AMINE OXIDASE AND AMINE METABOLISM

H. BLASCHKO

Department of Pharmacology, Oxford University

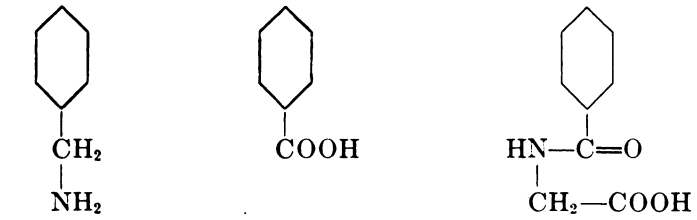
In the present review the attempt is made to correlate what is known of the metabolism of amines in the living organism with the properties and distribution of the enzyme amine oxidase. Our knowledge as to the functional significance of this enzyme is far from complete, but it seems worth while to set out what is known and what must be left to further study.

HISTORICAL

Work on the fate of amines in the living animal preceded the discovery by Miss Hare (now Mrs. Bernheim) in 1928 of an enzyme that catalyses the oxidative deamination of tyramine (86). During the nineteen thirties, there arose out

of the study of the metabolism of adrenaline *in vitro* (33) and of the simple aliphatic amines (134) the concept of amine oxidase, an enzyme system which catalyses an analogous oxidation reaction of a great number of amines (112, 34, 135).

Early work on amine metabolism. The first clear indication that amines are broken down in deamination reactions came from a study of the fate of benzylamine in the organism. In 1877, Schmiedeberg (155) showed that in the dog benzylamine given by mouth was excreted as hippuric acid (benzoylglycine). He recognised that this involved the intermediate formation of benzoic acid and arrived at the generalisation that not only benzylamine,



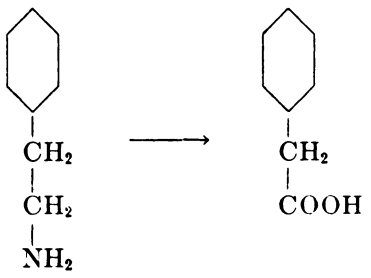
benzylamine *benzoic acid* *hippuric acid*

but "probably all the mono-amino bases in which the nitrogen is not directly linked to a benzene ring, i.e., all bases which contain the atomic grouping —CH_2—NH_2 are broken down in the organism with the formation of ammonia". This statement is still valid today for very many compounds which belong to this class.

Later, both Schmiedeberg (156) and Minkowski (126) isolated free benzoic acid. Minkowski incubated minced rabbit tissues with benzylamine and found that benzoic acid was formed; thus it was shown that the deamination reaction had also occurred *in vitro*. The quantitative aspects of the metabolism of benzylamine were studied by Mosso (127); he gave the amine subcutaneously to a dog and recovered over 90% as hippuric acid in the urine.

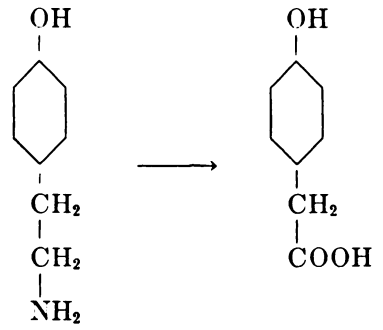
This work on benzylamine appears to have been forgotten by the time other amines were investigated.

The next higher homologue of benzylamine, phenylethylamine, was examined by Guggenheim and Löffler (85). They gave 1 g. of phenylethylamine hydrochloride to a rabbit by mouth and recovered 0.7 g. of crude phenylacetic acid in the urine, that is, 87% of the theoretical amount.



β-phenylethylamine phenylacetic acid

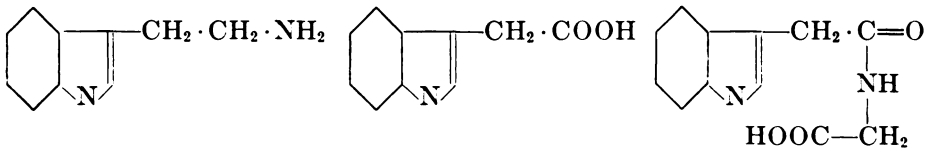
Earlier, already in 1910, Ewins and Laidlaw (65) had found that tyramine, perfused through the liver of the cat or the rabbit or through the rabbit's uterus, was destroyed and p-hydroxyphenylacetic acid was recovered, in about 70% of the maximum possible yield.



tyramine p-hydroxyphenylacetic acid

No breakdown of tyramine could be demonstrated in the perfused lung. Ewins and Laidlaw, discussing these results, wrote: "The results suggest that plain muscle having a rich sympathetic supply can effect the transformation of amine to acid; while plain muscle having a poor or no sympathetic supply is unable to do so." This connexion between adrenergic nerve supply and amine oxidase activity is a topic which will be discussed more fully below.

