bis(dimethylamino)sebacic acid, *NN'*-diglycyldiaminosuberic acid and *NN'*-diglycyldiaminosebacic acid were all readily soluble in water, but were less active than diaminosuberic acid. It is also possible that permeability considerations play an important part in the assimilation of these compounds.

SUMMARY

1. Methods are described for the synthesis of six $\alpha\alpha'$ -diaminodicarboxylic acids, homologues of $\alpha\alpha'$ -diaminopimelic acid.

2. These amino acids and some of their derivatives have been tested as antibacterial agents.

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Diaminopimelic Acid Decarboxylase in Cells and extracts of *Escherichia coli* and *Aerobacter aerogenes*

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$\alpha\alpha'$ -Diaminopimelic acid is a constituent of many bacteria and blue-green algae, but is absent from all other organisms so far examined. It has been found in certain cellular proteins, in insoluble cell-wall fractions, in the free state and in an extracellular peptide (Work, 1951; Work & Dewey, 1953; Gendre & Lederer, 1952; Holdsworth, 1952; Salton, 1953; Blass, Lecomte & Macheboeuf, 1951; Work, 1949; Pauletta & Defranceschi, 1952; Powell & Strange, 1953). In order to gain some knowledge of the metabolism of diaminopimelic acid, a search was made for a bacterial enzyme which would attack it. The possibility of decarboxylation was first considered, and *Escherichia coli* was the organ-

ism originally chosen since it was known to contain a large number of amino acid decarboxylases (Gale, 1946). Using the acidic conditions of growth and enzyme assay found by Gale to produce optimum decarboxylase activity, no decarboxylation of diaminopimelic acid was obtained. Attention was then directed to the transamination reaction which occurs in an alkaline medium (Feldman & Gunsalus, 1950). Under alkaline conditions, freeze-dried cell suspensions of *Esch. coli* converted diaminopimelic acid into lysine and cadaverine, and it was apparent that decarboxylation, and not transamination, had taken place.

The difference in pH optima for these two successive decarboxylations, and the differential distribution of the two activities in various strains of *Esch. coli* suggested the existence of two enzymes, one decarboxylating diaminopimelic acid and the other decarboxylating lysine (Dewey & Work, 1952a).

This paper describes the enzyme diaminopimelic acid decarboxylase, which converts diaminopimelic acid into lysine and carbon dioxide. The enzyme was first investigated in a strain of *Esch. coli*, but later a strain of *Aerobacter aerogenes* proved to be a richer and more convenient source of the enzyme. The optimum conditions for assay and production of the enzyme in both organisms are described, together with the preparation and properties of cell-free extracts from *Aero. aerogenes*. The work has already been described in brief (Dewey & Work, 1952b; Dewey, Hoare & Work, 1953).

METHODS

Organisms

Escherichia coli. This was a strain from the collection of this Medical School. It was a faecal type, giving the following reactions: methyl red, positive; Voges-Proskauer (reaction for acetoin formation), negative; indole, positive; no growth in citrate.

Aerobacter aerogenes ('strain 1' from the collection of the Medical Research Council Unit for Chemical Microbiology, Cambridge) gave the following reactions: methyl red, negative; Voges-Proskauer, positive; indole, negative; growth in citrate. (These differential coliform tests were carried out as recommended in Report no. 71, 'The Bacteriological Examination of Water Supplies', London: H.M. Stationery Office, 1939).

Growth media

Except where otherwise stated, the following media were used:

Broth contained: proteose peptone (Difco Laboratories Inc., Detroit 1, Michigan), 5 g.; yeast extract (Marmite Food Extract Co. Ltd., London, E.C. 3), 2 g.; Oxoid 'Lab-Lemco' (Oxo Ltd., London, E.C. 4), 1 g.; NaCl, 5 g.; glucose (autoclaved separately), 5 g.; water to 1 l.

Minimal medium 1. This contained: K_2HPO_4 , 7 g.; KH_2PO_4 , 3 g.; trisodium citrate, 0.5 g.; $(NH_4)_2SO_4$, 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.01 g.; $FeSO_4 \cdot 7H_2O$, trace; glucose (autoclaved separately), 2.0 g.; water to 1 l. (final pH 7.4).

Minimal medium 2. This was similar except that the glucose concentration was 20 g./l. and $MgSO_4 \cdot 7H_2O$, 0.05 g./l.

