J. Physiol. (1949) 108, 427–439 577.155.3.547.233:612.146

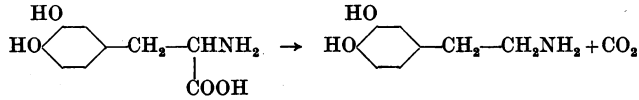
ENZYMIC FORMATION OF PRESSOR AMINES

By H. BLASCHKO, PAMELA HOLTON and G. H. SLOANE STANLEY

From the Department of Pharmacology, University of Oxford

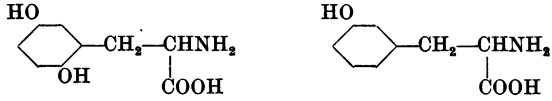
(*Received* 7 *July* 1948)

Pressor amines can be formed by the decarboxylation of amino-acids. Dopa decarboxylase is a mammalian enzyme which catalyses a reaction of this type, the formation of β-(3 : 4-dihydroxyphenyl)-ethylamine from the L-form of β-(3 : 4-dihydroxyphenyl)-alanine (Holtz, Heise & Lüdtke, 1938):



3:4-Dihydroxyphenylalanine 3:4-Dihydroxyphenylethylamine

Two new substrates of the enzyme are described in this paper, namely, 2 : 5-dihydroxyphenylalanine and meta-tyrosine (*m*-hydroxyphenylalanine). Both these amino-acids give rise to pressor amines on decarboxylation, and some of the properties of these amines are also described.



2:5-Dihydroxyphenylalanine Meta-tyrosine (*m*-hydroxyphenylalanine)

MATERIAL AND METHODS

The preparation of the tissue extracts and the plan of the manometric experiments differed in no way from the methods already described (Blaschko, 1942). Two organs known to contain dopa decarboxylase were used, the rat's liver and the guinea-pig's kidney. The latter organ gives extracts with high enzymic activity. The amino-acids were incubated with the tissue extracts in an atmosphere of nitrogen at a temperature of 37·5°.

The pressor action of the amines formed was studied on the arterial blood pressure of the spinal cat. DL-meta-tyrosine was kindly prepared by Dr H. R. Ing by the method described by Blum (1908). Meta-tyramine (*m*-hydroxyphenylethylamine) hydrochloride, prepared in the laboratory of Messrs Burroughs Wellcome and Company at Tuckahoe, N.Y., was made available through the kindness of Dr C. H. Kellaway.

2 : 5-dihydroxyphenylalanine has recently been synthesized by Dr A. Neuberger (1948). We are grateful to him for a sample of the racemic product, as well as of the two stereoisomers. He also gave us a sample of 2 : 5-dihydroxyphenylethylamine hydrochloride; this is also a new substance.

RESULTS

(1) *Observations with* 2 : 5-*dihydroxyphenylalanine*

(*a*) *Carbon dioxide formation from* L-2 : 5-*dihydroxyphenylalanine.* Carbon dioxide was formed when DL-2 : 5-dihydroxyphenylalanine was incubated under anaerobic conditions with tissue extracts containing dopa decarboxylase. The rate of CO_2 formation was compared with that which occurred when DL-3 : 4-dihydroxyphenylalanine was used as substrate. A typical experiment of this kind is shown in Fig. 1, in which 0·4 ml. of a M/50 solution of amino-acid was added to each flask. The rate of the reaction was slower with the 2 : 5-acid. At the end of the incubation, which lasted for 27 min., 86·5 μl. of CO_2 had been formed. At this time the reaction had come to a standstill. 86·5 μl. of CO_2 correspond to 0·48 moles CO_2 per mole of amino-acid added. This result suggests that only one of the two stereoisomers had given rise to carbon dioxide, probably that with the L configuration.

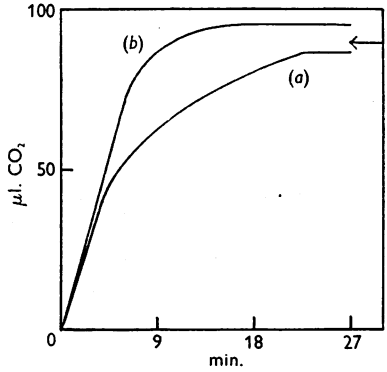


Fig. 1. Decarboxylation of 2 : 5-dihydroxyphenylalanine (*a*) and 3 : 4-dihydroxyphenylalanine (*b*) by an extract of guinea-pig's kidney. Abscissa: time in minutes; ordinate: μl. CO_2 formed. The arrow denotes half a molecule of CO_2 per molecule of DL-amino-acid added.