Ewins and Laidlaw also studied the fate of tryptamine (β-indolethylamine) (66). They isolated indoleacetic acid as the breakdown product of tryptamine in the perfused rabbit's liver; as the metabolic end product in the dog's urine they isolated what they thought was indoleaceturic acid, a condensation product of indoleacetic acid and glycine. Here again, deamination was the metabolic pathway.



tryptamine indoleacetic acid indoleaceturic acid

Similar experiments were carried out by different observers on aliphatic amines, but no characteristic breakdown products were isolated, except that with *iso*amylamine Guggenheim and Löffler (85) obtained evidence for the formation of *iso*valeric acid in the perfused rabbit's liver.

In all this older work it was assumed that the deamination of the amines was a hydrolytic reaction

$$R{-}CH_2{-}NH_2 + H_2O \longrightarrow R{-}CH_2OH + NH_3$$

i.e., a reaction in which an alcohol was formed; the alcohol was then further oxidised to the corresponding carboxylic acid. The possibility of an oxidative

breakdown of the amine with formation of the aldehyde was first discussed after the discovery of "tyramine oxidase" (9); this reaction can be considered as analogous to the formation of keto acids from amino-acids as catalysed by the amino-acid oxidases (113).

PART I

Distribution of Amine Oxidase

Intracellular distribution of amine oxidase. The mammalian liver is the most convenient source of the enzyme. There are small differences in the activity and the substrate specificity in different species, but the livers of the cat, the pig, the ox, the sheep, the rabbit, the guinea-pig and the rat have been used by different authors. The most characteristic property of the enzyme is its localization in the insoluble cell constituents. For instance, the first preparations of "adrenaline oxidase" (33) were prepared by following Warburg's description of "Körnchensuspension" (177). The methods of obtaining defined fractions of the formed elements of the liver cell have since been improved and they have been applied to the study of amine oxidase. Miss Hawkins has determined the enzymic activity of various fractions of rat liver obtained when homogenates were subjected to high-speed centrifugation in isotonic and hypertonic sucrose (91). Enzymic activity was found to be present only in the particulate matter; centrifugation in 0.25M-sucrose sedimented all enzymic activity. At least 50% of the total enzymic activity was found in the mitochondria fraction. Separation of this fraction from the crude homogenate resulted in an almost threefold purification of the enzyme when activity was determined per unit of protein nitrogen. At least 25% of the total enzymic activity sedimented less readily and was found in the microsome fraction. Probably the mitochondria and microsomes account for the total amine oxidase activity of the liver homogenates (see also 48).

That amine oxidase is present in two different fractions of the particulate matter of liver cells, may explain the observation by Bernheim and Bernheim (7) that saline extracts of rabbit's liver when subjected to low-speed centrifugation will yield two fractions, one in the sediment which can be easily washed by resuspending in saline and re-centrifugation (27), and one which remains in the supernatant.

These observations make us ask: can amine oxidase be considered as a homogeneous entity? At present, it cannot be decided if the two fractions are preformed in the cell or if they arise as artefacts during the preparation of the homogenates.

Action of lytic agents on amine oxidase. Amine oxidase activity does not depend upon the structural integrity of the particles. This can be easily demonstrated when enzymic activity is measured before and after treatment of homogenates or similar preparations with agents which destroy mitochondria. Lysolecithin, a substance formed in the hydrolysis of lecithin by the lecithinase of bee or cobra venom, is a very effective lytic agent of this kind. When enough lysolecithin is added to a homogenate of guinea-pig's liver, the appearance of the homogenate changes; it becomes transparent, almost like plasma. The amine oxidase activity

of a preparation thus treated remains unchanged. Amine oxidase is not the only enzyme found in mitochondria which is unaffected by treatment with lysolecithin: the rate of oxidation of indophenol before and after treatment with lysolecithin is also unchanged (16). In spite of the changed appearance of the lysolecithin-treated preparations, however, the enzymic activity is not entirely detached from particulate matter. The writer carried out an experiment with Dr. F. Himmelweit, in which a lysolecithin-treated guinea-pig liver preparation was passed through gradocol membranes; it was found that most of the activity passed through a filter of pore size 1.64 μ, but was retained on a filter of pore size 0.88 μ. The effect of lysolecithin was to disperse the large particles (mitochondria); the amine oxidase activity was now attached to particles of smaller size.

