLE I

THE EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON THE EXTRACTION OF Nb^{95} WITH METHYLDIOCTYLAMINE IN XYLENE

HCl, *M*	Nb^{95} Extracted, %
2	4.0
3	2.4
4	2.2
6	21.5
8	99.3
9.6	100.0
(control) 9.6 (no MDOA)	0.04

TABLE II

THE EFFECT OF HCl CONCENTRATION ON THE EXTRACTION OF Ta^{182} WITH METHYLDIOCTYLAMINE IN XYLENE

HCl, *M*	Ta^{182} Extracted, %
2.91	0.25
4.85	0.11
7.28	0.55
8.85	1.10
10.10	1.35
11.20	1.40

It has been found that Nb^{95} does not extract appreciably from nitric acid concentrations up to 10.6 *M* and from sulfuric acid concentrations up to 12 *M*. Ta^{182} does not extract from nitric acid concentrations up to 10.6 *M* nor from higher concentrations of sulfuric acid, but appears to extract appreciably from 2 *M* H_2SO_4. The extraction behavior of these elements in dilute sulfuric acid is being investigated.

(1) E. L. Smith and J. E. Page, *J. Soc. Chem. Ind.*, **67**, 48 (Feb., 1948).

Using the procedure given above, Nb^{95}–Ta^{182} tracer mixtures have been separated and nonradioactive Nb–Ta separations at one milligram/milliliter concentrations have been effected.

OAK RIDGE NAT. LAB. G. W. LEDDICOTTE
OAK RIDGE, TENN. F. L. MOORE

RECEIVED FEBURARY 19, 1952

THE DIPOLE MOMENT OF DECABORANE

Sir:

In the course of a comprehensive investigation of decaborane,[1] $B_{10}H_{14}$, we have measured the dielectric constant of benzene solutions and have obtained a surprising value for its dipole moment which should be of considerable interest to those concerned with the structures and bonding of the boron hydrides.

Preliminary experiments showed that even short exposure to moist air led to variable results. Therefore the decaborane and benzene were purified with great care and stored under vacuum conditions. The solutions were prepared in a dry box and transferred to the dielectric cell without exposure to the air. The dielectric constants of solutions ranging in concentration from 0.0071345 to 0.018691 mole fractions of decaborane were measured at 25°, using a heterodyne-beat oscillator equipped with a precision condenser and operating at a frequency of 1.79 megacycles. The dielectric constant of benzene was taken to be 2.2773 at 25°. Subsequently the densities of the solutions were determined pycnometrically and values were calculated for the polarization of decaborane according to the method described by Smyth.[2] Graphical extrapolation to infinite dilution gives the value 297.0 for the total molar polarization of $B_{10}H_{14}$ in benzene solution. If the sum of the electronic and atomic polarizations is assumed to be 1.05 times the molar refraction this sum equals 43.9, and the dipole moment of $B_{10}H_{14}$ is then calculated to be 3.52 ± 0.02 Debye.

Mole fraction $B_{10}H_{14}$	Total molar Polarization of $B_{10}P_{14}$
0.0071345	295.5
.0073878	295.1
.0078698	294.8
.013943	293.3
.018691	291.2

This is an unexpectedly high value in the light of the Kasper, Lucht and Harker model[3] for the structure of decaborane and present theories of electronegativity and bonding. It appears, however, that high polarity may be characteristic of asymmetric boron hydrides having a number of hydrogen bridge bonds. This is supported by a recent microwave investigation of pentaborane, B_5H_9, by Pimentel and associates.[4] They report a dipole

(1) We wish to thank Dr. A. E. Newkirk of the Research Laboratory of the General Electric Company for providing the decaborane and for information concerning the characteristics of this compound.

(2) C. P. Smyth, "Dielectric Constant and Molecular Structure" (The Chemical Catalog Co.), Reinhold Publ. Corp., New York, N. Y., 1931.

(3) J. S. Kasper, C. M. Lucht and D. Harker, *Acta Cryst.*, **3**, 436 (1950).

