Studies in Valine Biosynthesis

III. BIOLOGICAL DISTRIBUTION OF A DIHYDROXY ACID DEHYDRASE*

ROBERT L. WIXOM, JOHN H. WIKMAN, AND GEORGE B. HOWELL

From the Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock, Arkansas

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The steps in the pathway of formation of valine are: decarboxylation of pyruvate to an acetaldehyde-thiamine pyrophosphate intermediate which condenses with another molecule of pyruvate to yield α-acetoacetic acid, migration of the α-methyl carbon to form α-keto-β-hydroxyisovaleric acid, reduction of this acid to produce α,β-dihydroxyisovaleric acid, dehydration to yield α-ketoisovaleric acid, and, finally, transamination to form valine. The reactions in the biogenesis of isoleucine are parallel to those of valine. Instead of pyruvate, α-ketobutyrate condenses with acetaldehyde to form α-hydroxy-α-acetylbutyric acid. Migration of the ethyl group from the α to the β position gives the carbon skeleton of isoleucine. Reduction and dehydration yield the keto acid analogue of isoleucine. These pathways have been demonstrated primarily with Neurospora crassa, Escherichia coli, and Saccharomyces cerevisiae (cf. 1-4).

The last biosynthetic step for these two amino acids is transamination, a reaction that is known to occur in mammalian tissues. With the exception of threonine and lysine, each of the essential amino acids can be replaced by their corresponding α-keto acid analogues for a growth response in weanling rats (5). Similar nutritional relationships have been observed in lactic acid bacteria (6). Therefore, it was desirable to test mammalian tissues and lactic acid bacteria for the possible presence of the next to last step of valine biosynthesis, namely the dehydration reaction.

As a clue to the valine pathway, the presence of the dihydroxy acid dehydrase has been briefly reported in four new microorganisms (7) and higher plants (4). Herein is presented evidence for an even more widespread distribution of the dehydrase in those microorganisms that can grow on ammonium salts. In those microorganisms (7) and higher plants (4), the dehydrase has been briefly reported in four new microorganisms (8) (cf. 1-4).

EXPERIMENTAL PROCEDURE

Rat Growth Studies—A valine- or isoleucine-deficient rat diet was prepared with the mixture of nineteen crystalline amino acids as designed by Rose, Oesterling, and Womack (8) (Mixture XXIII) with the omission of the desired amino acid. The other components of the basal diet were in grams per 100-g diet:

- sucrose, 15
- salt mixture of Jones and Foster, 4
- cellulose, 2
- choline chloride, 0.2
- inositol, 0.1
- vitamin A and D concentrate (3000 U.S.P. units of vitamin A and 425 U.S.P. units of vitamin D), 0.06
- corn oil, 1.55
- The vitamins in milligrams per kilogram of diet were: thiamine-HCl, 5
- riboflavin, 10
- pyridoxine-HCl, 5
- niacinamide, 5
- calcium pantothenate, 25
- p-amino-benzoic acid, 300
- 2-methyl-1,4-naphthoquinone, 2
- folic acid, 5
- biotin, 0.1
- vitamin B12, 0.03
- and α-tocopherol, 2.5

The oily dihydroxy acids were dissolved in acetone, and the solution was distributed among the experimental groups (four per group) and housed in individual cages for the 2-week growth experiment.

Source of Microorganisms—The microorganisms were grown in the laboratory or were obtained commercially or by gift. Lactobacillus arabinosus 17-5, ATCC 8014, Lactobacillus casei, ATCC 7469, Streptococcus faecalis, ATCC 8043, and Leuconostoc mesenteroides P-60, ATCC 8042 were handled, in general, as described by Henderson and Snell (9), except for omission of NaCl in Salts A and substitution of potassium citrate for sodium citrate (10). For the growth experiments (Figs. 3 and 4), 2.0 ml of isoleucine-deficient basal media, a 16-hour incubation at 37°C, and 0.10 NaOH for titration of the lactic acid formed were used. For the enzyme assay which required the large-scale growth of these four organisms, the amino acid mixture of the basal media was replaced by 5.0 ml of 10% casein hydrolysate plus 20 mg of L-tryptophan per 100 ml of basal media. A rapidly growing inoculum (10 ml) was aseptically added to 400 ml of this modified media. After an 18-hour incubation at 37°C, the log phase cells were harvested by centrifugation at 3°C, washed with cold 0.9% NaCl solution, and resuspended. Approximately 3 to 6 g of wet packed cells were obtained per 400 ml of media.