Effects similar to those seen with lysolecithin have also been obtained with bile salts. However, these substances inhibit amine oxidase, and their effect on the enzyme can only be studied after they have been removed by dialysis.

The localization of amine oxidase in the mitochondria is of interest in view of the fact that these particles contain the cytochrome system, whereas amine oxidase *in vitro* does not require the cytochrome system as a "terminal oxidase". However, we do not know if the same is true in the living cell, and the possibility that in the cell the amine oxidase reaction is linked to the cytochrome system will be discussed below. Indications for the participation of the cytochrome system may be found in experiments by Govier *et al.* (81).

In addition, the amine oxidase reaction results in the formation of two toxic metabolites, both of which are removed by reactions which take place in the mitochondria. One of these metabolites is ammonia which is removed in the mitochondria by urea synthesis, the other is an aldehyde which is further metabolised in reactions known to be linked with the cytochrome-cytochrome oxidase system. The location of amine oxidase in the mitochondria thus ensures that these two end products of the enzymic reaction are rendered harmless not far from the site where they are formed.

Amine oxidase in regenerating rat liver. Hawkins and Walker (93) have studied the regeneration of amine oxidase activity after removal of about two thirds of the liver. During the first two days after the operation a rapid increase in liver weight took place without any increase in the total amine oxidase activity. During this period, therefore, the enzymic activity per unit of weight decreased, and this decrease was made good during the following week. It is known that a lively cell division takes place on the second day after partial hepatectomy; it seems that during this period of cell division the enzyme already present is redistributed with the preformed cytoplasmatic particles (microsomes and mitochondria) over the regenerating liver cells, and the resulting "dilution" of enzyme is followed by a new formation only after the second day when the cytoplasmatic particles multiply.

This interpretation is supported by observations on hepatectomised rats which received 0.1 mg. of colchicine 24 hours after the operation. In these animals the liver weight did not change much during the 24 hours following upon the ad-

ministration of colchicine. This indicates that colchicine had inhibited the new formation of liver cells. At the same time, no dilution of the enzyme occurred; the concentration of amine oxidase per unit of liver weight even rose slightly.

Distribution of amine oxidase in vertebrates. The enzyme has been found in all classes of vertebrates that have been examined (34): mammals, birds, reptiles, amphibians and teleosts. One elasmobranch species has also been examined; this is *Torpedo marmorata*, where the enzyme has been found in liver and kidneys; very little enzymic activity seems to occur in the electric organ (16).

Amine oxidase is found in many different tissues. Our knowledge of the distribution in vertebrates is mainly derived from experiments carried out on mammalian tissues. In the earlier work, enzymic activity has chiefly been studied in organs in which much enzyme is found, but interest has since shifted to tissues in which enzymic activity is relatively low. When data on amine oxidase activity obtained under different conditions are compared, it must be borne in mind that in the earlier work less efficient methods of extraction were used. Extracts were usually prepared by grinding the tissue with sand; they were then centrifuged. In this procedure an unknown amount of enzyme is lost in the sediment. Homogenates give a truer picture of total enzymic activity. The difficulties of determining total enzymic activity in tough, fibrous tissue are not yet fully overcome.

Very high enzymic activity is always found in the liver; species differences may occur, but have not been systematically studied; the guinea-pig's liver has very high enzymic activity. The kidneys also give very active preparations, but in the rat enzymic activity of the kidney is relatively low. Very active preparations can also be obtained from the pancreas and the intestine (34, 14). In sheep, the rumen, abomasum, duodenum and ileum have been examined and found to contain amine oxidase; in the duodenum and ileum, the muscular coat is richer in enzyme than is the mucous membrane (16).

In the suprarenal gland amine oxidase is present in the medulla as well as in the cortex (14, 150, 116). The enzyme occurs in other glands, *e.g.*, in the thyroid gland (14) and in the Harderian gland of the rabbit (169). It has been found in the lung (34a), including the human lung (117), but it is not known if the enzyme content of the lung is in the alveoli, in the bronchioles, or in the blood vessels. Amine oxidase has also been found in the spleen (14).

