

intermediate on the following grounds: (1) oxalacetate as assayed by the method of Neish³ does not accumulate. (2) The oxalacetic decarboxylating activity (both spontaneous and enzymatic) of these preparations is too low to account for the rapid rate of pyruvate formation from I (Fig. 1). (3) Addition of malic dehydrogenase to the incubation mixture (Fig. 1) indicates the absence of free oxalacetate. These observations do not exclude an enzyme-bound form of oxalacetate from participating in the reaction as shown.

The decarboxylation of I to form propiolic acid (CH₂CCOOH) has been ruled out as the latter compound is not active in this system. The possibility of an enzyme-propionate complex seems remote as the formation of such a bound form might be expected to be reversible and result in the incorporation of radioactivity when C¹⁴O₂ was present. Further purification and studies on the mechanism of the reaction are in progress.

(3) W. J. P. Neish, in D. Glick, "Methods of Biochemical Analysis," Vol. 5, Interscience Publishers, Inc., New York, N. Y., 1957, p. 168.

(4) Fellow of The Jane Coffin Childs Memorial Fund for Medical Research. This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

NATIONAL INSTITUTE OF ARTHRITIS
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ROLE OF BIOTIN IN CARBAMYLATION REACTIONS

Sir:

Biotin deficiency in *Streptococcus lactis* 8039 is associated with a loss of ability to convert ornithine and carbamyl phosphate to citrulline.¹ Carbamyl phosphate is involved in the conversion of aspartate to N-carbamylaspartate,² a precursor of pyrimidines, and a study of biotin sulfone-inhibition of the growth of *Lactobacillus arabinosus* 17-5 suggested a possible role of biotin in pyrimidine biosynthesis.³

In this investigation, a comparison of cell-free extracts of normal and biotin-deficient *L. arabinosus* revealed that the latter have a greatly diminished ability to carbamylate aspartate as well as ornithine⁴ as indicated in Table I. Specificity of this effect is indicated by the lack of an effect of biotin deficiency upon α -ketoglutarate-aspartate transaminase activity. Biotin or heat-inactivated extracts of normal cells do not restore activity to biotin-deficient cell extracts. Normal activity can be restored to biotin-deficient cells in a biotin supplemented growth medium in a few hours.

Protein synthesis in the presence of biotin is essential for the formation of the ornithine-citrulline enzyme in biotin deficient cells of *S. lactis*.⁵ Purification of this enzyme from extracts of *S. lactis* by

(1) J. M. Estes, J. M. Ravel and W. Shive, *THIS JOURNAL*, **78**, 6410 (1956).

(2) M. E. Jones, L. Spector and F. Lipmann, *ibid.*, **77**, 819 (1955).

(3) J. M. Ravel and W. Shive, *Arch. Biochem. Biophys.*, **54**, 314 (1955).

(4) Previously reported inability of *L. arabinosus* to effect this reaction resulted from arginine inhibition of active enzyme formation; in contrast, *S. lactis* requires arginine for optimal enzyme synthesis.

(5) R. P. Sand, J. M. Ravel and W. Shive, *J. Biol. Chem.*, in press.

TABLE I

EFFECT OF BIOTIN-DEFICIENCY ON ENZYME ACTIVITY

Enzyme system	Activity of cell-free extracts, ^a μ moles of product formed/mg. protein/hr.	
	Control	Biotin-deficient
Ornithine carbamylation ^b	144	16
Aspartate carbamylation ^c	18	1.3
α -Ketoglutarate-aspartate ^d transaminase	2.2	2.6

^a From *L. arabinosus* grown in amino acid medium¹ containing no arginine or uracil and 20 or 0.3 μ g./ml. of biotin, respectively. ^b Extract incubated with carbamyl phosphate, 20 μ moles; tris-(hydroxymethyl)-aminomethane buffer, 10 μ moles; ornithine, 10 μ moles in 1 ml. at pH 8.3 for 30 minutes at 35°. Citrulline determined colorimetrically.⁶ ^c As in *b* with aspartate in lieu of ornithine; pH 7.5; incubated at 25°; passed through a Dowex-50 column before carbamylaspartate determined.⁷ ^d Determined as previously described.⁸

ammonium sulfate fractionation (60 to 75% saturation) at pH 7, heat treatment (65° for 5 minutes), ammonium sulfate fractionation (50 to 75% saturation) at pH 8.5; and chromatography on diethylaminoethylcellulose gave a preparation with an activity of 73,000 (μ moles citrulline produced/mg. protein/hr.). Assayed with *Saccharomyces cerevisiae* after hydrolysis with 3.6 N sulfuric acid, the purified preparation and the original cell extract (activity, 960) gave 0.1 and 3 μ g., respectively, of biotin/mg. protein. Unless biotin is present in the enzyme in a form which does not yield an active form of biotin upon acid hydrolysis, biotin apparently is not a component of the enzyme but exerts its effect during enzyme synthesis presumably in the formation of groups necessary for the transfer of a carbamyl group.

