Studies on Bacterial Amino-acid Decarboxylases

3. DISTRIBUTION AND PREPARATION OF CODECARBOXYLASE

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Studies of the *l*(+)-lysine and *l*(−)-tyrosine decarboxylases have shown that these enzymes can be reversibly resolved into a specific protein portion and a hitherto undescribed coenzyme (Gale & Epps, 1944; Epps, 1944). The present communication deals with the distribution and isolation of this coenzyme substance which we have tentatively called 'codecarboxylase'.

METHODS

Figures have been published showing the variation of velocity of decarboxylation with the concentration of codecarboxylase for a given amount of apo-enzyme in the cases of *l*(+)-lysine decarboxylase (Gale & Epps, 1944) and of *l*(−)-tyrosine decarboxylase (Epps, 1944). In either case the velocity of decarboxylation bears an approximately linear relation to the amount of codecarboxylase added as long as this amount is considerably less than the saturation value for the apo-enzyme used. Consequently this velocity can be used as a measure of the amount of coenzyme present during test, and comparative measurements can be made as long as other conditions remain constant and the same amount of the same preparation of apo-enzyme be used for any one series of comparisons.

We have used this as a basis for the investigation of distribution and purification of codecarboxylase. The rate of decarboxylation is measured manometrically as previously described. In these studies we have made measurements with the apo-enzymes of both lysine decarboxylase and tyrosine decarboxylase, so that a double check should be provided on the results and also on evidence as to the identity of the coenzyme for these two enzymes. Sufficient enzyme was prepared in both cases to cover each full series of results; the apo-enzyme portions were prepared and made up in solutions of such strength that 1 ml. should contain 8 units of enzyme when saturated with coenzyme. 0·5 ml. of this preparation was used per test and measured portions of the coenzyme preparations were taken, such that the CO_2 evolution/5 min. was less than 200 μl., thus ensuring that the rate fell on the linear part of the velocity-coenzyme concentration curve. Tests were carried out at 30° and at the optimum pH of 6·0 for lysine, or 5·5 for tyrosine, decarboxylase. An arbitrary unit of coenzyme is defined as that amount of coenzyme which increases the rate of decarboxylation by the apo-enzyme by 100 μl./5 min. As a measure of the amount of substance in preparations, the C content was estimated by the wet combustion method of Van Slyke & Folch (1940). The activity, P, of coenzyme preparations is defined as μl. CO_2 liberated from substrate, due to presence of coenzyme/hr./mg. C of coenzyme. P is calculated from the formula $P = 12(R - r)/W$, where $r = \mu$l. CO_2 liberated/5 min. in presence of apo-enzyme alone, and $R = \mu$l. CO_2 liberated/5 min. in presence of apo-enzyme plus W mg. C of coenzyme preparation, provided that the value of R falls within the limits defined above. The values of P, and number of units, can be used only for comparative purposes, for tests carried out with a standard amount of a given apo-enzyme preparation.

DISTRIBUTION OF CODECARBOXYLASE

A survey of various animal, plant, yeast and bacterial cells has been made on the assumption that the active principle in each case is the same substance ('codecarboxylase'). A suitable amount of the tissue to be assayed was ground as finely as possible in water, and the suspension was then boiled for 2 min. and again ground. Measured portions were taken for estimation of the coenzyme activity, by addition to apo-enzyme, and of C content. In the manometric estimation of coenzyme activity it is necessary to equilibrate for a constant time, not less than 15 min., before the addition of substrate. Table 1 gives results obtained with both lysine and tyrosine apo-decarboxylases, expressed as units of coenzyme/mg. C of tissue, placed in descending order of activity.

The table shows the wide distribution of coenzyme activity. Positive results have been obtained with all the rat tissues tested; with all bacteria tested, whether possessing potential amino-acid decarboxylases (as *Esch. coli*, *Strep. faecalis*, etc.; Gale, 1940) or not (as *Ps. aeruginosa*; Gale, 1942), with *Sarcina lutea*, etc.; brewer's and baker's yeasts; and with the two plant tissues tested. The only biological substance found to give completely negative results was urine. In some cases (those marked with an asterisk in the table) the assays are only very approximate, as the impossibility of making homogeneous suspensions makes it very difficult to measure out accurate portions for assay. The substances are listed in the order of their activity towards lysine decarboxylase but it can be seen that, with few exceptions, the order of activities is the same when the coenzyme activity is assayed with regard to tyrosine decarboxylase. Amongst the substances having an activity towards lysine decarboxylase of more than 1 unit/mg. C, the most easily obtainable under present circumstances is

brewer's yeast and this has been selected as the most convenient source for isolation of the co-decarboxylase.