Cultures of Lactobacillus lactis 39A and Lactobacillus bulgaricus GA were obtained from Paul-Lewis Laboratories. They were washed, centrifuged, and ruptured as described elsewhere. Escherichia coli, ATCC 9637 was grown in 1-liter Erlenmeyer flasks for 18 hours at 25°C on a rotary shaker in the media of Davis and Mingioli (11). Frozen cells of Aerobacter aerogenes, ATCC 12409 and Pseudomonas aeruginosa, ATCC 10145 were a highly appreciated gift from Dr. B. L. Hutchings, Lederer Laboratories Division, American Cyanamid Company. Frozen cells of Serratia marcescens (Shears strain GW) were obtained from Difco Laboratories, Inc. Spray-dried cells of Micrococcus lysodeikticus, ATCC 4698, which were grown by the procedure of Beers (12), were obtained from Miles Chemical Company.

Lyophilized cells of Bacterium cadaveris, NCTC 6578, Clostrid-
The purity of the various batches was checked by the refractive index of the intermediate ethyl oc-α-oxid esters, by elemental analysis of the quinine salt of the α, β-dihydroxy acid, a growth stimulation should be observed if the dihydroxy acid is converted by dehydration and amination to the essential amino acid. Fig. 1 shows the results of the nutritional experiment to test DL-α, β-dihydroxyisovaleric acid as a possible valine precursor in the weanling rat. Positive growth was found with the complete diet (Curve A, +2.87 g per day). Omission of valine led to a steady weight loss (Curve B, −0.64 g per day). Rats in Group C, which received the valine-deficient diet plus an equimolar amount (2.29%) of DL-α, β-dihydroxyisovaleric acid, failed to grow (−0.61 g per day).

Essentially the same nutritional experiment was repeated with the isoleucine precursor, α, β-dihydroxy-β-methyl-n-valeric acid. Fig. 2 shows the growth rate for weanling rats with this substance. Whereas the rats grew at a rate of +3.16 g per day on the complete diet (Curve A), omission of isoleucine led to weight loss and death by the 9th day. Supplementation of the isoleucine-deficient diet with 0.90% of the resolved α, β-dihydroxy-β-methyl-n-valeric acid (2) led likewise to a decline in weight, and death by the 9th day. Meister and White have demonstrated that the keto analogue of L-isoleucine supported rat growth (16). Although this dihydroxy acid is a known intermediate in isoleucine formation in certain microorganisms, its failure to stimulate growth suggests the absence of detectable dihydroxy acid dehydrase in the rat.

Such growth studies are open to the question of whether or not the test substance is absorbed. This possibility cannot be completely eliminated, but it is unlikely in view of the positive growth response with α-keto acids, here and elsewhere, or with α-hydroxyisovaleric acid (5).

