*OCCURRENCE OF ORNITHINE δ-TRANSAMINASE: A DICHOTOMY**

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Against the background of the biochemical unity (cf. Kluyver and van Niel[1]) of all forms of life, instances of "biochemical diversity" have held a persistent attraction for students of biochemistry and physiology and of taxonomy and evolution. One striking example of such diversity can be seen in the dissimilar modes of ornithine synthesis in *Neurospora crassa* and *Escherichia coli:*[2] in *N. crassa* ornithine arises by transamination of glutamic γ-semialdehyde,[3] whereas in *E. coli* ornithine synthesis proceeds via several acetylated intermediates.[4]

The differences between the two ornithine pathways are reflected in the results of enzymatic studies: an enzyme that mediates the reversible transamination between glutamate and glutamic γ-semialdehyde could be demonstrated in extracts of *N. crassa*[5, 6] (and of some other micro-organisms[6]) but not in those of *E. coli*.[6] The enzyme from *N. crassa* has been named "ornithine δ-transaminase," after the reverse of the ornithine-yielding biosynthetic reaction (see Fig. 1).

The differences in the mode of ornithine synthesis and in ornithine δ-transaminase activity between *N. crassa* and *E. coli* encouraged the present, more systematic, study of the distribution of this enzymatic activity. Attention was initially focused on various fungi and eubacteria; subsequently some other organisms of interest were included in the survey.

The organisms were selected partly on the basis of their taxonomic position and partly on the basis of their ability to grow on simple, defined media (free of ornithine or glutamic γ-semialdehyde). Accordingly, to the extent that such media were used, this study is concerned with constitutive, rather than adaptive, ornithine δ-transaminase activity.

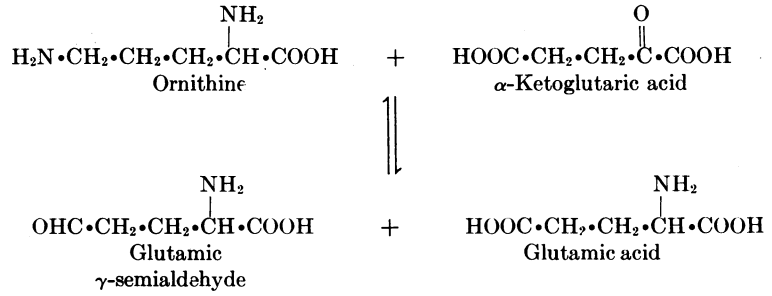

FIG. 1. Reaction catalyzed by ornithine δ-transaminase

For the survey of this activity, the organisms employed were appropriately disrupted, and the clarified cell-free extracts obtained were subjected to a diagnostic color reaction and bioassay.

MATERIALS AND METHODS

Organisms.—The strains identified by ATCC numbers were obtained from the American Type Culture Collection (Washington, D.C.). *Saccharomyces cerevisiae*

(Fleischmann's bakers' yeast, Standard Brands, Inc.), spinach leaf, and calf liver came from commercial sources. The remaining organisms were kindly provided, as indicated below, by various individuals, to whom the authors are very greatly indebted. *Micrococcus lysodeikticus* was received from Dr. L. Gorini, *Bacillus subtilis* from Mrs. M. Bonner, *B. megaterium* from Dr. P. B. Cowles, *Mycobacterium ranae* from Dr. M. I. Bunting, *N. crassa* from Dr. P. St. Lawrence, *N. sitophila* (thiamine-requiring mutant[7]) from Mrs. M. Bonner, *Coprinus lagopus* from Dr. H. Papazian (who isolated this organism), *Chlamydomonas reinhardi* (isolated by Dr. G. M. Smith) from Dr. W. T. Ebersold, *Chlamydomonas moewusii* (isolated by Dr. L. Provasoli) from Dr. R. R. L. Guillard, *Tetrahymena pyriformis* from Dr. G. W. Kidder, *Hydrogenomonas ruhlandii*[8] from Dr. L. Packer, *Pseudomonas fluorescens*[9] from Dr. W. Vishniac, *E. coli* strain B and *Aerobacter aerogenes* from Mrs. M. Bonner, *Serratia marcescens*[10] from Dr. M. I. Bunting, *Proteus vulgaris* from Mrs. M. Bonner, *Salmonella enteritidis* from Dr. A. D. Mandel, and *Anacystis nidulans*[11] from Dr. J. Myers.

