reduction[3] are inefficient and give only small yields.

In a typical preparation, 50 ml. of a solution 0.6 *N* in hydrochloric acid of tin(II) chloride containing 0.46 mmole of tin were introduced into a reaction flask connected to a series of traps suitable for the collection of the condensable products. The reaction system was swept with nitrogen, and the nitrogen stream was maintained throughout the reduction. From a dropping tube, 20 ml. of a 5% aqueous sodium borohydride solution were added over a period of 20 minutes. At this point the reduction was considered complete. The produced gases were passed through a trap held at −23° to remove most of the water vapor and through a trap kept at −196° to collect the stannane. The crude product was purified by fractionation through a trap maintained at −112°. The yield of stannane was 0.39 mmole or 84% based on the amount of tin taken.

The product was identified by its vapor pressure at −105° (39 mm.) and at −78° (198 mm.), values which are in agreement with those reported by Paneth.[4] The identification was verified by permitting 0.75 mmole of stannane to decompose into the elements. After seven days the decomposition was judged complete and 1.27 mmoles of hydrogen were found compared to 1.50 mmoles expected for stannane.

It has been observed that the yield of the hydride is dependent upon the acid concentration, the effect of which is still being investigated, and upon the concentration of tin. This latter effect is illustrated by the following data:

Mg. of tin per ml. of soln.	Yield of stannane, %
11	9
6	18
4	25
3	37
1	84

By an analogous reaction small but significant yields of bismuthine have been obtained. The preparation of these hydrides and those of related elements is under continuing study.

(3) F. Paneth and E. Rabinowitsch, *Ber.*, **57B**, 1877 (1924).

(4) F. Paneth, W. Haken and E. Rabinowitsch, *ibid.*, **57B**, 1898 (1924).

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THE SYNTHESIS OF PROTOPORPHYRIN FROM δ-AMINOLEVULINIC ACID IN A CELL-FREE EXTRACT[1]

Sir:

We have recently reported that δ-aminolevulinic acid can replace the two substrates, "active" succinate and glycine for porphyrin synthesis.[2] In order to investigate porphyrin synthesis in greater detail we have begun to fractionate duck red blood cell preparations which we have previously demonstrated are capable of synthesizing protoporphyrin *in vitro*. We have found that not only can homogenized duck erythrocyte preparations synthesize protoporphyrin from δ-aminolevulinic acid but that a soluble cell-free extract of the duck red blood cell can effect the synthesis. It can be seen from Table I, that the supernatant fluid obtained on high speed centrifugation (12×10^3 to 100×10^3 *g*) is almost as active as the homogenate and that even a lyophilized preparation of the cell-free extract still retains its synthetic activity. On the other hand, although the whole red blood cell or a gently hemolyzed preparation of duck erythrocytes can synthesize protoporphyrin from glycine and succinate,[3-5] the ability to convert these substrates to protoporphyrin is lost on disruption of the structure (Table I). It would appear that on homogenization the functional activity of only those enzymes that are involved in the condensation of succinate with glycine is lost.

TABLE I

COMPARISON OF C^{14} ACTIVITIES OF HEMIN SYNTHESIZED FROM δ-AMINOLEVULINIC ACID-5-C^{14} (0.05MC./MM.) AND SUCCINIC ACID-2-C^{14} (0.05 MC./MM.) IN DIFFERENT RED BLOOD CELL PREPARATIONS[a]

Expt.	Substrate, acid	Red cell preparation	C^{14} activity in hemin sample, c.p.m.
1	Succinic (0.05 mM.)[b]	Hemolyzed	390
	Succinic (0.05 mM.)[b]	Homogenized	7
2	δ-Aminolevulinic (0.013 mM.)	Hemolyzed	4300
	δ-Aminolevulinic (0.013 mM.)	Homogenized	4500
3	δ-Aminolevulinic (0.009 mM.)	Homogenized	2200
	δ-Aminolevulinic (0.009 mM.)	Supernatant (12 × 10^3 *g*)	1600
	δ-Aminolevulinic (0.009 mM.)	Supernatant (47 × 10^3 *g*)	1600
	δ-Aminolevulinic (0.009 mM.)	Supernatant (100 × 10^3 *g*)	1500
4	δ-Aminolevulinic (0.009 mM.)	Supernatant (12 × 10^3 *g*)	2000
	δ-Aminolevulinic (0.009 mM.)	Lyophilized prepn.	1500

[a] Each preparation represented 25 ml. of duck blood and prepared as previously described.[3,4] [b] Plus 0.33 mM. of non-radioactive glycine.

Further proof that δ-aminolevulinic acid is indeed the precursor for porphyrin synthesis was obtained by degrading a hemin sample synthesized from δ-aminolevulinic acid-5-C^{14}.[6,7] The δ-carbon atom of the latter compound should label the same carbon atoms of protoporphyrin as those which we have previously found arise from the α-carbon atom of glycine[6] since the latter carbon atom is the

(1) This work was supported by grants from the National Institutes of Health, United States Public Health Service (RG-1128(C5)), from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, and from the Rockefeller Foundation.

(2) D. Shemin and C. S. Russell, THIS JOURNAL, **76**, 4873 (1953).

(3) D. Shemin, I. M. London and D. Rittenberg, *J. Biol. Chem.*, **173**, 799 (1948); **183**, 757 (1950).

(4) D. Shemin and S. Kumin, *ibid.*, **198**, 827 (1952).

(5) I. M. London and M. Yamasaki, *Federation Proc.*, **11**, 250 (1952).

(6) J. Wittenberg and D. Shemin, *J. Biol. Chem.*, **185**, 103 (1950).

