

pH 13, the carboxyl group is not split off during the condensation. In a subsequent note we studied other cases of condensation of aldehydes with  $\beta$ -oxo acids with liberation of carbon dioxide within the pH range 3-11 at room temperature,<sup>3</sup> and extended our research to the condensation of aldehyde ammonias with  $\beta$ -oxo acids.<sup>4</sup> We, therefore, were the first to observe that the products of the reactions in question depend on the pH, and that in this instance within an approximately physiological pH-range the aldol condensation is coupled with the liberation of carbon dioxide, a fact which Henze<sup>5</sup> failed to notice in his study on the condensation of methylglyoxal with acetoacetic acid.

The condensation of methylglyoxal with  $\beta$ -oxo acids represents a special case of these "syntheses under physiological conditions." According to our former results it was to be expected that the condensation studied by Schechter, Green and LaForge should occur in the examined pH-range with spontaneous decarboxylation.

(3) Schöpf and Thierfelder, *Ann.*, **518**, 127 (1935).

(4) Schöpf and Lehmann, *Ann.*, **518**, 1 (1935); *cf. Angew. Chem.*, **50**, 779, 797 (1937), summary, with special reference to p. 783.

(5) Henze, *Z. physiol. Chem.*, **189**, 121 (1930). According to *ibid.*, **193**, 88 (1930), and **200**, 104 (1931), the carboxylic acid referred to above is described as being the first product of reaction; *Z. physiol. Chem.*, **214**, 281 (1933), subsequent heating to 50° has been prescribed to speed liberation of carbon dioxide.

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RECEIVED MAY 4, 1950

#### SPECIFICITY OF UREASE ACTION: A CORRECTION Sir:

In a letter under the above title<sup>1</sup> we reported partial hydrolysis of commercial C. P. biuret by the enzyme urease. Puzzled by the incompleteness of this reaction we have continued its study and have reached a definite conclusion that biuret is not hydrolyzed by urease. We were misled by the assurance of the manufacturer that the biuret was C. P. and also by the excellent agreement of elementary analysis with the calculated composition. Thus material as received gave N, 34.86, biuret monohydrate being calculated to give 34.7. After drying at 115°, as recommended, the loss in weight was 14.8%, 14.9% being calculated for the monohydrate. The residue analyzed as follows: N, 40.8 (40.8); C, 23.5 (23.3); H, 4.65 (4.85), the parenthesized figures referring to calculation for biuret. However, a later analysis of material which was dried in high vacuum at 40° gave N, 37.3; C, 23.4; H, 4.77, which does not agree with that of any mixture of biuret and its monohydrate.

It has been now established that the composition is apparently only accidentally that of biuret and that the material is a rather complex mixture of several compounds. In particular, it contains about 10% of total nitrogen which is hydrolyzed

(1) *THIS JOURNAL*, **72**, 634 (1950).

by urease<sup>2</sup> and which is contained in a compound that is more soluble in water than is biuret. Extraction with water suggests that this material is present in biuret as a solid solution rather than as separate crystals. After tedious fractional crystallizations from alcohol a material has been repeatedly obtained with a rather sharp melting point at 110 to 115°, which is somewhat lowered by additions of urea. Analysis of a typical preparation gave N, 44.6; C, 21.2; H, 6.2, but the composition appears to vary slightly with the method of purification. Its molecular weight, determined by the freezing point lowering method in water, ranges from 70 to 85. Total hydrolysis by urease gives nitrogen content of 33 to 41%. Infrared spectrum, obtained in Nujol suspension, is indistinguishable from that of urea. Although some of the above data are inconsistent with this conclusion, the most probable interpretation of the data is that the isolated substance is not a pure chemical compound but is a loose compound or solid solution of urea and something else with a similar elementary composition. Therefore our results to date do not prove the existence of another substrate for urease besides urea.

(2) Hydrolysis of 33% of total biuret nitrogen reported in the previous communication was obtained on making the solution with excess biuret and was evidently due to the preferential solubility of the hydrolyzable compound.

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#### SYNTHETIC MEMBERS OF THE FOLINIC ACID GROUP

Sir:

In attempts to synthesize certain derivatives of folic acid which might possess activity in replacing folic acid for *Lactobacillus casei*<sup>1</sup> and for *Leuconostoc citrovorum* 8081,<sup>2,3</sup> we have found that highly active material can be produced from folic acid as follows: Folic acid (500 mg.) is treated with sufficient (approximately 5 cc.) formic acid (98%) containing 20% acetic anhydride to effect solution of the folic acid. The reaction mixture is heated for one hour at 50°, and the resulting crude formylfolic acid is obtained by evaporation of the excess reagent in a frozen state under reduced pressure. The crude formylfolic acid and 2 g. of ascorbic acid are dissolved in 50 cc. of water, and the pH is adjusted with sodium carbonate to 7.2-7.6. The mixture is hydrogenated in the presence of platinum oxide as a catalyst until approximately 1 mole of hydrogen per mole of folic acid is consumed. After hydrogenation, the material is not highly active in the assays, but after autoclaving the reaction mixture at 120° for one hour, an amount of the reaction mixture equivalent to 0.00004 to 0.00001  $\gamma$  of the original folic acid per

(1) Bond, *et al.*, *THIS JOURNAL*, **71**, 3852 (1949).

(2) Säuberlich and Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(3) Bardos, *et al.*, *THIS JOURNAL*, **71**, 3852 (1949).

cc. of assay medium elicits a half-maximal response with *Leuconostoc citrovorum* 8081. Simultaneously a number of inactive principles are formed in the reaction mixture. Application of the above procedure to folic acid without prior formylation produces material which possesses some activity. Similarly, omission of ascorbic acid greatly reduces the activity of the reaction mixture.