In the male sex organs, amine oxidase occurs in the testes (14), in the prostate gland and in the seminal vesicles (189). In the uterus, activity is high (14), but seems to be related to the functional state of the organ; enzymic activity is often low in the uteri of virgin rabbits (157). In a sheep which had been pregnant for 113 days, amine oxidase occurred not only in the placenta, but also in the chorion; little if any activity was present in the amnion (16). The human placenta (124) and that of other mammals (172) have been shown to have amine oxidase activity.

The heart also contains amine oxidase, but there exist marked species differences (14). Bernheim and Bernheim studied the disappearance of tyramine in the presence of slices of heart tissue from different species. They found that in

some species (the rat, the rabbit and the guinea-pig) tyramine disappeared rapidly, whereas in others (cat, dog) the inactivating mechanism, probably amine oxidase, was much less potent (8). The human heart also contains amine oxidase (115).

Blood vessels have been studied by Thompson and Tickner (173); they found the enzyme in many of the blood vessels of the rabbit. The enzymic activity was high in the aorta and the renal arteries; most peripheral arteries gave preparations of lower enzymic activity. Low enzymic activity was found in veins, which confirms findings by Schapira (150). The inner half of the wall of the aorta yielded preparations as active as the outer half. In the rabbit's ear, amine oxidase has been found (150); it is present in the blood vessels of the ear (173). This is of particular interest because the observations of Gaddum and Kwiatkowski (73) on the effect of ephedrine were carried out on the blood vessels of the perfused rabbit's ear.

Skeletal muscle contains little enzymic activity, although it would seem desirable to obtain better data for this tissue with improved methods of extraction. It seems possible that the weak enzymic activity of muscle extracts can be accounted for by the enzyme present in the blood vessels contained in the tissue.

Blood is without amine oxidase activity.

Pugh and Quastel were the first to study amine oxidase in nervous tissue (134); they chiefly studied the enzyme in brain. The human brain also contains amine oxidase (15). The white matter does not contain much enzyme, and the basal ganglia are more active than the cortex. A systematic study of the enzyme in peripheral nervous tissue has not been made. In the stellate ganglion of the ox, amine oxidase has been found (183). Amine oxidase also occurs in the superior cervical and stellate ganglia of the dog; much less activity is found in the nodose ganglion (16).

The search for amine oxidase in other structures with adrenergic innervation has shown that amine oxidase occurs in the nictitating membrane and iris of cats and rabbits (145).

This list of organs and tissues with amine oxidase activity is probably not complete. It shows how widespread the enzyme is in mammalian tissues. It is difficult to make a general statement about the tissues which contain the enzyme; it is probably safe to say that it occurs in glands, plain muscle and nerve tissue.

Very little work has been done on the development of amine oxidase during growth. Epps has shown that in newborn human babies the mean enzymic activity of the kidneys—cortex as well as medulla—is about one half of the mean for adults (50; see also 190). In babies aged three months the figures for enzymic activity were as high as in adults. In the liver and the mucous membrane of the ileum Epps found no difference in the mean enzymic activity in adults and newborn babies; a surprisingly large number of extracts of duodenal mucosa from newborn babies were without any amine oxidase activity; those that were enzymatically active had a mean activity which was similar to that found in adults.

Amine oxidase in invertebrates. Only a few species representing different invertebrate phyla have been studied (34). The enzyme was not found in all phyla. No enzyme was found in annelids; this is of interest in connexion with the finding of chromaffine cells in species like the leech and the earthworm (132, 76).

Two echinoderms were examined, and both were found to contain amine oxidase; these were *Asterias rubens L.* and *Echinus esculentus L.* The enzyme was also present in the *Mollusc, Patella vulgata L.*, but not in *Helix aspersa.*

A more detailed study has since been made of the molluscan enzyme in two species of cephalopods, *Sepia officinalis* and *Octopus vulgaris.*

Sepia and *Octopus* contain in their hepatopancreas (shortly called "liver") the richest source of amine oxidase yet described (21, 30). In *Sepia*, the enzyme was present, in order of decreasing activity, in the liver, the "kidneys", the posterior salivary glands, the anterior salivary glands and ink sac; no activity was found in the musculature of the mantle. In *Octopus*, the order was: liver, posterior salivary glands, anterior salivary glands and brain. Like the mammalian enzyme, the amine oxidase of *Octopus* liver was associated with the particulate matter; after prolonged centrifugation all enzymic activity was present in the sediment.

