hydrogenase[10] activity, which is absent from the rat liver preparations used here.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE
BALTIMORE 5, MARYLAND ROBERT G. LANGDON[11]

(10) N. O. Kaplan, S. P. Colowick and E. F. Neufeld, *J. Biol. Chem.*, **205**, 1 (1953).

(11) This work was conducted during the tenure of a Lederle Medical Faculty Award.

VITAMIN B_{12}. XXVI. DEGRADATION OF FACTOR III TO 5-HYDROXYBENZIMIDAZOLE AND DERIVATIVES AND BIOSYNTHESIS OF FACTOR III

Sir:

Factor III was isolated from fermented sewage.[1] It has been reported to have hematological activity similar to that of vitamin B_{12}, and appeared to differ from vitamin B_{12} by having an unknown moiety in place of 5,6-dimethylbenzimidazole.[2] Friederich and Bernhauer[3] have now reported 5-hydroxybenzimidazole as a degradation product of Factor III.

Last year, through the generosity of Professor Dr. K. Bernhauer, we received samples of Factor III. We have independently identified 5-hydroxybenzimidazole as a part of the molecule. We have also prepared two crystalline cobalt complexes from 5-hydroxybenzimidazole and Factor B[4] by biosynthesis. One of these appears to be Factor III from comparison with the substance isolated from sewage.

Factor III was hydrolyzed with 6 *N* hydrochloric acid for 20 hours at room temperature, and the hydrolysate was subjected to paper electrophoresis in 0.5 *N* acetic acid containing a little cyanide. Material showing bright blue-white fluorescence under ultraviolet light separated from the pigments present. On paper chromatography in a butanol–acetic acid–water system,[5] it separated into two spots (I and II) of unequal intensity with R_f values of 0.16 and 0.22. Further hydrolysis of combined I and II with 6 *N* hydrochloric acid at 95° for 24 hours gave a new substance (IV) with an R_f value of 0.46. Hydrolysis of Factor III, or of the fluorescent materials I and II, with 6 *N* hydrochloric acid at 150° for 21 hours gave another fluorescent substance (V) with an R_f value of 0.65, and phosphate ion was detected in the hydrolysates. These same hydrolysis conditions degrade vitamin B_{12} to isomers of ribazole phosphate, ribazole and 5,6-dimethylbenzimidazole.[6]

In the case of Factor III, it was assumed that the substances obtained were isomers of a riboside phosphate (I and II), the riboside (IV) and the base (V). The base was isolated as a crystalline picrate, m.p. mainly 220–225°, which was converted to a polymorphic crystalline hydrochloride, m.p. mainly 185–190°. There was not sufficient material for elemental analysis, so a detailed study of spectra was made.

The absorption spectrum of V seemed to eliminate purines, pyrimidines, pyridines and alkylbenzimidazoles and indicated an hydroxybenzimidazole structure. The previously undescribed 5-hydroxybenzimidazole was prepared by demethylation of 5-methoxybenzimidazole[7] with hydrobromic acid; m.p. 220–222°; *Anal.* Found: C, 62.26; H, 4.60; N, 20.90. The absorption spectrum ($\lambda_{max.}^{0.1N\ NaOH}$, 2500 (485); 3050 (573): $\lambda_{max.}^{0.1N\ HCl}$, 2400–2500 (shoulder); 2870 (528)) was in good agreement with that of base V. The picrate and hydrochloride of synthetic 5-hydroxybenzimidazole had wide melting point ranges, but mixed melting points with the isolated salts showed no depression. The infrared spectra of the hydrochlorides showed them to be identical.

Factor B and synthetic 5-hydroxybenzimidazole were combined microbiologically using *Escherichia coli* 113-3,[8] and two red crystalline compounds were isolated. One of these did not separate from Factor III on mixed paper chromatograms. This result confirmed the chemical evidence that 5-hydroxybenzimidazole is present in Factor III and is not an artifact. The structure and synthesis of the other degradation products of Factor III are being studied further.

(1) (a) W. Friederich and K. Bernhauer, *Angew. Chem.*, **65**, 627 (1953); (b) K. Bernhauer and W. Friederich, *ibid.*, **66**, 776 (1954).

(2) W. Friederich and K. Bernhauer, *Z. Naturforschung.*, **9b**, 686 (1954).

(3) W. Friederich and K. Bernhauer, *Angew. Chem.*, in press.

(4) J. E. Ford and J. W. G. Porter, *Brit. J. Nutrition*, **1**, 326 (1953).

(5) C. E. Carter, *ibid.*, **72**, 1466 (1950).