Table 1. *Distribution of codecarboxylase in various tissues, cells and fluids*

Tissue	Carbon (mg./ml.)	Lysine codecarboxylase Units/ml.	Lysine codecarboxylase Units/mg. C	Tyrosine codecarboxylase Units/ml.	Tyrosine codecarboxylase Units/mg. C
Rat skeletal muscle	3·65	5·6	1·54*	1·19	0·325*
B. subtilis	2·85	4·3	1·5	1·7	0·6
Staph. aureus	1·72	2·55	1·48	0·6	0·35
Rat liver	4·2	5·1	1·22	1·52	0·36
Esch. coli	5·65	6·3	1·12	2·1	0·37
Brewer's yeast	11·6	11·9	1·03	3·4	0·29
Ps. aeruginosa	4·45	3·4	0·765	1·3	0·29
Rat kidney	6·3	4·0	0·63	1·0	0·16
Sarcina lutea	4·2	1·7	0·41	0·65	0·155
Rat heart	4·8	1·85	0·385*	0·51	0·106*
Rat lung	2·1	0·80	0·38	0·22	0·105
Baker's yeast	9·3	3·15	0·34	1·1	0·12
Brassica oleracea	1·16	0·40	0·29*	0·1	0·07*
Pisum sativum	5·25	1·25	0·24*	0·34	0·065*
Rat gut	7·3	1·65	0·23*	0·45	0·062*
Rat brain	13·7	2·9	0·21	0·9	0·065
Strep. faecalis	5·4	0·7	0·13	0·18	0·033
Milk (cow)	49·8	2·15	0·043	0·61	0·012
Blood (rat)	24·3	0·9	0·037*	0·11	0·0045*
Urine (man)	—	—	—	—	—

* These analyses are approximate only (see text).

PRELIMINARY WORK ON PREPARATION OF CODECARBOXYLASE FROM DRIED BREWER'S YEAST

1. *Extraction from yeast.* An aqueous suspension of brewer's yeast, boiled for 5 min., is a good source of coenzyme; but if the suspension is centrifuged most of the activity is carried down with the sediment. If the yeast is boiled in N/10 HCl no activity is extracted from the sediment; while if the boiling is in N/10 NaOH, there is some loss, but about half the activity remains in the supernatant liquid after removal of the sediment. The extraction of coenzyme from the cell is thus assisted by mildly alkaline conditions. Table 2, A, shows the effect of NaOH concentration upon the extent of extraction at 100° for 15 min.; concentrations above N/10 NaOH appear to have a destructive effect. By the use of material extracted in this way, it was found that the Ba salt of the coenzyme is soluble in water, while much inactive material can be precipitated by the addition of barium salts. We thought therefore that a step in the purification could be combined with the extraction process, if the latter were made with dilute barium hydroxide solution instead of NaOH. Table 2, B, shows the extraction obtained by boiling 1 g. dried yeast in 5 and 10 ml. $Ba(OH)_2$ solutions of various strengths, and the results show that 10 vol. N/28 $Ba(OH)_2$ extract twice as much as 5 vol., while stronger solutions appear to be destructive. Study of the time of extraction in N/28 $Ba(OH)_2$ (Table 2, C) shows that rapid destruction of coenzyme activity takes place if the heating process is prolonged for more than a few minutes. Consequently we tested the effect of cold extraction of brewer's yeast which had been dried at room temperature and then ground to a fine powder. Table 2, D, shows that extraction at room temperature in N/18 $Ba(OH)_2$ is the most efficient process so far employed and that there is no loss on standing for 30 min. Later experience has shown that satisfactory extraction is consistently obtained by the use of 10 vol. 0·75% $Ba(OH)_2 \cdot 8H_2O$, on standing for 30 min. at room temperature, and that there is no significant deterioration in activity if the mixture is left for 3–4 hr. at room temperature.

