the apo-enzyme is obtained with an amount of codecarboxylase corresponding to 0.06 μ g. C/ml. Conversion of these values into molecular concentrations must await the final purification and characterization of the coenzyme molecule. The shape of the velocity-coenzyme concentration curve would suggest that the non-protein moiety of the enzyme behaves as a coenzyme rather than as a prosthetic group.

SUMMARY

1. The lysine decarboxylase activity of coliform organisms grown in 2 % glucose-casein-digest-broth withstands drying of the organisms in acetone.

2. The enzyme can be extracted from the acetone-dried organisms by incubation with borate buffer at pH 8.5.

3. The partial purification of the enzyme is described; when *Bact. cadaveris* is used as a source, the preparation is specific for *l*(+)-lysine.

4. The enzyme is optimally active at pH 6.0, has a Michaelis constant of approximately 0.0015M and

will attack only *l*(+)-lysine, and possibly hydroxy-lysine, among the common amino-acids. The integrity of both amino groups in the lysine molecule is essential for activity.

5. The decarboxylation is quantitative and the product is cadaverine.

6. The enzyme can be resolved into specific apo-enzyme and a hitherto undescribed codecarboxylase by repeated precipitation with ammoniacal ammonium sulphate (66 % saturation).

7. The apo-enzyme can be reactivated by codecarboxylase preparations but by no other coenzyme or prosthetic group.

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REFERENCES

- Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
 Gale, E. F. (1940). *Biochem. J.* **34**, 392, 846, 853.
 — (1941). *Biochem. J.* **35**, 66.
 — & Epps, H. M. R. (1942). *Biochem. J.* **36**, 600.
 — (1943). *Nature, Lond.*, **152**, 327.
 — (1944). *Biochem. J.* **38**, 250.
 Neuberger, A. & Sanger, F. (1944). *Biochem. J.* **38**, 125.
 Stotz, E., Harter, C. J. & King, C. G. (1937). *J. biol. Chem.* **119**, 511.
 Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.
 Woods, D. D. & Clifton, C. E. (1937). *Biochem. J.* **31**, 1774.

Studies on Bacterial Amino-acid Decarboxylases

2. *l*(-)-TYROSINE DECARBOXYLASE FROM *STREPTOCOCCUS FAECALIS*

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This paper deals with the isolation, partial purification and some of the properties of *l*(-)-tyrosine decarboxylase from *Streptococcus faecalis*. Gale (1940b) showed that *l*(-)-tyrosine is the only one of 16 naturally occurring amino-acids to be decarboxylated by this organism, although it was found later that *l*-3:4-dihydroxyphenylalanine is also decarboxylated (Gale, personal communication). In the present paper an attempt is made to discover whether *l*-3:4-dihydroxyphenylalanine acts as substrate for *l*(-)-tyrosine decarboxylase or whether a separate enzyme is involved.

METHODS

Organisms. Of the faecal streptococci used in this study, one strain was isolated from human stools by Dr Crowley

of the Cambridge University Pathology Department; one by Dr Cruickshank of the Group Laboratory, the North-Western Hospital; one was the laboratory stock strain; the rest were isolated by the author.

Substrates. The *l*(-)-tyrosine used for the quantitative experiments was prepared by Merck and Co.; for all other experiments it was isolated from a tryptic digest of casein and thrice recrystallized: *l*(-) and *dl*-3:4-dihydroxyphenylalanine were supplied by Roche Products Ltd. The purification of the enzyme was worked out with *l*(-)-tyrosine as substrate, but in some cases parallel experiments were made with *l*-3:4-dihydroxyphenylalanine. The enzyme is referred to as *l*(-)-tyrosine decarboxylase throughout; data supporting the theory that a single enzyme is responsible for the decarboxylation of both these amino-acids are presented later.

Method of estimating and expressing enzyme activity. The activity of the enzyme preparations was estimated as for

l(+)-lysine decarboxylase (Gale & Epps, 1944a) in Warburg manometers. Unless otherwise stated, the reaction temperature was 30°, the gas phase air, the buffer 2 ml. M/5 acetate pH 5.5 and the substrate (in side-bulb) 0.5 ml. M/30 *l*(-)-tyrosine suspension or 0.5 ml. M/15 *l*-3:4-dihydroxyphenylalanine solution. Precautions were taken against progressive dissociation of the enzyme (see below) by addition to the main compartment of the manometer cup of excess (0.2 ml.) of a purified preparation of co-decarboxylase.

