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)
				107						

* 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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REFERENCES

Numbers on left in brackets refer to reference numbers in Tables 2-6

- (1) Bailey, K. (unpublished).
- (2) Bailey, K., Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 360.
- (3) Bergmann, M. & Niemann, C. (1936). *J. biol. Chem.* **115**, 77.
- (4) Block, R. J. (1939). *J. biol. Chem.* **128**, 181.
- (5) Chibnall, A. C. (1939). *Protein Metabolism of the Plant*, p. 117. New Haven: Yale University Press.
- (6) Chibnall, A. C., Rees, M. W., Williams, E. F. & Boyland, E. (1940). *Biochem. J.* **34**, 285.
- Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
- Epps, H. M. R. (1945). *Biochem. J.* **39**, 42.
- (7) Folin, O. & Marenzi, A. D. (1929). *J. biol. Chem.* **83**, 89.
- Gale, E. F. (1940). *Biochem. J.* **34**, 392, 846.
- Gale, E. F. (1941). *Biochem. J.* **35**, 66.
- Gale, E. F. & Epps, H. M. R. (1944). *Biochem. J.* **38**, 232.
- (8) Gordon, A. H., Martin, A. J. P. & Synge, R. L. M. (1943). *Biochem. J.* **37**, 313.
- (9) Harington, C. R. & Neuberger, A. (1936). *Biochem. J.* **30**, 809.
- Hills, G. M. (1940). *Biochem. J.* **34**, 1057.
- (10) Looney, J. M. (1926). *J. biol. Chem.* **69**, 519.
- (11) Lugg, J. W. H. (1938). *Biochem. J.* **32**, 775, 2114, 2123.
- (12) Macpherson, H. T. (1944). (Unpublished.)
- (13) Osborne, T. O., Van Slyke, D. D., Leavenworth, C. S. & Vinograd, M. (1915). *J. biol. Chem.* **22**, 259.
- (14) Tristram, G. R. (1939). *Biochem. J.* **33**, 1271.
- (15) Vickery, H. B. & Leavenworth, C. S. (1928). *J. biol. Chem.* **79**, 377.
- Woods, D. D. & Clifton, C. E. (1937). *Biochem. J.* **31**, 1774.

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

importance to determine if the remaining decarboxylases contain a codecarboxylase moiety and if it is possible to differentiate between the two types of enzyme by the action of inhibitors.

In the work to be described, the course of the investigation of each enzyme was, first, to obtain an active cell-free preparation from a suitable source; second, to determine the pH of optimum activity; third, to investigate the variation of velocity of reaction with substrate concentration so that subsequent experiments could be carried out with a saturation concentration of substrate; fourth, to attempt to resolve the enzyme and determine the nature of the coenzyme if resolution was successful, and finally, the optimum experimental conditions having been found, to investigate the action of various common inhibitors upon the activity.

l(+)-ARGININE DECARBOXYLASE

Source of enzyme. Gale & Epps (1944*a*) found that a strain of *Esch. coli*, National Collection of Type Cultures (N.C.T.C.), no. 86, possesses the decarboxylases of *l*(+)-arginine, *l*(+)-lysine, *l*(-)-histidine and *l*(+)-ornithine. Acetone treatment of thick washed suspensions of the organism destroys the ornithine enzyme without affecting the activity of the other three. The arginine decarboxylase can be extracted from the acetone powder under the same conditions as those described for lysine decarboxylase. Although it is comparatively simple to destroy the arginine enzyme and obtain a preparation which specifically decarboxylates lysine, no procedure has yet been found whereby the arginine enzyme can be separated in an active state from the lysine one.

Preparation of cell-free enzyme. *Esch. coli* is grown for 24 hr. at 25° in casein-tryptic-digest containing glucose to a final concentration of 2%. The organism is centrifuged, washed once with water, made into a thick cream in water and treated with 5 vol. cold acetone. The coagulum is filtered on a Buchner funnel, washed once with acetone and once with ether; it is then allowed to dry in air, and is powdered. The dry powder is incubated at 37° in M/45 borate buffer pH 8.5 (1 ml. to 20 mg. powder) for 2 hr. The suspension is then centrifuged and the opalescent supernatant used as enzyme preparation.