Table 2. *Conditions for extraction of codecarboxylase from dried brewer's yeast*

A. *Extraction with* NaOH *at* 100°

(Tubes contained 1 g. dried yeast in 5 ml.; incubated at 100° for 15 min. and sediment centrifuged down; 0·2 ml. supernatant for test.)

Conc. NaOH ...	N/5	N/10	N/15	N/20	N/50
Units of coenzyme/ml. extract	2·95	4·80	4·40	3·45	2·40

B. *Extraction with* $Ba(OH)_2$ *at* 100°

(Tubes contained 1 g. dried yeast in (*a*) 5 ml., (*b*) 10 ml.; incubated at 100° for 15 min. and sediment centrifuged down; 0·2 ml. supernatant taken for test.)

Conc. $Ba(OH)_2$ ...		N/3·7	N/9	N/18	N/28	N/37
Units of coenzyme/ml. extract:	(*a*)	—	3·4	3·0	2·4	2·5
	(*b*)	0·4	1·5	1·6	2·55	—

C. *Time of extraction with* N/28 $Ba(OH)_2$ *at* 100°

(Extraction with 10 vol. N/28 $Ba(OH)_2$, with heating at 100° before centrifuging.)

Time of heating (min.)	1	5	10	15	20	25	30	45	60
Units of coenzyme/ml. extract	5·4	4·4	3·2	2·5	1·6	1·1	0·98	0·55	0·4

D. *Extraction with* $Ba(OH)_2$ *at room temperature*

(In each case 5 g. dried yeast were ground up with 50 ml. $Ba(OH)_2$ solution at room temperature; left for (*a*) 5 min., (*b*) 30 min. and sediment then centrifuged down; 0·2 ml. supernatant taken for test.)

Conc. $Ba(OH)_2$ ...		N/9	N/18	N/28	N/55	0
Units of coenzyme/ml. extract:	(*a*)	—	—	3·9	—	—
	(*b*)	4·20	7·80	4·36	0·94	0·64

2. *Barium salt.* The Ba salt of the coenzyme is soluble in water but is precipitated by alcohol (ethanol or methanol). Addition of alcohol to the crude $Ba(OH)_2$ extract results in the precipitation of the coenzyme accompanied by much inactive

material, including a certain amount of protein-like substance. It is better to remove this protein-like material by alcohol precipitation first. Consequently the crude $Ba(OH)_2$ extract is first treated with H_2SO_4 to remove the Ba as sulphate and the material is then poured into alcohol to remove the protein. The latter will flocculate cleanly only if the pH lies between 6·8 and 7·0; hence the extract is first acidified to pH 3 with 25% H_2SO_4, left to stand for about 15 min. and the pH then readjusted to 6·8 with 10% NaOH. On pouring the mixture into alcohol, the $BaSO_4$ is carried down with the protein, leaving the active substance in the alcoholic supernatant liquid. The active substance can now be precipitated from the alcohol by the addition of concentrated barium acetate solution. Washing of the precipitate so obtained does not remove the active material, which appears to be firmly adsorbed on the precipitate, and some purification can be achieved at this stage by exhaustive washing of the precipitate with hot water. After such treatment the active substance can be redissolved by decomposing the precipitate with N/10 H_2SO_4. Table 3

Table 3. *Optimum concentration of alcohol for precipitation of* Ba *salts*

(Dried yeast extracted with 10 vol. 0·75% $Ba(OH)_2 \cdot 8H_2O$ at room temperature; sediment centrifuged down; supernatant adjusted to pH 3 with 25% H_2SO_4; left to stand 15 min. and readjusted to pH 6·8 with 10% NaOH. 2 ml. portions taken, poured into alcohol and precipitate centrifuged down and discarded; saturated Ba acetate added to supernatant until precipitation complete; precipitate washed in 10 ml. water; precipitate decomposed in 1 ml. N/10 H_2SO_4; vol. adjusted to 2 ml., and 0·1 ml. taken for test.)

Activity of initial crude extract = 12·3 units codecarboxylase/ml.

