C^{14}.[6] The purified AMP had no detectable contaminant by paper chromatography and assayed 99–100% AMP by enzymic deamination. Its specific activity was 46000 c.p.m./μM.

Incubation of pigeon liver homogenate with the test compound was done under the conditions previously described, except that the homogenate was centrifuged for 5 minutes at 500 *g* and only the supernatant was used.[1] Comparison of AMP and adenine as precursors of RNA yielded the results summarized in Table I.

TABLE I

INCORPORATION OF AMP-4,6-C^{14} INTO RNA

Each flask had 6 ml. of a 20% homogenate, incubated for 2 hours under air at 36° with labeled precursors. RNA was obtained as a mixture of mononucleotides after alkaline hydrolysis.

Precursor	μM.	% of added c.p.m. in RNA	RNA (c.p.m./mg.)	R.S.A.[a] $\times 10^4$
Adenine-8-C^{14}	0.8	0.26	1900	3.1
AMP-4,6-C^{14}	2.1[b]	0.24	107	8.2

[a] Relative specific activity = (specific activity RNA adenine)/(specific activity precursor adenine). [b] Includes endogenous AMP.

From these data AMP may be considered to be at least as effective a precursor of RNA as is adenine in this system.

Evidence that AMP is incorporated into RNA with the ribose-phosphate bond intact was obtained using P^{32}-labeled AMP prepared by Eggleston's method.[7] The resulting AMP, which was chromatographically homogeneous, had a specific activity of 5×10^6 c.p.m./μM. and assayed 99–101% AMP enzymically. The experiment was carried out in the same manner as that described in Table I, but the alkaline hydrolyzate was separated into its constituent mononucleotides by ion-exchange chromatography.[8] The results are summarized in Table II.

TABLE II

INCORPORATION OF AMP-P^{32} INTO RNA MONONUCLEOTIDES

Incubations as in Table I, with 6 μM. of adenine-4,6-C^{14} (A) or with 2 μM. of AMP-P^{32} (B).

	Specific activity, c.p.m./μM.	
	A	B[a]
Isolated nucleotide		
Cytidylic acid	26	2780
Adenylic acid	48800	720
Uridylic acid	390	5100
Guanylic acid	1760	3060

[a] There was one, as yet, unidentified fraction isolated that contained a significant amount of P^{32} in experiment B that was not present in experiment A.

These data demonstrate that the AMP was actually incorporated into the framework of the RNA molecule because the P^{32} was recovered, for the most part, in non-adenine containing nucleotides. This is so because after alkaline hydrolysis the phosphorus which was incorporated into RNA from the 5′-nucleotide should be found esterified with the 3′-position of the adjacent nucleoside residue. The differences in specific activities indicate strongly that the P^{32} was not incorporated as inorganic phosphate split off the AMP.

ARGONNE CANCER RESEARCH HOSPITAL AND
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CHICAGO EUGENE GOLDWASSER
CHICAGO 37, ILLINOIS

RECEIVED OCTOBER 10, 1955

(6) The author is indebted to Dr. E. L. Bennett of the University of California Radiation Laboratory for generous gifts of the crude acid-soluble nucleotides and a sample of adenine-4,6-C^{14}.

(7) L. V. Eggleston, *Biochem. J.*, **58**, 503 (1954).

(8) W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

THE CRYSTALLINE COMPOUND AMMONIA-BORANE,[1] H_3NBH_3

Sir:

The ammonia addition compound of the borane group has been considered anomalous for a long time, since its molecular weight in liquid ammonia corresponds to the formula $B_2H_6 \cdot 2NH_3$[2,3] and since no crystalline forms of the compound have been obtainable for X-ray study.

Very recently the new and long sought monomeric compound ammonia-borane, H_3NBH_3, has been prepared from the "diammoniate of diborane," $B_2H_6 \cdot 2NH_3$, as a white, ether soluble solid which gives a definite X-ray powder pattern. An easier synthesis of the compound is achieved from lithium borohydride. The reactions are carried out in diethyl ether at room temperature in accordance with the following equations.