Growth and harvesting of organisms

The organisms were grown either in 160 ml. of medium in Roux bottles, or in 2-3 l. batches in suitable glass bottles aerated either by a slow stream of air or by rapid rotation on inclined rollers. Inocula were 24 hr. cultures grown on an amount of medium representing 1-2.5% of the final volume used. Unless otherwise stated, *Esch. coli* was grown at 37° and harvested after 12.5 hr.; *Aero. aerogenes* was grown at 25° and harvested after 24 hr. The organisms were harvested by centrifuging, washed with water and acetone-dried by the

method of Gale & Epps (1944). The dried cells were stored at -10°.

Assays of enzyme activity

Buffers. All buffers were made with glass-distilled water and unless otherwise stated were 0.1 M mixtures of K_2HPO_4 and KH_2PO_4 . Assays on *Esch. coli* and *Aero. aerogenes* preparations were carried out at pH 7.2 and 6.8 respectively.

Substrates. Natural diaminopimelic acid was prepared from toxin-extracted *Corynebacterium diphtheriae* (Work, 1951), and also from the culture fluid of a lysine auxotroph of *Esch. coli* (Work & Denman, 1953); solutions (0.025 M) in buffers of appropriate pH were used. L-Lysine monohydrochloride (0.033 M) was also dissolved in buffer.

Manometric technique. Decarboxylation was followed manometrically in the Warburg apparatus at 37°; the gas phase was air unless otherwise stated. The main compartment contained either 40 mg. of dried cell preparation suspended in 2 ml. buffer or a cell-free enzyme preparation extracted from 40 mg. of dried cells dissolved in 2 ml. of buffer; 0.1 ml. of an aqueous solution of barium pyridoxal phosphate (0.1 mg./ml.) was also added. Substrate solution (0.5 ml.) was tipped from a side arm after equilibration. The reaction rate was measured from the slope of the linear portion of the plot of gas evolution against time. At pH 6.8 and 7.2 significant amounts of CO_2 were retained in the aqueous phase and were not measured in rate determinations. The enzyme activity was expressed as $Q_{CO_2} = \mu l.$ CO_2 evolved/hr./mg. of dried cells at 37° (not corrected for CO_2 retention). To obtain a quantitative estimation of CO_2 evolution, 0.1 or 0.2 ml. of 8 N- H_2SO_4 was placed in the second side arm and was tipped into the main compartment when the gas evolution had ceased.

Chemical estimations

Total N was measured by the micro-Kjeldahl method. The optical density (E) at 280 m μ , measured in a Beckman spectrophotometer, was used for rough estimations of protein in solution.

Paper chromatography

The enzymic reaction products were examined after deproteinization. When acid had been tipped, the precipitated protein was removed by centrifuging and the solution neutralized with $Ba(OH)_2$; in other cases, 1 vol. of ethanol was added, and the mixture was boiled and centrifuged. Supernatant solutions (equiv. 40-50 $\mu l.$ of reaction mixture) were examined by paper chromatography for ninhydrin-reacting components. Phenol (NH_3 atmosphere) was used as one solvent, and collidine-lutidine (Dent, 1948) or butanol-acetic acid (Campbell, T. S. Work & Mellanby, 1951) was the second solvent used when two-dimensional chromatograms were required.

RESULTS

PROPERTIES OF DIAMINOPIMELIC ACID DECARBOXYLASE IN WHOLE-CELL SUSPENSIONS OF *ESCH. COLI* AND *AERO. AEROGENES*

Products of decarboxylation

Both the organisms under investigation contained lysine decarboxylase when grown on the lysine-

containing nutrient broth. The decarboxylation of diaminopimelic acid was the rate-controlling step; the lysine formed did not accumulate but was rapidly converted into cadaverine by the lysine decarboxylase, which was considerably more active than diaminopimelic acid decarboxylase, even under the neutral conditions of assay. The rate of carbon dioxide evolution was thus proportional to the diaminopimelic acid decarboxylase activity, and 2 moles of carbon dioxide were evolved/mole of diaminopimelic acid decarboxylated. Using a suspension of dried *Esch. coli* grown on broth, the observed gas evolution (498 μ l.) was 89 % of the theoretical amount (560 μ l.) expected from the removal of 2 moles of carbon dioxide from 0.5 ml. of 0.025M diaminopimelic acid. The gas evolved was shown to be carbon dioxide since no volume change occurred when the reaction was carried out in flasks containing 20 % (w/v) potassium hydroxide in the centre wells. The other reaction product, cadaverine, was identified by paper chromatography. Added L-lysine was also quantitatively converted into cadaverine, proving the activity of lysine decarboxylase at pH 6.8; no reaction occurred with D-lysine. *Esch. coli*, when grown on minimal medium 1, had negligible amounts of lysine decarboxylase (Dewey & Work, 1952a), and the gas evolution corresponded to the loss of 1 mole of carbon dioxide/mole of diaminopimelic acid; paper chromatography showed that lysine was the only ninhydrin-reacting end product.