Vol. methanol used for precipitation	Units co-enzyme/ml.	Vol. ethanol used for precipitation	Units co-enzyme/ml.
1	10·9	1	10·6
2	10·9	2	9·8
3	9·4	3	8·4
4	5·3	4	3·5
5	4·1	5	2·1

shows the effect of ethanol concentration upon the yield of coenzyme, from the crude extract stage to the decomposition of the washed precipitate. Methanol gives better yields and cleaner precipitates than ethanol and the highest yields are obtained by the use of twice the volume of methanol. A volume of either ethanol or methanol equal to the volume of aqueous solution gives equally good yields, but removes little protein in the first precipitation, so that the Ba precipitate contains much inactive material.

3. *Lead and silver salts.* Both the Ag and Pb salts of the coenzyme are insoluble at pH 7 in water, but decomposition of these salts with H_2S leads to adsorption of the coenzyme on the metallic sulphides. No efficient method of elution has been discovered. The active material can be regenerated from the Pb salt by decomposition with H_2SO_4 and from the Ag salt with HCl, but the high sensitivity of the enzymes to the presence of Ag (Gale & Epps, 1944; Epps, 1944) makes this latter procedure unsatisfactory. Table 4 shows that precipitation of the Pb salt begins about pH 5·5 but is not complete until neutrality is reached; the Ag salt is precipitated at neutrality also.

Table 4. *Precipitation of lead and silver salts of codecarboxylase*

(Extract adjusted to pH 1 with HNO_3, and excess Pb acetate added to 2 ml. portions; pH then raised in separate samples and precipitates collected at pH 3, 5·5, 6·5, 7·0 and 8·0 respectively. Precipitates decomposed in 1 ml. N/10 H_2SO_4; 1 ml. water added and 0·2 ml. taken for test.)

Precipitation at pH ...	3	5·5	6·5	7·0	8·0
Units of coenzyme/ml. after decomposition of Pb salt	0	0·12	0·48	0·48	0·52

(Experiment repeated, using $AgNO_3$ as precipitating agent. In this case the precipitates were centrifuged off and the supernatant treated with NaCl to remove Ag, neutralized, and the volume adjusted; 0·25 ml. of supernatant taken for test.)

Precipitation at pH ...	3	4	6	7·5	9·0
Units of coenzyme/ml. of supernatant	0·5	0·5	0·45	0	0

4. *Alumina* C_γ *adsorption.* From crude extracts adsorption of the active material occurs on alumina C_γ suspension at pH 4·0. Elution has not been achieved. More highly purified preparations can be eluted by strong phosphate at pH 7·0, but there is no significant purification.

5. *Solubility in organic solvents, etc.* The active material can be extracted from acid solution with *n*-butanol, *iso*butanol, phenol, *m*- or *p*-cresol but not with amyl alcohol. The partition coefficient between phenol and water is increased in favour of the phenol by saturation of the water phase with ammonium sulphate. The active material is precipitated by phosphotungstic acid but not by picric or picrolonic acid.

METHOD OF PURIFICATION OF CODECARBOXYLASE FROM DRIED BREWER'S YEAST

Table 5 shows the steps in the preparation of codecarboxylase adopted as a result of the preliminary investigations described. The yields and degree of purification over each stage were followed by measuring units of codecarboxylase and *P* values under standard conditions, with both lysine apo-

decarboxylase and tyrosine apo-decarboxylase. As each step was completed, measurements were made of the volume of the preparation, its activity as codecarboxylase with both apo-enzymes, and its C content. The steps in the preparation of codecarboxylase from 1 kg. of dried brewer's yeast are as follows.

1. 10 l. of 0·75% $Ba(OH)_2.8H_2O$ solution are prepared by gentle warming. The dried and powdered yeast (1 kg.) is then added in small amounts with vigorous stirring to form a homogeneous suspension. The operation is spread over about 30 min., any lumps being broken up with the fingers. The addition of a few drops of caprylic alcohol as wetting agent is helpful.