Test for Conversion of Dihydroxy Acid to Amino Acid in Lactic Acid Bacteria—Many α-keto acids in the presence of pyridoxal in the media can be utilized for amino acid formation and thus stimulate the growth of several lactic acid bacteria (6, 17). Thus, as in the rat, these bacteria may be used to test the possible transformation of α, β-dihydroxy-β-methyl-n-valeric acid (isolated isomer from quinine resolution (2)) to isoleucine. Two kinds of experiments were run on each of four organisms. Growth was followed by titration of the lactic acid produced after 16 hours of incubation at 37°. The left graphs in Figs. 3 and 4 show the anticipated growth response to increasing increments of isoleucine with L. arabinosus and L. mesenteroides. The horizontal line in the same figures indicated the complete lack of cell growth in the absence of isoleucine and the presence of its neutralized dihydroxy acid analogue. The right hand graphs of Figs. 3 and 4 show the data when suboptimal amounts of isoleucine plus a 10, 20, 50, or 100 times factor of the dihydroxy acid were both added to the media. The observed zero slopes...
FIG. 1. Test for growth response of a dihydroxy acid with rats. Valine precursor experiment. Curve A (•—•), basal diet plus 2.00% DL-valine; Curve B (O—O), valine-deficient basal diet; Curve C (▲—▲), basal diet plus 2.29% DL-α,β-dihydroxyisovaleric acid; and Curve D (△—△), basal diet plus 1.18% α-ketoisovaleric acid.

FIG. 2. Test for growth response of a dihydroxy acid with rats. Isoleucine precursor experiment. Curve A (•—•), basal diet plus 1.60% DL-isoleucine; Curve B (O—O), isoleucine-deficient basal diet; and Curve C (▲—▲), basal diet plus 0.90% α,β-dihydroxy-α-methyl-β-valeric acid (quinine isomer).

indicate both the lack of dehydrase formation by an induced synthesis and the lack of inhibitory action of this acid. Although the β-hydroxy analogue of valine is a microbial inhibitor (18), evidently the substitution of a hydroxyl group for the α-amino group gives a compound without antimicrobial activity. As the α-hydroxyisovaleric acids and other hydroxy acids are absorbed and utilized by lactic acid bacteria (19), the factor of cell permeability for the dihydroxy acid is an unlikely explanation.

The above two experiments have also been performed with S. faecalis and L. casei with exactly the same results. Thus, whereas these four organisms have transaminases, which enable them to utilize α-keto acids, the above evidence suggests the absence of the dehydrase to transform the dihydroxy acid to the α-keto acid. Since such a conclusion for the rat and these bacteria is based on negative evidence, direct assays of tissue homogenates or microbial extracts were also undertaken.

**Keto Acid Formation by Rat and Other Vertebrate Tissues**—The general conditions for the dehydrase assay of S. cerevisiae extracts were described in “Experimental Procedure.” To repeat in part, the assays of rat tissue homogenates in Table I were performed in 12-ml centrifuge tubes in an air atmosphere in a 37° water bath with slow oscillatory shaking. These 1:10 homogenates were prepared in isotonic sucrose with a Potter-Elvehjem type homogenizer with a Teflon pestle. Each assay was corrected for the necessary enzyme and substrate blanks. Whereas most tissues were negative, kidney and liver homogenates gave a quite low, but reproducible, keto acid production. On the assumption that these figures are representative of the whole organs, these low apparent reaction rates are still sufficient to supply the rat with the required daily valine intake. Before accepting this implication at face value, other experiments were run.

Table II presents the keto acid formation, measured in the usual manner, when the rat homogenates or microbial extracts

### Table I

**Keto acid formation by rat tissues**

| Tissue source | Activity | Specific activity
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmole keto acid/0.3 ml homogenate</td>
<td>μmole keto acid/mg protein</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.28</td>
<td>0.034</td>
</tr>
<tr>
<td>Liver</td>
<td>0.06</td>
<td>0.011</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Heart</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Brain</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Lung</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Yeast extract*</td>
<td>0.94*</td>
<td>0.364*</td>
</tr>
</tbody>
</table>

* The keto acid production by 0.3 ml of a 1:10 centrifuged bakers’ yeast extract is included here as a reference point.

Fig. 3. Tests for growth response of a dihydroxy acid with Lactobacillus arabinosus. •—•, The amount of L-isoleucine added to the isoleucine-deficient basal media; O—O, the test α,β-dihydroxy-α-methyl-β-valeric acid (quinine isomer). In the right hand graph, each tube contained the indicated suboptimal amount of L-isoleucine plus 0, 1, 10, 20, 50, or 100 times that amount of the dihydroxy analogue of isoleucine. Growth was followed by titration after a 16-hour incubation at 37°.