Optimum pH. Fig. 1 shows the variation of velocity of decarboxylation with reaction pH. The optimum pH is approximately 5.25 and subsequent experiments were carried out in the presence of M/5 acetate buffer pH 5.25.

Effect of substrate concentration. Fig. 2 shows the variation of velocity of decarboxylation with arginine concentration. The Michaelis constant is 0.00075M which agrees well with the value of 0.00056M previously recorded for the activity in the intact organism (Gale, 1940). Subsequent experiments were carried out with an initial arginine concentration of 0.011M.

Experimental conditions. Decarboxylation was followed in Warburg manometers containing 0.5 ml. enzyme pre-

paration, 1.5 ml. M/5 acetate buffer pH 5.25 and (in the side-bulb) 0.5 ml. M/15 arginine. Experiments were carried out at 30° and the CO₂ evolution studied for 10 min. after the addition of substrate following equilibration.

Fig. 1. Activity-pH curves for arginine, ornithine and glutamic acid decarboxylases. Δ-Δ Arginine. ●-● Ornithine. ○-○ Glutamic acid. Contents of cups: 0.5 ml. enzyme; 0.5 ml. substrate solution; 2.0 ml. buffer. Temp. 30°.

Fig. 2. Effect of substrate concentration on arginine and ornithine decarboxylase activity. Contents of cups: 0.5 ml. enzyme; 1.5 ml. M/5 buffer; (0.5 ml. codecarboxylase in case of ornithine); 0.5 ml. substrate.

Resolution and recombination with codecarboxylase. *l*(+)-Lysine and *l*(-)-tyrosine decarboxylases can be reversibly resolved by treatment with alkali and the specific proteins precipitated by ammoniacal ammonium sulphate (Gale & Epps, 1944*a*; Epps, 1944). The arginine decarboxylase preparation was therefore precipitated with 2 vol. saturated ammonium sulphate solution containing 2.5% by volume 0.880 ammonia, and the activity of the precipitate towards arginine tested with and without codecarboxylase. Codecarboxylase was prepared according to Gale & Epps (1944*b*). The precipitation was repeated under the same conditions and also in the presence of higher ammonia concentrations. Table 1 shows that resolution does occur if the enzyme is precipitated by ammoniacal ammonium sulphate and that the activity of the apo-enzyme is restored by the presence of codecarboxylase. The decrease in enzyme units (1 unit being the amount of enzyme which will liberate 100 μl. CO₂ from

Table 1. *Reversible resolution of l(+)-arginine decarboxylase*

Preparation	Vol. of preparation (ml.)	CO ₂ produced (μl. CO ₂ /5 min./ml.)		Increase due to codecarb-oxylase (%)	Total units
		Alone	+ codecarb-oxylase		
1. Initial extract	24	1260	1260	0	302
2. First precipitation with 2 vol. 2.5% ammoniacal (NH ₄) ₂ SO ₄	15	1250	—	—	188
3. Second precipitation with 2 vol. 2.5% ammoniacal (NH ₄) ₂ SO ₄	14	1150	1275	11.1	179
4. Third precipitation with 2 vol. 2.5% ammoniacal (NH ₄) ₂ SO ₄	10	1060	1275	20.3	127
5. Precipitation with 2 vol. 3.3% ammoniacal (NH ₄) ₂ SO ₄	10	945	1225	29.6	122
6. First precipitation with 2 vol. 10% ammoniacal (NH ₄) ₂ SO ₄	10	350	800	129	80
7. Second precipitation with 2 vol. 10% ammoniacal (NH ₄) ₂ SO ₄	10	150	645	330	65

arginine in 5 min. at pH 5.25 and 30°) measured in the presence of codecarboxylase shows that there is some destruction of enzyme during the precipitation. Two consecutive precipitations of the preparation with 2 vol. saturated ammonium sulphate containing 10% ammonia result in the preparation of a protein whose activity towards arginine is increased 330% by the addition of excess codecarboxylase. Fig. 3 shows the dependence on code-

The organism used for these investigations was *Cl. septicum* Pasteur III, N.C.T.C. no. 547.

