$C_{21}H_{29}NO_5$: C, 67.2; H, 7.8; N, 3.7. Found: C, 67.2; H, 7.8; N, 3.9], characterized as the **acetate** (m.p. 206–208°; reported[5] m.p. 210°.)

Reduction of the carbonyl group in tetrahydrodemethoxycolchicine to methylene was effected by preparing the **dimethylmercaptol** [m.p. 190°; $[\alpha]^{25}D$ −160° (*c*, 0.96, ethanol); *Anal.* Calcd. for $C_{23}H_{33}NO_4S_2$: C, 61.2; H, 7.4; S, 14.2. Found: C, 61.4; H, 7.5; S, 14.0] which on heating with Raney nickel gave hexahydrodemethoxydesoxycolchicine[7] [m.p. 183.5–184°; $[\alpha]^{25}D$ −162° (*c*, 1.10, ethanol); *Anal.* Calcd. for $C_{21}H_{29}NO_4$: C, 70.2; H, 8.1; OCH_3, 25.9. Found: C, 70.1; H, 8.2; OCH_3, 26.0]. Titration with perbenzoic acid showed the presence of 1.07 double bonds.

Phosphorus pentoxide in refluxing xylene degraded hexahydrodemethoxydesoxycolchicine to the desacetamido compound which was directly hydrogenated (1.1 moles of hydrogen absorbed) to octahydrodemethoxydesoxydesacetamidocolchicine (II) [m.p. 49–50°; $[\alpha]^{25}D$ 0° (*c*, 1.01, ethanol); *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7; OCH_3, 30.8. Found: C, 75.4; H, 8.7; OCH_3, 30.9]. Titration with perbenzoic acid showed the presence of 1.02 double bonds and gave a crystalline *oxide* (m.p. 115–116°; *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.7; H, 8.2. Found: C, 71.6; H, 8.3). The ultraviolet and infrared absorption spectra of the various degradation products above were determined and found to be compatible with the assigned structures.

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(7) A compound of this empirical formula and m.p. 182–183° was isolated in small amounts by Bursian (ref. 5) from the hydrogenation of colchicine. However, Kemp and Tarbell (ref. 6) reported none of this material among the hydrogenation products of colchicine.

(8) American Cancer Society Postdoctoral Fellow.

COMPARISON OF CITROVORUM FACTOR AND A SYNTHETIC COMPOUND WITH LEUCONOSTOC CITROVORUM GROWTH ACTIVITY

Sir:

By the application of purification procedures to desiccated liver powder we have obtained concentrates of the citrovorum factor (I) with substantially the same activity, weight for weight, for *Leuconostoc citrovorum* 8081 as the recently reported compound resulting from the formylation and reduction of pteroylglutamic acid[1] (II). The differences in the microbiological activity of the respective acid degradation products and the absorption spectra lead us to believe that the synthetic compound[2] is not the citrovorum factor as obtained from liver.

The method used for the preparation of our concentrates was based on that previously described,[3] extended and modified to include Florisil and Dowex 1 chromatograms. The use of barium and silver precipitations was eliminated. The product (I) which was obtained by fractional precipitation from methanol of eluates from Al_2O_3 columns was found by assay with *Leuconostoc citrovorum* 8081 to contain 176 CF units[3] per γ. Under the same assay conditions II contained 152 units per γ.

When assayed for folic acid activity using *Streptococcus faecalis* R, 1γ of I was equivalent to 0.648 γ pteroylglutamic acid (PGA) while 1γ of II had a value of 0.572γ PGA. When stored at *p*H 2.0, for 20 hours at 23°, both materials showed 96–97% loss of citrovorum activity. However, as is the case with much cruder materials,[3] I exhibited 32% loss of PGA activity when assayed with *Streptococcus faecalis* R; on the other hand, II showed an enhanced PGA activity of approximately 13%. This increase in activity was found consistently and could not be ascribed to errors inherent in the microbiological assay.

While both materials in 30% ethanol containing 0.03% NH_3 showed a maximum at approximately the same wave length, there was a very marked difference in the intensity. At a concentration of 10 mg/l. I exhibited 38.6% T at 286 mμ as compared with 24.6% T at the same wave length for II. Assuming both I and II have the same chromophoric group and if there is no great difference in their molecular weights, then I can be calculated to have a purity of approximately 70%.

(1) Brockman, Roth, Broquist, Hultquist, Smith, Fahrenbach, Cosulich, Parker, Stokstad and Jukes, THIS JOURNAL, **72**, 4325 (1950).

(2) Samples of crystalline free acid kindly supplied by Dr. Thomas H. Jukes, Lederle Labs. Division, American Cyanamid Company, Pearl River, N. Y.

(3) Keresztesy and Silverman, *J. Biol. Chem.*, **183**, 473 (1950).

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L-HISTIDINOL, A PRECURSOR OF L-HISTIDINE IN *Escherichia coli*

Sir:

Of three mutant strains of *Escherichia coli* responding to histidine, one (26-25) excretes a substance that satisfies the histidine requirement of the other two (26-24, 26-24D1). Strain 26-24D1 was derived from 26-24 and differs from it by more rapid utilization of the excreted substance. The latter was isolated from culture filtrates of 26-25 by adsorption on charcoal (Darco G-60) at *p*H 7.5, elution with decinormal hydrochloric acid in 85% ethanol, evaporation to dryness of the eluate, and precipitation with picric acid from aqueous solution. The resulting dipicrate, recrystallized from water (m.p. 194–197°. *Anal.* Calcd. for $C_{18}H_{17}O_{15}N_9$: C, 36.07; H, 2.86; N, 21.03. Found: C, 36.19; H, 3.03; N, 20.98), was treated with normal hydrochloric acid and the liberated picric acid removed with ether. On evaporating the aqueous phase to dryness, the active material (85 to 150 mg. per liter culture filtrate) was obtained as dihydrochloride and recrystallized twice from 95% ethanol. It sinters at 193° and melts at 197–199.5° on the micro-block, $[\alpha]^{20}D$ −3.0° (*c*, 5.0 in water). *Anal.* Calcd. for $C_6H_{13}ON_3Cl_2$: C, 33.66; H, 6.12; N, 19.63. Found: C, 33.67; H, 6.00; N, 19.53. These data, together with the fact that the dihydrochloride could be oxidized to L-histidine, indicated

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.); $[\alpha]^{21}D$ −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.

(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).

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.

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

(6) Kindly supplied by the American Cyanamid Company.

FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
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RECEIVED MARCH 9, 1951

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

(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. Nyc and H. K. Mitchell, THIS JOURNAL, **69**, 1382 (1947).