This was confirmed when the two stereoisomers were tested separately. Of each of the two isomers, 0·4 ml. of a M/100 solution were incubated with extract of guinea-pig's kidney. No CO_2 was formed from the dextrorotatory compound; from the laevorotatory isomer 94 μl. of CO_2 were formed, that is, 1·05 molecules of CO_2 per molecule of amino-acid added. It is known that dopa decarboxylase is specific for the L-configuration and does not act on D-3 : 4-dihydroxyphenylalanine (Holtz *et al.* 1938; Blaschko, 1942); our results therefore suggest that the laevorotatory form of 2:5-dihydroxyphenylalanine has the L-configuration. This is in agreement with the chemical evidence (Neuberger, 1948).

Dopa decarboxylase is thought to be responsible for the decarboxylation of 2 : 5-dihydroxyphenylalanine for these reasons:

(1) Decarboxylation of the 2 : 5-acid occurs in extracts of both rat's liver and guinea-pig's kidney, organs known to contain the enzyme.

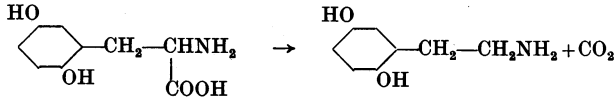
(2) The ratio

$$\frac{q_{CO_2}^{3:4\text{-acid}}}{q_{CO_2}^{2:5\text{-acid}}}$$

is very similar for both organs; in each the reaction is somewhat slower with the 2 : 5-acid.

(3) The rates of carbon dioxide formation, with both acids added together, were not additive; in one experiment with guinea-pig's kidney extract the amounts of CO_2 formed in 6 min. were: with M/500 L-2 : 5-dihydroxyphenylalanine, 25 μl.; with M/125 L-3 : 4-dihydroxyphenylalanine, 25·5 μl.; with both acids, 28 μl.

(*b*) *The pressor action of* β-(2 : 5-*dihydroxyphenyl*)-*ethylamine.* The experiments described above suggested that L-2 : 5-dihydroxyphenylalanine reacted in the tissue extracts, according to the equation:



L-2:5-Dihydroxyphenylalanine 2:5-Dihydroxyphenylethylamine

The product of decarboxylation is 2 : 5-dihydroxyphenylethylamine, a substance which had not been made by the organic chemist at the time when our first experiments were carried out. Since the amine was expected to have sympatheticomimetic properties, it was thought desirable to compare the activity of the substance formed by enzymic action with that of the synthetic product. Leaf & Neuberger (1948) prepared 2:5-dihydroxyphenylethylamine and we are indebted to them for the sample used in the following experiments.

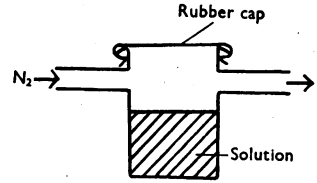


Fig. 2. Assay vessel. Half size. The vessel is closed by a rubber cap and nitrogen is passed through.

In aqueous solutions 2 : 5-dihydroxyphenylethylamine is easily oxidized, and it was necessary to protect the amine from oxidation during the course of the assay. The solutions containing the amine were kept in a glass vessel shown in Fig. 2. A rubber membrane was tied over the top and a slow stream of nitrogen was passed through the vessel. A weight was put on top of the rubber cap; this was removed when the needle attached to a syringe was plunged through the membrane in order to remove some of the solution for an injection.

Fig. 3 shows a record of the arterial blood pressure of the spinal cat. The effect of an intravenous injection of the 2 : 5-amine was compared with that of an equal amount of the corresponding 3 : 4-dihydroxyphenylethylamine. One mg. of each amine was given. The effect with each substance was a blood-pressure rise, but the type of response was different. After the injection of the 2 : 5-amine the rise was less steep and the maximum attained was less than that after an injection of the 3 : 4-amine. The rise of blood pressure due to the 3 : 4-amine was more reminiscent of an injection of adrenaline. The duration of the blood-pressure rise was more prolonged with the 2 : 5-amine.

The greater similarity of the blood-pressure response of the 3 : 4-amine to that of adrenaline is well shown on Fig. 4A, in which approximately equipressor doses of adrenaline and the two dihydroxyphenylethylamines were given. The different type of response to the 2 : 5-amine is evident.

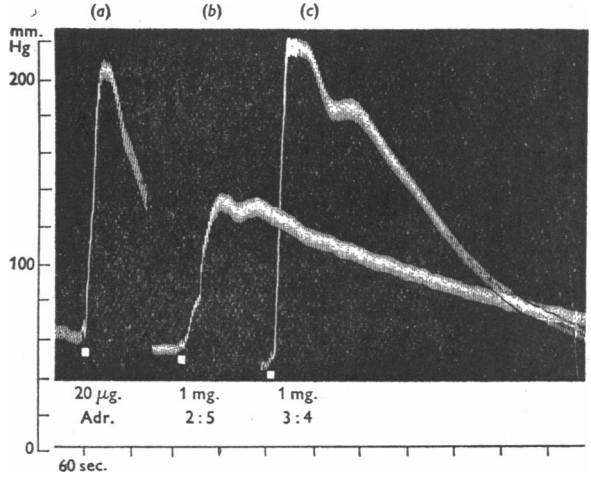


Fig. 3. Spinal cat; arterial blood pressure. The effect of intravenous injection of: (*a*) 20 μg. adrenaline; (*b*) 1 mg. 2 : 5-dihydroxyphenylethylamine (synthetic); (*c*) 1 mg. 3 : 4-dihydroxyphenylethylamine (synthetic).