2. The suspension is then centrifuged. We have used an International 13-l. serum centrifuge for this purpose, spinning for 15 min. at 2000 r.p.m. The sediment occupies about one-fifth of the total volume after centrifuging, and the collected supernatant is a slightly turbid, brown liquid. 25% H_2SO_4 is added to the supernatant until the pH is approx. 3 (bright yellow to bromocresol-green). The mixture is allowed to stand 15–30 min. at this stage and is then neutralized with 10% NaOH to approx. pH 6·8 (light green to bromothymol-blue). There is no need to remove the precipitate formed by this procedure. The mixture is now poured into 2 vol. methanol and stirred vigorously. A thick precipitate forms which flocks and settles within a few minutes. If the supernatant is cloudy, stirring the sediment and allowing it to settle again usually results in a perfectly clear yellow-brown supernatant which can be decanted. The settled sediment can be filtered under a small negative pressure on large Büchner funnels. Saturated barium acetate solution (usually 250–300 ml.) is now added with stirring to the clear alcoholic filtrate until precipitation is maximal. A fine precipitate forms which settles slowly. After 2–3 hr. the supernatant liquid can be decanted and discarded, and the sludge separated on a Sharples super-centrifuge. The material is stable from this stage onwards. The precipitated material is broken up in *c.* 1 l. hot distilled water to form a fine homogeneous suspension and centrifuged off again. The supernatant liquid is coloured yellow and the washing process is continued until the washings are colourless; five washings, with *c.* 600 ml. boiling water each time, are usually required. The washed precipitate is then suspended in *c.* 300 ml. N/2 H_2SO_4 and left to decompose for about 30 min. On centrifuging, a reddish supernatant is obtained which contains the active material. The white sediment is resuspended in 200 ml. N/2 H_2SO_4, centrifuged as before and the two supernatants collected. Table 5 shows that the whole of this step results in a purification of about 30 times with little loss of active material.

3. Phenol is melted with approx. 10% water. The acid extract from (2) is saturated with this phenol and then extracted 4 times with 20 ml. liquid phenol. This extraction seems to remove most of the flavin nucleotides, as the first extracts are coloured deep red and, if carried through the later steps, give fluorescent yellow solutions having the properties of flavin compounds. Some coenzyme is extracted at this stage, but reaches a limit at 7000–8000 units (lysine) beyond which no further extraction takes place. It has been found most satisfactory to discard this first extract, as little purification is obtained by its inclusion in the next stage. Some of the lost coenzyme can be recovered by shaking the phenol extract with one-tenth vol. water and adding the water extract to the aqueous mother-liquor.

4. The mother-liquor from the first phenol extraction is now saturated with $(NH_4)_2SO_4$ and re-extracted with liquid phenol: the saturation should be carried out in the presence of phenol and it is advisable to use good quality $(NH_4)_2SO_4$. Four extractions, each with *c.* 50 ml. phenol, will usually extract about 50% of the remaining active material, but further extraction is difficult. It has been found inadvisable to increase the scale of the preparation at this stage, as the percentage extraction decreases with the volume of the mother-liquor. The active material is removed from the phenol by the addition of 3 vol. ether and vigorous shaking with three or four successive amounts of 30 ml. water. The water layer becomes coloured yellow and extraction should be continued until the phenol-ether layer is colourless. The yields over this step seldom exceed 50% but a purification of 3–4 times is effected. The water extract is washed twice with equal volumes of ether to remove residual phenol.

5. The water extract is acidified with HNO_3 to pH 1 (red to *m*-cresol-purple) and extracted 10–12 times with an equal volume of *iso*butanol saturated with water. The butanol extract is coloured light yellow, while most of the coloured material remains in the water phase. Extraction with *n*-butanol results in more rapid and quantitative extraction but little purification, while *iso*butanol is far more specific in its extraction. The *iso*butanol extracts are combined, neutralized with NaOH to pH 7·0 (green to bromothymol-blue) and then extracted with 50 ml. N/10 NaOH. The coloured material and coenzyme activity pass quantitatively into the alkali, which separates cleanly only if the preliminary adjustment to pH 7·0 has been accurate—the emulsion which otherwise results is best separated by centrifuging. The yields from the *iso*butanol extraction vary from 50 to 65% with a purification of 2–3 times.

6. The alkaline extract is adjusted to pH 7·0 with HNO_3 and excess lead acetate is added, when the coenzyme is precipitated as its lead salt: it may

be necessary to readjust the pH to 7·0 with dilute alkali after the addition of the lead acetate. Precipitation is complete in 10–15 min. Some inactive yellow material is not carried down under these circumstances and decomposition of the lead precipitate shows purification with little loss.

7. The lead salt is decomposed in N/10 H_2SO_4 and the excess sulphate removed with barium acetate. Whenever $BaSO_4$ is precipitated in the presence of coenzyme, some active material is lost by adsorption. This is greatest if precipitation takes place at pH 5–6 and least in conditions more strongly acid than pH 1. There is little loss at this stage of the preparation but some purification results from absorption of pigment. The $BaSO_4$ is centrifuged off and washed, the washings being added to the first centrifugate.

