Resolution of the enzyme. It has previously been shown that *l*(+)-lysine decarboxylase from coliform organisms and *l*(-)-tyrosine decarboxylase from *Strep. faecalis* contain the same coenzyme, codecarboxylase, whose isolation from yeast has been described (Gale & Epps, 1944*a, b*; Epps, 1944). Dissociation of *l*(+)-lysine decarboxylase can be induced by precipitation of the protein with 66 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution containing 10 % NH_4OH and dissociation of *l*(-)-tyrosine decarboxylase by precipitation with ammoniacal $(\text{NH}_4)_2\text{SO}_4$ solution, by dialysis against distilled water at 0° or by keeping the enzyme preparation in the ice-chest for several days.

l(-)-Histidine decarboxylase preparations were subjected to the following treatments in attempts to dissociate the enzyme: (a) Protein was thrice precipitated by addition of 3 vol. saturated $(\text{NH}_4)_2\text{SO}_4$ solution containing 10 % NH_4OH . (b) Enzyme preparations were dialyzed at 0° for 24 hr. against distilled water and against 45 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution. (c) Enzyme preparations were kept in the ice-chest for periods up to 8 weeks. (d) Protein was precipitated at pH 3.0 with 40 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution and washed with 75 % saturated $(\text{NH}_4)_2\text{SO}_4$ solution. (e) The preparation was made *m* with respect to KCN, protein precipitated by addition of 3 vol. saturated $(\text{NH}_4)_2\text{SO}_4$ solution and the precipitate washed twice with 75 % saturated $(\text{NH}_4)_2\text{SO}_4$. After treatments (a), (b) and (c) there was only small

loss of activity while after treatments (d) and (e) the preparations were almost inactive; in no case was activity increased by addition of codecarboxylase preparation or a boiled aqueous suspension of *Cl. welchii* BW 21. It has also been shown that activity can be restored to *l*(-)-tyrosine decarboxylase apo-enzyme by addition of a boiled preparation of *l*(+)-lysine decarboxylase (Gale & Epps, 1944*b*), whereas addition of boiled *l*(-)-histidine decarboxylase preparation (≈ 13 units enzyme) has no such effect; the presence of codecarboxylase can, however, be shown in a suspension of *Cl. welchii* BW 21. These results suggest that codecarboxylase is not part of *l*(-)-histidine decarboxylase from *Cl. welchii* Type A.

SUMMARY

1. The extraction, purification and properties of *l*(-)-histidine decarboxylase from a strain of *Cl. welchii* Type A are described.
2. Acetone powders and enzyme preparations specifically attack *l*(-)-histidine, which is decarboxylated quantitatively to histamine.
3. It has not been possible to bring about dissociation of the enzyme, which would appear to be devoid of codecarboxylase.

I wish to express my thanks to Drs E. F. Gale and M. Dixon for advice, to Dr T. Mann for the gift of sulphanilamide derivatives and to the Medical Research Council for a full-time grant.

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Studies on Bacterial Amino-acid Decarboxylases

5. THE USE OF SPECIFIC DECARBOXYLASE PREPARATIONS IN THE ESTIMATION OF AMINO-ACIDS AND IN PROTEIN ANALYSIS

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Previous papers of this series have described specific enzyme preparations for the decarboxylation of *l*(+)-lysine (Gale & Epps, 1944), of *l*(-)-tyrosine (Epps, 1944) and of *l*(-)-histidine (Epps, 1945). The present communication shows the application of these and other preparations to the rapid analysis of protein hydrolysates for the amino-acids concerned.