Media.—With the exception of *Saccharomyces cerevisiae* and of *T. pyriformis* (see below), the organisms used were grown on the following chemically defined media: (*a*) Gorini and Lord's medium[12] containing glucose, acetate, glutamate, biotin, and salts; (*b*) Medium E[13] supplemented with glucose (0.2 per cent) and biotin (2.5 μg/liter);[14] (*c*) Medium E[13] with glucose (0.5 per cent); (*d*) Medium E[13] with glycerol (2.0 per cent); (*e*) Medium E[13] with sucrose (2.0 per cent); (*f*) Medium N with sucrose (2.0 per cent);[15] (*g*) Fries medium[16, 17] supplemented with sucrose (2.0 per cent) and thiamine (3.0 μg/ml); (*h*) glucose-glutamate-thiamine-salt medium, kindly communicated by Dr. H. Papazian; (*i*) Sager and Granick's medium;[18] (*j*) Guillard's medium;[19] (*k*) proteose-peptone (3.0 per cent) and glucose (0.5 per cent), as recommended by Dr. G. W. Kidder, whose helpfulness and advice in this connection are gratefully acknowledged; (*l*) Medium E[13] with sodium lactate (0.5 per cent); (*m*) Medium Z with sucrose (1.5 per cent);[20] (*n*) Medium E[13] with sodium succinate (0.5 per cent); (*o*) Labrum and Bunting's synthetic medium;[10] (*p*) Medium E[13] supplemented with glucose (0.25 per cent) and nicotinic acid (0.25 μg/ml); (*q*) Medium C of Kratz and Myers[11] with sodium succinate (0.5 per cent) and carbon dioxide (0.5 per cent in air, volume by volume).

Cultivation of Organisms and Preparation of Extracts.—The conditions used for the cultivation of the organisms are indicated in Table 1.—After cultivation, the organisms were harvested by centrifugation, except *T. pyriformis*, which was allowed to sediment by gravity. The organisms thus collected were washed in chilled 0.1 *M* phosphate buffer (pH 7.0) and resuspended and disrupted in the same buffer at 0°–5° C.

All the bacteria used, except *M. lysodeikticus* and *Arthrobacter histidinolovorans*, were disrupted by sonic vibration (applied for 30 minutes) in a 9-kc. Raytheon oscillator. These two bacteria and all the remaining organisms (except *T. pyriformis*, which was ground in the frozen state without abrasive) were disrupted by grinding with alumina (A-301, Alcoa); spinach leaf and calf liver were ground with sand. The resulting extracts were clarified by cold centrifugation, dialyzed against cold 0.1 *M* phosphate (pH 7.0) when necessary, and promptly tested as sources of enzyme activity. In general, the extracts obtained could be stored at −15° C. for several weeks without detectable serious deterioration. The protein content of

the extracts was of the order of 10 mg/ml. Protein was determined by the method of Lowry *et al.*[21]

Test for Ornithine δ-Transaminase Activity.—For preliminary screening, a color test was employed. This test, which depends on the formation of the yellow color resulting from the reaction of Δ^1-pyrroline-5-carboxylate with *o*-aminobenzaldehyde,[22] was carried out in the general manner described by Fincham.[5] Δ^1-Pyrroline-5-carboxylate is the spontaneously cyclized form of glutamic γ-semialdehyde,