8. Excess of barium acetate is added, the pH is adjusted to 7·0 and the barium salts are fractionated with ethanol. The concentration is made up to 30 % (by vol.) ethanol and the solution is left 30–60 min. to complete precipitation. The precipitate (8 *a*) is spun off and alcohol added to 50 % ethanol. After 60 min. the resulting precipitate (8 *b*) is spun off and the process repeated at 70 % ethanol (precipitate 8 *c*). In each case the precipitate is redissolved in water, adjusted to pH 6·8 and reprecipitated as the lead salts. The lead precipitates are washed and decomposed in N/10 H_2SO_4 for the activity test—this avoids the presence of 'foreign' C in the preparations for C analysis. Table 5 shows that the most active fraction is that precipitated by 30 % ethanol and that the yield represents some 40–50 % of the active material present at stage 7.

9. A further fraction, slightly less active than 8 *a*, can be obtained by taking the barium salt precipitated by 30–50 % ethanol, dissolving it in water and reprecipitating with 30 % ethanol.

10. The fractions 8 *a* and 9 are combined, precipitated with lead acetate and the lead salts decomposed in N/10 H_2SO_4. This final stage of the purification depends upon the precipitation of various impurities as their lead salts at a pH lower than that at which the lead salt of the coenzyme precipitates (cf. Table 4). The separation is conveniently done as follows: saturated lead acetate solution is added drop by drop to the solution of the crude coenzyme in N/10 H_2SO_4 until a turbidity forms; 3–4 more drops are added and the precipitate is left to coagulate before centrifuging down; another 3–4 drops of lead acetate are then added and the process is repeated until the addition of a drop of lead acetate produces no further precipitate. In this way the bulk of the coloured material is precipitated together with one-third to one-half of the coenzyme; this slow fractional precipitation is essential to avoid adsorption of most of the coenzyme on the coloured material. The supernatant remaining is coloured faintly yellow. N/10 NaOH is now added drop by drop until the pH reaches 6·8–6·9 and the precipitate left for 30 min. to coagulate. This precipitate is white and represents the purest preparation of codecarboxylase so far obtained. A further yield can usually be obtained by decomposing the lead salts of the coloured material obtained in the early part of this stage and repeating the whole process of fractional lead salt precipitation under the same conditions.

The yield of material obtained in stage 10 represents 3–5 % of that originally present in the dried yeast, and the purification is 12,000–15,000 times. The fractions are best kept as their lead salts, in which state they can be dried *in vacuo* and kept indefinitely, being regenerated for use by decomposition in N/10 H_2SO_4. The yield of lead salt from fraction 10 is approx. 10 mg./kg. of dried yeast extracted. This fraction, freed from lead, gives a

Table 5. *Purification of codecarboxylase from dried brewer's yeast*

	Apo-enzyme ...		Lysine decarboxylase				Tyrosine decarboxylase		
Stage	Preparation	Total vol. (ml.)	Units of activity/ ml.	Total units	mg. C/ ml.	P_{Lysine}	Units of activity/ ml.	Total units	$P_{Tyrosine}$
1	Yeast-baryta-suspension	10,500	13·3	140,000	38·0	420	3·3	34,000	104
	Supernatant	8,300	8·85	73,500	11·5	925	2·85	23,700	298
2	Ba precipitate decomposed	620	112	69,000	4·24	31,700	34	17,000	9,620
3	First phenol extract (discarded)	84	90	7,560	3·13	34,500	23	1,930	8,820
4	Second phenol extract	128	220	28,000	2·45	108,000	49	6,250	24,000
5	*iso*Butanol extract	138	140	18,000	0·58	284,000	32	4,400	66,000
6	First Pb salt (decomposition)	66	265	17,500	0·789	404,000	64·5	4,250	98,100
7	SO_4 removed with Ba	68	220	15,000	—	—	58	3,950	—
8	Ethanol fraction: Ba salt								
	(*a*) 0–30 % ethanol	12	515	6,200	0·346	1,800,000	175	2,100	608,000
	(*b*) 30–50 % ethanol	8	600	4,800	0·688	1,050,000	190	1,500	332,000
	(*c*) 50–70 % ethanol	5	140	700	0·475	355,000	31	205	104,000
9	0–30 % ethanol fraction of 8 *b*	4	620	2,480	0·46	1,620,000	210	780	550,000
	8 *a* plus 9	16	—	8,680	—	—	—	2,880	—
10	Final Pb fraction at pH 6·8	10	420	4,200	0·100	5,050,000	130	1,300	1,560,000