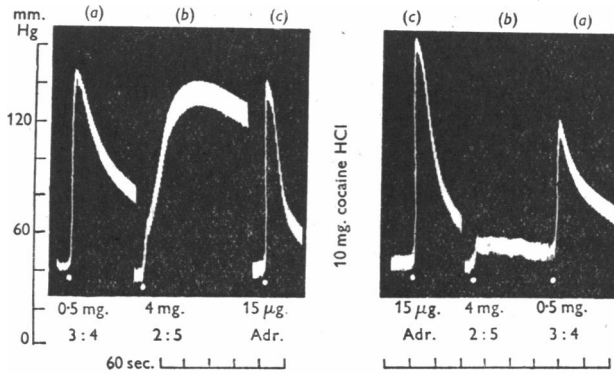


Fig. 4A. Fig. 4B.

Fig. 4. Spinal cat; arterial blood pressure. Fig. 4A shows the pressor actions on intravenous injection of: (*a*) 0·5 mg. 3 : 4-dihydroxyphenylethylamine (synthetic); (*b*) 4 mg. 2 : 5-dihydroxyphenylethylamine (synthetic); (*c*) 15 μg. adrenaline. Fig. 4B shows the action of the same doses, after an intravenous injection of 10 mg. cocaine hydrochloride.

In the same experiment 10 mg. of cocaine hydrochloride were injected. Fig. 4B shows that the effect of adrenaline was enhanced, that of the 3 : 4-amine slightly reduced and that of the 2 : 5-amine was almost abolished. In another experiment of this kind the result with the 2 : 5-amine was essentially

the same as that shown on Fig. 4, but the blood-pressure rise after the injection of the 3 : 4-amine was the same before and after cocaine.

In another experiment 2·5 mg. of ergotoxine ethanesulphonate were injected intravenously. Fig. 5 shows that the pressor action of the 2 : 5-amine was reversed.

(*c*) *Assay of pressor activity produced by enzyme action on 2 : 5-dihydroxyphenylalanine.* For this experiment three manometric flasks were set up, each containing in the main vessel 1·0 ml. of guinea-pig's kidney extract (corresponding to 0·5 g. of fresh tissue) and 0·6 ml. of M/15 sodium phosphate buffer

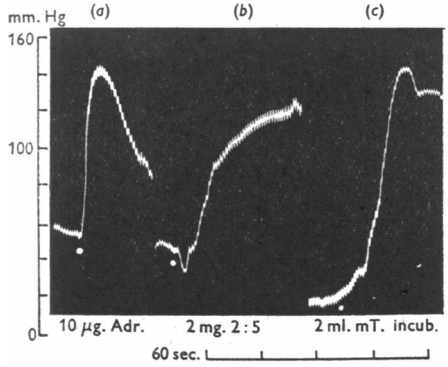


Fig. 5A.

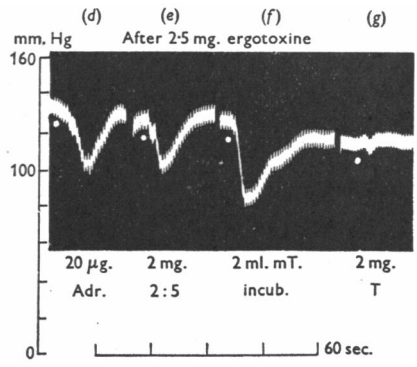


Fig. 5B.

Fig. 5. Spinal cat; record of arterial blood pressure. **Fig. 5A.** The effect of intravenous injection of: (*a*) 10 μg. adrenaline; (*b*) 2 mg. 2 : 5-dihydroxyphenylethylamine (synthetic); (*c*) 2 ml. of guinea-pig's kidney extract containing meta-tyramine produced by enzyme action. **Fig. 5B.** The effect of intravenous injection, after 2·5 mg. ergotoxine ethanesulphonate, of: (*d*) 20 μg. adrenaline; (*e*) same as (*b*); (*f*) same as (*c*); (*g*) 2 mg. tyramine.

(pH 7·4). The side bulb of the vessels contained 0·4 ml. of M/12·5 DL-2 : 5-dihydroxyphenylalanine. The amino-acid was added to the extracts after temperature equilibrium had been attained. The incubation was carried out in nitrogen at 37·5°. After 90 min., when the reaction had almost ceased, a total of 1008 μl. of carbon dioxide had been formed in the three flasks, as compared with a theoretical total formation of 1080 μl. The contents of the manometer flasks were then rapidly transferred into the same assay vessel (shown in Fig. 2). Each flask was washed once with 0·5 ml. of distilled water and the washings were added, giving a total volume of 7·5 ml. The amount of amine expected to be present in 2 ml. of the final solution was

$$\frac{1008 \times 153 \times 2}{22400 \times 7{\cdot}5} = 1{\cdot}84 \text{ mg.},$$

the molecular weight of the base being 153.