METHODS

Manometric arrangements. The method depends in every case upon the manometric measurement of the CO_2 liberated from the amino-acid by the specific decarboxylase at the optimum pH and 30°. Warburg manometers were used containing measured portions of amino-acid solution or protein

hydrolysate together with appropriate amounts (1.5–2.0 ml.) of buffer in the main cup, and in the side-bulb 0.5 ml. enzyme preparation made up in the same buffer. The buffers used in the various estimations were M/5 phosphate pH 6.0 for lysine and ornithine, M/5 citrate pH 5.5 for tyrosine, and M/5 acetate pH 4.5 for glutamic acid and histidine estimations. CO₂ retention is very slight at pH 5.5 or less and can be allowed for in a correction, but at pH 6.0 the CO₂ retained in solution may represent 8–10% of that liberated, and a calculated correction may not be accurate, particularly with protein hydrolysates where the final experimental pH may not be exactly that of the buffer used. Accordingly, it is advisable to use the 'acid-tip' manometric method (Woods & Clifton, 1937) to estimate total CO₂ liberated, when estimating lysine and ornithine. To ensure the complete liberation of retained CO₂ at the end of the experiment, 0.25 ml. 8N-H₂SO₄ has been used in the second side-bulb for this purpose. It is imperative that for the accurate estimation of the experimental CO₂ liberation the acid-tip be carried out in control manometers on all reagents.

Protein hydrolysates. With protein hydrolysates it is necessary to adjust the pH of the sample to the optimum pH of the enzyme to be used. It has been found satisfactory in practice if the hydrolysate is adjusted to approx. pH 5 before sampling: the use of strong buffers in the manometers then renders further adjustment unnecessary. The hydrolysate is made sufficiently concentrated for samples of 0.3–1.0 ml. to liberate in each case 100–400 μ l. CO₂. Equilibration is carried out for at least 15 min. to ensure the removal of preformed CO₂ from the specimen; this precaution is highly necessary at pH 6.0. The preparations for the estimation of lysine, tyrosine and histidine usually have no blank gas-output but the suspensions of organisms used for the estimation of glutamic acid or ornithine have a small blank which must be measured and subtracted from the total.

The protein hydrolysates were prepared, in the majority of cases, by the accurate weighing of approx. 1 g. pure protein of known moisture and ash content, followed by solution in HCl (36 g./100 ml.), addition of water to make the final HCl concentration 20 g./100 ml., and then 24 hr. hydrolysis under a reflux condenser. After hydrolysis the hydrolysates were evaporated to dryness *in vacuo* and most of the free HCl removed by dissolving five successive times in water followed by evaporation to dryness *in vacuo*. The dry material was then taken up in c. 15 ml. water, the pH adjusted to 5 using CO₂-free NaOH, and the volume then made up to a convenient value, usually 25 ml. In the case of haemoglobin, the precipitated humin was centrifuged down, washed five times with water at pH 5 and the washings added to the main hydrolysate;

in all other cases the humin was left in suspension. I am indebted to Dr G. R. Tristram for total N determinations on such hydrolysates as were used for this work alone. The hydrolysis of tyrocidine, kindly provided by Dr R. L. M. Synge, was carried out *in vacuo* under the conditions recommended by Gordon, Martin & Synge (1943). In some cases estimations of lysine and histidine were made on a solution of the bases prepared by electrodialysis of the hydrolysate. Electrodialysis was carried out by Dr H. T. Macpherson of this department by a method to be published shortly. The enzymic CO₂ evolution is more rapid in solutions of these simple bases than in the complete hydrolysates.

ENZYME PREPARATIONS

l(+)-Lysine decarboxylase. The enzyme preparation used in this case corresponds to stage 5 of the preparation from *Bact. cadaveris* (National Collection of Type Cultures no. 6578) described by Gale & Epps (1944). The enzyme when freshly prepared sometimes contains a trace of arginine decarboxylase activity but this is destroyed if the preparation is kept 2–3 days in the ice-chest; the lysine activity remains unimpaired for 3–4 weeks in the ice-chest. The preparation should always be tested for this point, and not used until 0.5 ml. gives no CO₂ output from arginine under the experimental conditions over a period of 20 min. A convenient strength for the preparation is 4–6 units of enzyme per ml., of which 0.5 ml. is used per test. The preparation is virtually specific for *l(+)-lysine*, but it is probable that hydroxylysine is slowly attacked.