Establishment of optimum conditions for enzyme assay

Using *Esch. coli* grown on broth, acetone drying of the cells was essential in order to obtain maximum diaminopimelic acid decarboxylase activity. These dried cells showed no respiration, and as the rate of carbon dioxide evolution was not changed by substituting air for nitrogen, all assays were carried out with air as the gas phase.

The optimum pH for the decarboxylation of diaminopimelic acid by dried *Aero. aerogenes* was found to be 6.8 and this pH was used in all tests on this organism. Enzyme activity was halved by increasing the buffer strength to M, but was not affected by reducing the concentration below 0.1M.

The rate of decarboxylation of diaminopimelic acid by dried *Esch. coli* grown on broth was doubled by adding either pyridoxal phosphate or a boiled suspension of *Esch. coli*. Different batches of *Aero. aerogenes* grown on minimal medium 1 showed different degrees of activation by pyridoxal phosphate; in some batches there was no measurable effect, in others up to 80 % increase in activity was obtained.

Effect of variation of growth conditions on enzyme activity

The diaminopimelic acid decarboxylase activities of *Esch. coli* and *Aero. aerogenes* were affected differently by the phase of growth at which they were harvested. With *Esch. coli* (Fig. 1) enzyme activity was greatly influenced by growth time, although the maximal activity attainable on a given medium was almost independent of growth temperature. With *Aero. aerogenes* grown at 25° on either type of minimal medium, growth time had little effect on diaminopimelic acid decarboxylase activity (Table 1), but lysine decarboxylase activity increased with duration of growth. Similar results were obtained with cells grown on broth.

Changes in composition of the growth medium were made with the following aims: (a) to raise the concentration of diaminopimelic acid decarboxylase; (b) to reduce the concentration of lysine decarboxylase; (c) to increase the total yield of cells. Replacement of broth by minimal medium had no effect on diaminopimelic acid decarboxylase

Fig. 1. Effect of growth time and growth temperature on diaminopimelic acid decarboxylase activity in *Esch. coli*: ▲, cells grown at 37° on nutrient broth and tested at pH 7.2; cells grown at 18° (×), at 25° (○) and at 30° (●), on a salt-glucose medium (Stephenson, 1949), tested at pH 6.8.

Table 1. *Effect of growth time on decarboxylase activities of Aero. aerogenes*

For conditions, see text.

Medium	Growth time (hr.)	Yield of dried cells (g./l.)	Q_{CO_2} at pH 6.8	
			Diamino-pimelic acid	Lysine
Minimal 1	22	0.42	9.4	—
	24	0.45	9.8	—
	26	0.44	9.4	—
	28	0.42	9.9	—
Minimal 2	15	0.98	6.8	1.5
	24*	1.37	7.8	3.2
	28	1.48	6.4	4.2

* Near end of logarithmic growth.

(Table 2), but virtually eliminated lysine decarboxylase activity in *Esch. coli* (assayed at pH 7.2), and considerably reduced it in *Aero. aerogenes* (assayed at pH 6.8). Diaminopimelic acid decarboxylase activity of both organisms was not changed by the addition of pyridoxin or diaminopimelic acid to the growth medium (Table 2) and in *Aero. aerogenes* it was unaffected by changes in pH between 6.0 and 7.6 (Table 3). *Esch. coli* grown on broth showed a sixfold drop in rate of decarboxylation of diaminopimelic acid with rise in pH from 5.0 to 8.0. As lysine decarboxylase activity was shown by Gale & Epps (1944) to decrease markedly over the same pH range, the apparent decrease in activity of *Esch. coli* may be partly attributed to lysine decarboxylase becoming the rate-limiting factor in the tests made on cells grown at higher pH.

The most effective method of raising the yield of cells without increasing lysine decarboxylase was to increase the glucose concentration of the minimal

medium. This had no effect on diaminopimelic acid decarboxylase, although the figures in Table 3 showed an apparent fall. In effect, this was an unexplained fall in activity which occurred in the cells of the stock culture at the time that the change from minimal medium 1 to 2 was made.