TABLE 1

OCCURRENCE OF ORNITHINE δ-TRANSAMINASE ACTIVITY

Organism	Cultivation Conditions: Medium*	Temperature (° C.)	Agitation	Duration (Days)	Gram Character
Activity Found					
Micrococcus lysodeikticus, S	*a*	25	+	3	Positive
Arthrobacter histidinolovorans, ATCC 11442†	*b*	25	+	2	Variable
Bacillus subtilis, Marburg‡	*c*	37	+	2	Positive
Bacillus licheniformis, ATCC 11560	*c*	30	+	2	Positive
Bacillus pumilus, ATCC 7061	*b*	30	+	2	Positive
Bacillus megaterium, C	*c*	30	+	2	Positive
Mycobacterium ranae, 10	*d*	37	+	2	Positive
Saccharomyces cerevisiae, Gebrüder Mayer	..	..	..	..	Positive
Torulopsis utilis, ATCC 9950	*e*	30	+	1	Positive
Neurospora crassa, 74A	*f*	30	+	2	....
Neurospora sitophila, S-1090a	*g*	30	−	3	....
Coprinus lagopus	*h*	25	−	7	....
Chlamydomonas reinhardi, 137C§	*i*	25	−	6	....
Chlamydomonas moewusii§	*j*	25	−	6	....
Tetrahymena pyriformis, W†	*k*	25	+	3	....
Spinach leaf	..	..	..	..	....
Calf liver	..	..	..	..	....
Activity Not Detected					
Hydrogenomonas ruhlandii‖	*l*	30	+	1	Negative
Pseudomonas fluorescens, Tr-23	*c*	30	+	2	Negative
Azotobacter agile, ATCC 9104	*m*	30	+	2	Negative
Agrobacterium radiobacter, ATCC 6466	*c*	30	+	2	Negative
Alcaligenes faecalis, ATCC 8750	*n*	37	−	2	Negative
Escherichia coli, ATCC 9637‡	*c*	37	+	1	Negative
Escherichia coli, B‡	*c*	37	+	1	Negative
Aerobacter aerogenes, Yale	*c*	37	+	1	Negative
Erwinia carotovora, ATCC 8061	*c*	30	+	2	Negative
Serratia marcescens, HY	*o*	37	+	2	Negative
Proteus vulgaris, Yale	*p*	37	+	1	Negative
Salmonella enteritidis, ETS 64	*c*	37	+	2	Negative
Anacystis nidulans§	*q*	39	+	5	Negative

* The letters in this column refer to the subsection "Media" under "Materials and Methods."

† This organism was classified as exhibiting the transaminase activity on the basis of the color reaction only, since the bioassay was obscured by the relatively large amounts of proline present in the enzyme preparations derived from this organism; the proline (found even in dialyzed preparations) appears to arise from protein breakdown.

‡ This result was unchanged when the medium used for the cultivation of this organism was supplemented with 0.2 per cent N-Z-Case (Sheffield) and 0.2 per cent yeast extract (Difco).

§ This alga was cultivated with illumination.

‖ The same result was obtained when this organism was grown autotrophically (rather than heterotrophically).

which in turn is produced through the δ-transamination or ornithine. The yellow color is due to a dihydroquinazolinium compound, which is dissociable into its original components.[22] Since the reaction with *o*-aminobenzaldehyde is not uniquely specific for Δ^1-pyrroline-5-carboxylate,[23] enzymatic reaction mixtures that showed a yellow color were subjected to bioassay with *E. coli* mutant strain 55-25. This strain gives a growth response either to Δ^1-pyrroline-5-carboxylate[22, 24] or to proline,[22] or, presumably, to proline peptides.[25] As far as is known, L-Δ^1-

pyrroline-5-carboxylate is the only compound that gives both a yellow color with *o*-aminobenzaldehyde and a growth response with strain 55-25.