l(-)-Tyrosine decarboxylase. Many strains of streptococci produce tyrosine decarboxylase when grown in 2% glucose-casein-tryptic-digest (Gale, 1940) and a preparation of the enzyme has been worked out by Epps (1944). The organism used for this work has been a group D *Streptococcus* National Collection of Type Cultures (N.C.T.C.) no. 6782. The organism also contains arginine dihydrolase (Hills, 1940), but the activity of this enzyme is destroyed by acetone-drying of thick suspensions, while the tyrosine activity is unimpaired. Of the common amino-acids only *l(-)-tyrosine* is decarboxylated by the acetone powder under the experimental conditions used (in acetate buffer at pH 5.5), although dihydroxyphenylalanine is also attacked. The powder retains its activity for some months in the dry state. For the estimation a suspension of 50 mg. powder per ml. is made up in M/5 acetate buffer pH 5.5 and 0.5 ml. used per test.

l(-)-Histidine decarboxylase. Many strains of *Cl. welchii* possess decarboxylases attacking histidine and glutamic acid (Gale, 1941) and a specific preparation of the histidine enzyme has been worked out by Epps (1945). The organism used in this work has been *Cl. welchii* BW 21 (N.C.T.C. no. 6785)

dried in acetone and extracted as described by Epps (1944b). The crude yellow extract can be used as a source of specific enzyme, a convenient amount being 0.5 ml. of an extract obtained from a suspension of 50 mg. dry organism per ml. It has been our custom to test freshly prepared extracts against glutamic acid but no activity has yet been found in any extract prepared exactly as described.

l(+)-Glutamic acid decarboxylase. Although most strains of *Cl. welchii* possess the decarboxylases of histidine and glutamic acid (Gale, 1941), the strain S.R. 12 (N.C.T.C. no. 6784) is specific for *l(+)-glutamic acid*. Consequently a thick suspension of this organism grown for 12 hr. in 2% glucose casein-digest at 37° can be used for the estimation of *l(+)-glutamic acid*. The total crop from 800 ml. of medium should be made up into c. 25 ml. suspension in M/5 acetate pH 4.5 and 0.5 ml. used for test. As this preparation consists of intact organisms it was thought that fermentation reactions might invalidate the results, but if the organism is well washed before use neither the carbohydrate remaining in protein hydrolysates nor glucose is attacked under the experimental conditions used. Acetone drying of the cells results in the loss of 85–95% of the activity. The preparation is specific for the natural isomer, consequently results on protein hydrolysates tend to be low owing to racemization occurring with glutamic acid under the hydrolysis conditions used. Synthetic β -hydroxy-glutamic acid was also attacked.

l(+)-Ornithine decarboxylase. Washed suspensions of *Cl. septicum* decarboxylate *l(+)-ornithine* alone of the common amino-acids (Gale, 1941), and can therefore be used for the estimation of this amino-acid in the absence of glucose. The carbohydrate of protein hydrolysates is not attacked. Drying the organisms in acetone results in the loss

of 80–100% of the activity. For these tests the organism used was *Cl. septicum* Pasteur III (N.C.T.C. no. 547) grown for 16 hr. in 2% glucose casein-tryptic digest: the crop from 800 ml. medium was made up to 25 ml. suspension, of which 0.5 ml. was used for test. The suspension steadily loses its activity on standing and should be used on the day of preparation. The most satisfactory results are obtained in M/5 phosphate at pH 6.0, but it is possible to estimate ornithine in the presence of glucose by using well-washed cells in citrate buffer at pH 5.5, when glucose is not fermented: the evolution of CO_2 from ornithine is slower under these conditions than in phosphate at pH 6.0.