The assay is shown on Fig. 6A. The final solution of the incubated extract had in 2 ml. a pressor action equivalent to more than 1·5 mg. and less than 2·0 mg. of the synthetic 2 : 5-dihydroxyphenylethylamine.

The degree of accuracy to be expected in an experiment of this kind is not very high, as the untreated tissue extracts cause a progressive deterioration of the spinal preparation; an injection of the tissue extract alone was without pressor response.

Fig. 6B shows that the pressor response in the incubated extracts was abolished after cocaine, as was the response to the synthetic 2 : 5-amine.

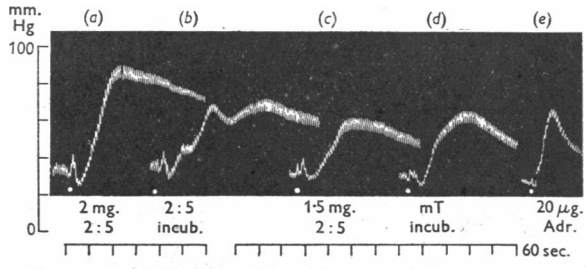


Fig. 6 A.

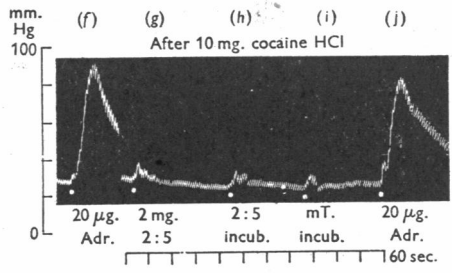


Fig. 6 B.

Fig. 6. Spinal cat; the effect on arterial blood pressure of intravenous injection of—Fig. 6A (*a*) 2 mg. 2 : 5-dihydroxyphenylethylamine (synthetic); (*b*) guinea-pig's kidney extract expected to contain 1·84 mg. 2 : 5-dihydroxyphenylethylamine; (*c*) 1·5 mg. 2 : 5-dihydroxyphenylethylamine; (*d*) meta-tyramine produced by enzyme action; (*e*) 20 μg. adrenaline. Fig. 6B, after 10 mg. cocaine hydrochloride: (*f*) same as (*e*); (*g*) same as (*a*); (*h*) same as (*b*); (*i*) same as (*d*); (*j*) same as (*e*).

(*d*) 2 : 5-*dihydroxyphenylalanine and amino-acid oxidases.* Experiments on 2 : 5-dihydroxyphenylalanine as substrate of oxidizing enzymes are not easy, since aqueous solutions of the amino-acid are not very stable under aerobic conditions. We have not been able to satisfy ourselves that the DL-form of the amino-acid was oxidized by an acetone-dried preparation of pig's kidney which had good D-amino-acid oxidase activity. Since it is known that both L-tyrosine and L-3 : 4-dihydroxyphenylalanine are substrates of the ophio-L-amino-acid oxidase (Zeller & Maritz, 1944), we have incubated the 2 : 5-acid with cobra

venom in oxygen: whereas both L-tyrosine and L-3 : 4-dihydroxyphenylalanine were rapidly oxidized, there was practically no oxygen uptake with DL-2 : 5-dihydroxyphenylalanine.

(*e*) 2 : 5-*dihydroxyphenylethylamine as substrate of amine oxidase.* Difficulty was also encountered when the 2 : 5-amine was examined as a substrate of oxidation. The solution of the amine was prepared immediately before use and added to the side bulb of the manometer flasks after these had been mounted and during the gassing with oxygen; the time allowed for temperature equilibration was reduced to 10 min. An acetone-dried powder of rabbit's liver served as source of the enzyme; 40 mg. of this powder in 1·6 ml. of M/15 sodium phosphate buffer (pH 7·4) was used. Six manometer flasks were set up; all contained the enzyme suspension plus 0·2 ml. of a neutralized M/50 solution of potassium cyanide. In the last three flasks two drops of octyl alcohol were added. The side bulbs of the three vessels contained 0·2 ml. of water, 0·2 ml. of M/20 tyramine hydrochloride or 0·2 ml. of M/20 2 : 5-dihydroxyphenylethylamine hydrochloride respectively. Under these conditions the oxygen uptake in the presence of tyramine was completely inhibited by octyl alcohol. The oxygen uptake with the 2 : 5-amine in the first two 3 min. intervals was approximately halved in the presence of octyl alcohol. At the end of the incubation, which was continued for 100 min., the total oxygen uptake in the absence of octyl alcohol was 140 μl.; in the presence of octyl alcohol it was 94 μl. In this flask the oxygen consumption had come to a standstill; in the flask without octyl alcohol the oxygen uptake between the 80 and 100 min. readings was 2·5 μl. The contents of both flasks which had contained the amine were darker than those of the blanks at the end of the incubation, but this darkening was less marked in the flask which contained octyl alcohol.