(6) (a) N. G. Brink and K. Folkers, THIS JOURNAL, **72**, 442 (1950); (b) N. G. Brink and K. Folkers, *ibid.*, **74**, 2856 (1952); (c) E. A. Kaczka and K. Folkers, *ibid.*, **75**, 6317 (1953).

(7) E. Ochiai and M. Katagu, *J. Pharm. Soc. Japan*, **60**, 543–550 (1940); *Chem. Abs.*, **35**, 1785 (1951).

(8) B. Davis and E. Mengioli, *J. Bact.*, **60**, 17 (1950).

RESEARCH LABORATORIES F. M. ROBINSON
CHEMICAL DIVISION I. M. MILLER
MERCK & CO., INC. J. F. MCPHERSON
RAHWAY, NEW JERSEY KARL FOLKERS

RECEIVED SEPTEMBER 12, 1955

ON THE ORIGIN OF THE METHYL GROUPS OF PHOSPHOLIPID CHOLINE IN THE RAT[1]

Sir:

We previously reported that the extent of incorporation of the carbon of the methyl group of methionine, labeled with C^{14}, to phospholipid choline is markedly reduced in the folic acid-deficient rat, and as a tentative explanation of this observation we suggested that in the folic acid-deficient rat the synthesis of the acceptor of the methyl group of methionine for choline formation is inhibited[2]. It is generally assumed that aminoethanol is the acceptor of three methyl groups of methionine *in vivo*, the formation of choline taking place via direct transmethylation from methionine. However, existing evidence does not indicate that folic acid or its biological derivative is a co-factor in the enzymatic reactions involving transmethylations from methionine, neither is folic acid or its derivative involved in the *in vivo* decarboxylation of serine to aminoethanol. It occurred to us, therefore, that the acceptor of the methyl group

(1) Aided by grants from the National Cancer Institute, U. S. Public Health Service, from the U. S. Atomic Energy Commission, AT-30-1 (1552), and by an Institutional grant from the American Cancer Society. *The isotopic material was obtained on allocation from the Atomic Energy Commission.*

(2) J. A. Stekol, S. Weiss, P. Smith and K. Weiss, *J. Biol. Chem.*, **201**, 299 (1953).

of methionine by transmethylation *in vivo* is not ethanolamine, and that in the *in vivo* synthesis of this acceptor, folic acid or its derivative is a co-factor. The data summarized briefly in Table I show that the extent of incorporation of choline-CH_3-C^{14} into the phospholipid choline is not inhibited by folic acid deficiency, whereas the incorporation of the carbon of the methyl group of methionine or of aminoethanol into choline was markedly reduced. The administration of amino-

TABLE I

THE UTILIZATION OF AMINOETHANOL-1,2-C^{14} AND THE EFFECT OF AMINOETHANOL, MONOMETHYLAMINOETHANOL, DIMETHYLAMINOETHANOL, AND *Citrovorum* FACTOR ON THE UTILIZATION OF METHIONINE-CH_3-C^{14} FOR THE SYNTHESIS OF PHOSPHOLIPID CHOLINE IN FOLIC ACID-DEFICIENT RATS[a]

Isotope injected	Supplement injected with the isotope	Per cent. of total activity in phospholipid choline: Normal	Per cent. of total activity in phospholipid choline: Folic acid-deficient
Choline-CH_3-C^{14}	None	73.0	76.0
Methionine-CH_3-C^{14}	None	30.0	18.4
Methionine-CH_3-C^{14}	Aminoethanol		17.8
Methionine-CH_3-C^{14}	Monomethylaminoethanol		20.0
Methionine-CH_3-C^{14}	Dimethylaminoethanol		38.8
Methionine-CH_3-C^{14}	*Citrovorum* factor	29.8	34.8
Aminoethanol-1,2-C^{14}	None	3.0	0.7

[a] Thirty-days old female rats were maintained for two months on a folic acid-free diet or on the same diet supplemented with folic acid. 1×10^{-3} mM. of the isotope per 100 g. wt. alone or together with 1×10^{-1} mM. of the non-isotopic amines was injected intraperitoneally in a single dose. *Citrovorum* factor (Leucovorin, Lederle), 0.1 mg., was injected two hours before the radiomethionine. All animals were sacrificed one hour after the injection of the isotopes. Phospholipid choline was isolated from the entire pooled carcasses (2–3 animals per pool).