A unit of enzyme activity is arbitrarily defined as that amount which will liberate 100 μ l. CO₂/5 min. from the appropriate substrate under the conditions outlined above. The activity is given as $Q_{CO_2}^C = \mu$ l. CO₂ evolved/mg. C preparation/hr.; the rates were calculated from the CO₂ output measured at 5 min. intervals during the first 15 min. after tipping the substrate into the main reaction vessel, and C was estimated by the micro-method of Van Slyke & Folch (1940).

1. PRELIMINARY WORK ON THE PURIFICATION OF THE ENZYME

Growth of the organism and preparation of a cell-free extract. For this preliminary work the cultures were grown at 37° for 15 hr. in broth consisting of a tryptic digest of casein to which marmite (0.1%) and glucose (1%) had been added. As in the preparation of *l*(+)-lysine decarboxylase, the bacteria were centrifuged off, washed once and made into a thick suspension with distilled water. The suspension was cooled in ice and stirred into 10 vol. ice-cold acetone. After half an hour the precipitate was filtered off on a small Büchner funnel and washed successively with acetone, a mixture of acetone and ether in equal parts and, finally, ether. When prepared under these conditions the enzyme content of the acetone powder is apparently greater than that of the original washed suspension (Table 1); this apparent increase may be compared with that observed by Davies (1943) during extraction of acetoacetic acid decarboxylase from acetone powders of *Cl. acetobutylicum*.

l(-)-Tyrosine decarboxylase can be extracted from the acetone powder by incubation with buffer at 37°, the optimum pH being 5.5. In the preparation of *l*(+)-lysine decarboxylase from coliform organisms, extraction was favoured by a growth time of 22–24 hr., but for *l*(-)-tyrosine decarboxylase equally good results were obtained with growth periods of 12–21 hr. The quantitative nature of the process varies widely with the particular organism used in the preparation of the acetone powder. From eight strains of faecal streptococci tested the amount of enzyme going into solution varied between 20–95% of the total; this appears to be due to strain differences and for further work an organism was chosen (*Strep. faecalis* Dunn) which gives consistent extraction of the order of 95%. Incubation of the powder at 37° for 18–21 hr. results

in optimal extraction of the enzyme without appreciable loss in activity, while incubation at room temperature for the same period is only half as effective. The rate of extraction of the enzyme is independent of the concentration of acetone powder between 10 and 50 mg./ml., but more concentrated suspensions do not centrifuge cleanly. The final conditions adopted were as follows: the powder (20 mg./ml.) was suspended in M/45 phosphate pH 5.5 and incubated at 37° for 21 hr., after which the suspension was centrifuged and the supernatant collected for further purification.

Adsorption of the enzyme. Acid-ethanol precipitation was found to be a useful step in the purification of *l*(+)-lysine decarboxylase. In the present case precipitation occurs if the enzyme is adjusted to pH 5.0 or less in the presence of 50% ethanol, but less than 22% of the *l*(-)-tyrosine decarboxylase activity is found in the precipitate and this treatment was abandoned. Adsorption will take place on to C_γ alumina; at pH 5.0, 72% of the enzyme is adsorbed but the remainder is apparently destroyed; elution cannot be effected by buffers over the range pH 4–10, by (NH₄)₂SO₄ solutions up to 20% saturation, or by phosphate solutions up to 2M at pH 7.0. The adsorbed enzyme loses 75% of its activity while standing at room temperature for an hour. Neither fuller's earth nor charcoal adsorb the enzyme under acid, neutral or alkaline conditions, and the maximum adsorption with kaolin was 20%.

Fig. 1. Variation of adsorption on to Ca₃(PO₄)₂ with pH and quantity of adsorbent used. ●—● Variation with pH: 1 ml. enzyme extract adjusted to the required pH and excess Ca₃(PO₄)₂ suspension added: portions of precipitate taken for estimation. ▲—▲ Variation with quantity of adsorbent: 1 ml. enzyme extract + 0.2 ml. 2 M-acetate pH 5.0; different amounts Ca₃(PO₄)₂ suspension (30 mg./ml.) added and portions of precipitate taken for estimation. Volume of Ca₃(PO₄)₂ expressed as % (by vol.) of original extract.