Amine oxidase in plants and alkaloid synthesis. In earlier experiments no amine oxidase activity in plants was detected (34, 179), but more recently, Werle and Roewer (182) have prepared enzymatically active extracts from plant tissue. They demonstrated enzymic activity in the leaves, stalks and roots of *Salvia uliginosa*, in *Colchicum bornmuelleri*, in *Cannabis indica* and *Momordica balsamina.* Ammonia formation as well as oxygen consumption were measured. Like the amine oxidase of animal origin, the enzyme in plant tissue was insensitive to cyanide.

The full significance of amine oxidase in plants seems not yet to have been recognized, but it seems worth while to discuss the possibility that the enzyme has an important function in alkaloid synthesis.

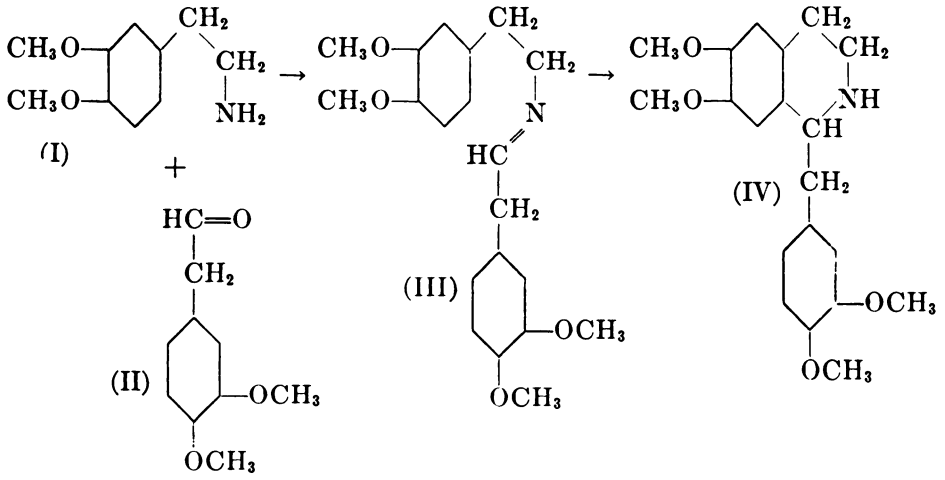
Organic chemists have for many years discussed theories of alkaloid synthesis, in order to explain the formation of the complex ring structures which are found in some of the plant alkaloids. One reaction which has been discussed is the condensation of an aldehyde with an amine:

$$R'CH_2 \cdot NH_2 + R''—CHO \longrightarrow R'CHN{:}CHR'' + H_2O$$

(186, 146).

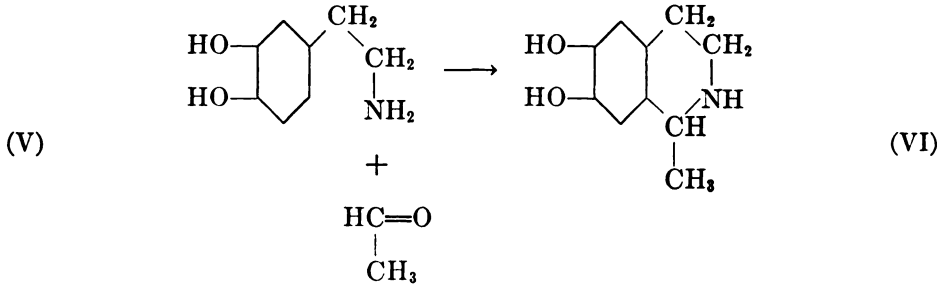
Reactions of this type can be studied *in vitro*, as they occur in the absence of any specific catalyst. However, catalysts must be assumed to occur which cause the amine and the aldehyde to be present at the same time. Amine oxidase is such a catalyst.

An example of such a condensation reaction which occurs *in vitro* and which may take place in alkaloid synthesis has been studied by Späth and Berger (168). In this reaction $R' = R''$. The condensation occurs between β-3:4-dimethoxyphenylethylamine (I) and the corresponding aldehyde, 3:4-dimethoxyphenylacetaldehyde (II). In the condensation reaction, the first product is believed to be compound (III) which rearranges itself to tetrahydropapaverine (IV).

