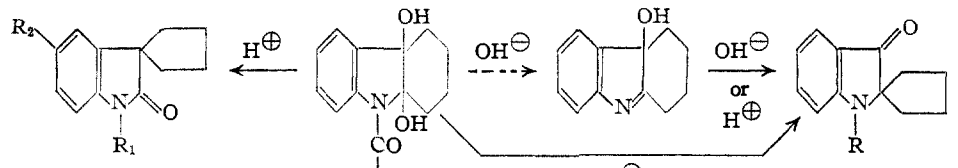

V. $R_1 = CH_3CO-$. $R_2 = H$
VI, $R_1 = CH_3$. $R_2 = H$
VII. $R_1 = CH_3$. $R_2 = NO_2$

I CH_3 II $OH^{\ominus}$

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.

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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.

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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).
(3) W. Shive and E. C. Roberts, *J. Biol. Chem.*, **162**, 463 (1946).
(4) K. C. Winkler and P. G. de Haan, *Arch. Biochem.*, **18**, 97 (1948).
(5) D. D. Woods, *Bull. soc. chim. biol.*, **30**, 730 (1948).
(6) Kindly made available by Dr. T. H. Jukes of Lederle Laboratories.
(7) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 703 (1948).
(8) E. Shaw and D. W. Woolley, *ibid.*, **181**, 89 (1949).
(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).

matic hydrolysis; odor of ammonia was observed after adding sodium carbonate; in the absence of buffers the *p*H changed from 7 to 8. Carbon dioxide was quantitatively measured by absorption by Ascarite on aeration; it was also qualitatively detected by the barium hydroxide test. Microcombustion of the C. P. biuret, obtained from Eimer and Amend, gave N, 34.86%, whereas $C_2N_3H_5O_2 \cdot H_2O$ gives N, 34.71%; the well-known biuret test was positive. Thus hydrolysis of biuret is definitely established; it occurred only in the presence of urease preparations as was shown by negative blanks. To establish that urease is responsible for this reaction the following experiments were performed. The ratio of ammonia produced in a 0.016 *M* solution of biuret to that in a 0.0019 *M* solution of urea by aliquot portions of a crude extract from jackbean meal was 1.5 at 20° and *p*H 7.0 in phosphate buffer. Crude extract from soya beans gave for this ratio 1.6. Crystalline enzyme prepared from jackbean meal by the method of Sumner and having a specific activity of 63,000 Sumner units gave a ratio of 1.6. On adding 10^{-4} *M* solution of Ag^+ to the enzyme its activity was reduced to that of the blank both for urea and biuret. In a solution containing 0.048 *M* of sodium sulfite and 0.013 *M* sodium bisulfite per liter in which the activity of urease toward urea is reduced[2] to one-fifth, the above ratio was still found to be 1.6. These ratios were reproducible to 10–15%. On adding to a 1.5% solution of urea 1.0% biuret the rate of production of ammonia by another preparation of crystalline enzyme (about 30,000 S. u. activity) was reduced by 11%. According to the data of Sumner, the production of ammonia is fastest in the 1.5% solution of urea, and thus the addition of biuret acted in the same manner as a further addition of urea, virtually eliminating the possibility that two different enzymes are responsible for the two reactions and showing that the two substrates compete for the same enzyme. The other experiments described above point in the same direction and we believe that hydrolytic activity of urease toward biuret has been established with a high degree of probability.

In 5×10^{-2} *M* solution of urea the production of ammonia is faster by a factor of 9 than in an equimolar solution of biuret. This is the partial explanation of the failure of Takeuchi[3] to detect hydrolysis of biuret by crude extracts from soya beans.

Preliminary determinations of both the ammonia and the carbon dioxide produced by urease in solutions of biuret indicate a ratio of 3:1, rather than 3:2, which would be obtained on complete hydrolysis, suggesting that some other products are being formed. The enzyme appears to become rapidly inactivated in hydrolyzing biuret, which suggests inhibition by some products of hydrolysis.

Urease occurs not uncommonly in living tissue and the need for elimination of urea from such tissue appears to be a weak justification for the presence of urease. The finding of another substrate toward which urease is active is therefore of interest as suggesting an additional biological reason for urease. We are therefore extending our experiments to derivatives of biuret and its homologs (carbonyl diurea, tetruret, etc.) as well as studying the mechanism of the biuret hydrolysis.

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RECEIVED NOVEMBER 23, 1949

(3) T. Takeuchi, *J. Coll. Agr., Tokyo, Imp. Univ.*, **1**, 1 (1909–1913).

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NEW BOOKS

Scientific and Technical Abbreviations, Signs and Symbols. By O. T. ZIMMERMAN, Ph.D., Professor of Chemical Engineering, University of New Hampshire, and IRVIN LAVINE, Ph.D., Formerly Professor of Chemical Engineering and Head of the Department, University of North Dakota. Industrial Research Service, Dover, N. H., 1948. xii + 476 pp. Illustrated. 14 × 22 cm. Price, $7.50.

In the days of Berzelius and Bunsen a scientist carried most of his science in his head and usually knew where to find the rest easily, but the time for abstract journals soon came, then science dictionaries, encyclopedias, monographs and special compilations such as the one here considered. Zimmerman and Lavine have done an extensive job of collecting the abbreviations, signs and symbols from A (aeronautics) to Z (zoology), with chemistry, physics, biology, botany, astronomy, mathematics, engineering, radio, electronics, hydrography, topography, meteorology, medicine, communications, machine and tool shopcraft, commerce, banking, military, navy, even plumbing and heating, in between, all well arranged by subjects and fields, in neat and readable tables. The sources drawn from are many, in particular the American Standards Association.

Any criteria of excellence or completeness are hard to establish; the reviewer could not find the kX. unit now substituting for Å. (A. U.), nor $[\alpha]^t_D$, nor *MR*D, all of which are commonly used by chemists, nor O. R. C., B. R. T. or B. L. E. in the section devoted to railway terms. The authors discuss favorably in the Preface the growing practice of omitting periods after abbreviations. The