Ca₃(PO₄)₂ was found to be a suitable adsorbent and Fig. 1 shows variation of adsorption with pH and with amount of Ca₃(PO₄)₂ added. The final conditions adopted consisted of addition to the extract of 1/5 vol. of 2M-acetate at pH 5.0 followed

by 1/5 vol. of neutral $\text{Ca}_3(\text{PO}_4)_2$ suspension (30 mg./ml.). The enzyme is best eluted with a solution of $(\text{NH}_4)_2\text{SO}_4$; buffer solutions of range pH 4–10 and solutions of Na_2SO_4 were unsatisfactory. The optimal eluent is 1 ml. 50 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution at pH 7.0/20 mg. $\text{Ca}_3(\text{PO}_4)_2$; elution was equally efficient at room temperature or at 37° over a period of 10 or 30 min.

Fractionation with ammonium sulphate. The protein is precipitated from the eluate by full saturation with $(\text{NH}_4)_2\text{SO}_4$. Owing to the small volume, this is best done by the addition of an equal volume of 50 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution followed by 40 g. solid $(\text{NH}_4)_2\text{SO}_4$ /100 ml. of the final solution, the resulting suspension being centrifuged at 3000 r.p.m. for 30 min. The precipitate is dissolved in distilled water and fractionated twice with saturated $(\text{NH}_4)_2\text{SO}_4$ solution, as below; further fractionation was abandoned since it led to even distribution of the enzyme among the fractions without significant increase in activity.

Repetition of adsorption on to $\text{Ca}_3(\text{PO}_4)_2$ and elution with $(\text{NH}_4)_2\text{SO}_4$ solution. After fractionation the enzyme can again be adsorbed on to $\text{Ca}_3(\text{PO}_4)_2$; the pH conditions are similar to those given above and the amount of adsorbent and eluent were related to the number of units remaining in the preparation. Optimal conditions are 75 mg. $\text{Ca}_3(\text{PO}_4)_2$ /10 units enzyme for adsorption and 1 ml. 25 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution/15 mg. $\text{Ca}_3(\text{PO}_4)_2$ for elution.

2. FINAL CONDITIONS FOR PURIFICATION

The strain of organism used in this preparation was *Strep. faecalis* Dunn*; Table 1 gives the volume, enzyme activity and C content at each stage of the purification.

4.5 l. of medium were inoculated, and incubated at 37° for 16 hr.; the culture was passed through a Sharples supercentrifuge and the bacteria washed

* Deposited in National Collection of Type Cultures: No. 6783.

and made into a thick suspension with distilled water (stage 1). The suspension was cooled in ice and stirred into 10 vol. of ice-cold acetone. After half an hour the precipitate was filtered off on a small Büchner funnel and washed successively with acetone, a mixture of acetone and ether in equal parts, and ether; it was then spread on a glass dish to dry.

The dry powder (20 mg./ml.) was suspended in M/45 phosphate pH 5.5 (stage 2 a) and incubated at 37° for 21 hr. At the end of this period (stage 2 b) the suspension was centrifuged at 3000 r.p.m. for 15 min. and the supernatant collected (stage 3). The pH of the extract was adjusted by addition of 2M-acetate pH 5.0 (1 ml. buffer to 5 ml. extract), and 3 ml. $\text{Ca}_3(\text{PO}_4)_2$ at pH 7.0 were added/10 ml. original extract; after 10 min. the precipitate was spun off and the supernatant discarded. The $\text{Ca}_3(\text{PO}_4)_2$ precipitate was washed once with distilled water before the enzyme was eluted with 50 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution; the elution was carried out in two stages, the volume of eluate used each time being 1 ml./20 mg. of $\text{Ca}_3(\text{PO}_4)_2$ used for adsorption and the suspension being left to stand for 10 min. before centrifuging. The combined eluates were diluted with an equal volume of 50 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution (stage 4); 40 g. solid $(\text{NH}_4)_2\text{SO}_4$ were added/100 ml. of preparation and the suspension was centrifuged at 3000 r.p.m. for 30 min.

The precipitate was dissolved in distilled water (stage 5) and sufficient saturated $(\text{NH}_4)_2\text{SO}_4$ solution added to bring to 50 % saturation (6 a). The precipitate was centrifuged off and the supernatant brought to 65 % (6 b) and then to 75 % saturation (6 c). The middle fraction (6 b) was again precipitated at 52, 58 and 63 % $(\text{NH}_4)_2\text{SO}_4$ saturation, and the precipitates were dissolved in water (stages 7 a, b and c).