(4) Professor G. C. Pimentel and associates describe this in a letter recently submitted to the *Journal of Chemical Physics*.

moment of 2.13 ± 0.04 debye for this compound which is believed to resemble decaborane by being asymmetric and having four hydrogen bridge bonds.[5]

We are continuing measurements on solutions of decaborane in other solvents.

(5) (a) K. Hedberg, M. E. Jones and V. Schomaker, THIS JOURNAL, **73**, 3538 (1951); (b) W. J. Dulmage and W. N. Lipscomb, *ibid.*, **73**, 3539 (1951).

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY A. W. LAUBENGAYER
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RECEIVED FEBRUARY 16, 1952

FRACTIONATION OF AN ACTH PREPARATION BY IONOGRAPHY

Sir:

The technique of ionography,[1,2,3,4] that is, electromigration on wet surfaces, was utilized to fractionate an ACTH preparation[5] obtained from pig pituitaries. This material was prepared by the acid acetone extraction method of Lyons[6] and further purified by treatment with 9% ammonium hydroxide solution and fractional acetone precipitation. The final material was freeze dried from aqueous solution. It was one half as active as Armour's LA la standard. The particular instrument employed was the Precision Ionograph.[7] The paper used was Eaton-Dikeman 613, 8 mm. in width. The experiments were conducted at 24–26° in a helium atmosphere.

The ionogram was dried on a glass plate by a stream of hot air. It was then passed through a saturated mercuric chloride solution of 95% ethyl alcohol containing 1 g. of brom phenol blue per 100 ml. of solution. The ionogram was again dried in a stream of hot air and then passed successively through several beakers containing saturated aqueous mercuric chloride solution until all the excess indicator was washed out of the paper strip. On re-drying the strip, the protein zone appeared as a dull green area which changed to a sharp deep blue color by passing the ionogram over concentrated ammonium hydroxide.

Using a veronal buffer of ionic strength 0.015 at *p*H 5.5, and applying a potential of 6 volts/cm. for three hours across the ends of the filter paper strips, the ACTH preparation separated into three fractions: a heavy-staining fraction "A" which moved to the negative pole, a light-staining fraction "B" which moved to the positive pole and a heavy-staining fraction "C" which did not move. As the *p*H of the buffer used to saturate the paper strips was increased to 6.0–6.6, the mobility of the heavy-staining fraction A was found to approach zero, indicating that its isoelectric point was in this region. This fraction contained 98% of the biological activity as determined by the adrenal ascorbic acid depletion test,[8] but only about 31% of the total input nitrogen. The isoelectric point of the light fraction B, which was found to have only 0.2% activity, but about 45% of the nitrogen was shown to be in the region of 4.2–4.8. The isoelectric point of fraction C, which contained 2% of the activity and 21% of the nitrogen, was shown to be in the region of 5.0–6.0. It would appear from these experiments, and others,[9] that the biological activity of ACTH is not uniformly distributed throughout the whole protein preparation.

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RECEIVED JANUARY 16, 1952

(1) H. J. McDonald, M. C. Urbin and M. B. Williamson, *Science*, **112**, 227 (1950).

(2) H. J. McDonald, M. C. Urbin and M. B. Williamson, THIS JOURNAL, **73**, 1893 (1951).

(3) H. J. McDonald, M. C. Urbin, E. P. Marbach and M. B. Williamson, *Federation Proc.*, **10**, 218 (1951).

(4) H. J. McDonald, M. C. Urbin and M. B. Williamson, *J. Colloid Sci.*, **6**, 236 (1951).

(5) The ACTH preparation (control X1-134-3) was supplied by G. D. Searle and Co. We are indebted to Dr. F. J. Saunders of G. D. Searle and Co. for the determinations of biological activity.

(6) W. R. Lyons, *Proc. Soc. Exptl. Biol. Med.*, **35**, 645 (1937).

(7) Manufactured by Precision Scientific Co., Chicago 47, Ill.

(8) M. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948).

(9) G. P. Hess, J. I. Harris, F. H. Carpenter and C. H. Li, THIS JOURNAL, **73**, 5918 (1951).

(10) Reuben Myron Strong Research Fellow.