Ascorbic acid, though not specific, appears to enhance the ability of several organisms to convert folic acid to a substance possessing folinic acid-like activity.

Application of the above procedure to N-pteroyl- $\alpha$ -glutamylglutamic acid produces a reaction mixture which gives two active bands on paper chromatograms developed with butanol-water containing acetic acid. One of these bands corresponds to the single band obtained with the reaction mixture derived from folic acid. If N-pteroyldi- $\gamma$ -glutamylglutamic acid is employed in the above procedure, three bands of active principles are obtained on paper chromatograms developed in the same solvent. Thus, the diglutamate gives rise presumably to the mono-glutamate and an  $\alpha$ -glutamylglutamate, and the triglutamate is converted presumably to the mono-glutamate, a  $\gamma$ -glutamylglutamate and a di- $\gamma$ -glutamylglutamate containing a modified pteroyl substituent. Pteric acid, itself, subjected to the above procedure forms material possessing slight activity.

The activity of the synthetic material derived from folic acid is destroyed by very dilute acid, peroxides, dilute chlorine solutions, and by dilute nitrous acid. Destruction in very dilute acid occurs with the formation of material which is still effective in promoting the growth of *Streptococcus faecalis* R and *Lactobacillus casei*.

Further investigations concerning the relationship of these synthetic members and the naturally occurring members of the folinic acid group are in progress.

THE BIOCHEMICAL INSTITUTE AND THE WILLIAM SHIVE  
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THE UNIVERSITY OF TEXAS THOMAS J. BARDOS  
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FOR RESEARCH, AUSTIN, TEXAS LORENE LANE ROGERS  
RECEIVED APRIL 20, 1950

#### ON NEUTRON DEFICIENT ISOTOPES OF BARIUM<sup>1</sup>

Sir:

While investigating the spallation reactions occurring when cesium is bombarded with 250 Mev. protons in the Rochester 130-inch cyclotron, two previously unreported positron emitting isotopes of barium were encountered.

Spectroscopically pure cesium chloride in a thin platinum tube was irradiated for periods of 30 and 90 minutes. The latter was used in an attempt

(1) This work was performed under atomic Energy Commission contract AT(30-1)-655.

to produce enough barium activity to make parent-daughter separations feasible. The decay of the barium fraction was followed in the usual manner and also by deflecting the positrons into a counter tube by means of the 4800 gauss field of a permanent magnet. The decay curves of the barium fraction were resolved into half-life periods of  $1.8 \pm 0.2$  hours,  $2.4 \pm 0.1$  days and  $11.5 \pm 0.2$  days. The latter is undoubtedly that assigned to Ba<sup>131</sup> by Katcoff<sup>2</sup> and Yu, Gideon and Kurbatov.<sup>3</sup>

A 31-hour cesium activity was isolated from the barium fraction. This is clearly the activity assigned to Cs<sup>129</sup> by Fink, Reynolds and Templeton.<sup>4</sup> Parent-daughter separations performed more than 24 hours after the bombardment failed to show any Cs<sup>129</sup> activity whereas earlier milkings did show the activity. This led to the conclusion that the 1.8-hour barium is Ba<sup>129</sup>. The 2.4-day barium activity had no observable daughter activity.

Energy studies have been made. The 2.4-day barium emits a positron of 3.1 Mev. energy as shown by the end-point in aluminum. Energies have not been obtained for Ba<sup>129</sup>. Measurements made when both Ba<sup>129</sup> (1.8-hour) and the 2.4-day barium were present showed a  $\beta^+$  energy of 3.9 Mev., the aluminum absorption curve of which could not be resolved. It is felt that this energy is a combination of the energies of both isotopes, indicating that  $\beta^+$  from Ba<sup>129</sup> is hard. On this assumption, the relative yields at the end of bombardment are approximately Ba<sup>129</sup>:Ba<sup><129</sup> = 19:1.

The results on these two isotopes<sup>5</sup> can be summarized.

Isotope	Half-life	Mode of decay and energy	Method of mass assign.
Ba <sup>129</sup>	$1.8 \pm 0.2$ hr.	$\beta^+$ (hard) exact energy unknown	Isolation of daughter Cs <sup>129</sup> (31-hr.)
Ba <sup>&lt;129</sup>	$2.4 \pm 0.1$ days	$\beta^+$ 3.1 $\pm$ 0.3 Mev.	

The coöperation of Professor Sidney S. Barnes and the staff of the 130-inch cyclotron in making these irradiations possible is gratefully acknowledged.

(2) S. Katcoff, *Phys. Rev.*, **72**, 1160 (1947).

(3) Fu-chun Yu, D. Gideon and J. D. Kurbatov, *ibid.*, **71**, 382 (1947).

(4) R. W. Fink, F. L. Reynolds and D. H. Templeton, *ibid.*, **77**, 614 (1950).

(5) Ba<sup>129</sup> and Ba<sup>128</sup> have been listed on the General Electric 1950 revised Chart of the Nuclides just received but as yet have not been reported in the literature. A subsequent private communication from Professor Templeton of the University of California Radiation Laboratory indicates that the results of Fink and Templeton are in agreement with those presented here.

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF ROCHESTER CHARLES C. THOMAS, JR.  
ROCHESTER, N. Y. EDWIN O. WIIG

RECEIVED APRIL 13, 1950

#### RADIOACTIVE ISOTOPES OF BARIUM<sup>1</sup>

Sir:

In this communication we report the results of some preliminary experiments concerning neutron deficient isotopes of barium.

(1) This work was supported by the Atomic Energy Commission.