The solution at stage 7 b was opalescent and faintly yellow; its pH was then adjusted by the

Table 1. Purification of l(–)-tyrosine decarboxylase (from *Strep. faecalis* Dunn)

Stage	Volume (ml.)	Activity towards tyrosine (μl./ml./5 min.)	Units	Dry wt. (mg. C./ml.)	$Q_{\text{CO}_2}^c$	Activity towards dihydroxy-phenylalanine (μl./ml./5 min.)	Activity ratio: tyrosine/dihydroxy-phenylalanine
1	28.5	1080	306	32.5	400	740	1.46
2 (a)	98	690	672	10.1	—	—	—
(b)	—	672	655	—	800	450	1.47
3	92	635	585	4.5	1,690	415	1.53
4	27.5	1570	446	3.63	5,040	1080	1.45
5	20	1720	344	3.30	6,220	1020	1.69
6 (a) 0–50 %	10	190	19	—	—	140	1.36
(b) 50–63 %	10	2600	260	1.70	18,400	1490	1.75
(c) 63–75 %	5	935	47	—	—	575	1.63
7 (a) 0–53 %	5	1590	79.5	2.42	7,900	905	1.75
(b) 53–58 %	5	2270	113.5	0.595	45,700	1410	1.61
(c) 58–63 %	5	765	37	0.67	13,700	475	1.61
8	4.5	1510	67	0.392	46,300	910	1.65

addition of 1/5 vol. 2M-acetate pH 5.0, and $\text{Ca}_3(\text{PO}_4)_2$ added, 75 mg./10 units of enzyme. After 10 min. the precipitate was centrifuged off and washed with distilled water. The enzyme was eluted by adding 1 ml. 25% saturated $(\text{NH}_4)_2\text{SO}_4$ solution/15 mg. of $\text{Ca}_3(\text{PO}_4)_2$ used for adsorption; after 10 min. the suspension was centrifuged and the supernatant (stage 8) collected. In this particular preparation the fractionation at stage 7 had been unusually successful and the final adsorption and elution did not result in a significant increase in enzyme activity, although the solution at stage 8 was water-clear. The final preparation (from 4.5 l. medium) contained 1.76 mg. C (c. 3.5 mg. protein) and the degree of purification was some 100-fold.

Ultra-violet adsorption of the enzyme preparation. A solution containing 0.267 mg. C/ml. (stage 8) in 25% saturated $(\text{NH}_4)_2\text{SO}_4$ showed a typical protein ultra-violet adsorption curve with an extinction $\log_{10} I_0/I$ at 270 $m\mu$ = 1.6, when $d = 2$ cm.

3. PROPERTIES OF *l*(-)-TYROSINE DECARBOXYLASE

Source of enzyme. The enzyme preparations used in these studies were stages 8 or 7b of various preparations from *Strep. faecalis* Dunn.

Variation of activity with pH and the nature of the buffer system. The optimum pH for the enzyme is 5.5 in acetate, phosphate or phthalate buffers, although the activity varies according to the buffer system used. pH curves for *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine in acetate and phosphate buffers are shown in Fig. 2. Discrepancies because of variation of the solubility of tyrosine with pH were avoided by keeping the tyrosine concentration that of a saturated solution at pH 5.5 (the iso-electric point) at the temperature used. The manometers were set up with 1 ml. substrate and 1.5 ml. buffer in the main compartment and 0.2 ml. enzyme, 0.1 ml. codecarboxylase preparation and 0.2 ml. buffer in the side-bulb. The activity of the enzyme at pH 5.5 was also measured in citrate and phthalate buffers (Table 2). The pH curve for

cell was also observed by Davies (1943) for acetoacetic acid decarboxylase from *Cl. acetobutylicum* and by us for *l*(+)-lysine decarboxylase from *Esch. coli* and *Bact. cadaveris* (Gale & Epps, 1944a).

Fig. 2. Variation of enzyme activity with pH. ●—● *l*(-)-tyrosine as substrate, M/5 acetate buffer. ○—○ *l*(-)-tyrosine as substrate, M/15 phosphate buffer. ▲—▲ *l*-3:4-dihydroxyphenylalanine as substrate, M/5 acetate buffer. △—△ *l*-3:4-dihydroxyphenylalanine as substrate, M/15 phosphate buffer.

Fig. 3. Variation of enzyme activity with substrate concentration. ●—● *l*(-)-tyrosine as substrate. ○—○ *l*-3:4-dihydroxyphenylalanine as substrate (different enzyme preparation).

