

Search for a dihydroxy acid dehydrase in rat and microbial tissues*

Valine can be replaced by its α -keto analogue for the growth of the weanling rat^{1,2}. This nutritional observation is generally interpreted as a conversion of the keto acid to the amino acid due to tissue transaminases.

The last steps in the biosynthesis *de novo* of valine in *Neurospora crassa*^{3,4}, *Escherichia coli*^{3,4}, *Torulopsis utilis*⁵ and *Saccharomyces cerevisiae*⁶ involves the dehydration of α,β -dihydroxyisovaleric acid to α -ketoisovaleric acid and its transamination to yield valine. Thus it is desirable to conduct experiments to test for the presence of the next to the last enzymic step in the rat.

A valine-free basal diet was obtained by using a mixture of 18 crystalline amino acids⁷ in a synthetic diet. The male weanling rats (four per group) on the basal diet steadily lost weight during the 2-week experimental period (Table I). Supplementation of the basal diet with valine produced growth. Addition of an equimolar amount of DL- α,β -dihydroxyisovaleric acid failed to stimulate growth. As observed by previous investigators^{1,2}, α -ketoisovaleric acid supported growth in the rats. Though this growth data is in accord with the presence of transaminase, it also suggests the absence of the dihydroxy acid dehydrase in the rat.

In addition direct assays for the dehydrase enzyme were performed (Table II).

TABLE I

EFFECT OF VALINE-RELATED COMPOUNDS ON THE GROWTH RATE OF WEANLING RATS

Supplement to the basal diet	Weight gain (g/day)
None	— 0.64
DL-Valine, 2.00%	+ 2.87
DL- α,β -Dihydroxyisovaleric acid, 2.29%	— 0.61
α -Ketoisovaleric acid, 1.18%	+ 1.56

TABLE II

DEHYDRASE ASSAYS

The complete dehydrase assay system contained 0.05 *M* tris(hydroxymethyl)aminomethane buffer, pH 7.4, 0.02 *M* MgCl₂, 0.02 *M* DL- α,β -dihydroxyisovalerate and 0.10–0.30 ml of tissue extract in a final volume of 1.0 ml. These components were incubated in triplicate for 30 min in a nitrogen atmosphere with constant shaking in a water bath at 37°. The keto acid measurement and assay corrections were described previously⁵.

Tissue source	Dehydrase activity (μ moles keto acid formed/0.3 ml extract)	Specific activity (μ moles keto acid formed/mg protein)
Rat-liver homogenate	0.00	0.000
Rat-kidney homogenate	0.00	0.000
<i>Lactobacillus casei</i>	0.00	0.000
<i>Lactobacillus arabinosus</i>	0.01	0.002
<i>Saccharomyces cerevisiae</i>	0.94	0.364
<i>Torulopsis utilis</i>	0.81	0.474
<i>Serratia marcesens</i>	0.72	0.171
<i>Bacterium cadaveris</i>	0.41	0.038
<i>Micrococcus lysodeikticus</i>	0.28	0.130

* A part of this communication was presented at the Fifth International Congress of Nutrition, Washington, D. C., September 1-7, 1960.

Addition of 0.3 ml of a 1:10 rat-liver and -kidney homogenate in 0.25 *M* sucrose failed to catalyze the formation of keto acid under the assay conditions developed for *Saccharomyces cerevisiae*⁶. Incubations at pH 6.8 and 8.0 under nitrogen were also negative.

Table II also contains the dehydrase assay on two groups of micro-organisms. The *Lactobacilli* were obtained by the incubation of inoculum for 16 h at 37° in 400 ml media containing casein hydrolyzate and tryptophan as the nitrogen source. After centrifuging, the 3–6 g of wet packed cells were suspended in 9 vol. cold 0.10 *M* phosphate buffer, pH 7.4, ruptured by a 40 min treatment in a Raytheon 10 Kc ultrasonic oscillator and centrifuged at 18,000 × *g* for 20 min at 3°. Triplicate assay tubes containing 0.3 ml of this supernatant for four different batches of each *Lactobacilli* were essentially negative.

Cell-free extracts of the bottom group (Table II) of 5 microorganisms, obtained from commercial sources, were prepared by similar procedures. Shorter sonic treatment for rupture of the last 4 organisms listed was possible. To maintain a linear response with enzyme concentration, 0.05–0.20 ml of these 5 extracts was used. Correction for the volume of this aliquot was made to facilitate comparisons in Table II.

The above *Lactobacilli*, like the rat, fail to grow with ammonium salts as the nitrogen source. While both can convert α -ketoisovaleric acid to valine due to the presence of transaminase⁸, they are devoid of detectable dihydroxy acid dehydrase activity. The last 5 microorganisms listed in Table II, in addition to *Escherichia coli* and *Neurospora crassa*³, grow on ammonium salts as the sole nitrogen source and can synthesize all of their amino acids. As each contains the dihydroxy acid dehydrase, the two terminal steps of valine formation is similar in these 7 organisms.

This investigation was supported by Grant No. G-2173 of the National Science Foundation and Grant No. RG-6626 (Cl) of the National Institutes of Health, U. S. Public Health Service.

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Received October 17th, 1960