intermediate on the following grounds: (1) oxalacetate as assayed by the method of Neish[3] 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 (CH⋮CCOOH) has been ruled out as the latter compound is not active in this system. The possibility of an enzyme-propiolate 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 $C^{14}O_2$ 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.

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RECEIVED MARCH 10, 1958

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 ornithine[4] 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 ammonium sulfate fractionation (60 to 75% saturation) at *p*H 7, heat treatment (65° for 5 minutes), ammonium sulfate fractionation (50 to 75% saturation) at *p*H 8.5; and chromatography on diethylaminoethylcellulose gave a preparation with an activity of 73,000 (μmoles 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 mμ 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.

TABLE I

EFFECT OF BIOTIN-DEFICIENCY ON ENZYME ACTIVITY

Enzyme system	Activity of cell-free extracts.[a] μmoles 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 medium[1] containing no arginine or uracil and 20 or 0.3 mμg./ml. of biotin, respectively. [b] Extract incubated with carbamyl phosphate, 20 μmoles; tris-(hydroxymethyl)-aminomethane buffer, 10 μmoles; ornithine, 10 μmoles in 1 ml. at *p*H 8.3 for 30 minutes at 35°. Citrulline determined colorimetrically.[6] [c] As in *b* with aspartate in lieu of ornithine; *p*H 7.5; incubated at 25°; passed through a Dowex-50 column before carbamylaspartate determined.[7] [d] Determined as previously described.[8]

(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. F. Sund, J. M. Ravel and W. Shive, *J. Biol. Chem.*, in press.

(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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RECEIVED FEBRUARY 20, 1958

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 authors[1] 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. Tal'rose and E. L. Frankevitch, *Doklady Akad. Nauk S.S.S.R.*, **111**, No. 2, 37 (1956).