Table 2. Variation of enzyme activity at pH 5.5 with nature of buffer system

Buffer system	pH	$\mu\text{l. CO}_2/5 \text{ min.}$
M/5 acetate	5.51	124
M/5 citrate	5.48	107
M/15 phosphate	5.48	98
M/20 phthalate	5.48	77

the intact organism is of similar shape (Gale, 1940b), although the optimum varies between pH 5.0 and 5.5, whereas the optimum for enzyme preparations at all stages was 5.5. This apparent shift of the optimum pH on extraction of the enzyme from the

Effect of substrate concentration. The variation of enzyme activity with substrate concentration is shown in Fig. 3. The greatest concentration of *l*(-)-tyrosine (0.0025M) is that of a saturated solution at pH 5.5 and 30°; in the presence of excess undissolved *l*(-)-tyrosine the rate of CO_2 production is further increased by c. 13%, but this increase must depend on other factors than enzyme-substrate affinity. The Michaelis constant of such a reaction cannot be calculated from the substrate concentration curve. For *l*-3:4-dihydroxyphenylalanine the enzyme gives a normal affinity curve, the value of the Michaelis constant (the half-saturation point) being 0.0023M.

Effect of reaction temperature. Table 3 shows the activity of the enzyme preparation at different temperatures; in order to avoid anomalies due to variation in the solubility of tyrosine with temperature the substrate consisted of 1 ml. saturated

Table 3. *Variation of enzyme activity with temperature*

Temp. (°)	Activity towards tyrosine (μ l./ml./5 min.)	Activity towards dihydroxy- phenylalanine	Activity ratio: tyrosine dihydroxy- phenylalanine
16.5	25.5	16.5	1.55
25	50	28.5	1.75
31	69	40	1.73
38	70	41	1.71

solution of *l*(-)-tyrosine at pH 5.5 and 16° (manometers set up as for Fig. 2). It appears that the activity does not increase significantly with a rise of temperature above 30°; this suggests that partial inactivation of the enzyme is taking place although the acetone powder is unaffected by prolonged incubation at 37° in the absence of substrate (Table 1) and the activity of the intact organism increases up to 44° (Gale, 1940b).

Fig. 4. Variation of activity with enzyme concentration. ●—● *l*(-)-tyrosine as substrate. ○—○ *l*-3:4-dihydroxyphenylalanine.

Effect of enzyme concentration. The rate of CO₂ production from both substrates is directly pro-

portional to the amount of enzyme used over the range included in manometric tests (Fig. 4).

Quantitative nature of the reaction and stereospecificity of the enzyme. Manometers were set up containing 0.5 ml. *m*/30 solution (or suspension) of the appropriate substrate and the course of the reaction followed. Since pH 5.5 is near the limit of effective buffering power of acetate, *m*/5 citrate was used in these experiments. The results are tabulated in Table 4 from which it appears that decarboxylation is quantitative and that the enzyme is specific for the *laevo*-isomers.

Isolation of reaction products. A Krebs's vessel incubated at 30° contained 30 ml. *m*/60 *l*(-)-tyrosine suspension, 30 ml. *m*/5 acetate pH 5.5 and 3 ml. enzyme preparation. The course of the reaction was followed in a control manometric experiment until gas evolution ceased (c. 90 min.); at the end of this period the contents of the Krebs's vessel were deproteinized with ferric sulphate, acidified to pH 1, concentrated *in vacuo* to a small volume and treated with benzoyl chloride as described by Gale (1940b). The resulting dibenzoyltyramine was twice recrystallized from ethanol. The product (120 mg.) melted at 170–171° (corrected) and gave on analysis (Weiler) C, 75.87; H, 5.54; N, 3.97%. Calc. for C₂₂H₁₉NO₃: C, 76.50; H, 5.54; N, 4.06%.

In another experiment, 30 ml. *m*/30 *l*-3:4-dihydroxyphenylalanine solution replaced the *l*(-)-tyrosine and 6 ml. enzyme preparation were used. At the end of the reaction period zinc hydroxide was used as a deproteinizing agent, since here boiling the solution with ferric sulphate led to formation of coloured oxidation products. The deproteinized solution was benzoylated with sufficient benzoyl chloride to give the tribenzoyl derivative. The tribenzoyldihydroxyphenylethylamine was thrice recrystallized from ethanol. Yield 94 mg., m.p. 140–141° (corrected). Analysis (Weiler), C, 78.20; H, 5.28; N, 3.28%. Calc. for C₂₉H₂₃NO₅: C, 79.50; H, 5.40; N, 3.44%.