Preparation of cell-free enzyme. Acetone-drying of the organisms destroys the ornithine enzyme in all cases tested, but it is possible to obtain a cell-free active extract by disintegrating thick suspensions of *Cl. septicum* by shaking with glass beads. 5 l. 2% glucose-casein-tryptic-digest are inoculated with a meat culture of *Cl. septicum*, a little sterile heart muscle and 1 drop of thiolacetic acid are added, and the culture is incubated for 16–18 hr. at 37°. The organisms are harvested on a Sharples super-centrifuge, washed once with water and made up into a thick cream in 15–20 ml. distilled water. This cream is then shaken for 2 hr. with glass 'ballotini' in a shaker constructed from the description by Curran & Evans (1942). A rate of shaking of approximately 400/min. has been adopted and 2 hr. is then sufficient to disintegrate the cells to give an active extract. The extract is filtered from the beads on a sintered-glass filter, the beads are washed with about 10 ml. water and the combined filtrates then centrifuged at 3000 r.p.m. for 30 min. The supernatant liquid appears white by reflected light but is clear yellow-brown by transmitted light and contains no intact cells. The enzyme is unstable and can be used during 24–48 hr. after preparation only: it also undergoes spontaneous resolution and must be tested in the presence of added codecarboxylase. When freshly prepared, 0.2 ml. of supernatant will liberate 200–300 μl. CO₂ from l(+)-ornithine in 5 min. but the activity rapidly deteriorates and 0.5 ml. supernatant is required for experiments conducted with preparations older than 20 hr.

Optimum pH. Fig. 1 shows the variation of velocity of decarboxylation with reaction pH; the optimum pH falls at 5.25, the same value as that obtained for the l(+)-arginine decarboxylase.

Effect of substrate concentration. The Michaelis constant, determined from Fig. 2 showing the variation of velocity of decarboxylation with ornithine concentration, is 0.004 M. The value determined for the activity of the intact organism is 0.0028 M. Subsequent experiments were carried out in the presence of an initial concentration of 0.017 M l(+)-ornithine.

Experimental conditions. Decarboxylation was followed in Warburg manometers containing 0.5 ml. enzyme preparation, 0.5 ml. codecarboxylase solution (excess), 1.5 ml. M/5 acetate buffer pH 5.25 and (in the side-bulb) 0.5 ml. M/10 l(+)-ornithine. Experimental temperature = 30°.

Reversible resolution. When the activity of the enzyme preparation alone is tested against l(+)-

Fig. 3. Effect of coenzyme concentration on rate of decarboxylation of arginine and ornithine by their apo-enzymes. ●—● Arginine. ○—○ Ornithine. Contents of cups: 0.25 ml. enzyme; 2.0 ml. M/5 acetate buffer; 0.5 ml. substrate. Temp. 30°. (Volume of codecarboxylase required for half-saturation tyrosine apo-carboxylase = 0.01 ml.)

carboxylase concentration of the rate of arginine decarboxylation by such a preparation of apo-enzyme. The codecarboxylase solution used was tested against tyrosine apo-decarboxylase, in which case 0.01 ml. gave half-saturation of the apo-enzyme with coenzyme.

l(+)-ORNITHINE DECARBOXYLASE

Source of enzyme. l(+)-Ornithine decarboxylase is found in the potential enzyme constitution of many coliform organisms and of *Cl. septicum*, but whereas the former usually possess several decarboxylases, strains of the latter specifically decarboxylate l(+)-ornithine (Gale, 1940, 1941, 1945).

ornithine, it is found to fall off very rapidly. Since the tyrosine decarboxylase of *Strep. faecalis* undergoes spontaneous dissociation into apo-enzyme and codecarboxylase (Epps, 1944a), we tested the effect of the addition of either boiled yeast suspension or codecarboxylase preparation (Gale & Epps, 1944b) to the ornithine decarboxylase preparation. We found that the activity can be restored to a large extent by either addition. After standing 24 hr. the ornithine decarboxylase has lost 75 % of its activity and the full activity can be restored by the addition of codecarboxylase: presumably the loss of activity is due to spontaneous resolution followed by destruction of the codecarboxylase. Fig. 3 shows the dependence of the rate of decarboxylation upon the concentration of codecarboxylase present. In an attempt to complete the resolution and obtain a completely inactive apo-enzyme preparation, the enzyme was dialyzed against distilled water and also precipitated by ammoniacal ammonium sulphate as described for arginine decarboxylase; all such treatments irreversibly inactivated the ornithine enzyme.