Fig. 4. Tests for growth response of a dihydroxy acid with Leuconostoc mesenteroides. See legend of Fig. 3 for other details.
were incubated simultaneously in various gas phases. The oxygen atmosphere enhanced the keto acid production by the liver and kidney homogenates, whereas the nitrogen gas phase obliterated the keto acid formation. These results are in striking contrast to those obtained with the five microbial extracts. The reaction proceeded to the same extent in all three gas phases for M. lysodeikticus. For the other four microbial extracts, the keto acid formation occurred at the same rate in air and nitrogen but was impaired in the oxygen atmosphere. In another experiment, the keto acid production by rat liver or kidney homogenate in the oxygen phase was found to increase in a linear manner over a 2-hour period. Thus, the keto acid production by rat homogenates may be due to the oxidation of the substrate to a keto acid by a nonoxidative reaction, i.e. a cleaving out a molecule of water from the dihydroxy acid.

Since Table II demonstrates that the dehydrase assay with rat homogenates must be run in a nitrogen atmosphere, this condition was used for the incubation of liver and kidney homogenates of 16 other vertebrates: Mammalia (mouse, pig, dog, rabbit, guinea pig, hamster, and cat); Aves (chick, turkey poult, and pigeon); Reptilia (turtle and snake); Amphibia (frog and toad); and Pisces (catfish and goldfish). All were negative or essentially negative for keto acid formation. Several modifications were also tested: (a) use of 4 times the above amount of tissue for rat and turkey tissues (0.6 ml of liver and kidney homogenate (1:5, weight per volume)); and (b) incubation with Tris and phosphate buffers at pH 6.8 and pH 8.0, in addition to the usual pH 7.4, for liver and kidney homogenates from the rat and chick. Each gave the same negative results. Thus, the dehydrase is absent from rat tissues by two different criteria and from 16 other vertebrates by the assay, in vitro.

### Table II

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Volume used</th>
<th>Activity in atmosphere of</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air</td>
<td>O$_2$</td>
<td>N$_2$</td>
<td></td>
</tr>
<tr>
<td>Rat liver homogenate</td>
<td>0.3</td>
<td>0.04</td>
<td>0.08</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Rat kidney homogenate</td>
<td>0.3</td>
<td>0.21</td>
<td>0.59</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>M. lysodeikticus extract</td>
<td>0.2</td>
<td>0.70</td>
<td>0.81</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae extract</td>
<td>0.2</td>
<td>0.70</td>
<td>0.63</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa extract</td>
<td>0.1</td>
<td>0.49</td>
<td>0.38</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>E. coli extract</td>
<td>0.2</td>
<td>0.59</td>
<td>0.77</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>A. aerogenes extract</td>
<td>0.1</td>
<td>0.44</td>
<td>0.40</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

The conditions for the assay in an air atmosphere are described in the legend for Table I and the text. For the last two columns, the reaction components were incubated in 10-ml beakers in a Dubnoff shaking water bath at 37°C with either a nitrogen or oxygen gas phase. Ten per cent rat homogenates or centrifuged microbial extract were used in the stated volumes. After correction for enzyme and substrate blanks, the activity data is expressed in units of micromoles of keto acid formed per 30 minutes.

Table III also presents the concentration of the dehydrase in extracts of eight bacteria and three yeasts, of which only E. coli (1) and S. cerevisiae (2) have been heretofore reported. As no growth occurred with these organisms, the dehydrase activity was determined as the micromoles of keto acid formed per 30 minutes under the stated conditions. Its concentration is expressed in units per 0.3 ml of the supernatant fluid of a microbial extract (1:10, weight per volume).