Specificity, inhibition and denaturation. It has already been stated that *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine are the only naturally occurring amino-acids to be decarboxylated by *Strep. faecalis*; the following compounds chemically related to tyrosine were tested as substrates for the enzyme preparation and as inhibitors of its reaction

Table 4. *Quantitative nature of decarboxylation of l(-)-tyrosine and l-3:4-dihydroxyphenylalanine and stereospecificity of enzyme preparation*

Results refer to total CO₂ output in μ l. after subtraction of the blank (0.5 ml. *m*/30 substrate = 373 μ l. CO₂).

Substrate	No. of experiments	Mean	Standard deviation	Range	% of calc.
<i>l</i> (-)-Tyrosine	9	357	11	343–372	96
<i>l</i> -3:4-Dihydroxyphenylalanine	5	148	3	343–353	94.5
<i>dl</i> -Tyrosine	11	174	9	172–192	47
<i>dl</i> -3:4-Dihydroxyphenylalanine	5	171	4	167–177	46

with *l*(-)-tyrosine: *l*-phenylalanine, *l*-3:4-dihydroxyphenylalanine, *l*-diiodotyrosine, *l*-tyrosine sulphonic acid, *N*-methyl-*dl*-tyrosine, methoxy-*l*-tyrosine, *N*-methyl-methoxy-*l*-tyrosine, *l*-thyroxine, *l*-thyronine (desiodothyroxine) and glycyl-tyrosine. *l*-3:4-Dihydroxyphenylalanine was attacked by the preparation; none of the others had any action either as substrate or inhibitor.

Table 5 shows the effect of general inhibitors; in those cases where an effect was observed, dilution below the molar concentrations whose negative

test; nitrite was removed by addition of excess of a concentrated urea solution which itself had no inhibitory effect on the enzyme.

Dissociation of the enzyme. During the early stages of this work it was observed that the activity of the enzyme preparations decreased rapidly during purification, so that the quantitative recovery from any stage was poor. This phenomenon does not occur with the intact organism nor with the dry acetone powder, which maintains its full activity for several months; it is only after extraction of the enzyme

Table 5. *Effect of enzyme inhibitors*

Inhibitor	Substrate: tyrosine		Substrate: dihydroxyphenylalanine	
	$-\log_{10}$ (molar conc.)	% inhibition	$-\log_{10}$ (molar conc.)	% inhibition
Ag (as AgNO ₃)	2	99	2	100
Cu (as CuSO ₄ ·5H ₂ O)	3	98	3	97
Hg (as HgCl ₂)	2	97	2	96
Fe (as Fe(NH ₄) ₂ (SO ₄) ₄ ·6H ₂ O)	1*	99	1*	98
Fe (as FeCl ₃)	2	99	2	100
Pb (as Pb(CH ₃ COO) ₂ ·3H ₂ O)	1	98	1	98
NaF	1	0	1	0
Iodoacetate	1	48	1	45
Na ₂ S	2	0	2	0
KCN	2	93	2	95
NaN ₃	1	13	1	15
NH ₄ OH	4	97	4	97
N ₂ H ₄	3*	98	3*	98
Baeyer 205 (antrypol)	2	75	2	73
Sulphanilamide	2	9	2	10
<i>p</i> -Phenylenediamine	2	41	2	40

* These experiments were carried out in an atmosphere of N₂, since dihydroxyphenylalanine took up O₂ even in absence of enzyme when these inhibitors were present.

logarithms are given in the table resulted in a lesser degree of inhibition. (NH₄)₂SO₄ in low concentrations has been found to have an inhibitory effect on the activity of preparations of *l*(+)-lysine decarboxylase, but solutions of (NH₄)₂SO₄ up to 33 % saturation in strength do not significantly affect the activity of *l*(-)-tyrosine decarboxylase.

Table 6 shows the effect of various treatments on the activity of the enzyme; the losses in activity are irreversible and accompanied by protein denaturation. Where necessary, the pH of the preparation was readjusted to 5.5 before samples were taken for

that deterioration begins. The isolation from brewer's yeast of a compound with the function of codecarboxylase for *l*(+)-lysine decarboxylase is described in a further paper in this series (Gale & Epps, 1944b); it was found that addition of this compound restored the activity of the *l*(-)-tyrosine decarboxylase preparations, whose deterioration was probably caused by the spontaneous and progressive dissociation of the enzyme into apo-enzyme and codecarboxylase.