l(+)-GLUTAMIC ACID DECARBOXYLASE

Source of enzyme. A strain of *Cl. welchii*, type A, S.R. 12, is specific for the decarboxylation of *l*(+)-glutamic acid but acetone-drying of the organisms results in 80–90 % destruction of the enzyme (Gale, 1945). Many coliform organisms possess a glutamic decarboxylase which is resistant to acetone-drying and can be extracted from dried cells in a manner similar to that employed for the extraction of the *l*(+)-lysine and *l*(+)-arginine decarboxylases. To obtain such an organism, rat faeces were plated out and a selection of coliform organisms isolated in pure culture. They were grown in 2 % glucose-casein-tryptic digest for 24 hr. at 25° and tested for glutamic acid decarboxylase activity (Gale, 1940). The most active of the isolations was taken for the following investigations.

Preparation of cell-free enzyme. The preparation was carried out exactly as described above for the preparation of *l*(+)-arginine decarboxylase from *Esch. coli*. The cell-free extract contained decarboxylases of *l*(+)-glutamic acid, *l*(+)-arginine and *l*(+)-lysine, but whereas the glutamic acid activity was stable for 14–20 days, the activities towards arginine and lysine disappeared within 7–8 days on standing in the ice-chest. For experiments on resolution, the glutamic acid enzyme was partially purified as follows: 3 % acetic acid was added to the supernatant preparation until the pH fell to 5.5, when one half-volume of alumina C₂ suspension (16 mg./ml.) was added and the enzyme adsorbed. The enzyme was twice eluted from the alumina with a volume of *m*/5 phosphate pH 7.0 equal to the volume of alumina suspension taken for adsorption. The combined eluates were precipitated with ammonium sulphate 50 g./100 ml. The precipitate was dissolved in water and the

calculated amount of saturated ammonium sulphate solution added to bring the final salt concentration to 40 % saturation. The precipitate was discarded and the ammonium sulphate concentration raised to 55 % saturation. This precipitate contains approximately half the total amount of glutamic acid enzyme originally present, no arginine activity and a very small activity towards lysine; this last activity disappears on standing in the ice-chest for a few days, after which a repetition of the ammonium sulphate fractionation gives a specific enzyme in the 40–55 % saturation fraction. For the experiments quoted below, other than those on resolution, the non-specific crude extract was used as enzyme source.

Optimum pH. Fig. 1 shows that the pH of optimum activity lies at approximately 4.25.

Effect of substrate concentration. Fig. 4 shows the variation of velocity of decarboxylation of *l*(+)-glutamic acid with substrate concentration, with (a) the intact organism

Fig. 4. Effect of glutamic acid concentration on glutamic decarboxylase activity. ●—● Washed suspension. ○—○ Cell-free enzyme. Contents of cups: 0.5 ml. enzyme; 2.0 ml. *m*/5 acetate buffer pH 4.25; 0.5 ml. substrate solution. Temp. 30°.

and (b) the cell-free extract as source of enzyme. The Michaelis constant, when the intact organism is used as enzyme source, is 0.005 *m*, but the affinity of the cell-free extract is considerably less, the Michaelis constant being approximately 0.027 *m*, though the fact that it is difficult to saturate the enzyme introduces doubt about the accuracy of this value. Subsequent experiments were carried out in the presence of an initial concentration of 0.17 *m*-*l*(+)-glutamic acid.

Experimental conditions. Decarboxylation was followed in Warburg manometers containing 0.5 ml. enzyme preparation, 1.5 ml. *m*/5 acetate buffer pH 4.25 and (in the side-bulb) 0.5 ml. *m*/2-*l*(+)-glutamic acid neutralized to pH 4.25.

Attempts at resolution. In an attempt to resolve the enzyme into specific protein and coenzyme, the following procedures were carried out and the resulting preparations tested alone, in the presence of codecarboxylase, and in the presence of boiled yeast suspension: (1) Precipitation with 2 vol. ammonium sulphate solution containing 2.5, 3.3 and 10 % ammonia respectively: no resolution and no destruction. (2) Dialysis against distilled water for 24 hr.: no resolution but 37 % loss of activity.