PREPARATION AND FRACTIONATION OF CELL-FREE EXTRACTS CONTAINING DIAMINOPIMELIC ACID DECARBOXYLASE

Extraction of enzyme

No diaminopimelic acid decarboxylase activity was extracted from dried *Esch. coli* after incubation for 2 hr. at 37° with buffers between pH 4 and 8. Disintegration of dried cells in 0.1M phosphate buffer (pH 6.8) produced active extracts which maintained their activity in solution after centrifuging for 40 min. at 25 000 g. The methods of disintegration were: shaking (10 min.) with ballo-

Table 2. *Effect of change in composition of growth medium on diaminopimelic acid decarboxylase activity*

For conditions of tests, see text.

Organism	Control medium	Test medium (wt. of nutrient added/100 ml. medium)	Q_{CO_2} control cells	Q_{CO_2} test cells
<i>Esch. coli</i>	Broth	Broth + diaminopimelic acid (100 mg.)	4.0*	4.0*
	Broth	Broth + pyridoxin (10 mg.)	4.8*	3.9*
	Broth	Minimal 1	3.7*	3.0
<i>Aero. aerogenes</i>	Broth	Minimal 1	22*	9.0
	Minimal 1	Minimal 1; (MgSO ₄ , 7H ₂ O increased to 10 mg.)	9.0	8.0
	Minimal 2	Minimal + pyridoxin (10 mg.)	5.3	6.2
	Minimal 2	Diaminopimelic acid added† (10 mg.)	5.2	5.3

* These figures are twice the Q_{CO_2} for diaminopimelic acid decarboxylase because of the presence of lysine decarboxylase in broth cultures.

† Concentrated mutant medium (Work & Denman, 1953).

Table 3. *Effect of pH and aeration of growth medium on diaminopimelic acid decarboxylase activity*

For conditions, see text.

Organism	Growth conditions	Initial pH	Final pH	Yield of dry cells (g./l.)	Q_{CO_2}
<i>Esch. coli</i>	Buffered broth. Normal aeration	8	8	0.40	1.0
		7	7	0.47	2.1
		6	6	0.40	4.5
		5	5	0.29	6.2
<i>Aero. aerogenes</i>	Minimal medium 1. Normal aeration	7.4	6.7	0.20	9.4
		6.9	6.8	0.24	9.5
		6.6	6.6	0.13	10.2
		6.0	6.4	0.11	9.7
<i>Aero. aerogenes</i>	Minimal medium 2. Normal aeration	7.6	6.0	1.14	6.1
		7.4	5.7	1.25	5.7
		6.9	5.0	0.92	6.7
		6.1	5.0	1.01	6.5
<i>Aero. aerogenes</i>	Minimal medium 2. Poor aeration*	7.4	6.0	0.80	5.4
<i>Aero. aerogenes</i>	Minimal medium 2. Vigorous aeration	7.4	5.2	1.26	5.2

* 250 ml. batches.

tini beads in a Mickle cell-disintegrator (Mickle, 1948); ultrasonic disintegration (Smith & Stumpf, 1946); grinding with an abrasive (Dockstader & Halvorson, 1950). Many of the cell-free extracts were prepared by the last method, using to 1 part of cells, 2 parts of Filter-cel (Johns Manville Co., Ltd., London), and 6 parts of buffer. The mixture was ground by hand for 15 min. in a pestle and mortar. On centrifuging, this gave a supernatant containing 86% of the original diaminopimelic acid decarboxylase activity.

Fractionation of enzyme

Since most of the lysine decarboxylase was extracted with the diaminopimelic acid decarboxylase, it was necessary to find a method of separating the two enzymes before further studies could be made. Separation was studied on cell-free extracts of *Aero. aerogenes* grown on broth so that measurable lysine decarboxylase would be present. Fractionation with inorganic salts was not possible because they readily inactivated diaminopimelic acid decarboxylase (Table 5). Fractional precipitation with acetone at -10° produced no separation. At 0° , the bulk of the lysine decarboxylase was precipitated between 45 and 50% (v/v) acetone, while only 11% of the original diaminopimelic acid decarboxylase was found in this fraction (Table 4).