At stage 8 of the preparation (above) the enzyme complex was 50 % dissociated, but more complete dissociation could be induced by suitable treatment; this consisted either in precipitating the protein at stage 6 *b* with 3 vol. of saturated (NH₄)₂SO₄ solution containing 3.3 % ammonia (cf. *l*(+)-lysine decarboxylase, Gale & Epps, 1944a) or in keeping the enzyme preparation in the ice-chest for 72 hr. and then dialyzing it overnight against glass-distilled water; in the former case about half the enzyme activity is lost by irreversible inactivation, while in the latter recovery is nearly quantitative on addition of the coenzyme.

Addition of boiled yeast or codecarboxylase preparation restores the activity of the apo-enzyme

Table 6. *Denaturation of the enzyme*

Conditions of treatment	Activity remaining (%)	
	Substrate: tyrosine	Substrate: dihydroxy- phenyl- alanine
55° for 5 min. (pH 5.0)	57	53
60° for 5 min. (pH 5.0)	19	21
pH 4.5 for 10 min.	64	63
50% ethanol at pH 5.0 (10 min.)	6	6
10 ⁻⁴ M-KMnO ₄ at pH 5.5 (10 min.)	76.5	73
NaNO ₂ (0.1 M) at pH 5.5 (10 min.)	44	46

Table 7. *Restoration of activity to apo-enzyme*

Manometer no.	...	1	2	3	4	5	6	7	8	9
Additions in ml.										
Apo-enzyme		0.2	0.2	0.2	0.2	—	—	—	0.2	0.2
Codecarboxylase preparation		—	0.2	—	0.2	0.2	0.2	—	—	0.2
M/30 Tyrosine		—	—	0.5	0.5	0.5	—	0.5	—	—
M/15 Dihydroxyphenylalanine		—	—	—	—	—	—	—	0.5	0.5
M/5 Acetate pH 5.5		2.8	2.6	2.3	2.1	2.3	2.8	2.5	2.3	2.1
CO ₂ output, μ l./5 min.		0	0	17	126	0	0	0	4	82

towards both *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine (Table 7), but none of the following had any significant effect: coenzyme I, coenzyme II preparation, cocarboxylase, aneurin, flavin adenine dinucleotide, flavin mononucleotide, riboflavin, muscle adenylic acid, adenosine, nicotinamide, nicotinic acid. Fig. 5 shows the activity obtained

Fig. 5. Restoration of activity to apo-enzyme *l*(-)-tyrosine as substrate.

when varying amounts of a purified preparation of codecarboxylase were added to the *l*(-)-tyrosine decarboxylase apo-enzyme; the shape of the curve suggests a loose association of the coenzyme type rather than a prosthetic group linkage and half-saturation of the enzyme is given by 0.60 μ g. C of codecarboxylase/ml. The distribution of the codecarboxylase of *l*(+)-lysine decarboxylase and its probable identity with the coenzyme of *l*(-)-tyrosine decarboxylase are dealt with elsewhere (Gale & Epps, 1944b).

4. *l*(-)-TYROSINE AND *l*-3:4-DIHYDROXY-PHENYLALANINE AS ALTERNATIVE SUBSTRATES FOR *l*(-)-TYROSINE DECARBOXYLASE

Holtz, Credner & Walter (1940) and Blaschko (1942) have shown that in animal tissues *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine are decarboxylated by different enzymes. Does the same hold for

bacteria or is a single enzyme concerned? The evidence can be summarized as follows: 1. The activity towards both substrates of the preparation described above was estimated during purification to an 100-fold extent, and there was no significant difference between the values obtained for the activity ratios (for the values in the last column of Table 1, mean = 1.58, standard deviation = 0.11). 2. The response of the activities of enzyme preparations towards *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine with changes in pH, temperature and enzyme concentration are shown in Fig. 2, Table 3 and Fig. 4 respectively; there is general similarity between the pairs of results. The variation of activity with substrate concentration is shown in Fig. 3. 3. The effects of general inhibitors are very similar towards the two substrates (Table 5). 4. The effects of exposing the enzyme to various denaturing processes are similar towards the two activities (Table 6). 5. When the concentration of each substrate was sufficient to saturate the enzyme, the rate of CO₂ production from a mixture of *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine was intermediate between those recorded for each substrate alone (Table 8). 6. Gale (1940a, b; 1941) showed that *l*(-)-tyrosine can be decarboxylated only by *Strep. faecalis* and *Cl. aerofetidium* among numerous organisms tested. From a survey of several of the same organisms it appears that only *Strep. faecalis* and *Cl. aerofetidium* possess activity towards *l*-3:4-dihydroxyphenylalanine. An acetone powder was prepared from a culture of *Cl. aerofetidium* grown anaerobically overnight at 37° in 2% glucose broth to which meat had been added (Gale, 1941). The pH curves were determined for the decarboxylation of *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine (Fig. 6). At the optimum pH (5.5) the activity ratio was 2.7. The variation of enzyme activity with substrate concentration was determined (Fig. 7); the Michaelis constant for *l*-3:4-dihydroxyphenylalanine is 0.0012 M. The result of a mixed substrate experiment is shown in Table 8.

