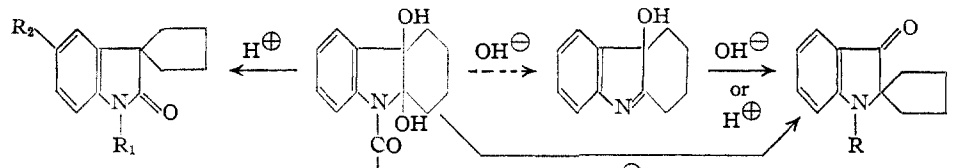

V, R_1 = CH_3CO—, R_2 = H
VI, R_1 = CH_3, R_2 = H
VII, R_1 = CH_3, R_2 = NO_2

I

II

III, R = H
IV, R = CH_3CO—

C, 77.81; H, 7.67). The smooth nitration furnished VII, identical with the product from I (pale yellow prisms, m. p. 142°. *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.41; H, 5.7. Found: C, 63.57; H, 5.96).

Further reactions and rearrangements in this series will be reported shortly.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY J. B. PATRICK*
CAMBRIDGE, MASSACHUSETTS BERNHARD WITKOP‡

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* Harvard University Graduate School.
‡ Harvard University Faculty 1948–.

FORMATION OF 4-AMINO-5-CARBOXAMIDOIMIDAZOLE DURING GROWTH OF *ESCHERICHIA COLI* IN THE PRESENCE OF 4-AMINOPTEROYLGLUTAMIC ACID

Sir:

When *Escherichia coli* is grown in the presence of amounts of 4-aminopteroylglutamic acid just sufficient to inhibit multiplication slightly, 4-amino-5-carboxamidoimidazole accumulates in the medium, and has been isolated from it. This is the same substance which was found by Stetten and Fox[1] when this and other bacteria were grown in the presence of sulfadiazine or sulfapyridine. It was identified by Shive, *et al.*,[2] and recognized as the probable precursor in the biosynthesis of hypoxanthine.

The accumulation of the imidazole through the intervention of the antimetabolite of folic acid is of importance in consideration of the mode of action of sulfonamide drugs and of folic acid. Thus, inhibition analysis has led to the conclusion that *p*-aminobenzoic acid participates in several reactions, of which the first to be affected by sulfanilamide derivatives is the formation of methionine, the next is concerned with purine formation,[3] and less sensitive processes, presumably the synthesis of folic acid,[4] are then retarded. On the other hand, Woods[5] has concluded that the primary action of the sulfonamides is the inhibition of folic acid formation, and that synthesis of purines and of methionine are secondary events in which that vitamin participates. The present finding would favor the latter view. Since the folic acid antagonist leads to the accumulation of the same imidazole as do the *p*-aminobenzoic acid antimetabolites, the latter presumably act by creating a deficiency of folic acid, which in turn is responsible for the failure in purine formation.

The demonstration was conducted as follows: *E. coli* was grown in the manner of Stetten and Fox[1] except that sulfadiazine was omitted and 0.2 mg. per cc. of 4-aminopteroylglutamic acid[6] was added. Judged colorimetrically, about the same amount of diazotizable amine accumulated as when sulfadiazine was the inhibitor. Isolation of the base was accomplished as in[1] except that 2.5 times as much mercury salt was used and the ether extraction was omitted. Final separation was made on paper strips with butanol–diethylene glycol–water solvent, in an atmosphere containing ammonia,[7] in which the imidazole showed R_F of 0.5. Identity of the isolated substance with synthetic 4-amino-5-carboxamidoimidazole[8] was established by comparison of (a) the R_F in the solvent just mentioned, (b) the absorption spectra in the ultraviolet region at *p*H 2.0 and 11.0, and (c) the melting points (with decomposition) of the picrate. In every case the behavior of the known and the unknown was the same.

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH D. W. WOOLLEY
NEW YORK, N. Y. R. B. PRINGLE[9]

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(1) M. R. Stetten and C. L. Fox, *J. Biol. Chem.*, **161**, 333 (1945).
(2) W. Shive, W. W. Ackermann, M. Gordon and M. E. Getzendaner, THIS JOURNAL, **69**, 725 (1947).
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(5) D. D. Woods, *Bull. soc. chim. biol.*, **30**, 730 (1948).
(6) Kindly made available by Dr. T. H. Jukes of Lederle Laboratories.
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(9) Fellow of the National Institutes of Health.

SPECIFICITY OF UREASE ACTION

Sir:

Urease has been repeatedly cited[1] as a strictly specific enzyme which hydrolyzes only urea. In the course of experiments with substances related to urea we have observed a hydrolysis of biuret $H_2N—\overset{\overset{O}{\|}}{C}—NH—\overset{\overset{O}{\|}}{C}—NH_2$ by urease preparations.

As much as 33% of nitrogen initially contained in solutions of biuret was identified as ammonia (by Nessler technique[2]) after prolonged enzy-

(1) Sumner and Sommers, "Enzymes," Academic Press, New York, N. Y., p. 156.
(2) Ambrose, Kistiakowsky and Kridl, THIS JOURNAL, **72**, 317 (1950).