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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RECEIVED MARCH 16, 1951

(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
NEW YORK, N. Y. D. W. WOOLLEY

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).

in the ureido carbon atom (equivalent to position 2 of orotic acid assuming closure to form a 6 membered ring). After growth in each instance, the washed cells were dried, ground, and treated with 5% trichloroacetic acid at 100° for 30 minutes. The liberated nucleic acids were degraded by hydrolysis in 1 *N* HCl at 100° for one hour.[6] This procedure in our experience with *Lactobacillus bulgaricus* 09 yields free purines and variable proportions of pyrimidine nucleosides and pyrimidine nucleotides. The nucleic acid hydrolysates were chromatographed on paper against a tertiary butyl alcohol–HCl mixture.[6] The eluted purine compounds were chromatographed further on Dowex-50[7] to satisfactory absorption ratios at 262/248 mμ. The eluted pyrimidine components similarly were chromatographed further on Dowex-1[8] to satisfactory absorption ratios at 278/262 mμ. The purified purines and pyrimidine derivatives isolated, as well as aliquots of the orotic acid and ureidosuccinic acid used to promote growth in the two experiments, were counted with the results summarized in Table I.

TABLE I

SUMMARY OF RADIOACTIVE ISOTOPE STUDIES

Growth factor	Compound studied	Activity counts/μM/min.
Orotic acid	Orotic acid	11,650 (0.143)
	Adenine	14 (0.390)
	Guanine	19 (1.62)
	Uridylic acid	7,500 (0.586)
	Cytidyclic acid	7,950 (0.120)
Ureidosuccinic acid	Ureidosuccinic acid	12,060 (1.03)
	Adenine	0 (1.08)
	Guanine	17 (2.00)
	Uridylic acid	7,600 (1.21)
	Cytidine	7,000 (1.08)

All samples were counted on 1.33 sq. cm. plates with a windowless Q-gas counter. Figures in parentheses indicate the amount of compound in μM counted.

(6) J. D. Smith and R. Markham, *Biochem. J.*, **46**, 509 (1950).

Although interpretation of the data in Table I is complicated by a small factor of dilution in the pyrimidine derivatives isolated, it would appear that the following facts have been established: (a) as in the rat,[9,10] orotic acid serves as a source of the pyrimidine components of ribonucleic acid, and, more significantly, (b) at least in the present system, ureidosuccinic acid is an acyclic biological precursor of the pyrimidine ring.

(7) W. E. Cohn, *Science*, **109**, 377 (1949).

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

(9) S. Bergström, H. Arvidson, E. Hammarsten, N. A. Eliasson, P. Reichard, and H. v. Ubisch, *J. Biol. Chem.*, **177**, 495 (1949).

(10) L. L. Weed, M. Edmonds, and D. W. Wilson, *Proc. Soc. Exptl. Biol. & Med.*, **75**, 192 (1950).

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BOOK REVIEWS

Physical Aspects of Organic Chemistry. Fourth Edition. By WILLIAM A. WATERS, Sc.D., M.A., Ph.D. (Cantab.); M.A. (Oxon.); F.R.I.C.; Fellow of Balliol College, Oxford; University Lecturer and Demonstrator in Organic Chemistry, Oxford University. D. Van Nostrand Company, Inc., 250 Fourth Avenue, New York, N. Y., 1950. xii + 539 pp. 15 × 22 cm. Price, $8.00.

The present edition of this book seems to be quite in keeping with the author's expressed intention of presenting the theories of organic chemistry in the framework of their historical development. Representative portions of the book which clearly adhere to this aim are to be found in Chapter XIV, which gives an excellent treatment of the evolution of the concepts of tautomerism, prototropy, etc., and in the material of Chapter XVII dealing with the beginnings of aromatic substitution theory. This historical approach has been rendered more valuable by the judicious incorporation of direct quotations from many classical papers in the field.

However, the impression was gained by this reviewer that, in certain sections of the volume at least, far more emphasis has been placed on the earlier phases of development of the science than on the more instructive and striking discoveries of the past twenty years. This stress on early papers at the expense of very recent work could lead to a mistaken impression of the present level of advancement of physical organic theory. The lack of updating is particularly apparent in the discussion of ionic reactions in general. Specific examples of this point are to be found in the consideration of the Friedel–Crafts reaction as an addition–elimination process, the complete neglect of a possible ionic formulation (given by Criegee) for the reaction of lead tetraacetate with 1,2-glycols, and the overzealous presentation of the theory of alternating polarity to explain the reactivity of conjugated systems. In contrast to this treatment of ionic reactions, though, the corresponding sections involving free radical reactions have received a fairly thorough and stimulating discussion.

One rather undesirable feature resulting from the process of revision in this new edition is the presentation of a given subject in an incomplete form at an early point in the book (*e.g.*, the section on the addition of halogens to olefins, on pp. 216–218), and the completion of this topic with new and pertinent data in a later chapter (pp. 320–322). Integration of both new and old references at one point would have led to a more forceful exposition.

Some mild criticism might be directed too at the hazy nature of certain portions of the treatment of reaction rate theories given in Chapter V. Hammett's ρ–σ correlation is given a very minimum of attention, and no clear distinction is made between the collision and transition-state theories. Also, a regrettable tendency to discuss solution reactions in terms of the collision theory is evident.

A very fine feature of the book is to be found in the authoritative treatment given to chapters dealing with the evaluation and significance of the physical properties of organic molecules. Here one finds a uniquely valuable discussion of the theoretical and experimental techniques em-