The enzyme tests were carried out in 4-inch test tubes in a total volume of 0.5 ml. of aqueous solution. The complete system contained 0.05 millimole phosphate (pH 7.0), 0.5 mg. L-ornithine monohydrochloride (Mann Research Laboratories), 1.25 mg. α-ketoglutaric acid (neutralized with sodium carbonate), 2.5 μg. pyridoxal phosphate, 0.5 mg. *o*-aminobenzaldehyde,[26] and enzyme preparation containing about 2 mg. (range, 1–3 mg.) protein. Pyridoxal phosphate was included, because it was found[27] to stimulate the activity of partially purified ornithine δ-transaminase from *N. crassa*. In parallel with each tube containing a complete system, three corresponding control tubes were prepared, from which ornithine, α-ketoglutarate, or enzyme preparation were respectively omitted.

The tubes were incubated at 25° C. for approximately 10 hours and examined for yellow color. Samples giving a positive color test were bioassayed with strain 55-25, according to the method previously described.[22] A growth response dependent on the simultaneous presence of ornithine and α-ketoglutarate in an enzymatic digest was taken to indicate that the enzyme preparation involved has ornithine δ-transaminase activity. The respective enzymes responsible for this activity may or may not be identical in different organisms.[28–30]

The sensitivity of the procedures used is estimated to be such that one-sixth of the lowest transaminase activity found could have been detected on the basis of the color test, and one-third on the basis of the growth response. The following figures will illustrate the range of activities encountered. The mean activity corresponded to about 0.05 μmole L-Δ^1-pyrroline-5-carboxylate formed per tube under the conditions given above. The lowest and highest values found were approximately 10 and 500 per cent of the mean (for *B. megaterium* and *B. subtilis*, respectively; see below). Since crude enzyme preparations were used, the quantitative activity values obtained are perhaps best considered minimum values.

In a survey of an enzymatic activity, it is possible to obtain misleading results, for example, through inadequately specific detection methods ("false positives"); or through insufficiently sensitive assay techniques or unsuitable extraction and preservation procedures ("false negatives"); or (in various conceivable ways) through interfering enzyme activities or inhibitors. With the aid of the methods described, it was endeavored to avoid such misleading results.

RESULTS AND DISCUSSION

The results of the present survey are summarized in Table 1. A clear-cut, consistent distribution pattern of the transaminase activity will be noted. Table 1 shows that ornithine δ-transaminase activity is present in all the gram-positive bacteria, the (gram-positive) yeasts and other fungi, the green algae, the protozoon, and the higher plant and animal tissues examined. In contrast, this transaminase activity could not be detected in any of the gram-negative bacteria or in the (gram-negative[31]) blue-green alga tested. Thus, as judged by the absence of this enzyme activity, the gram-negative bacteria and blue-green algae appear to constitute a group of organisms set apart from other forms of life.

The distribution of ornithine δ-transaminase activity, therefore, is in harmony with available taxonomic information: a relationship between bacteria and blue-

green algae has been inferred, on various grounds, by a number of investigators (cf. Stanier;[32] Pringsheim[31]).

Within the bacteria, the presence or absence of the transaminase activity is seen to correlate with gram-positive (including gram-variable[33]) or gram-negative character, respectively. This correlation is in line with other significant differences between the gram-positive and the gram-negative bacteria (see, for example, Bartholomew and Mittwer;[34] Mitchell and Moyle;[35] Salton[36]).

The presence of ornithine δ-transaminase activity in calf liver supplements the earlier findings of this activity in other mammals.[37, 38] Furthermore, evidence from nutritional or tracer experiments is consistent with the occurrence of this activity in protozoa,[39] mammals,[40] and higher plants.[41]

The results of the present investigation are of special interest in conjunction with the remarkable and comprehensive information available on the occurrence of α,ε-diaminopimelic acid.[42–48] This amino acid gives rise to lysine in *E. coli* by decarboxylation (see review by Work[49]). Since lysine is a homologue of ornithine (which is producible through the transaminase activity now studied), the information on diaminopimelic acid seems particularly relevant.