(7) D. Shemin and J. Wittenberg, *ibid.*, **192**, 315 (1951).

source of the δ-carbon atom of the δ-aminolevulinic acid.[2] It can be seen from Table II that the same C^{14} distribution pattern was found in protoporphyrin synthesized from δ-aminolevulinic acid-5-C^{14} and from glycine-2-C^{14}; 50% of the C^{14} activity resides in the pyrrole rings and 50% in the methene bridges.

TABLE II

DISTRIBUTION OF C^{14} ACTIVITY IN PROTOPORPHYRIN SYNTHESIZED FROM δ-AMINOLEVULINIC ACID-5-C^{14} AND FROM GLYCINE-2-C^{14}

Fragments of porphyrin	Molar activity (%) in fragments of porphyrin synthesized from δ-Aminolevulinic acid-5-C^{14}, %	Glycine-2-C^{14} (ref. 6), %
Protoporphyrin	100	100
Pyrrole rings A + B (methylethylmaleimide)[a]	24.5	24.6
Pyrrole rings C + D (Hematinic acid)[a]	25.2	25.3
Pyrrole rings A + B + C + D	49.7	49.9
Methene bridge carbon atoms	50.3	50.1

[a] Obtained from protoporphyrin as previously described.[6,7]

Our finding that δ-aminolevulinic acid is an intermediate in porphyrin synthesis has been confirmed in two recent communications.[8,9]

(8) A. Neuberger and J. J. Scott, *Nature*, **172**, 1093 (1953).
(9) E. I. B. Dresel and J. E. Falk, *ibid.*, **172**, 1185 (1953).

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FIRST STEP IN THE HYDROLYSIS OF PENTABORANE

Sir:

Normally, the identification of intermediate products resulting from the hydrolysis of boron hydrides is difficult to obtain because of the ease with which they hydrolyze; however, by immobilizing the water molecules we were able to obtain evidence for the occurrence of such intermediates in the case of diborane.[1,2] Now, by means of the mass spectrometer and with the technique of using "bound water" in silica gel[1] we have succeeded in identifying the first step in hydrolysis pentaborane.

Purified pentaborane gas was passed through a shallow bed of silica gel containing only bound water, and then led directly into a Consolidated Engineering model 21-103 mass spectrometer. Mass spectra of the gaseous products were taken immediately and after intervals of 20, 40 and 60 minutes exposure time of pentaborane to the silica gel. By stripping the mass spectrum for pure pentaborane from these spectra, we obtained a residual pattern whose peak heights as a function of mass numbers (m/e) is illustrated in Fig. 1.

The height of the residual peaks based upon a relative peak height of 100 at $m/e = 59$ (highest peak height in pentaborane spectrum) was largest in the spectrum obtained after the 20 minutes exposure time, and decreased with time. The residual pattern in the spectrum taken immediately after the pentaborane was exposed to the silica gel was very small. The amount of hydrogen generated in the hydrolytic reaction increased with time. Mass spectra taken immediately and at short intervals after mixing pentaborane and water vapor showed no residual pattern except for hydrogen.

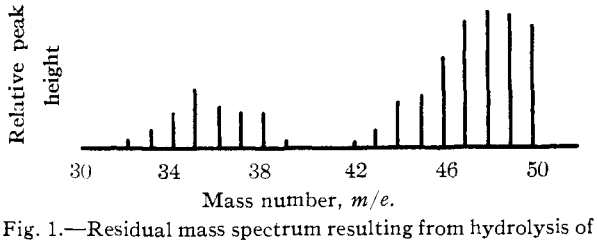

Fig. 1.—Residual mass spectrum resulting from hydrolysis of pentaborane.

The residual pattern given above is very similar to the mass spectrum of tetraborane[3] for which the principal monoisotopic species is B_4H_6. We believe the first step in the hydrolysis of pentaborane to be the removal of a borine by the "bound water" with subsequent generation of hydrogen.[1] From a comparison of the models for pentaborane[4] and tetraborane[5] the removal of any one of the four basal borons of pentaborane would leave the tetraborane skeleton.

That the "bound water" in silica removes a borine from pentaborane can also be demonstrated by boron and hydrogen balances of what is retained on the silica and what is recovered in the volatile portion of the products. By adding water to the silica after all volatile material has been pumped off the solid, one can measure the amount of active hydrogen gasometrically and the amount of boron by titration with mannitol and standard base. Silica must be filtered from the solution before titration of the boron. In one experiment pentaborane from a carbon tetrachloride slush (−23°) was passed through a bed of silica at room temperature into a liquid nitrogen trap. The noncondensable gas (hydrogen) was compressed into a gasometer by means of a toepler pump. The amount of hydrogen gas generated during the pentaborane hydrolysis was approximately the same as that found after the addition of water to the silica. The ratio of active hydrogen to boron in the silica was found to be slightly greater than unity. Thus the over-all ratio of hydrogen (hydride) to boron accounted was *ca.* 2.5 instead of the expected value of 3.0 for borine. This difference can be attributed to errors of measurement because of the small amounts of material involved. The material caught in the liquid nitrogen trap was found to be a mixture of pentaborane and an unstable product which decomposed at room temperature over a period of hours to give hydrogen and a fibrous-looking solid boron hydride.

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(1) I. Shapiro and H. G. Weiss, *J. Phys. Chem.*, **57**, 219 (1953).
(2) H. G. Weiss and I. Shapiro, THIS JOURNAL, **75**, 1221 (1953).
(3) To be published.
(4) K. Hedberg, M. E. Jones and V. Schomaker, *Proc. Natl. Acad. Sci.*, **38**, 679 (1952).
(5) M. E. Jones, K. Hedberg, and V. Schomaker, THIS JOURNAL, **75**, 4116 (1953); C. E. Nordman and W. N. Lipscomb, *ibid.*, **75**, 4116 (1953).
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