(a) From Lithium Borohydride.

$$LiBH_4 + NH_4Cl \xrightarrow{\text{Diethyl ether}} LiCl + H_3NBH_3 + H_2$$

$$2LiBH_4 + (NH_4)_2SO_4 \xrightarrow{\text{Diethyl ether}} Li_2SO_4 + 2H_3NBH_3 + 2H_2$$

(b) From the "Diammoniate of Diborane".[4]

$$[H_2B(NH_3)_2^+][BH_4^-] + NH_4Cl \xrightarrow[\text{trace of } NH_3]{\text{Diethyl ether}} [H_2B(NH_3)_2^+][Cl^-] + H_3NBH_3 + H_2$$

Although the theoretical amount of hydrogen is produced in the reactions using lithium borohydride, the yields of ether soluble ammonia-borane have been about 45%; an amorphous ether insoluble compound which appears to be the "diammoniate" is produced also.

Molecular weight measurements in dioxane, by freezing point depression, and in diethyl ether, by vapor pressure depression, indicate that ammonia-borane is a monomer (theory: 30.88; found: 31 ± 4).

Anal. Calcd. for H_3NBH_3: H (hydridic), 9.79; B, 35.0; N, 45.4. Found: H (hydridic), 9.73; B, 35.1; N, 45.6.

The properties of crystalline ammonia-borane contrast sharply with those of the classical "di-

(1) The designation of BH_3 as borane is consistent with the system of nomenclature for boron compounds, which was presented by G. W. Schaeffer and T. Wartik at the 125th meeting of the American Chemical Society, Kansas City Missouri.

(2) A. Stock and E. Pohland, *Ber.*, **58**, 657 (1925); H. I. Schlesinger and A. B. Burg, THIS JOURNAL, **60**, 290 (1938).

(3) D. R. Schultz, S. G. Shore and R. W. Parry, to be published.

(4) This laboratory will soon publish accumulated chemical and physical evidence which indicates that the so-called "diammoniate of diborane" is actually a borohydride with a probable structural formula of $[H_2B(NH_3)_2^+][BH_4^-]$. The formulation is consistent with the experimental observations and is used in analogy to the reaction with $LiBH_4$.

ammoniate." The solid splits out hydrogen slowly at room temperature. Only slight evolution of hydrogen occurs upon solution of the solid in distilled water; in the presence of aqueous acid, hydrogen is evolved rapidly. It has a negative temperature coefficient of solubility in ether. At room temperature a white solid, believed to be the "diammoniate of diborane" and perhaps some $(H_2NBH_2)_x$, is slowly deposited from ether solution. A trace quantity of hydrogen is liberated also.

The relative intensities and interplanar spacings, which were obtained from the X-ray powder pattern of the crystalline material, are tabulated below.

Intensity	d, Å.	Intensity	d, Å.	Intensity	d, Å.
VVS	3.72	S	2.13	MW	1.66
VS	3.65	MS	2.09	MS	1.60
S	2.63	W	1.86	VW	1.40
MS	2.52	W	1.82	W	1.37

Acknowledgment.—This research was conducted under Contract AF33(616)-8 with the United States Air Force, the sponsoring agency being the Aeronautical Research Laboratory of the Wright Air Development Center, Air Research and Development Command.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN
SHELDON G. SHORE
ROBERT W. PARRY

RECEIVED SEPTEMBER 13, 1955

IN VITRO CONVERSION OF SQUALENE TO LANOSTEROL AND CHOLESTEROL[1]

Sir:

According to a scheme of sterol biogenesis proposed in 1953,[2] squalene cyclizes to an intermediate having the structure of a 4,4′,14-trimethylcholestane derivative while the formation of cholesterol occurs subsequently by removal of the three methyl substituents. As proposed, the scheme also provided an explanation for the origin of lanosterol from a triterpenoid precursor. Recently the synthesis of lanosterol from acetic acid and the conversion of this sterol to cholesterol have been shown to occur in homogenates of rat liver.[3] These findings have furnished strong support for the view that C_{30} sterols are intermediates in the biosynthesis of cholesterol from squalene. During the present study of squalene metabolism we have found that in one type of liver preparation squalene is converted to a mixture of sterols with cholesterol predominating while in another preparation lanosterol accumulates.