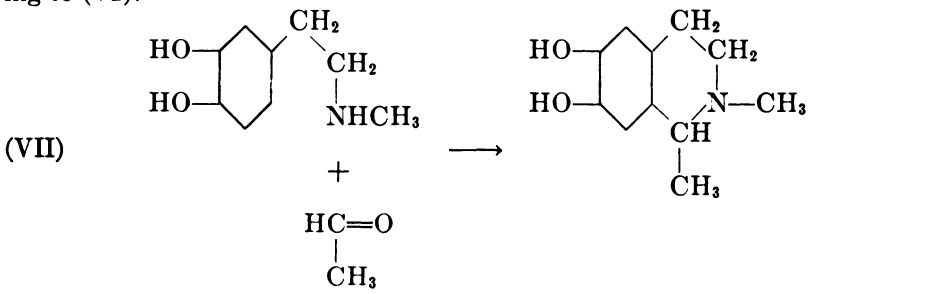


The amine (I) is a substrate of the mammalian amine oxidase (14); the aldehyde (II) can therefore be produced by amine oxidase. This must mean that during the enzymic reaction both (I) and (II) are present simultaneously. It thus appears that the presence of amine oxidase creates the conditions in which alkaloid synthesis by condensation can take place.

In the living cell, condensations may also occur between amines and aldehydes in which the radicals R′ and R″ are not identical. Two condensations have been studied by Schöpf & Bayerle (158) in which the aldehyde is acetaldehyde. In the first reaction the aldehyde condenses with hydroxytyramine (V); the product isolated is 1-methyl-6:7-dihydroxy-1:2,3:4-tetrahydroisoquinoline (VI):



This condensation reaction is interesting, because it is known that hydroxytyrmaine occurs in plants (154). In the second reaction acetaldehyde was incubated with epinine (VII); the reaction product was the N-methyl derivative corresponding to (VI).



Oxidation of amines and the formation of indoleacetic acid in plants. Kenten and Mann (110) have recently extended the list of plants which yield preparations that deaminate amines. They do not consider the identity of this system with the amine oxidase of animals as fully established. Pea and lupin seedlings gave active preparations. The authors discuss the possibility that the oxidation of tryptamine to the corresponding aldehyde is a step in the biosynthesis of the growth hormone indoleacetic acid in plant tissue. For the formation of this acid in mammalian metabolism see the work of Ewins and Laidlaw (66) discussed on p. 417.

PART II

Properties of Amine Oxidase

Preparations of amine oxidase. Amine oxidase activity has often been studied in fresh tissue extracts. The enzyme used to be considered as rather labile because preparations had poor keeping qualities; it was also believed to be destroyed by organic solvents, *e.g.*, by acetone. However, it was found in 1945 that treatment with cold acetone yielded powders (*e.g.*, from mammalian liver or kidney) which contained most, if not all, the enzymic activity of the tissue. The amine oxidase activity of the acetone-dried powders is present in the insoluble residue.

This observation has corrected our views on the stability of amine oxidase: an acetone-dried powder of rabbit's liver prepared in November 1945 and kept standing in the laboratory without any precautions had still retained more than one third of its initial activity in November 1951.

The acetone-dried powder has proved a useful preparation. It is a material of known enzymic activity. It can be easily washed, a procedure which reduces the "enzyme blank". It is very suitable for the study of the reversibility of inhibitions: enzyme suspensions can be treated with a known concentration of inhibitor; reversible inhibitors can then easily be removed by washing and centrifugation (67).

Amine oxidase also withstands freeze-drying, but little work has been done with this preparation (16). In a preparation from guinea-pig liver the activity was in the insoluble material. The freeze-dried material differed in one respect from the acetone-dried powder: washing increased the enzymic activity. Possibly the treatment with acetone removes an inhibitor of the enzyme which is retained in the freeze-dried preparation.

The amine oxidase activity of *Octopus* liver is destroyed by treatment with acetone for unknown reasons; freeze-drying destroys much but not all enzymic activity. There is no indication that the loss of activity is due to the splitting off of a prosthetic group.

Homogeneity of amine oxidase. Attempts to obtain purer specimens of amine oxidase have not been carried very far, although some purification has been reported (112, 1). At present we cannot say if one and the same enzyme is responsible for the oxidation of many of the amines which have been studied. Two sets of observations can be quoted as supporting the homogeneity of amine oxidase (34):

(1) oxidation of representatives of the different types of substrates occurs in all organs in which amines are oxidised;
(2) two substrates present at the same time are not oxidised at an additive rate, but the rate of oxidation of such a mixture is intermediate between the rates of oxidation of the two amines when tested separately.