Fractional extraction with acetone. By making use of the lower solubility of lysine decarboxylase in acetone-buffer mixtures it was possible to extract preferentially diaminopimelic acid decarboxylase from disintegrated cells. By grinding dried *Aero. aerogenes* with abrasive and 0.1M phosphate buffer (pH 6.8) containing 55% (v/v) acetone, centrifuging the extract and adding acetone to 80% (v/v)

to the supernatant, a precipitate was obtained which, after removal of acetone, contained 40% of the original diaminopimelic acid decarboxylase but only 1% of the lysine decarboxylase of the cells. After reducing the lysine decarboxylase content by growing the cells on minimal medium 1, a diaminopimelic acid decarboxylase preparation was obtained containing no demonstrable lysine decarboxylase. However, recovery of diaminopimelic acid decarboxylase from these cells was only 20%; this figure was restored to 40% by reducing the acetone content of the extraction mixture to 48% (v/v). Variation of the pH between 5.7 and 7.2 had no effect on the enzymic activity of the preparation, although the total protein extracted rose with increasing pH. Outside these limits, the activity fell sharply. Therefore, pH 5.8 was chosen for subsequent extractions.

The standard partially purified cell-free preparation of diaminopimelic acid decarboxylase was made in the following manner from *Aero. aerogenes* grown on minimal medium. In a mortar of uniform curvature dried cells (1 g.) were mixed to a thick paste with 5–6 ml. of extraction mixture (acetone, 48 ml.; 0.025M phosphate buffer (pH 5.8), 52 ml.). Filter-cel (1 g.) was added in successive small portions and grinding was continued for 15 min. The contents of the mortar were washed into centrifuge tubes with extraction mixture (overall volume used, 75 ml.), the tubes were covered and centrifuged for 20 min., at 3000 rev./min. using an International Centrifuge size 1 (Gallencamp and Co., London). All these operations were carried out at $+2^{\circ}$. The supernatant liquid (66 ml.) was cooled to -10° and 100 ml. of acetone at -10° were added. After standing for 10 min. the precipitate was collected by centrifuging and freed from acetone *in vacuo* at $+2^{\circ}$. This precipitate was dissolved in 10 ml. of 0.1M buffer (pH 6.8). This solution (1 ml., containing 0.344 mg. N) produced carbon dioxide from diaminopimelic acid at $473 \mu\text{l./hr.}$ Since the original cells contained 6.5% N and had Q_{CO_2} 11.0, the purification of enzyme achieved was 8.1 (based on N content). Total enzyme recovery was 43%.

Table 4. *Relative solubilities of diaminopimelic acid and lysine decarboxylases in mixtures of acetone with 0.1M potassium phosphate buffer (pH 6.8)*

Starting solution; 20 ml. of cell-free buffer extract from 500 mg. of acetone-dried *Aero. aerogenes*; acetone at 0° added slowly with stirring. Solution centrifuged, supernatant solution further fractionated. Solids freed from acetone at 0° and tested for enzyme activity.

Acetone concn. range (% v/v)	Percentage recovery		
	Diaminopimelic acid decarboxylase	Lysine decarboxylase	Protein ($E_{280 \text{ m}\mu}$)
0–35	0	1	3
35–40	0	1.5	2
40–45 *	0	4	3
45–50	11	40	24
50–55	24	3	25
55–60 *	19.5	2.5	11
60–65	5.5	1	8.9
65–70	3.5	0	4

* Brackets indicate separate experiments. Mean figures given in overlap ranges.

Properties of partially purified diaminopimelic acid decarboxylase from *Aero. aerogenes*

The properties of the enzyme were investigated in the standard preparation made as described in the previous section. The amount of enzyme used in each test was 1 ml.

Enzyme characteristics. The effect of change in pH on observed enzyme activity is shown in Fig. 2. The curve has a sharp maximum at pH 6.8, which is similar to that shown by dried-cell suspensions. Since no corrections have been made for increasing retention of carbon dioxide with rise in pH, the curve does not represent the true effect of pH on

Fig. 2. Effect of pH on the activity of diaminopimelic acid decarboxylase in purified extracts of *Aero. aerogenes*.

enzyme activity, but only the observed effect which is a combination of changes in enzyme activity and carbon dioxide retention. Because of the sharpness of the peak, the true optimum would not be far from the observed value.

The retention of carbon dioxide has prevented any calculation of Michaelis constant, but judging from the observed velocities, the substrate concentration giving half-maximum velocity is about 0.0028 M.

A straight-line relationship was found between enzyme concentration and rate of carbon dioxide evolution. The plot of the two functions did not pass through the origin, but cut the ordinate at a point representing 30 μl. CO₂/hr. This may represent the carbon dioxide held by the buffer before saturation is reached.