(6) R. M. Archibald, *J. Biol. Chem.*, **156**, 121 (1944).

(7) S. B. Koritz and P. P. Cohen, *ibid.*, **209**, 145 (1954).

(8) N. E. Tonhazy, N. G. White and W. W. Umbreit, *Arch. Biochem.*, **28**, 36 (1950).

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DETERMINATION OF PROTON AFFINITY AND BOND DISSOCIATION ENERGY BY ION IMPACT METHOD

Sir:

Until recently there were no experimental methods for the determination of the proton affinity of saturated molecules. A method for such a determination has been proposed by the authors¹ and it consists in investigating reactions between ions and molecules in the ionization chamber of a mass spectrometer. If, under the experimental conditions, a reaction can be observed, one may conclude that the reaction has no activation energy (to within an accuracy of 1-2 kcal./mole) and that it is either thermoneutral or exothermic. If a reaction is not observed one may conclude that it is endothermic. After a number of reactions have been investigated, the above criterion can be used to set up a series of inequalities and to determine thereby the limits within which either the proton

(1) V. L. Talrose and E. I. Frankovich, *Doklady Akad. Nauk S.S.S.R.*, **111**, No. 2, 37 (1956).

TABLE I

Values to be determined	Upper and lower limits, kcal./mole	Reactions by which upper and lower limits were obtained	Mean values, kcal./mole	Data obtained by the electron impact method
$P_{\text{H}_2\text{O}}$	171	$\text{H}_2\text{O} + \text{C}_2\text{H}_2^+ \not\rightarrow \text{H}_3\text{O}^+ + \text{C}_2\text{H}_2$	169 ¹	...
	167	$\text{H}_2\text{O} + \text{H}_2\text{S}^+ \rightarrow \text{H}_3\text{O}^+ + \text{HS}$		
$P_{\text{CH}_3\text{OH}^a}$	183	$\text{CH}_3\text{OH} + \text{NH}_3^+ \not\rightarrow \text{CH}_3\text{OH}_2^+ + \text{NH}_2$	180	...
	177	$\text{CH}_3\text{OH}^+ + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{OH}_2^+ + \text{OH}$		
$P_{\text{C}_2\text{H}_5\text{OH}^a}$	202	$\text{C}_2\text{H}_5\text{OH} + \text{C}_6\text{H}_6^+ \not\rightarrow \text{C}_2\text{H}_5\text{OH}_2^+ + \text{C}_6\text{H}_5$	193	...
	185	$\text{C}_2\text{H}_5\text{OH}^+ + \text{H}_2\text{O} \rightarrow \text{C}_2\text{H}_6\text{OH}_2^+ + \text{OH}$		
$P_{\text{H}_2}^a$	79	$\text{H}_2 + \text{C}_2\text{H}_2 \not\rightarrow \text{H}_3^+ + \text{C}_2\text{H}$	70	...
	61	$\text{H}_2^+ + \text{H}_2 \rightarrow \text{H}_3^+ + \text{H}$		
$P_{\text{CH}_4}^a$	129	$\text{CH}_4^+ + \text{H}_2\text{O} \not\rightarrow \text{CH}_5^+ + \text{OH}$	122	...
	114	$\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3$		
$P_{\text{C}_2\text{H}_6}^a$	61	$\text{C}_2\text{H}_6 + \text{H}_2^+ \not\rightarrow \text{C}_2\text{H}_7^+ + \text{H}$	< 61	...
	...			
$P_{\text{C}_3\text{H}_8}^a$	61	$\text{C}_3\text{H}_8 + \text{H}_2^+ \not\rightarrow \text{C}_3\text{H}_9^+ + \text{H}$	< 61	...
	...			
$P_{\text{C}_2\text{H}_4}^a$	165	$\text{C}_2\text{H}_4^+ + \text{C}_3\text{H}_8 \not\rightarrow \text{C}_2\text{H}_5^+ + \text{C}_3\text{H}_7$	157	156.6 ⁴
	149	$\text{C}_2\text{H}_4 + \text{C}_3\text{H}_8^+ \rightarrow \text{C}_2\text{H}_5^+ + \text{C}_3\text{H}_7$		
$P_{\text{C}_3\text{H}_6}^a$	183	$\text{C}_3\text{H}_6 + \text{NH}_3^+ \not\rightarrow \text{C}_3\text{H}_7^+ + \text{NH}_2$	> 183	185 ⁴
	121	From spectroscopic data ³		
$D(\text{C}_2\text{H}-\text{H})^a$	116	$\text{H}_2\text{O} + \text{C}_2\text{H}_2^+ \not\rightarrow \text{H}_3\text{O}^+ + \text{C}_2\text{H}$	> 116	...
	...			
$D(\text{C}_2\text{H}_3-\text{H})^a$	97	$\text{H}_2\text{O} + \text{C}_2\text{H}_4^+ \not\rightarrow \text{H}_3\text{O}^+ + \text{C}_2\text{H}_3$	> 97	> 96 ⁵
	...			