RESULTS

Table 1 gives the CO_2 evolution measured in the estimation of standard solutions of amino-acids by the above enzyme preparations under the conditions described. In most cases, the evolution with and without the use of the acid-tip measurement of retained CO_2 is given. In the estimation of tyrosine, glutamic acid and histidine error introduced by a calculated correction of the retained CO_2 is no greater than that involved in the acid-tip technique. For estimations at pH 6.0 of lysine and ornithine it appears that CO_2 retention depends upon the amount of protein present (lysine decarboxylase, being a purified enzyme preparation, contains much less protein than the thick suspension of organisms used for ornithine estimations) and it is sufficiently large to make calculated corrections unreliable; it is therefore advisable to estimate CO_2 retention manometrically in these cases. For each amino-acid the accuracy of the method has been tested over a substrate range of 75–560 μl . and, as would be expected, the experimental error tends to increase

Table 1. CO_2 evolved from amino-acid solutions in presence of specific decarboxylase preparations

Amount of substrate	Lysine pH 6.0		Ornithine pH 6.0		Tyrosine pH 5.5		Glutamic acid pH 4.5		Histidine pH 4.5	
	Evolved (μl .)	Total (μl .)	Evolved (μl .)	Total (μl .)	Evolved (μl .)	Total (μl .)	Evolved (μl .)	Total (μl .)	Evolved (μl .)	Total (μl .)
373 μl .										
Mean	344	366	327	367	359	369	366	—	359	364
No. of observations	19	10	10	10	14	5	12	—	14	3
Standard-deviation	4.7	5.1	2.8	5	14	12	3.5	—	6.5	—
Range	337–354	359–373	313–321	356–373	339–382	351–382	361–374	—	346–365	—
Mean (% of calc.)	92*	98*	87*	98*	96*	97*	98*	—	96*	97*
<div>% of calculated</div>										
560 μl .	90	99	83	98	95	98	97	—	95	—
373 μl .	92	98	87	98	96	99	98	—	96	—
300 μl .	93	98	88	96	90	96	98	—	96	—
224 μl .	93	98	88	97	91	96	97	—	96	—
150 μl .	91	96	88	96	98	104	96	—	92	—
75 μl .	90	92	97	105	86	91	96	—	101	—

* Values taken for calculations of the analyses in the following examples. 'Total' CO_2 is 'evolved CO_2 ' + retained CO_2 .

with decreasing amount of substrate. For reasonable accuracy it is desirable that samples should be chosen which will evolve 100–400 μ l. CO_2 . The widest deviations from the mean in the series are found in the tyrosine values, as there is a considerable sampling error involved in pipetting tyrosine suspensions. In protein hydrolysates, however, unless these are highly concentrated, the tyrosine is usually held in solution by the other amino-acids and the error is then no greater than that observed in the estimations of the other amino-acids.

Tables 2–6 give results obtained by the application of these methods to analysis of protein hydrolysates, with values recorded in the literature for comparison. In some cases analyses were carried

out in parallel with determinations by other methods by Prof. A. C. Chibnall and co-workers; these results are recorded in italics. In general there is good agreement with values obtained by other methods. It is improbable that an error of less than 3–5 % can be ascribed to any manometric method of this nature, but the advantage here lies first in the speed with which estimations can be carried out, and second in the fact that they can be performed on the crude hydrolysate without any treatment other than the adjustment of pH. In most cases CO_2 evolution is complete within 30 min. after tipping in the contents of the side-bulb and it is possible with ease to analyze six hydrolysates for one amino-acid per day. As a general rule each analysis was

Table 2. *Lysine content of proteins estimated by l(+)-lysine decarboxylase*

Protein	Total vol. (ml.)	Total N (mg.)	Experimental sample (ml.)	CO_2 liberated (μ l.)	Mean CO_2 (μ l.)	Corrected CO_2 (μ l./ml.)	Total lysine-N (mg.)	N content (%)		Literature reference no.*
								Found	Literature	
Synthetic mixture	50	296	0.75 (10–7)†	42 42.5 43	42.5	40.5	2.5	0.85	(0.845)	—
	—	—	0.75 (10–4)†	70.5 73 71	71.5	39.0	2.4	0.82	(0.845)	—
Cocksfoot grass	50	143	0.5	76 79 77	77	158	9.87	6.91	6.2	(14)
Fibrin	25	185	0.5	298 297 299	298	610	19.2	10.4	11.3	(3)
Horse haemoglobin	50	182	0.75	234 232 241	235.5	320	19.9	10.95	9.4	(15)
	200	672	1.0 (4–5)	224 220 220	221	226	70.4	10.47	9.4	(15)
Globin (base solution)	200	64	1.0 (10–1)†	226 229 230	228	23.3	5.8	9.1	9.4	(15)
Gliadin	25	157	0.75	30 27 33	30	41.5	1.25	0.79	0.7	(13)
Edestan	25	198	0.5	88 83 94 91	89	182	5.7	2.88	—	—
Edestin (base solution)	250	296	1.0 base (4–1)†	259 261 264	261	66.3	20.7	2.44	2.44	—
Wool (base solution)	200	77	1.0 (20–1)†	191 198 199	196	10.0	2.5	3.25	3.0	(4)