It is known that amine oxidase is cyanide-insensitive, but inhibited by octyl alcohol. The experiment described indicates that the oxygen uptake in the presence of cyanide was partly due to amine oxidase; this part of the oxygen uptake was abolished in the presence of octyl alcohol.

(2) *Observations with meta-tyrosine*

(*a*) *Formation of carbon dioxide.* Only the racemic form of meta-tyrosine was at our disposal. Formation of carbon dioxide occurred when the amino-acid was added to extracts of guinea-pig's kidney or rat's liver. In one experiment, in which 1·2 ml. of guinea-pig's kidney extract (equivalent to 600 mg. of fresh weight of tissue) were used, 79 μl. of CO_2 were formed in 9 min. from 0·4 ml. of M/50 DL-meta-tyrosine added. This corresponds to 0·44 moles of CO_2 per mole of amino-acid added. This and all our subsequent experiments suggest that only the amino-acid with the L-configuration was a substrate of the enzyme.

The evidence that dopa decarboxylase was responsible for the decarboxylation of meta-tyrosine is the same as for 2 : 5-dihydroxyphenylalanine. The

reaction was catalysed by extracts of organs which contain this enzyme; the q_{CO_2} ratios in rat's liver and guinea-pig's kidney extracts were similar, as shown in Table 1. In an experiment, in which the 3 : 4-acid and meta-tyrosine were added together, the rate of decarboxylation was approximately the same as with either substrate alone.

TABLE 1. Rates of decarboxylation of 3:4-dihydroxyphenylalanine and meta-tyrosine by tissue extracts

Organ	$q_{CO_2}^{3:4\text{-acid}}$ (*A*)	$q_{CO_2}^{\text{meta-tyrosine}}$ (*B*)	Ratio: (*A*)/(*B*)
Guinea-pig's kidney	1·70	1·58	1·08
Rat's liver	0·65	0·55	1·17

(*b*) *Assay of pressor activity produced by incubating meta-tyrosine with guinea-pig's kidney extract.* Two manometer vessels, each containing 1·0 ml. of guinea-pig's kidney extract, 0·6 ml. of M/15 sodium phosphate buffer (pH 7·4) and 0·4 ml. of a M/12·5 solution of DL-meta-tyrosine were incubated anaerobically for 1 hr.; after this time the formation of CO_2 had come to a standstill. The formation of carbon dioxide did not quite reach the theoretical amount of 358 μl.; the reaction stopped after about 300 μl. of CO_2 had been formed. Each vessel was washed with 0·2 ml. of distilled water, and the extracts and washings from both flasks were pooled in a small centrifuge tube. Unlike the 2 : 5-amine, meta-tyramine is not destroyed by heating, and the centrifuge glass was therefore placed in boiling water for 5 min. The resulting protein precipitate was spun down on a centrifuge, and the supernatant fluid was used in the assay. The result of the assay is shown on Fig. 7. From the amount of CO_2 formed we expected 2 ml. to contain 1·67 mg. of meta-tyramine; the tracings show that the amount found was equivalent to more than 1 mg., and less than 2 mg., of tyramine. The extract without added meta-tyrosine, which had served as the blank in the manometric experiment, was also boiled. The supernatant fluid was tested and found to be without pressor effect.

This experiment was repeated after synthetic meta-tyramine had become available. In the manometric experiment the CO_2 formation was 321 μl., as compared with the theoretical figure of 358 μl. Fig. 8 shows the blood pressure response to 1 mg. of meta-tyramine, compared with the response to the extract boiled after incubation with meta-tyrosine. The amount of meta-tyramine expected to be formed is 0·9 mg., if the figure of 321 μl. of CO_2 is used in the calculation; if the theoretical figure of 358 μl. of CO_2 is used the amount expected is 1·0 mg. This shows that both size and shape of the blood-pressure response with the amine produced by enzyme action are those of the synthetic meta-tyramine.

(*c*) *Pharmacological properties of meta-tyramine.* The properties of meta-tyramine have not been studied since Barger & Dale (1910) found it to be approximately equi-active with tyramine. This is confirmed by the experiment

reported in the preceding section. After cocaine, the pressor action of meta-tyramine was abolished (Fig. 6); in this respect, therefore, meta-tyramine does not differ from tyramine.

Meta-tyramine differs from tyramine in its action on the blood pressure after a dose of ergotoxine. Fig. 5 shows a reversal of the blood-pressure action of meta-tyramine after ergotoxine, whereas the action of tyramine was abolished. In this experiment we used meta-tyramine produced by enzyme action; we have since repeated the experiment with synthetic meta-tyramine; although the reversal was not as marked as in the experiment of Fig. 5, a reversal of the blood-pressure effect after ergotoxine was also seen with the synthetic meta-tyramine.