Addition of pyridoxal phosphate caused variable increases in activity of different preparations, the magnitude of the effect being similar to that observed with the suspension of the particular batch of cells from which the enzyme preparation was made. Addition of boiled cell suspensions of dried *Aero. aerogenes* had no effect on enzyme activity in the presence of added pyridoxal phosphate. The chelating agent 'Versene' (ethylenediaminetetraacetic acid) was without effect on enzyme activity, suggesting that no metal cofactor is required.

Partial irreversible inactivation occurred on dialysis. This could be prevented by the addition of 2:3-dimercaptopropanol ($M \times 10^{-3}$) to the dialysis liquid. No dissociation of pyridoxal phosphate resulted from dialysis.

Table 5 shows the effect of various salts and enzyme inhibitors on decarboxylase activity. The most potent inhibitors were cupric sulphate and *p*-chloromercuribenzoate. Because of the susceptibility to copper, the enzyme was at times inhibited by water from the laboratory copper still, necessitating the use of glass-distilled water throughout. The inhibition caused by *p*-chloromercuribenzoate was reversed to the extent of 70 % by adding glutathione 5 min. after mixing enzyme and inhibitor (Table 6). No inhibition was obtained with glycine, DL-alanine, DL-valine, DL-isoleucine, or DL-aspartic acid when present in concentrations four times that of the substrate. High concentrations of DL-glutamic acid inhibited the enzyme; the degree of inhibition was dependent on the molar ratio of glutamic acid to diaminopimelic acid (a ratio of 10/1 gave 50 % inhibition). For tests on inhibition by homologues of diaminopimelic acid see Table 7.

Specificity. Diaminopimelic acid decarboxylase is highly specific for the naturally occurring form of substrate (Table 7). The mean value (263 μl.) for eleven estimations of carbon dioxide evolution from 0.005 m-moles of natural diaminopimelic acid was 94 % of the theoretical. Various unfractionated samples of synthetic diaminopimelic acid gave

Table 5. *Action of inhibitors on partially purified diaminopimelic acid decarboxylase*

For conditions of tests, see text.

Inhibitor	Molar concentration giving 50 % inhibition
Ammonium sulphate	2.5×10^{-1}
Magnesium sulphate	1.0×10^{-1}
Copper sulphate	3.0×10^{-5}
Potassium cyanide*	4.2×10^{-4}
Semicarbazide	1.2×10^{-4}
Iodoacetamide	2.0×10^{-3}
Iodosobenzoate	1.4×10^{-4}
<i>p</i> -Chloromercuribenzoate†	5.0×10^{-5}
<i>p</i> -Arsinosobenzamide	1.0×10^{-3}

* Reversed by dialysis.

† Reversed by glutathione (see Table 6).

Table 6. *Reversal by glutathione of p-chloromercuribenzoate inhibition of diaminopimelic acid decarboxylase*

0.3 ml. of 0.1 M glutathione added to one of two duplicate Warburg flasks 5 min. after adding the inhibitor. The resulting enzyme activity is expressed as % of control.

<i>p</i> -Chloromercuribenzoate concentration (M)	% Enzyme activity present	
	No glutathione	Glutathione added
Nil	100	95
3.75×10^{-5}	68	72
1.25×10^{-4}	4	74
3.75×10^{-4}	0	70

carbon dioxide output 45–48 % of theoretical and the reaction mixture contained lysine and unchanged diaminopimelic acid. The enzyme had no effect on any of the common naturally occurring amino acids. Several members of the homologous series of diaminodicarboxylic acids and related substances were also tested with negative results.

Use of diaminopimelic acid decarboxylase for the identification of diaminopimelic acid in natural materials

Diaminopimelic acid could not be estimated enzymically in acid hydrolysates of micro-organisms owing to enzyme inhibition by constituents of the acid hydrolysate, even after complete removal of mineral acid by ion-exchange resins or trioctylamine

(Hughes & Williamson, 1951). After fractionation in an electrodialysis cell (Work & Dewey, 1953), diaminopimelic acid in the neutral fraction was completely decarboxylated as shown by examination of the reaction products by paper chromatography. Under the conditions used, there was a variable loss of diaminopimelic acid to the cathode compartment, therefore the true content of diaminopimelic acid could not be estimated. Some figures obtained with a few organisms are given in Table 8; they are not corrected for cathode losses and are thus only minimal values.

The figures on the *Streptomyces* sp. are included since the organism was shown by paper chromatography to contain an unidentified amino acid differing only slightly from diaminopimelic acid in

Table 7. *Specificity of diaminopimelic acid decarboxylase for structural analogues of diaminopimelic acid and the effect of analogues as inhibitors of the enzyme*

Inhibition experiments were conducted with analogue and diaminopimelic acid at equimolar concentrations.

