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

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(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.

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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.

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