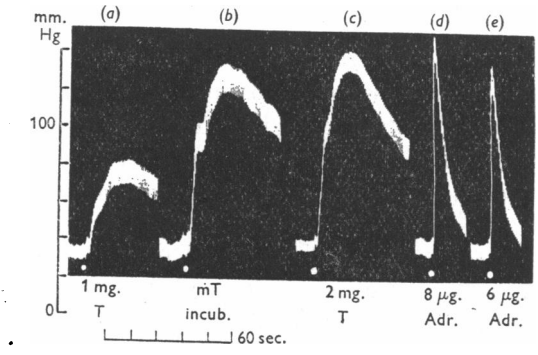


Fig. 7.

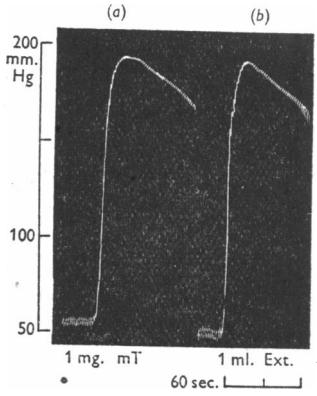


Fig. 8.

Fig. 7. Spinal cat; arterial blood pressure. Assay of meta-tyramine produced by enzyme action. Effect of intravenous injection of: (*a*) 1 mg. tyramine; (*b*) 2 ml. boiled guinea-pig's kidney extract (calculated to contain 1·67 mg. of meta-tyramine); (*c*) 2 mg. tyramine; (*d*) 8 μg. adrenaline; (*e*) 6 μg. adrenaline.

Fig. 8. Spinal cat; arterial blood pressure. Meta-tyramine produced by enzyme action compared with synthetic meta-tyramine. Effect of intravenous injection of: (*a*) 1 mg. meta-tyramine (synthetic); (*b*) 1 ml. of guinea-pig's kidney extract incubated with meta-tyrosine (expected to contain 0·9 mg. of meta-tyramine).

(*d*) *Meta-tyrosine as a substrate of amino-acid oxidases.* DL-Meta-tyrosine was found to be a substrate of both L-amino-acid oxidase and D-amino-acid oxidase. In an experiment with ophio-oxidase (3·2 mg. of cobra venom in 1·6 ml. of M/15 sodium phosphate buffer of pH = 7·4 and 0·4 ml. of a M/50 solution of DL-meta-tyrosine), there was an uptake of 78·5 μl. of oxygen in 14 min., after which time the reaction came to a standstill. This corresponds to about 0·9 moles of oxygen per mole of substrate added.

For the experiment on D-amino-acid oxidase we used an acetone-dried powder of pig's kidney (2 g. of powder extract with 20 ml. of M/15 sodium

phosphate buffer). Of this extract, 0·6 ml. was incubated with 1·4 ml. of a M/25 solution of DL-meta-tyrosine. There occurred a rapid uptake of oxygen, 40 μl. of O_2 being consumed within the first 15 min. The reaction was not followed to completion.

(*e*) *Meta-tyramine as substrate of amine oxidase.* We find that meta-tyramine is a substrate of amine oxidase. This is in agreement with Randall (1946), who used fresh extracts of guinea-pig's and cat's liver as source of the enzyme. We incubated an acetone-dried preparation of rabbit's liver with both tyramine and meta-tyramine in the presence of oxygen and we found that the rate of oxygen uptake was about the same with both amines.

DISCUSSION

Our experiments suggest that both L-meta-tyrosine and L-2 : 5-dihydroxyphenylalanine are substrates of dopa decarboxylase. This observation allows us to formulate the conditions governing substrate specificity of the enzyme with greater precision than has so far been possible. Most of the information available was related to the side chain of the molecule: it is known that on the introduction of an *N*-methyl group or of a hydroxyl group in the β position, the compounds are no longer acted on by the enzyme (Blaschko, Holton & Sloane Stanley, 1948; Blaschko, 1942).

As to the importance of the ring structure, it is known that tyrosine is not a substrate of dopa decarboxylase. The substrate specificity of the enzyme must therefore be dependent either upon the number of phenolic hydroxyl groups or on the presence of a hydroxyl group in a given position. Our results show that, irrespective of the number of hydroxyl groups, all three amino-acids with the hydroxyl group in the meta-position are substrates of the enzyme. The presence of the hydroxyl group in the para-position is not essential.