colourless or faintly yellow solution. The figures quoted for the recombination of the specific proteins of lysine and tyrosine decarboxylases with codecarboxylase refer to preparations of the latter corresponding to fraction 10 (Gale & Epps, 1944; Epps, 1944).

Ultra-violet absorption spectra. Fig. 1 shows the ultra-violet absorption spectra of codecarboxylase solutions containing (1) 0·052 mg. C/ml. (neutral) and (2) 0·058 mg. C/ml. in N/10 H_2SO_4. There is an absorption band with a peak at 265mμ but no marked absorption in the 350–450mμ range, so that the substance does not appear to be a flavin derivative. Reduction of the substance with cysteine or hydrosulphite does not alter the shape of the absorption curve significantly.

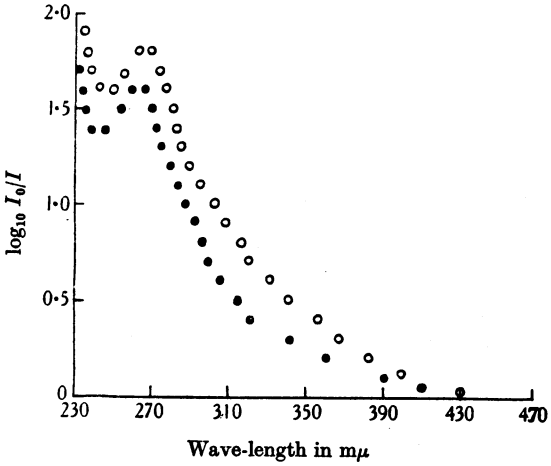

Fig. 1. Ultra-violet absorption spectra ($d=1$). o Solution containing 58 μg. C/ml. in 0·1 N-H_2SO_4. • Solution containing 52 μg. C/ml., neutral.

Empirical composition. Analysis of lead salt of fraction 10 (Weiler): C, 16·00; H, 2·31; N, 3·59; Pb, 69·8%; P, nil; S, nil.

Stability towards acid and alkali. Suitable amounts of the codecarboxylase, freed from lead, were dissolved in N/2 and N/10 NaOH and H_2SO_4 respectively and placed in a boiling water-bath. Samples were removed at intervals for test of codecarboxylase activity. Table 6 shows that the activity is not affected by 4 hr. at 100° in N/2 or N/10 NaOH. In N/10 acid at this temperature the activity is rapidly destroyed.

Table 6. *Stability to acid and alkali of codecarboxylase preparations*

(Activities expressed in units of codecarboxylase/ml. before and after treatment with acid or alkali.)

Time at 100° (min.)	N/10 H_2SO_4		N/10 NaOH	
	Lysine decarboxylase	Tyrosine decarboxylase	Lysine decarboxylase	Tyrosine decarboxylase
0	380	31·5	380	31·5
30	207	22·1	—	—
60	110	11·3	—	—
90	66	6·6	—	—
120	22	2·0	—	—
240	—	—	365	32·1

Stability towards nitrous acid, etc. Solutions of codecarboxylase left to stand with HNO_2 or HOBr, or shaken in alkali with benzoyl chloride, followed by removal of the reagents, show no decrease of coenzyme activity.

Identity of the coenzymes of lysine and tyrosine decarboxylases. Whenever possible, preparations have been assayed for coenzyme activity with both lysine and tyrosine apo-decarboxylases. It can be seen from Table 1 that the distribution of the coenzyme amongst various tissues is the same whether the assay is carried out with the lysine or the tyrosine apo-enzyme. Table 5 shows that the stages of the purification are the same for lysine codecarboxylase as for tyrosine codecarboxylase and that, for any one state, the purification achieved in one case is parallel to that obtained in the other. Table 6 shows that the stability of the coenzyme to hydrolysis is the same whichever apo-enzyme is used for assay. It would appear probable from these sets of data that the codecarboxylase is identical for the two enzyme systems studied. If this be so, then it should be possible to activate the apo-enzyme of one with the coenzyme moiety of the other. Table 7 shows the results of an experiment in which this has been accomplished. Preparations were made of *l*(+)-lysine decarboxylase (Gale & Epps, 1944) from *Bact. cadaveris* and purified to the highest degree so far obtained ($Q^{C}_{CO_2}=33{,}500$) and of *l*(−)-tyrosine