(3) Dialysis against 50 % saturated ammonium sulphate solution containing 2 % ammonia for 18 hr.: no resolution and no inactivation. (4) Precipitation with 2 vol. saturated ammonium sulphate solution at pH 3 and 1: no resolution; complete destruction at pH 1. The glutamic acid decarboxylase thus resembles the *l*(-)-histidine decarboxylase of *Cl. welchii* in being resistant to resolution; in the latter case Epps (1945) was able to show the absence of codecarboxylase in the enzyme preparation, since the addition of the freshly boiled preparation to tyrosine apo-decarboxylase gave no increase in tyrosine decarboxylase activity. A similar experiment has been carried out with glutamic decarboxylase. Tyrosine apo-decarboxylase was prepared according to Epps (1944) and its activity towards tyrosine tested in the presence and absence of codecarboxylase. Two preparations of *l*(+)-glutamic acid decarboxylase were then boiled for 5 min. and the boiled materials added to the tyrosine apo-enzyme as sources of codecarboxylase. Table 2

Table 2. *Absence of codecarboxylase in l*(+)-glutamic acid decarboxylase

(Conditions as for other decarboxylases, concentration of tyrosine 0.005 M, pH 5.5, temp. = 30°.)

Additions to tyrosine apo-decarboxylase	CO ₂ from tyrosine (μ l./5 min.)
None (control)	4
Excess codecarboxylase	66
Crude extract (=7 units glutamic acid decarboxylase)	50
Purified glutamic acid decarboxylase preparation (=5 units)	6

shows that the crude extract, containing the glutamic acid, arginine and lysine decarboxylases, gives a good codecarboxylase response but the partially purified specific *l*(+)-glutamic acid decarboxylase, prepared as described above, gives no significant response. From these results it is highly probable that *l*(+)-glutamic acid decarboxylase, extracted from this organism, does not contain codecarboxylase as part of the enzyme molecule.

ACTION OF INHIBITORS

Cell-free preparations of all six known amino-acid decarboxylases are now available. Of the six enzymes, the decarboxylases of *l*(+)-lysine, *l*(+)-arginine, *l*(+)-ornithine and *l*(-)-tyrosine have been resolved into specific proteins and codecarboxylase as coenzyme; the decarboxylases of *l*(-)-histidine and *l*(+)-glutamic acid have not been resolved and probably do not contain codecarboxylase as coenzyme. In the following section the action of various common inhibitors on the cell-free preparations of the six enzymes, under conditions of optimal activity in each case, is described. In all cases the

action was followed in manometers at 30°, the substrate tipped in from the side-bulb after 15 min. equilibration, during which time the enzyme was incubated in the presence of buffer, inhibitor and, if necessary, codecarboxylase. Table 3 lists the percentage inhibition obtained in each case with concentrations of inhibitor between M/100 and M/100,000.

Silver appears to be the inhibitor most generally active against the whole group of enzymes, but the tyrosine decarboxylase is not very sensitive to either silver or mercury. The group as a whole is not very sensitive to *copper* with the exception of the *l*(+)-ornithine decarboxylase, which is completely inhibited by a concentration of 10^{-5} M. and 27 % inhibited by 10^{-6} M. Cu: this may be correlated with the higher sensitivity of this enzyme to oxidation by *permanganate*. *Iron* has little effect on any of the enzymes, but the codecarboxylase proteins are markedly more sensitive to it than the others. All the decarboxylases are *cyanide*-sensitive, that of histidine the least so, but this is not correlated with an equal sensitivity to *azide*, which seriously poisons only the lysine enzyme, but slightly inhibits the arginine and ornithine enzymes also. It has been shown that the cyanide inhibition of the lysine enzyme is reversible (Gale & Epps, 1944a). Epps (1945) has shown that the *l*(-)-histidine enzyme is 51 % inhibited by M/100 *sulphanilamide*; the other enzymes of the group are not affected to the same extent, although a 33 % inhibition is obtained with the ornithine enzyme.