### Table III

<table>
<thead>
<tr>
<th>Family and genus</th>
<th>Growth by min</th>
<th>Solubility</th>
<th>Dehydrase conc.</th>
<th>Protein conc.</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus arabinosus</td>
<td>* 0.01</td>
<td>9.6</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>* 0.00</td>
<td>6.0</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus lactis</td>
<td>* 0.00</td>
<td>11.9</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus hulgarinus</td>
<td>* 0.00</td>
<td>11.8</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>* 0.00</td>
<td>5.5</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leuconostoc mesenteroides</td>
<td>* 0.00</td>
<td>3.2</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+ 10</td>
<td>8.5</td>
<td>0.780</td>
<td></td>
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</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>+ 10</td>
<td>32.5</td>
<td>0.015</td>
<td></td>
<td></td>
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<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Escherichia coli</td>
<td>+ 30</td>
<td>1.42</td>
<td>7.0 676</td>
<td></td>
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<tr>
<td>Aerobacter aerogenes</td>
<td>+ 5</td>
<td>4.38</td>
<td>8.0 130</td>
<td></td>
<td></td>
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<tr>
<td>Serratia marcescens</td>
<td>+ 20</td>
<td>0.72</td>
<td>14.0 0.171</td>
<td></td>
<td></td>
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<tr>
<td>Bacterium cadaveris</td>
<td>+ 20</td>
<td>0.41</td>
<td>36.2 0.035</td>
<td></td>
<td></td>
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<tr>
<td>Micrococaceae</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus lysodeikticus</td>
<td>+ 40</td>
<td>1.53</td>
<td>11.7 0.436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillaceae</td>
<td>+ 10</td>
<td>0.07</td>
<td>39.0 0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascomycetous fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>+ 40</td>
<td>0.94</td>
<td>8.8 0.364</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried brewer’s yeast</td>
<td>+ 10</td>
<td>0.23</td>
<td>4.8 0.159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torulopsis utilis, frozen</td>
<td>+ 20</td>
<td>0.81</td>
<td>5.7 0.474</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torulopsis, dried</td>
<td>+ 10</td>
<td>3.80</td>
<td>19.0 0.945</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The lactic acid bacteria were treated for 20 and 40 minutes in the sonic oscillator.
† Dried brewers’ yeast extract was prepared by autolysis.
noted in Table III, each organism can grow on ammonium salts as the main nitrogen source, an indication that they can synthesize all of their amino acids. Although B. cadaveris and M. lysodeikticus used here were grown on a crude media, they can be grown on a defined media with ammonium salts as the nitrogen source (20, 21). Two plant tissues are mentioned here for later comparison (4). Chlorella pyrenoidosa and spinach leaf had an activity of 0.24 and 0.32 and a specific activity of 0.290 and 0.304, respectively, in the same units of Table III. Again, there is a parallel presence of the dehydrase and the ability to grow on inorganic nitrogen.

**DISCUSSION**

In the classical approach of nutrition, amino acids are classified as essential or nonessential with respect to their required dietary presence for growth of the weaning test animal. The nonessential amino acids are manufactured by biosynthetic reactions in the tissues. Hence, detection of the dihydroxy acid dehydrase, an enzyme in a biosynthetic pathway with an equilibrium favoring product formation (1, 2), may help elucidate some of the qualitative comparative aspects of amino acid nutrition. The evidence herein indicates the absence of the dehydrase in 17 vertebrates and six lactate acid bacteria. In this respect the rat, mouse, man, pig, dog, chick (5), fish (22), insect (5), protozoan (5), and lactate acid bacteria (23, 24), have a known nutritional requirement for valine and isoleucine for the purpose of growth or nitrogen balance. By extrapolation, this lack of the dehydrase suggests, but does not prove, that all vertebrates have a similar nutritional need for valine and isoleucine. Although this absence does not exclude biosynthesis by some different pathway, this possibility is rendered quite remote by the widespread microbial (Table III) and plant (4) distribution of the dehydrase. Thus, the consistent pattern of both valine and isoleucine as essential amino acids in the above species is a reflection of the deletion of the dehydrase and perhaps earlier enzymes in the sequence of parallel biosynthetic steps.