It must be borne in mind that these criteria are sometimes misleading, and it remains to be seen if the present picture will have to be modified when more purified specimens of amine oxidase become available. It is known that amine oxidases from different sources have differences in substrate specificity. This has been interpreted as an indication of the existence of different amine oxidases (1, 188). There is good evidence that a specific catalyst of the amine oxidase type with distinct properties is responsible for the oxidation of mescaline in the rabbit's liver (7, 23, 170), but even here the evidence is not entirely unambiguous. A separation of amine oxidase into two distinct enzymes has recently been reported (183); one of these is said to oxidise tyramine, the other aliphatic amines, *e.g.*, *n*-butylamine. More detailed reports of these experiments will have to be awaited before it can be said if amine oxidase is a complex which is composed of more than one enzyme.

We also do not know whether oxidation involves a sequence of reactions, *e.g.*, of hydrogen transfers interposed between substrate and molecular oxygen. It is possible that several steps are necessary, and each of these may require a specific catalyst. This will be discussed more fully below.

Oxidative deamination catalysed by amine oxidase. The reaction catalysed by "tyramine oxidase" was originally formulated thus (9):

$$(1) \qquad R{-}CH_2{\cdot}NH_2 + O_2 = R{-}CH{:}NH + H_2O_2$$

$$(2) \qquad R{-}CH{:}NH + H_2O = R{-}CHO + NH_3$$

In this sequence the formation of the imino compound was followed by its hydrolysis. Richter (143) replaced this formulation by equations (3) and (4) in order to allow for the fact that tertiary amines, *e.g.*, hordenine, were oxidised by the enzyme:

$$(3) \qquad R{-}CH_2{\cdot}\overset{+}{N}HR'R'' + O_2 = R{-}CH{:}\overset{+}{N}R'R'' + H_2O_2$$

$$(4) \qquad R{-}CH{:}\overset{+}{N}R'R'' + H_2O = R{-}CHO + \overset{+}{N}H_2R'R''$$

The overall reaction is the sum of (3) and (4):

$$(5) \qquad R{-}CH_2{\cdot}\overset{+}{N}HR'R'' + O_2 = R{-}CHO + \overset{+}{N}H_2R'R'' + H_2O_2$$

In this formulation it is assumed that the ionised form of the amine reacts with the enzyme. We shall see that this is supported by experimental evidence: the amines which are substrates of amine oxidase are chiefly present as ions at the pH at which the enzyme is active. Amines which are not ionised to any appreciable extent are not oxidised.

Equation (5) gives a true picture when catalase is absent. In crude tissue extracts catalase is usually present, and hydrogen peroxide reacts thus:

(6) $$H_2O_2 = H_2O + \tfrac{1}{2}O_2$$

The overall equation of the enzymic reaction in the presence of catalase is thus (5) + (6):

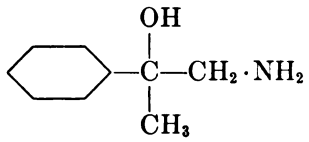
(7) $$R{-}CH_2\cdot\overset{+}{N}HR'R'' + \tfrac{1}{2}O_2 = R{-}CHO + \overset{+}{N}H_2R'R''$$

Equation (7) is reasonably well fulfilled under conditions met with in experiments with crude tissue extracts. Sometimes, however, the oxygen uptake exceeds half a molecule of oxygen for each molecule of amine added. Two factors contribute to this excessive consumption of oxygen:

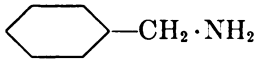
(a) some of the hydrogen peroxide formed is not decomposed according to reaction (6), but is used up in the oxidation of some organic material: such "peroxidatic" reactions are known to be catalysed by the almost ubiquitous enzyme catalase (109); the apparent increase of the rate of the amine oxidase reaction by alcohol (96) is probably explained by a peroxidatic oxidation of alcohol;

(b) the aldehyde formed is further oxidised to the corresponding carboxylic acid; such reactions are likely to occur in tissues like liver that contain an aldehyde oxidase.

Zeller (188) has recently suggested that in the histaminase reaction the primary oxidation product is: $R\text{-}CH{:}CH\cdot\overset{+}{N}HR'R''$, which rearranges itself to: $R\text{-}CH_2\cdot CH{:}\overset{+}{N}R'R''$. This interpretation is also discussed for amine oxidase; he points out that the compound



where there is no hydrogen atom in the β position, is not a substrate of amine oxidase (166). However, the fact that benzylamine



and many of its derivatives are oxidised by amine oxidase is not easily reconciled with this interpretation.