Hydrazine is an inhibitor of all the enzymes except those of histidine and glutamic acid; a concentration of M/1000 inhibits the first four enzymes in the table 91–100 % but has little or no effect on the other two at this concentration. *Hydroxylamine* is an inhibitor which is again more effective against the first four decarboxylases; 10^{-4} M inhibits the histidine and glutamic enzymes 50 % but the remainder 91–100 %. *Semicarbazide*, 10^{-4} M inhibits the lysine, ornithine and tyrosine enzymes 97–100 %, arginine enzyme 78 %, histidine enzyme 52 % and the glutamic acid enzyme 3 %. There is some evidence then that these three inhibitors are more effective against the four enzymes which are known to require codecarboxylase as coenzyme than against the two enzymes in which the presence of codecarboxylase has not been demonstrated. Since these inhibitors are believed to act by combination with keto-groups, it may be that the activity of the codecarboxylase enzymes is dependent upon the presence of such a group in the enzyme molecule.

Gale & Epps (1944a) found that lysine decarboxylase activity is inhibited by the presence of fairly high concentrations of salts such as ammonium sulphate, sodium sulphate, sodium chloride, etc. Epps (1944, 1945) did not find any such effect

Table 3. *Action of inhibitors on cell-free amino-acid decarboxylases*

Decarboxylase substrate		<i>l</i> (+)- Lysine	<i>l</i> (+)- Arginine	<i>l</i> (+)- Ornithine	<i>l</i> (-)- Tyrosine	<i>l</i> (-)- Histidine	<i>l</i> (+)- Glutamic acid
Codecarboxylase present in enzyme?		+	+	+	+	-	-
Inhibitor	-log ₁₀ molar concentration	Inhibition (%)					
AgNO ₃	5	98	—	98	—	13	4
	4	100	35	100	—	100	20
	3	100	100	100	39	100	81
	2	100	100	100	99	100	100
HgCl ₂	5	98	14	—	—	—	—
	4	100	100	35	—	58	27
	3	100	100	100	—	94	100
	2	100	100	100	97	100	100
CuSO ₄	5	—	—	100	—	—	—
	4	46	7	100	—	—	4
	3	89	51	100	98	61	10
	2	98	100	100	100	92	33
FeSO ₄	4	30	—	16	—	—	—
	3	82	23	32	26	0	0
	2	100	54	67	66	15	0
KMnO ₄	5	10	—	53	24	—	6
	4	100	17	98	100	15	41
	3	100	100	100	100	94	100
	2	100	100	100	100	100	100
KCN (neutral)	4	81	22	40	—	—	93
	3	93	98	77	64	20	100
	2	100	100	96	93	97	100
NaN ₃	4	61	7	36	—	—	—
	3	98	21	51	—	—	10
	2	100	100	97	0	0	27
Sulphanilamide NH ₂ OH	2	15	21	35	9	51	0
	5	95	91	50	43	—	7
	4	100	95	98	97	54	42
	3	100	100	100	100	100	96
NH ₂ .NH ₂	5	95	62	80	—	—	0
	4	100	71	92	30	0	9
	3	100	91	100	98	0	13
	2	100	100	100	100	10	43
Semicarbazide	6	42	—	—	—	0	0
	5	76	45	77	90	33	0
	4	98	78	97	97	52	3
	3	100	94	100	100	77	6
	2	100	100	100	100	89	24
	1	43	34	86	0	0	15
(NH ₄) ₂ SO ₄	1	43	34	86	0	0	15

for the decarboxylases of tyrosine or histidine. Of the remaining three enzymes, the ornithine decarboxylase is very sensitive to the presence of salt, *m*-(NH₄)₂SO₄ producing 86 % inhibition; arginine decarboxylase is affected to approximately the same extent as the lysine enzyme, while the glutamic acid decarboxylase is comparatively insensitive. It would seem that this salt effect is not due to resolution of the protein-codecarboxylase system, for (a) it cannot be reversed to any extent by the addition of excess codecarboxylase, and (b) the magnitude of the salt sensitivity is not correlated

with either the presence of codecarboxylase in the enzyme or the ease with which the enzymes containing codecarboxylase can be resolved.

SUMMARY

1. Methods for obtaining cell-free preparations of *l*(+)-arginine, *l*(+)-ornithine, and *l*(+)-glutamic acid decarboxylases are described.