These observations show that there is close similarity between the decarboxylation of *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine by bacteria, and that the criteria laid down by Dixon (1938) for the identity of two enzymes are satisfied. Further evidence for a single enzyme being concerned is provided by the restricted and identical distribution of

Table 8. *Mixed substrate experiments*

(Substrates, 0.5 ml. M/30 *l*-tyrosine suspension and 0.5 ml. M/15 *l*-3:4-dihydroxyphenylalanine solution; activity as μ l. CO₂/5 min.)

	Tyrosine	Dihydroxy-phenylalanine	Tyrosine + dihydroxy-phenylalanine
0.2 ml. Enzyme preparation (<i>Strep. faecalis</i> Dunn)	106	58	93
20 mg. Acetone powder (<i>Cl. aerofetidium</i>)	57	21	43

Fig. 6. Variation of activity with pH: *l*(-)-tyrosine decarboxylase from *Cl. aerofetidium* as for Fig. 4, with 50 mg. acetone powder in each manometer vessel. ●—● *l*(-)-tyrosine as substrate, M/5 acetate buffer. ○—○ *l*(-)-tyrosine as substrate, M/15 phosphate buffer. ▲—▲ *l*-3:4-dihydroxyphenylalanine as substrate, M/5 acetate buffer. △—△ *l*-3:4-dihydroxyphenylalanine as substrate, M/15 phosphate buffer.

the two activities throughout certain bacterial species, by the constancy of the activity ratio during purification of the enzyme preparation, by the similarity of the effects of changes in experimental temperature, by the similar action of a wide range of inhibitors and denaturing agents and by the results of mixed substrate experiments. The difference in the activity ratios and Michaelis constants of enzyme preparations from *Strep. faecalis* and *Cl. aerofetidium* is not necessarily surprising when

the different nature of these organisms is considered. Taking all these factors together, it seems highly probable that only one enzyme is concerned in the

Fig. 7. Variation of activity with substrate concentration: *l*(-)-tyrosine decarboxylase from *Cl. aerofetidium*. Each manometer vessel contained, for *l*(-)-tyrosine, 2.3 ml. M/5 acetate pH 5.5 and 0.2 ml. acetone powder suspension (100 mg./ml.). 2.1 ml. buffer and 0.4 ml. enzyme were used with *l*-3:4-dihydroxyphenylalanine. 0.5 ml. of the appropriate substrate was contained in the side-bulb. ●—● *l*(-)-tyrosine as substrate. ○—○ *l*-3:4-dihydroxyphenylalanine.

decarboxylation of *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine by bacteria.

SUMMARY

1. The extraction, purification and properties of *l*(-)-tyrosine decarboxylase from *Strep. faecalis* are described.

2. The enzyme dissociates spontaneously during purification, and preliminary evidence is presented that the coenzyme involved is the same as that of *l*(+)-lysine decarboxylase.

3. The enzyme preparations are specific for *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine, which they decarboxylate quantitatively to the corresponding amines.

4. The evidence suggests that, in contrast to the mammalian decarboxylases, a single enzyme is responsible for the bacterial decarboxylation of *l*(-)-tyrosine and *l*-3:4-dihydroxyphenylalanine.

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REFERENCES

- Blaschko, H. (1942). *J. Physiol.* **101**, 337.
 Davies, R. (1943). *Biochem. J.* **37**, 230.
 Dixon, M. (1938). *Enzymologia*, **5**, 198.
 Gale, E. F. (1940a). *Biochem. J.* **34**, 392.
 — (1940b). *Biochem. J.* **34**, 846.
 — (1941). *Biochem. J.* **35**, 66.

- Gale, E. F. & Epps, H. M. R. (1944a). *Biochem. J.* **38**, 232.
 — (1944b). *Biochem. J.* **38**, 250.
 Holtz, P., Credner, K. & Walter, H. (1940). *Hoppe-Seyl. Z.* **282**, 111.
 Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.