The enzyme systems for the over-all synthesis of cholesterol from acetic acid, as shown by Bucher and McGarrahan,[4] are constituents of the microsome and supernatant fraction of rat liver homogenates. A conversion of squalene to digitonin precipitable steroids by liver extracts has been reported by Dituri, *et al.*[5] In the present experiments with C^{14} squalene, two liver preparations were used. Rat liver was homogenized[6] and centrifuged for 40 minutes at 100,000 × g and the microsomes from 40 ml. of homogenate were combined with 10 ml. of supernatant (Preparation A). The microsomes from another 95 ml. of homogenate were suspended in 20 ml. of supernatant, subjected to sonic oscillation (9 Kc.) for 15 seconds and, after addition of another 20 ml. of supernatant, centrifuged at 100,000 × g for 40 minutes. The resulting particle-free supernatant is designated Preparation B. The source of C^{14} squalene was liver and intestinal tissue of rats which had received 1-C^{14} acetate by injection, and were killed 10 minutes later. The radioactive hydrocarbon was characterized as squalene as described previously.[7] It was suspended in supernatant and incubated with the two liver preparations for three hours at 37°. The petroleum ether extracts containing the unsaponifiable material were first chromatographed on active alumina and the sterol fraction rechromatographed on partially deactivated alumina after the addition of non-isotopic lanosterol and cholesterol as carrier. Lanosterol and cholesterol thus separated were recrystallized three times without loss of radioactivity. Also, the specific activity of one sample of cholesterol was not diminished by purification through the dibromide. DPN[8] which is essential for the incorporation of acetate into cholesterol in homogenates[9] had no marked effect on the yields of sterols formed from squalene in these preparations, nor did its presence significantly alter the ratio of C^{14} recovered in the two sterols. As shown in Table I about ten times as much C^{14} is found in cholesterol as in lanosterol when the enzyme preparation contains intact microsomes, while in the particle-free preparation the C^{14} content of cholesterol is insignificant compared to that of lanosterol. Thus, both the cyclization of squalene to lanosterol and demethylation to cholesterol take place when the

TABLE I

	Microsomes + Supernatant, (Prep. A)[a]		Microsome Extract + Supernatant, (Prep. B)[b]	
	+ DPN (6.5 mg.) c.p.m.	... c.p.m.	+ DPN (6.5 mg.) c.p.m.	... c.p.m.
C^{14} squalene added	5700	5700	11400	11400
C^{14} squalene recovered	2400	2380	2200	3040
Lanosterol	62	72	145[d]	133
Cholesterol	789[c]	566	<10	<10

[a] 14.5 ml. of preparation A per experiment. [b] 22.5 ml. of preparation B per experiment. [c] Specific activities 17.7, 18.2; 23.0 and 18.0 c.p.m./mg. on successive crystallizations. [d] Specific activities 8.7, 10.7; 9.5 and 11.5 c.p.m./m.g. on successive crystallizations.

(1) Supported by grants-in-aid from the National Science Foundation and the Life Insurance Medical Research Fund.

(2) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953); W. G. Dauben, *et al.*, *ibid.*, **75**, 3038 (1953).

(3) R. B. Clayton and K. Bloch, *Fed. Proc.*, **14**, 194 (1955).

(4) N. L. R. Bucher and K. McGarrahan, *ibid.*, **14**, 187 (1955).

(5) F. Dituri, F. Cobey, J. V. B. Warms and S. Gurin, *ibid.*, **14**, 203 (1955).

(6) N. L. R. Bucher, THIS JOURNAL, **75**, 498 (1953).

(7) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 129 (1953).

(8) DPN is diphosphopyridine nucleotide.

(9) I. D. Frantz and N. L. R. Bucher, *J. Biol. Chem.*, **206**, 471 (1954).