Compounds of the type	Source	Decarboxylation (%)	Inhibition (%)
$\text{HOOC} \cdot \text{CH}(\text{NH}_2) \cdot (\text{CH}_2)_x \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$			
$x=0$. Diaminosuccinic acid	Synthetic	0	0
$x=1$. Diaminoglutaric acid	Synthetic	0	0
$x=2$. Diaminoadipic acid	Synthetic ¹	0	0
$x=3$. Diaminopimelic acid	Synthetic ¹	45–48 (mean 46)	—
	<i>C. diphtheriae</i> ²	90–98 (mean 94)	—
	<i>Esch. coli</i> (mutant medium) ³	85–95 (mean 90)	—
$x=4$. Diaminosuberlic acid	Synthetic ¹	0	0
$x=5$. Diaminoazelaic acid	Synthetic ¹	0	0
$x=6$. Diaminosebacic acid	Synthetic ¹	0	0
$x=8$. 1:10-Diaminodecan-1:10-dicarboxylic acid	Synthetic ¹	0	0
$x=10$. 1:12-Diaminododecan-1:12-dicarboxylic acid	Synthetic ¹	0	0
Other compounds			
ϵ -C-Methyllysine	Synthetic ⁴	0	0
$\alpha\epsilon$ -Diamino- β -hydroxypimelic acid	<i>Pseudomonas tabaci</i> toxin ⁵	0	15
Lanthionine	Wool ⁶	0	0
meso-Lanthionine	Subtilin ⁷	0	0
Pyridine-2:6-dicarboxylic acid (dipicolinic acid)	<i>B. megatherium</i>	0	45
	germinating spores ⁸		
'Cysteine sulphinic acid'	L-Cystine ⁹	0	0

¹ Simmonds, 1954.

² Work, 1951.

³ Work & Denman, 1953.

⁴ McLaren & Knight, 1951.

⁵ Woolley, Schaffner & Braun, 1952.

⁶ Horn, Jones & Ringel, 1941.

⁷ Alderton & Fevold, 1951.

⁸ Powell, 1953.

⁹ Lavine, 1936.

Table 8. *Diaminopimelic acid content of the neutral fraction obtained in electrodialysis cell from hydrolysates of micro-organisms, estimated by diaminopimelic acid decarboxylase*

Organism	Amount tested (equiv. dry wt. of cells in mg.)	Diaminopimelic acid	
		mg. found assuming 94 % decarboxylation	g./100 g. dry wt. of organism
<i>Corynebacterium diphtheriae</i>	80	0.40	0.50
<i>C. diphtheriae</i>	100	0.53	0.53
<i>C. diphtheriae</i>	160	0.70	0.45
<i>Brucella abortus</i>	100	0.54	0.54
<i>Shigella dysenteriae</i>	100	0.19	0.19
<i>Rhodopseudomonas palustris</i>	180	0.55	0.31
<i>Streptomyces</i> sp.*	200	0	0
<i>Streptomyces</i> sp.* + 1.2 mg. diaminopimelic acid	200	1.2	0

* See text.

its chromatographic properties. This amino acid was not decarboxylated and was subsequently isolated and identified as a branched-chain higher homologue of diaminopimelic acid (Work, 1953).

DISCUSSION

The estimation of diaminopimelic acid decarboxylase activity in micro-organisms is complicated by two factors. First, the retention of carbon dioxide in the neutral buffer prevented a true measure of enzyme activity from the rate of gas evolution. Because of the variable proportions of carbon dioxide dissolved during the course of the reaction, no correction was made for this retention. The second difficulty in measuring the activity in whole cells is the presence of lysine decarboxylase. When lysine is rapidly decarboxylated, diaminopimelic acid decarboxylation becomes the rate-limiting step; but where the lysine decarboxylase was not very active, there was no method of assessing the meaning of the Q_{CO_2} in terms of rate of decarboxylation of diaminopimelic acid.

Preparation of purified cell-free extracts of diaminopimelic acid decarboxylase has been rendered difficult by the low enzyme activity in the bacteria examined. By comparison with lysine decarboxylase, where Gale (1946) reports $Q_{CO_2} > 400$, the values of 10 or less found for diaminopimelic acid decarboxylase are negligible. The change from *Esch. coli* to *Aero. aerogenes* as a source of enzyme had many advantages. The enzyme in the latter organism was not only more active; it also had a slightly lower pH optimum, which decreased carbon dioxide retention in buffer. In *Aero. aerogenes* the time of harvesting was without effect on enzyme activity, while with *Esch. coli* growth time was so critical that, in order to obtain reproducible results, a machine had to be constructed to give a uniform inoculum at a specified time (Dewey & Wallace, 1953).