* These numbers refer to those preceding the References at the end of this paper.

† Indicates solution reduced in volume: thus (10–7) = 10 ml. reduced to 7 ml.

Table 3. *Histidine content of proteins estimated by l(-)-histidine decarboxylase*

Protein	Total vol. (ml.)	Total N (mg.)	Experimental sample (ml.)	CO ₂ liberated (μl.)	Mean CO ₂ (μl.)	Corrected CO ₂ (μl./ml.)	Total histidine-N (mg.)	N content (%)		Literature reference no.*
								Found	Literature (3-96)	
Synthetic mixture	50	296	0.75	95 89 88	91	126	11.8	3.98	(3.96)	—
Cocksfoot grass	50	143	1.87	78 77 79	78	43.5	4.08	2.86	2.3	(14)
Fibrin	25	185	0.75	128 131 138	132	184	8.65	4.67	3.95	(3)
	50	147.5	0.5 (15-5)†	101 104 104 105	103.5	72	6.75	4.57	3.95	(3)
Horse haemoglobin	50	182	0.75	176 178 175	176	244	22.95	12.5	12.56	(15)
Haemoglobin (base solution)	200	303	1.0 (2-1)†	204 196 200	200	102.5	38.5	12.71	12.51	—
Gliadin	25	157	0.75	81 85 89	85	117	5.53	3.53	3.30	(12)
Edestan	25	198	0.75	115 122 118	118	139	6.51	3.35	—	—
Edestin (base solution)	250	296	1.0 base (9-1)†	145 145 145	145	67.2	31.1	3.66	4.1	—
Insulin	2.5	11.5	0.5	97 100 100 99	99	207	0.97	8.44	8.4	—
Ovalbumin	100	436	0.5 (6-2)†	141 141 141	141	98	18.4	4.23	4.2	—

* † See Table 2.

carried out in triplicate and, in calculation, corrections are applied to the gas evolution in conformity with the figures determined and given in Table 1. In all cases the methods were tested in the first place on a synthetic mixture of amino-acids, resembling hydrolyzed insulin in constitution, which had been boiled with HCl under the usual conditions before analysis; results obtained with this mixture are placed at the head of the table for each amino-acid. The widest deviation from theoretical was obtained with glutamic acid and it is with this series of results in general that we find the widest deviations from values reported in the literature. This may be due in part to the racemization oc-

curing with glutamic acid during hydrolysis. In the case of gliadin, Chibnall, Rees, Williams & Boyland (1940) have shown that 2-3 % of the glutamic acid is present in the *d*(-)-form, which is not attacked by the enzyme.

Contrary to the usual custom, tyrosine has been estimated on the acid hydrolysates, as we find that alkaline hydrolysis, although preventing the destruction of tyrosine, results in a high degree of racemization; thus an alkaline hydrolysate of insulin gave a tyrosine content of 8.3 % compared with the value of 10.5 % estimated on an acid hydrolysate and with 12.5 % total tyrosine estimated by Harington & Neuberger (1936).