^a Detailed results will be published in the near future.

affinity P or the dissociation energy D lies. If the bond dissociation energies and the ionization potentials are known, the proton affinity may be estimated; if the proton affinities are known, the dissociation energy D may be estimated. The values obtained by the authors are listed in Table I. Proton affinities for unsaturated compounds can also be measured by the electron impact method. The results of such measurements are quoted for comparison in Table I.

The determination of P_{CH_4} by this method as published in THIS JOURNAL² seems to be erroneous.

(2) F. W. Lampe and F. H. Field, THIS JOURNAL, **79**, 4244-4245 (1957).

(3) R. Cherton, *Bull. Soc. Sci. Liège*, **11**, 203 (1942).

(4) J. L. Franklin, *Trans. Faraday Soc.*, **48**, 443 (1952).

(5) T. Cottrell, "The Strengths of Chemical Bonds, London," 1954.

This is due to the fact that F. W. Lampe and F. H. Field failed to identify the CD_4H^+ in the ionized $\text{CD}_4\text{-H}_2$ mixture. On the other hand, our experiments have shown that CH_5^+ are formed in the ionization of $\text{CH}_4\text{-H}_2$ mixture. As can be seen from our data¹ Lampe and Field had not taken into account the fact that the cross sections for proton transfer may be as large as those for H transfer. Otherwise they would have seen their mistake, since if the reaction $\text{CH}_4 + \text{H}_2^+ \rightarrow \text{CH}_5^+ + \text{H}$ did not take place that would mean that $P_{\text{CH}_4} < 61$ kcal./mole and the method would not be self-consistent.

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BOOK REVIEWS

Progress in Semiconductors. Volume 1. ALAN F. GIBSON, B.Sc., Ph.D., General Editor, Prof. R. E. BURGESS, Vancouver, B.C., American Editor, and Prof. P. AIGRAIN, Paris, European Editor. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1956. vii + 220 pp. 15.5 × 23.5 cm. Price, \$8.00. **Volume 2.** vii + 280 pp. 16 × 24.5 cm. Price, \$10.50.

These are the first two of an annual series of volumes reviewing the general field of semiconductors. The editor points out in the preface to Volume 1 that about a thousand papers are published per year in this area, and has instigated this series as a means of helping semiconductor workers keep up-to-date. Each volume contains a number of review articles on some aspect of semiconductors (seven papers

in the first, eight in the second volume), by authorities from England, France, Canada, Switzerland and U.S.A. The subjects treated to date are: Recent Advances in Silicon, The Germanium Filament in Semiconductor Research, Theory of the Seebeck Effect in Semiconductors, The Electrical Properties of Phosphors, The Design of Transistors to Operate at High Frequencies, Photo-Magneto-Electric Effect in Semiconductors, Field Effect in Semiconductors (first volume) and Semiconductor Alloys, Properties of the III-V Compound Semiconductors, Radiation Effects in Semiconductors, Lifetimes of Free Electrons and Holes in Solids, The Production of High-Quality Germanium Single Crystals, Impurities in Germanium, High Electric Field Effects in Semiconductors, Theories of Electro-