Turning to organisms that can grow on an inorganic nitrogen source, N. crassa and E. coli have long been studied to elucidate the steps of valine and isoleucine biosynthesis (1-4). Only the literature related to organisms other than these two will be discussed here. The over-all formation of valine has been studied by the radioisotope incorporation approach or by isotope competition method in T. utilis (25-28), S. cerevisiae (29-31), and A. aerogenes (32) with results that are consistent with the inability of both vertebrates and microorganisms to grow on valine and isoleucine.

Thus, the consistent pattern of both valine and isoleucine as essential amino acids in the above species is a reflection of the deletion of the dehydrase and perhaps earlier enzymes in the sequence of parallel biosynthetic steps.

The third area of evidence is the correlation of the presence of the dehydrase in microorganisms (Table III) and plants (4) with their ability to grow on ammonium salts, i.e., to synthesize all of their amino acids. Several of these dehydrases have been found to possess a similar specificity with seven dihydroxy acids tested as substrates. Thus, the results of this study are consistent with the conclusion that the last two steps of valine formation, whenever it occurs, are the same in the various biological systems examined. Furthermore, the earlier knowledge of the microbial a-acetolactate formation suggests that all steps in the pathway may be identical, but such a hypothesis requires further experiments to exclude alternative interpretations.

Although this paper has focused on the distribution of the dehydrase, there are wider implications for the comparative aspects of amino acid biosynthesis. Lysine formation proceeds via a-aminoadipic acid in N. crassa and other fungi; but in E. coli, other bacteria, actinomycetes, green algae, and higher plants, the key intermediate is a-ε-diaminopimelic acid (37, 38). Ornithine (and arginine) biosynthesis in N. crassa and T. utilis begins by reduction of glutamic acid to glutamic γ-semialdehyde which undergoes transamination to form ornithine (39). This over-all transformation in E. coli proceeds by first N-acetylation, then reduction, transamination, and finally, decylation to yield ornithine (39). In contradistinction to these examples, the evidence to date is consistent with a single pathway of valine biosynthesis in the diverse biological systems studied.

**SUMMARY**

A dihydroxy acid dehydrase, which catalyzes a step in the biosynthesis of valine and isoleucine, has been examined with respect to its distribution in diverse biological systems. By the criteria of both growth response and dehydrase assay *in vitro*, there was no detectable conversion of the α,β-dihydroxy acids to the α-keto acids in the rat, Lactobacillus arabinosus, Lactobacillus casei, Leuconostoc mesenteroides, and Streptococcus faecalis. The dehydrase assay was also negative for liver and kidney homogenates of 16 other vertebrates and two other lactate acid bacteria. In both the vertebrates and microorganisms studied to date, there is an inverse correlation between the presence of the dehydrase and a nutritional requirement for valine.

In addition to the three microorganisms and plants previously known to have this enzyme, this paper reports its presence in *Pseudomonas aeruginosa, Pseudomonas fluorescens, Aerobacter aerogenes, Serratia marcescens, Bacterium cadaveris, Micrococcus lysodeikticus, Clostridium kluyveri, brewers' yeast, and Torulopsis utilis.* As the dihydroxy acid dehydrase catalyzes a known step in the middle of a pathway, at least the last two steps in valine formation in the above organisms are identical with the biosynthetic steps in Neurospora crassa, Escherichia coli, and Saccharomyces cerevisiae. Further implications of the presence of this enzyme are discussed.

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St. Regis Paper Company, Rhinelander, Wisconsin for the contribution of Torula yeast; to Dow Chemical Company for a gift of DL-threonine and DL-phenylalanine; and to Du Pont Chemical Company for a gift of L-lysine and DL-methionine.

Addendum—Myers (40) has recently described some properties of a purified dihydroxy acid dehydrase from E. coli and concludes that this enzyme is probably common for both the valine and isoleucine pathways of biosynthesis.

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