The diaminopimelic acid decarboxylase content of *Aero. aerogenes* was independent of all changes made in the growth medium. It is thus likely that this enzyme is part of the constitutive equipment of the cell and is not an adaptive enzyme as are the six bacterial amino acid decarboxylases described by Gale (1946). The products of decarboxylation of these six amino acids are mostly bases, without known major metabolic function; they are probably by-products formed as an adaptive defence mechanism of the cell against acidic environments (Gale, 1946). On the other hand, the product of decarboxylation of diaminopimelic acid is the 'essential' amino acid lysine. Davis (1952) has shown that in *Esch. coli* (ATCC 9637) diaminopimelic acid is a precursor of lysine; here the enzyme responsible for the conversion is diaminopimelic acid

decarboxylase, since it is missing from certain lysine auxotrophs of *Esch. coli* that accumulate diaminopimelic acid in their culture media (Dewey & Work, 1952b). The activity of diaminopimelic acid decarboxylase at neutrality and its presence in the cell at early phases of growth also distinguish it from the adaptive amino acid decarboxylases, which have acid pH optima and are formed towards the end of cell division. King (1953) has reported that valine and leucine are decarboxylated at pH 7 by *Proteus vulgaris* grown in the presence of either one of these amino acids. It is thus evident that both the neutral and the polar amino acids are susceptible to decarboxylation by bacteria, and that certain bacterial amino acid decarboxylases will operate at neutral reaction.

Although rigorous proof of the requirement for pyridoxal phosphate has not been obtained, the activation of the enzyme by pyridoxal phosphate and the reversible inhibition by cyanide suggest that this substance is the coenzyme. The studies on inhibition indicate that diaminopimelic acid decarboxylase is a very sensitive SH enzyme.

The high specificity of the enzyme for the natural substrate is similar to that of the other bacterial amino acid decarboxylases; the true optical specificity has not yet been investigated, as the three diastereo isomers of diaminopimelic acid are not yet available. Only half of the synthetic diaminopimelic acid was attacked, and the reaction mixture contained lysine and unchanged diaminopimelic acid; this indicates that the enzyme has a definite optical specificity. The lysine formed from decarboxylation of both natural and synthetic diaminopimelic acid was the L-form, since it was further decarboxylated to cadaverine by the bacterial lysine decarboxylase which was specific for L-lysine and unable to attack the D-form.

The use of diaminopimelic acid decarboxylase as an analytical tool for the estimation of diaminopimelic acid has been prevented by the easy inhibition of the enzyme, although, when a suitable means for quantitative removal of inhibitors is found, the enzymic method of assay should be applicable to biological materials. However, the method has already been of use in checking chromatographic evidence for the presence of diaminopimelic acid in micro-organisms (Work & Dewey, 1953) and, because of its specificity, it has helped in the identification of a naturally occurring homologue of diaminopimelic acid (Work, 1953).

SUMMARY

1. An enzyme decarboxylating diaminopimelic acid has been studied in single strains of *Escherichia coli* and of *Aerobacter aerogenes*.

2. The reaction products are L-lysine and carbon dioxide.

3. Changes in growth conditions indicated that the enzymic activity of the bacteria was unaffected by pH, composition or degree of aeration of the growth medium. In *Aero. aerogenes*, but not in *Esch. coli* it was also unaffected by phase of growth.

4. The pH optimum was 7.2 for *Esch. coli* and 6.8 for *Aero. aerogenes*.

5. Partially purified cell-free extracts of enzyme were obtained from *Aero. aerogenes* by fractional acetone extraction. The properties of the enzyme in such extracts were investigated.

6. Pyridoxal phosphate was required for maximum activity, and cyanide caused inhibition, which was reversed by dialysis.

7. The enzyme was strongly inhibited by copper and other SH reactive inhibitors. Glutathione reversed *p*-chloromercuribenzoate inhibition.

8. The enzyme was highly specific for natural diaminopimelic acid. Of those tested, no other naturally occurring amino acid or natural or synthetic homologue of diaminopimelic acid was decarboxylated. Synthetic diaminopimelic acid was decarboxylated to half the extent for the natural product.

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