Table 4. *Tyrosine content of proteins estimated by l(-)-tyrosine decarboxylase*

Protein	Total vol. (ml.)	Total N (mg.)	Experimental sample (ml.)	CO ₂ liberated (μl.)	Mean CO ₂ (μl.)	Corrected CO ₂ (μl./ml.)	Total tyrosine-N (mg.)	N content (%)		Literature reference no.*
								Found	Literature	
Synthetic mixture	50	296	0.3	180 180 177	179	.610	19.25	6.5	(6.65)	—
Cocksfoot grass	50	143	1.0	100 124 115	113	118	3.68	2.6	2.3	(11)
Fibrin	25	185	0.75	226 226 215	222	.308	4.84	2.61	2.56	(1)
Horse haemoglobin	50	182	0.75	62 58.5 57	59	82	2.55	1.41	1.43	(7)
Gliadin	25	157	0.75	110 112 124	115	160	2.50	1.59	1.33	(10)
Edestan	25	198	0.75	160 159 157	159	221	3.45	1.78	1.85	(11)
Tyrocidin	3.1	7.5	0.5	141 133	137	285	0.56	7.4	6.8	(8)
Insulin	200	561	0.5	109 118 112	113	235.5	29.5	5.05	6.05	(9)

* See Table 2.

Table 5. *Glutamic acid content of proteins estimated by l(+)-glutamic acid decarboxylase*

Protein	Total vol. (ml.)	Total N (mg.)	Experimental sample (ml.)	CO ₂ liberated (μl.)	Mean CO ₂ (μl.)	Corrected CO ₂ (μl./ml.)	Total glutamic-N (mg.)	N content (%)		Literature reference no.*
								Found	Literature	
Synthetic mixture	50	296	0.5	508 508 498	505	1040	32.5	11.0	(11.85)	—
Cocksfoot grass	50	143	0.5	153 154 149	152	313	9.75	6.85	10.7	(5)
Fibrin	25	185	0.4	379 365 366	370	944	14.75	7.98	8.25	(3)
Horse haemoglobin	50	182	0.75	186 192 191	190	257	8.05	4.42	3.76	(2)
Gliadin	25	157	0.15	349 344 340	346	2350	36.7	23.4	25.3	(6)
Edestan	25	189	0.3	355 363 353	357	1215	18.98	10.06	10.44	(2)
Tyrocidin	3.1	7.5	0.5	135 132	133.5	273	0.53	7.06	7.2	(8)

* See Table 2.

Table 6. *Ornithine content of proteins estimated by l(+)-ornithine decarboxylase*

Protein	Total vol. (ml.)	Total N (mg.)	Experimental sample (ml.)	CO ₂ liberated (μl.)	Mean CO ₂ (μl.)	Corrected CO ₂ (μl./ml.)	Total ornithine-N (mg.)	N content (%)		Literature reference no.*
								Found	Literature	
Synthetic mixture	50	296	0.5	0	0	0	0	0	(0)	—
Insulin	200	561	0.5	0	0	0	0	0	0	—
Haemoglobin	50	182	0.5	0	0	0	0	0	0	—
Tyrosidine	3.1	7.5	0.5	113	110	253	0.984	13.11	13.2	(8)

* See Table 2.

SUMMARY

The manometric estimation of *l*(-)-histidine, *l*(+)-lysine, *l*(-)-tyrosine, *l*(+)-glutamic acid, and *l*(+)-ornithine in solution and in protein hydrolysates by the use of preparations of the specific amino-acid decarboxylases is described. Typical analyses

are quoted to indicate the accuracy of the method.

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Studies on Bacterial Amino-acid Decarboxylases

6. CODECARBOXYLASE CONTENT AND ACTION OF INHIBITORS

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Six amino-acid decarboxylases have as yet been found in bacteria: these enzymes are specific for the decarboxylation of *l*(+)-lysine, *l*(-)-tyrosine, *l*(-)-histidine, *l*(+)-arginine, *l*(+)-ornithine, and *l*(+)-glutamic acid (Gale, 1940, 1941). Of the three of these enzymes which have been studied in a cell-free and partially purified condition, two, namely, the

decarboxylases of *l*(+)-lysine and *l*(-)-tyrosine, can be resolved into specific protein portions (apoenzyme) and a common codecarboxylase (Gale & Epps, 1944a; Epps, 1944), while the third, *l*(-)-histidine decarboxylase of *Cl. welchii*, cannot be resolved and apparently does not contain codecarboxylase (Epps, 1945). Consequently it is of