ethanol or of monomethylaminoethanol together with radiomethionine did not improve the utilization of methionine methyl group for choline formation in the deficient animals. However, the administration of either the *Citrovorum* factor or dimethylaminoethanol together with radiomethionine promptly enhanced the incorporation of the methyl group of methionine into choline. Injection of the *Citrovorum* factor into normal animals did not increase the extent of transfer of the methyl group of methionine to choline. Increasing the period of the *in vivo* reaction to 20 or 48 hours did not improve the utilization of the methyl group of methionine on administration of aminoethanol or monoaminoethanol in folic acid-deficient rats. These data strongly suggest that choline is synthesized in the rat not by transfer of three methyl groups of methionine to aminoethanol, but by transfer of one methyl group of methionine to dimethylaminoethanol as the direct acceptor. The data further indicate that the *de novo* synthesis of the two methyl groups of dimethylaminoethanol is mediated by a folic acid derivative, and that folic acid or its derivatives are not involved in the transfer of the methyl group of methionine to dimethylaminoethanol.

The composition of the diets, the procedures for the isolation of choline, and the radio assay procedure employed were the same as previously reported.[2]

THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH PHILADELPHIA 11, PA.
JAKOB A. STEKOL
SIDNEY WEISS
ETHEL I. ANDERSON

RECEIVED AUGUST 5, 1955

STEREOISOMERIC TETRA-*o*-TOLYLSILANES[1]

Sir:

In connection with an investigation of highly-substituted aromatic organosilicon compounds,[2] stereoisomers of a novel type have been isolated.[3] The aromatic groups of tetra-*o*-tolylsilane are not free to rotate about the carbon–silicon bonds and it is possible to construct no fewer than eight models of the molecule, representing four *meso* compounds and two racemic pairs.

Using as starting materials SiX_4 or $(o\text{-}CH_3C_6H_4)_3$-SiX (where —X is —Cl, —OCH_3, or —OC_2H_5) and $o\text{-}CH_3C_6H_4Li$, four different compounds have been isolated which appear to be tetra-*o*-tolylsilane stereoisomers. These melt at 145°, 228°, 300° and 344°. A fifth material which melts at 270° may also be a stereoisomer.[4] We wish to draw particular attention to the 145° and 228° isomers.

145° Isomer.—*o*-Tolyllithium (1.16 moles) in ether was slowly added to methyl silicate (0.20 mole). After 117 hours at room temperature and 35 hours of reflux (all under nitrogen), the reaction mixture was hydrolyzed.[2a] Crystalline products could be isolated only after distillation of the crude syrups. The fraction boiling 200–210° (1 mm.) (50% yield if tetra-*o*-tolylsilane) was extracted repeatedly with hot methanol. The cooled extracts slowly deposited solids. The solids from the first extracts melted as low as 45°, but those from later extracts 110–120°.[5] Six recrystallizations of the latter material raised the melting point to 143–145°, and five additional recrystallizations to 144.8–145.7° (2 g., 3%). The recrystallization solvents were methanol–benzene (9:1), ligroin, methanol, ethanol, propanol-2 and acetic acid. Considerable amounts of low melting solids and syrups also were obtained. These appeared to be mixtures of tetra-*o*-tolylsilanes. *Anal.* Calcd. for $C_{28}H_{28}Si$: C, 85.66; H, 7.19; Si, 7.15. Found: C, 85.18; H, 7.05; Si, 7.15. *Ultraviolet data.*[6] $\lambda_{max.}$ in mμ (and ϵ) for cyclo-

(1) The authors gratefully acknowledge the financial support of the Research Corporation.

(2) (a) H. Gilman and G. N. R. Smart, *J. Org. Chem.*, **15**, 720 (1950); (b) **16**, 424 (1951); **19**, 441 (1954).

(3) The absorption spectra of triphenylmethyl radicals, Crystal Violet ions, and related types have been interpreted in terms of the existence of stereoisomeric forms (G. N. Lewis, T. T. Magel, and D. Lipkin, THIS JOURNAL, **64**, 1774 (1942)). The polymorphism of certain tri-1-naphthylboron–amine addition compounds has been attributed to the restricted rotation of the naphthyl groups (H. C. Brown and S. Sujishi, *ibid.*, **70**, 2793 (1948)).

(4) The principal position isomers have been prepared and are different from the tetra-*o*-tolylsilanes.

(5) Melting points are uncorrected.

(6) The absorption curves for the 145° and 228° isomers are experimentally indistinguishable in the region 290–212 mμ. Above 290 mμ, the solutions are transparent. Below 246 mμ, there is a rapid rise in absorption continuing into the vacuum ultraviolet (ϵ at 212 mμ, 60,000). These spectra are related to those of tetraphenylsilane and tetra-*p*-tolylsilane.