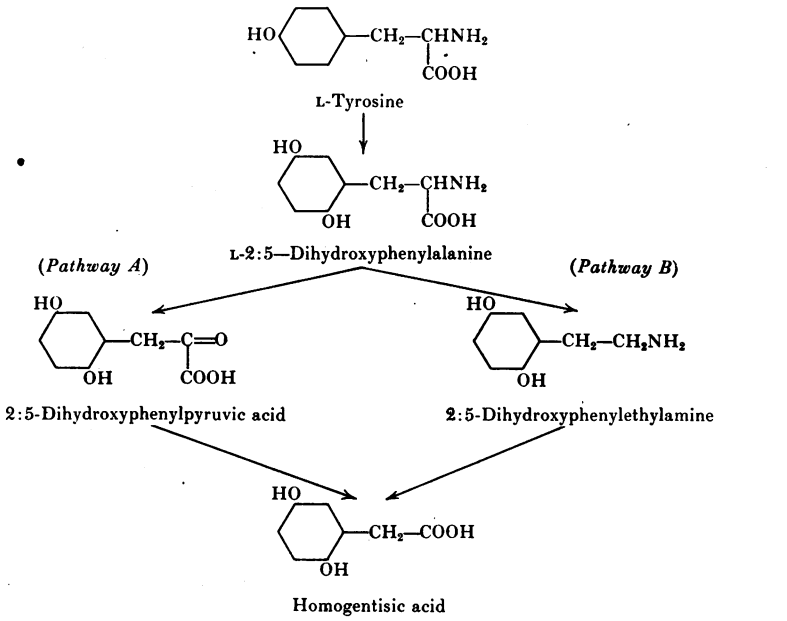
Are the two decarboxylation reactions described in this paper of physiological importance? There are indications that 2 : 5-dihydroxyphenylalanine may occur in the breakdown of tyrosine. This follows from observations on alkaptonuria, a metabolic disorder in which another 2 : 5-dihydroxy derivative, homogentisic acid, is excreted. Homogentisic acid is considered a normal product of tyrosine metabolism in man. Pathways of homogentisic acid formation from tyrosine were discussed by Neubauer (1928); two of these pathways involved the occurrence of 2 : 5-dihydroxyphenylalanine as an intermediate metabolite.

Neuberger, Rimington & Wilson (1947) have recently shown that 2 : 5-dihydroxyphenylalanine is a possible precursor of homogentisic acid. They found that in a case of human alkaptonuria at least 40% of the ingested DL-form of 2 : 5-dihydroxyphenylalanine was converted into homogentisic acid. They suggested that with the L-form the percentage conversion would be much higher, and they considered the possibility of the L-isomer as a normal intermediate between L-tyrosine and homogentisic acid.

Such an assumption would require that the D-form of the 2 : 5-acid does not significantly contribute to the formation of homogentisic acid. One would therefore expect:

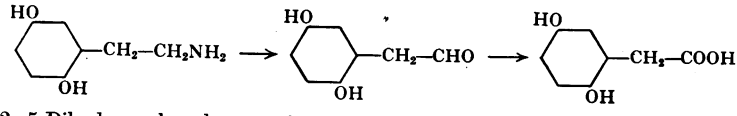
(1) the breakdown of the 2 : 5-acid to be catalysed by an enzyme specific for the L-configuration; and,

(2) the inversion of the D-isomer in the body to be slow.



Scheme 1. Pathways of formation of homogentisic acid.

Our results show that pathway (*B*) fulfils the first of these conditions. It has been established that the L-isomer is decarboxylated, whereas the corresponding D-isomer is not. We have also shown that the amine formed is a substrate of amine oxidase. Therefore, the amine would be further metabolized to the corresponding aldehyde which, on further oxidation, would give homogentisic acid:



Scheme 2. Formation of homogentisic acid from 2 : 5-dihydroxyphenylethylamine.

In their discussion of homogentisic acid formation Neuberger *et al.* (1947) suggest that L-2 : 5-dihydroxyphenylalanine is further metabolized by oxidative deamination (Pathway (*A*) of Scheme 1). This is the more orthodox path of

amino-acid breakdown in mammals; it also requires stereospecific enzymes as catalysts. It remains, however, to be shown that 2 : 5-dihydroxyphenylalanine is a substrate of amino-acid oxidases. The L-form of the acid, unlike those of tyrosine, meta-tyrosine and 3 : 4-dihydroxyphenylalanine, is not a substrate of the L-amino-acid oxidase of cobra venom; experiments with mammalian enzymes were not conclusive.

More recently, on the basis of observations in alkaptonuria, Neuberger (1948) also considers pathway (*B*) as more likely than pathway (*A*).

In their first paper on dopa decarboxylase, Holtz *et al.* (1938) discussed the possibility that a decarboxylation reaction was a normal occurrence in the breakdown of L-amino-acids and preceded oxidative deamination; they suggested that L-amino-acid oxidase might not be one enzyme but might consist of two: an amino-acid decarboxylase plus an amine oxidase. This possibility was put to an experimental test and not confirmed when it was found that the decarboxylation reaction was specific for 3 : 4-dihydroxyphenylalanine and that other amino-acids were not metabolized in the same way. The interpretation given to the substrate specificity of dopa decarboxylase was that the enzyme might catalyse an intermediate step in the biosynthesis of adrenaline and sympathin (Blaschko, 1939, 1942, 1949). The experiments described in this paper show that dopa decarboxylase may have another role in the metabolism of tyrosine.