Table 7. *Activation of tyrosine apo-decarboxylase by coenzyme moiety of lysine decarboxylase*

Manometer no. ...	1	2	3	4	5	6	7
	Additions (ml.)						
M/5 Acetate pH 5·5	1·5	1·5	1·5	1·5	1·5	1·5	1·5
M/15 Tyrosine	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Tyrosine apo-decarboxylase	0·5	0·5	0·5	0·5	—	—	—
Codecarboxylase (excess)	—	0·5	—	—	0·5	—	—
Boiled tyrosine decarboxylase preparation (8 units)	—	—	0·5	—	—	0·5	—
Boiled lysine decarboxylase preparation (15 units)	—	—	—	0·5	—	—	0·5
Water	0·5	—	—	—	—	—	—
μl. CO_2/5 min.	15	227	156	160	0	3	0

decarboxylase from *Strep. faecalis* (Epps, 1944). The specific protein of the tyrosine decarboxylase was then precipitated and freed from codecarboxylase (Epps, 1944). The activity of this specific protein was then tested towards tyrosine alone and in the presence of codecarboxylase (preparation 8 *a*); a boiled preparation of tyrosine decarboxylase (8 units), and a boiled preparation of lysine decarboxylase (15 units). The values in Table 7 show that the apo-enzyme can be reactivated towards its substrate by the presence of the codecarboxylase preparation, or of the boiled inactive preparations of either enzyme. Thus the lysine decarboxylase preparation contains a substance which will act as the codecarboxylase of the tyrosine decarboxylase.

SUMMARY

1. Codecarboxylase is widely distributed amongst animal tissues, plant tissues, yeasts and bacteria.

2. Codecarboxylase can be extracted from dried brewer's yeast by mildly alkaline solutions.

3. A method is described for the purification of codecarboxylase extracted from dried brewer's yeast. An over-all purification of 15,000 times can be achieved.

3. The resulting preparation is colourless or faintly yellow, contains C, H, N but no P or S, and has a single absorption band in the ultra-violet at 265mμ.

4. The coenzyme activity is stable to alkaline hydrolysis but is rapidly destroyed by N/10 H_2SO_4 at 100°.

5. Evidence is presented that *l*(+)-lysine decarboxylase and *l*(−)-tyrosine decarboxylase have the same codecarboxylase.

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A Liver Storage Test for the Assessment of Vitamin A

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The introduction of chemical methods for the determination of vitamins led most investigators to prefer these methods to biological assays. The chemical methods are simpler, cheaper and require less time. The physiologist, however, is not only interested in the quantity of vitamin given, but in its utilization by the organism. This question can be answered by the biological assay. Often, especially in the investigation of liver oils, there are considerable differences among the chemical, physical and biological results of vitamin A determinations (Coward, Dyer, Morton & Gaddum, 1931; Edisbury, Gillam, Heilbron & Morton, 1932; Morgan, Edisbury & Morton, 1935; Pritchard, Wilkinson, Edisbury & Morton, 1937; Robinson, 1938; Embree, 1939; Lunde & Kringstad, 1940). These differences have brought about such uncertainty regarding the value of the vitamin A potency of some materials that in these cases it is now agreed to consider only the results of bioassay as reliable. Nevertheless, great disadvantages of the methods of bioassay for vitamin A lie in the time and effort needed for their performance; and this is especially true of the curative growth test, the method most commonly used.

The difficulties in the determination of the vitamin A content of biological materials are even greater, if these materials contain both vitamin A and carotene, or even carotene alone. The usual chemical method for carotene determination, that of Guilbert (1934), is not exact enough, because α-, β-, and γ-carotene and kryptoxanthin are all determined together, and although all are precursors of vitamin A they differ in their biological potency. Because of the different amounts of the various carotenes present in different plant foods, this kind of determination cannot give the real vitamin A