Investigations of the comparative biochemistry of ornithine and lysine metabolism promise to be rewarding in view of the available results of pathway studies with *N. crassa* and *E. coli*. In *N. crassa* there is evidence that both ornithine and lysine are formed from ω-semialdehydes: ornithine is formed from glutamic γ-semialdehyde,[3] and lysine appears to arise from (the recently obtained)[50] α-aminoadipic δ-semialdehyde.[50–53] In *E. coli*, on the other hand, ornithine and lysine are formed via N-acylated intermediates:[54] for ornithine the acyl group involved is acetyl,[2, 4, 55, 56] and for lysine the acyl group is succinyl.[57] It is thus seen that the homologous amino acids ornithine and lysine are produced through one set of (at least partly) analogous reactions in *N. crassa* and through another in *E. coli*.

Diaminopimelic acid functions not only as an intermediate in lysine synthesis in *E. coli* but also as a characteristic cell-wall constituent in this and other microorganisms.[36, 43, 47, 49] To what extent the two roles of diaminopimelic acid (as lysine precursor and as cell-wall constituent) are associated in various forms of life is an interesting question of comparative biochemistry.

The above-mentioned investigations of the distribution of diaminopimelic acid have revealed a pattern which is consistent with, and complemented by, the pattern of occurrence of ornithine δ-transaminase activity, as manifested in the present results. The two distribution patterns are compared, as far as possible, in Figure 2.

The occurrence of α,ε-diaminopimelic acid, in one or another isomeric form, appears to be confined (with one reported exception;[44, 58] see below) to the bacteria and blue-green algae.[32, 42, 49] Diaminopimelic acid was found in all the bacteria and blue-green algae tested, except in a group of gram-positive bacteria (Group 2 of Fig. 2), including certain cocci[42, 45] and lactobacilli.[45, 46] The gram-positive bacteria in which diaminopimelic acid was found are represented as Group 1 in Figure 2. Diaminopimelic acid could not be detected in any of the fungi, non-blue-green algae, protozoa, or higher forms of life examined,[42, 43] except for a green alga, *Chlorella ellipsoidea*, which seems to contain a relatively low amount of the amino acid.[44, 58]

The distribution studies of ornithine δ-transaminase activity and of diaminopimelic acid are seen to point to two similar, but not identical, dichotomies. As shown in Figure 2, the two points of division split the range of organisms into three parts: except in the (relatively small) central area, the transaminase activity and diaminopimelic acid would seem to be mutually exclusive. Thus the available information indicates that the fungi, non-blue-green algae, protozoa, and higher plants and animals all are similar in possessing the transaminase activity but lacking diaminopimelic acid; in contrast, the gram-negative bacteria and blue-green algae appear to lack the transaminase activity but to contain diaminopimelic acid. The central area in Figure 2 is occupied by a group of gram-positive bacteria (as represented by *B. subtilis*) which have an intermediate position in that they exhibit the transaminase activity *and* contain diaminopimelic acid. A second group of gram-positive bacteria (Group 2, as represented by *M. lysodeikticus*) have affinities to the higher organisms on the basis of both criteria.[59]

In view of the highly suggestive and complementary distribution patterns of ornithine δ-transaminase activity, as revealed by the present study, and of diaminopimelic acid, as discovered by other authors, it might not be unduly optimistic to expect that such surveys will help to shed light on taxonomic, and perhaps even on evolutionary, relationships among micro-organisms and other forms of life.