2. *l*(+)-Arginine decarboxylase has optimum activity at pH 5.25 and Michaelis constant 0.00075*M*; *l*(+)-ornithine decarboxylase, pH 5.25, 0.0039*M*; *l*(+)-glutamic acid decarboxylase, pH 4.25, 0.027*M*.

3. *L*(+)-Arginine decarboxylase can be resolved into apo-enzyme and codecarboxylase by precipitation with ammoniacal ammonium sulphate solution. *L*(+)-Ornithine decarboxylase resolves spontaneously on standing into apo-enzyme and codecarboxylase. It has not been possible to resolve *L*(+)-glutamic acid decarboxylase and there is no evidence that this enzyme contains codecarboxylase.

4. The action of common enzyme inhibitors on six amino-acid decarboxylases in a cell-free state is

described. The four enzymes containing codecarboxylase are markedly more sensitive to inhibition by iron and hydrazine than the two in which it has not been possible to demonstrate the presence of codecarboxylase.

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REFERENCES

- Curran, H. R. & Evans, F. R. (1942). *J. Bact.* **43**, 125.
 Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
 Epps, H. M. R. (1945). *Biochem. J.* **39**, 42.
 Gale, E. F. (1940). *Biochem. J.* **34**, 392, 846.
 Gale, E. F. (1941). *Biochem. J.* **35**, 66.
 Gale, E. F. (1945). *Biochem. J.* **39**, 46.
 Gale, E. F. & Epps, H. M. R. (1944*a*). *Biochem. J.* **38**, 232.
 Gale, E. F. & Epps, H. M. R. (1944*b*). *Biochem. J.* **38**, 250.

The Lead Content of Urinary and Biliary Calculi and Gall-bladder Bile

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The report by Trumper & Gordy (1941) of a renal calculus containing 3% of lead, and a subsequent paper by Wood (1941), who examined a number of urinary calculi, both recent and from a collection made in 1850-60, and found lead present spectroscopically in all except two, suggested a further investigation of the lead content of urinary calculi. Two groups were examined, one of 17 stones removed at operation over the last few years, the other of 16 stones, a representative selection from a pre-1900 collection of bladder calculi in the museum of the Pathology Department, one of the seven dated stones belonging to 1830, the remaining six to 1860-80. In view of the possible importance of the bile as a channel of lead excretion (Aub, Fairhall, Minot & Reznikoff, 1926), biliary calculi removed at operation or *post mortem*, and bile obtained from the gall-bladder *post mortem*, were also analyzed.

EXPERIMENTAL

The stones were dried, powdered, and a portion taken for qualitative analysis. From 1 to 3 g. of the remainder were analyzed for lead by the method previously described (Tompsett & Anderson, 1935), with the modification advised for materials containing considerable calcium phosphate (Tompsett, 1939).

Lead was estimated in bile by the method described for blood (Tompsett & Anderson, 1935, 1939).

The results of the analyses of urinary calculi are shown in Table 1, of gall-stones in Table 2, and of bile in Table 3. The stones in Table 1 are grouped

by the results of qualitative analysis according to the scheme given by Harrison (1937).

Table 1. *Lead content of urinary calculi*

Type	Lead content (mg./100 g.) of	
	Recent stones	Old stones
Renal calculi:		
Oxalate	7.5, 5.4, 3.7, 11, 10.5	—
Oxalate and phosphate	7.6, 10.5, 9	—
Phosphate and carbonate	11, 29, 8.4	—
Vesical calculi:		
Uric acid or urate	0.66, 0.33	1.6, trace, 0.44
Phosphate	5.5	—
Phosphate and urate	2.7	3.5, 3.6, 3.2
Phosphate, urate and carbonate	1.3, 11	—
Phosphate and oxalate	—	7.7, 19, 9.65, 5.2, 6.3, 2.7
Phosphate, oxalate and urate	—	10.8, 9.2, 10.6
Urate and oxalate	—	3.7
Mean, excluding pure uric acid or urate stones	8.9	7.3
Mean, all stones excluding pure uric acid or urate stones	8.2, standard deviation 5.15	

Table 2. *Lead content of biliary calculi*

Type of calculus	Lead content (mg./100 g.)
Multiple faceted	0.61, 2.2, 0.45, 4
Cholesterol-pigment	0.3, 11, 6.1
'Pure' cholesterol	1.1
'Pure pigment'	65, 13.5