that the active substance was L-histidinol[1] (L-2-amino-3-[4(or 5)-imidazolyl]-1-propanol). The identity was established by comparison of the isolated dihydrochloride and dipicrate with a synthetic sample of L-histidinol dihydrochloride[2] and the dipicrate prepared from it. The corresponding melting points were identical and mixed melting points undepressed. Natural and synthetic L-histidinol dihydrochloride give the same response (equivalent to that of 75% of their weight of L-histidine dihydrochloride) with strain 26-24D1, which was used as assay organism in the isolation.

Excretion of L-histidinol by one mutant and utilization by others suggest that this compound is an intermediate in the biosynthesis of histidine in *E. coli*. That L-histidinol is utilized slowly by 26-24 does not invalidate this interpretation, since an analogous phenomenon encountered with shikimic acid, a common precursor of aromatic metabolites, has been explained.[3] Factors affecting the rate of utilization of L-histidinol are being further investigated.

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[1] P. Karrer, M. Suter and P. Waser, *Helv. Chim. Acta*, **32**, 1936 (1949). Reported for dihydrochloride: m.p. 193-195° (uncor.); $[\alpha]^{21}\text{D}$ −3.98° (water).

[2] Generously furnished by Professor P. Karrer.

[3] B. D. Davis, *J. Biol. Chem.*, in press.

[4] U. S. Public Health Service Research Fellow.

ENZYMATIC SYNTHESIS OF FOLIC ACID BY THE ACTION OF CARP THIAMINASE

Sir:

Carp thiaminase destroys thiamine by cleaving off the thiazole moiety and uniting the pyrimidine portion of the vitamin to some unknown substance in the enzyme preparation.[1] Sealock and Davis[2] have shown that this latter material can be replaced by nitro-aniline, which is alkylated on the amino group by the pyrimidylmethyl part of thiamine. They have suggested the similarity of this reaction to transmethylation. If this view be correct, then other amines might be alkylated by other suitably constituted quaternary salts when catalyzed by this enzyme. In this way certain other metabolically essential substances might be formed, such as pteroic acid and its derivatives, which are amines alkylated with a substituted methyl group.

2-Amino-4-hydroxy-6-pteridylmethyl-(4′-methyl-5′-hydroxyethylthiazolium) bromide (a pteridine analog of thiamine) was formed by the stepwise reaction of α,β-dibromopropionaldehyde with "thiamine thiazole"[3] and then with 2,4,5-triamino-6-hydroxypyrimidine as in a related synthesis leading to folic acid[4]; although the compound was rather unstable, it was obtained analytically pure.

Solutions of thiaminase were made from fresh carp viscera as previously described.[1,5] These were incubated for one hour at 30° with the 2 substrates, *viz.*, the thiazolium salt and the amine. PAB yielded pteroic acid, and PABG gave pteroylglutamic acid, as judged microbiologically. Either substrate alone with the enzyme gave no new folic acid. Without enzyme, the 2 substrates yielded small amounts of folic acid, but this was greatly augmented by the enzyme. Thus 4 cc. of carp extract plus 5 mg. each of PAB and thiazolium salt yielded 10 gamma pteroic acid; enzyme blank 1.8 gamma; substrate blank 0.6 gamma. The *p*H dependence and the need for a dialyzable component were similar to those for thiaminase activity.[1]

The specificity of the enzyme was directed to the thiazolium part of the molecule, because no synthesis of folic acid was observed with the corresponding pyridinium salt,[6] which can be used in the chemical synthesis of this vitamin.[4]

Because the natural occurrence of the thiazolium salt is unknown there is no proof that this is the mode of biosynthesis of folic acid. Rather, it is offered as experimental evidence for a new kind of biosynthetical mechanism in which the driving force resides not in a phosphate bond, but in a quaternary ammonium ion which is reduced to a tertiary amine during the reaction. From the existing information about relationships of folic acid and vitamin B_{12}, it is quite possible that the dialyzable coenzyme of carp thiaminase may contain this vitamin, and efforts to learn about this are in progress.

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[1] L. O. Krampitz and D. W. Woolley, *J. Biol. Chem.*, **152**, 9 (1944).

[2] R. R. Sealock and N. C. Davis, *ibid.*, **177**, 987 (1949).

[3] "Thiamine thiazole" was kindly supplied by Dr. G. A. Emerson.

[4] M. E. Hultquist, E. Kuh, D. B. Cosulich, M. J. Fahrenbach, E. H. Northey, D. R. Seeger, J. P. Sickels, J. M. Smith, Jr., R. B. Angier, J. H. Boothe, B. L. Hutchings, J. H. Mowat, J. Semb, E. L. R. Stokstad, Y. SubbaRow, and C. W. Waller, *Ann. N. Y. Acad. Sci., Supplement*, **48**, 1 (1947).

[5] D. W. Woolley, *J. Biol. Chem.*, **141**, 997 (1941).

[6] Kindly supplied by the American Cyanamid Company.

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BIOLOGICAL PRECURSORS OF THE PYRIMIDINES

Sir:

Lactobacillus bulgaricus 09 has been found to require either orotic acid or ureidosuccinic acid as an essential growth factor.[1,2,3,4] The following experiments with *Lactobacillus bulgaricus* 09 are concerned with the role of these compounds in the biogenesis of the pyrimidine components of ribonucleic acid.

Lactobacillus bulgaricus 09 was grown in 500 ml. amounts of pyrimidine-free basal medium (2) containing, in the first experiment, 5 mg. of added orotic acid (5) labelled in position 2 with C^{14} and, in the second experiment, with 15 mg. of added DL-ureidosuccinic acid[5] (aseptic addition) labelled

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[1] L. D. Wright, J. W. Huff, H. R. Skeggs, K. A. Valentik and D K. Bosshardt, THIS JOURNAL, **72**, 2312 (1950).

[2] L. D. Wright, K. A. Valentik, D. S. Spicer, J. W. Huff and H. R. Skeggs, *Proc. Soc. Exptl. Biol. & Med.*, **75**, 293 (1950).

[3] O. P. Wieland, J. Avener, E. M. Boggiano, N. Bohonos, B. L. Hutchings and J. H. Williams, *J. Biol. Chem.*, **186**, 737 (1950).

[4] The microbiological activity previously reported for 5-(carboxymethylidine)-hydantoin could not be confirmed with more carefully prepared preparations. Evidence is now available that this activity was due to contaminating orotic acid.

[5] J. F. Nye and H. K. Mitchell, THIS JOURNAL, **69**, 1382(1947).