There is no previous study of 2 : 5-dihydroxyphenylethylamine, but the corresponding *N*-methyl derivative has been made by Buck (1932) and its pharmacological actions have been briefly described (Hjort, 1934). Like the primary amine, the secondary amine proves less active on the blood pressure after cocaine, but Hjort (1934) did not observe a reversal of the pressor effect after ergotoxine with the *N*-methylated amine.

As far as meta-tyrosine is concerned, there is no record of its occurrence in mammals; *m*-hydroxybenzoic acid has been found by Lederer (1941) in the dried scent glands of the Canadian beaver (*Castor fiber* L.). Since meta-tyramine is a substrate of amine oxidase it is conceivable that *m*-hydroxybenzoic acid is derived from meta-tyrosine.

A comparison of the substrate specificity of dopa decarboxylase with that of the L-tyrosine decarboxylase of *Streptococcus faecalis* shows that the bacterial enzyme differs from the mammalian enzyme not only in its high affinity for tyrosine, but also in its low affinity for 2 : 5-dihydroxyphenylalanine. Recent experiments have shown (Sloane Stanley, unpublished) that the bacteria are unable to decarboxylate 2 : 5-dihydroxyphenylalanine; meta-tyrosine is decarboxylated by an acetone-dried preparation of the bacteria.

SUMMARY

1. L-2 : 5-Dihydroxyphenylalanine and L-meta-tyrosine (*m*-hydroxyphenylalanine) are decarboxylated in extracts of guinea-pig's kidney and of rat's liver; it is likely that these reactions are catalyzed by the enzyme dopa decarboxylase.

2. The amines formed in the decarboxylation reaction are 2 : 5-dihydroxyphenylethylamine and meta-tyramine (*m*-hydroxyphenylethylamine); they have pressor action, and the amounts of amine formed in the decarboxylation reaction have been determined by assay on the arterial blood pressure of the spinal cat. The pressor action of 2 : 5-dihydroxyphenylethylamine is reversed after ergotoxine; it is decreased after cocaine. The pressor action of meta-tyramine is abolished by cocaine; after ergotoxine, the pressor action of meta-tyramine is reversed.

3. The decarboxylation of 2 : 5-dihydroxyphenylalanine is of interest in connexion with the formation of homogentisic acid in alkaptonuria.

4. The common feature in the three substrates of dopa decarboxylase examined is the hydroxyl group in the meta-position to the side chain.

5. Both 2 : 5-dihydroxyphenylethylamine and meta-tyramine are substrates of amine oxidase.

6. Meta-tyrosine is oxidized by D-amino-acid oxidase of pig's kidney and by the L-amino-acid oxidase of cobra venom; 2 : 5-dihydroxyphenylalanine does not appear to be oxidized by the cobra venom enzyme.

The authors are grateful to Prof. J. H. Burn for help and advice on the pharmacological aspects of this work.

One of us (G. H. S. S.) is grateful to the Medical Research Council for a personal grant.

REFERENCES

Barger, G. & Dale, H. H. (1910). *J. Physiol.* **41**, 19.
Blaschko, H. (1939). *J. Physiol.* **96**, 50*P*.
Blaschko, H. (1942). *J. Physiol.* **101**, 337.
Blaschko, H. (1949). Adrenaline and sympathin. In *The Hormones. Physiology, Chemistry and Applications*, vol. II, chapter 25*b*. New York: Academic Press Inc.
Blaschko, H., Holton, P. & Sloane Stanley, G. H. (1948). *Biochem. J.* **42**, xlviii.
Blum, L. (1908). *Arch. exp. Path. Pharmak.* **59**, 269.
Buck, J. S. (1932). *J. Amer. chem. Soc.* **54**, 3661.
Hjort, A. M. (1934). *J. Pharmacol.* **52**, 101.
Holtz, P., Heise, R. & Lüdtke, K. (1938). *Arch. exp. Path. Pharmak.* **191**, 87.
Leaf, G. & Neuberger, A. (1948). *Biochem. J.* **43**, 606.
Lederer, E. (1941). *Bull. Soc. Chim. biol., Paris*, **23**, 1457.
Neubauer, O. (1928). *Handbuch der normalen und pathologischen Physiologie*, **5**, 860. Berlin: Springer.
Neuberger, A. (1948). *Biochem. J.* **43**, 599.
Neuberger, A., Rimington, C. & Wilson, J. M. G. (1947). *Biochem. J.* **41**, 438.
Randall, L. O. (1946). *J. Pharmacol.* **88**, 216.
Zeller, E. A. & Maritz, A. (1944). *Helv. chim. Acta*, **27**, 1888.