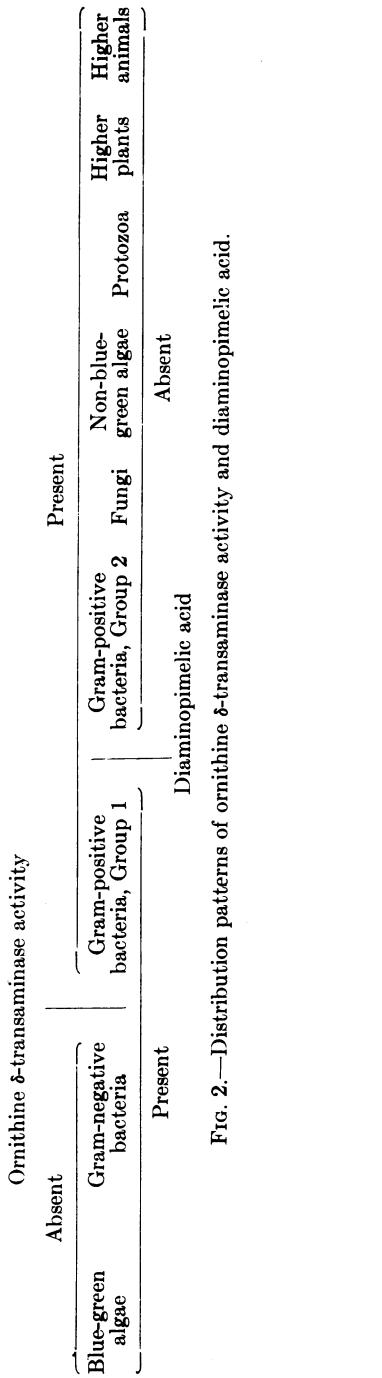

FIG. 2.—Distribution patterns of ornithine δ-transaminase activity and diaminopimelic acid.

SUMMARY

The difference in ornithine synthesis and the concomitant difference in ornithine δ-transaminase activity between *N. crassa* and *E. coli* prompted an investigation of the occurrence of this activity among various organisms.

The transaminase activity was not detected in extracts of any of the gram-negative bacteria or of the blue-green alga tested but was uniformly found in extracts of the gram-positive bacteria, fungi, non-blue-green algae, the protozoon, and the higher plant and animal tissues examined. Thus, in harmony with available information, the group containing the gram-negative bacteria and blue-green algae appears to be set apart from other organisms.

The distribution pattern found is particularly interesting in comparison with the known distribution pattern of α,ε-diaminopimelic acid. The two patterns may well reflect two similar, but not identical, dichotomies in the biological world. A group of certain gram-positive bacteria are intermediate forms in that they occupy a region between the two points of division indicated by the two distribution patterns.

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† The results reported here are taken from a dissertation presented by W. I. Scher, Jr., for the degree of Master of Science in Yale University.

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[57] N-Succinyl-L-α,ϵ-diaminopimelic acid has recently been identified as a precursor of L-α,ϵ-diaminopimelic acid in *E. coli* by C. Gilvarg, *Biochim. et Biophys. Acta*, **24**, 216, 1957. Accordingly, the terminal phase of lysine synthesis in this organism appears to be represented by the following sequence: N-succinyl-L-α,ϵ-diaminopimelic acid, L-α,ϵ-diaminopimelic acid, *meso*-α,ϵ-diaminopimelic acid, L-lysine (cf. Antia *et al.*, *op. cit.*, and Work, in *Amino Acid Metabolism*, ed. W. D. McElroy and B. Glass [Baltimore, Md.: Johns Hopkins Press, 1955], p. 462).

[58] T. Fujiwara and S. Akabori, *J. Chem. Soc. Japan*, **75**, 990, 1954.

[59] The position of the gram-positive bacteria is further highlighted by the thought-provoking findings of Powell and Strange (*op. cit.*) with *Bacillus sphaericus*. An asporogenous strain of this species was shown to be devoid of detectable diaminopimelic acid. Similarly, this amino acid could not be demonstrated in vegetative cells of the sporogenous parent of this strain but was demonstrated in the spores. Accordingly, the asporogenous strain might be placed in Group 2 of Fig. 2, whereas the sporogenous one should probably be placed in Group 1.