Amine oxidase and riboflavin deficiency. The mechanism of hydrogen transfer mediated by amine oxidase must remain unknown until the chemical composition of the enzyme (or of its prosthetic group) is known. The reaction catalysed is analogous to the oxidative deamination of D-amino acids by D-amino acid oxidase. Like amine oxidase, D-amino acid oxidase is cyanide-insensitive. It is a

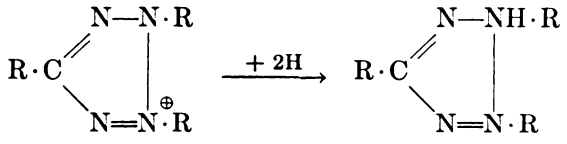
flavoprotein, and its prosthetic group is flavin-adenine-dinucleotide (FAD). In riboflavin deficiency the D-amino acid oxidase activity of rat liver extracts is known to be greatly reduced (2, 147). Miss Hawkins has recently compared the amine oxidase and D-amino acid oxidase activity of livers from riboflavin-deficient rats (92). She found that after a fortnight on the deficient diet the D-amino acid oxidase activity of liver homogenates from deficient rats had fallen to about one fifth of that from plus-riboflavin controls, whereas the amine oxidase activity was still about 50% of that of the control animals. Administration of riboflavin to the deficient animals resulted in a rapid rise of D-amino acid oxidase activity to normal values; the rise in amine oxidase activity was very small. These observations do not allow us to answer the question whether or not amine oxidase is a flavin enzyme; one is left with the impression that flavin is important, but not as directly as in D-amino acid oxidase. However, it is possible that amine oxidase is a flavin enzyme less ready to give up its flavin. Or, possibly, flavin is essential for synthesis of enzyme protein. In the presence of inositol in the diet riboflavin was more effective in restoring amine oxidase activity, but the role of inositol is not yet understood.

Amine oxidase and histaminase. Werle and Pechmann (181) believe that pyridoxal is a constituent of histaminase (see also 162). This is based on the observation that carbonyl reagents which inhibit pyridoxal enzymes, *e.g.*, the amino acid decarboxylases, also inhibit histaminase. They suggest that the histaminase reaction is initiated by a transfer of the amino groups from the diamine to pyridoxal phosphate. Pyridoxamine phosphate is then believed to be oxidised to pyridoxal phosphate by a second catalyst, possibly a flavin enzyme.

There are many similarities between histaminase and amine oxidase, but it seems very unlikely that amine oxidase contains pyridoxal. In contrast to histaminase, amine oxidase is not inhibited by carbonyl reagents, and in a few experiments on pyridoxine-deficient rats, no lowering of amine oxidase activity was observed (164).

Anaerobic reduction of dyes. In 1937, while studying the reduction of redox indicators by the amine oxidase system, Mrs. Philpot discovered the strong inhibitory action of methylene blue and toluylene blue on the enzyme (129). Two other dyes were reduced by the amine oxidase system in the Thunberg tube under anaerobic conditions; these were: o-bromophenolindophenol and o-cresolindophenol. Neither potassium indigosulphonate nor potassium indigotetrasulphonate was reduced. She thus determined the potential of the amine oxidase system as between -0.046 and $+0.195$ V.

The amine oxidase system of rabbit liver will anaerobically reduce triphenyltetrazolium chloride (TTC) (32). This substance forms a red reduction product in the presence of a fresh amine oxidase preparation and tyramine. When the rate of reduction of TTC is compared with the oxygen consumption of the same preparation under aerobic conditions, it is found that the rate of the anaerobic reaction in the presence of TTC is much slower than that of the aerobic reaction in the absence of TTC. (See 114.).



R = C_6H_5

In the anaerobic reduction of methylene blue by dehydrogenases, a flavin enzyme intervenes between the dye and the reduced coenzyme (pyridine nucleotide) (see 178). Similarly, for the reduction of TTC by enzymes requiring coenzyme I, a flavin enzyme is essential (37). In experiments on the amine oxidase system it has been found that the enzyme preparations lose their ability to reduce TTC more rapidly than their aerobic activity in the manometric experiment. Well washed, acetone-dried preparations will also not reduce TTC, although they are active when tested in the manometric experiment. These observations suggest that in the anaerobic reduction of TTC amine oxidase and an additional enzyme system are involved. This raises the question whether in the living organism the enzyme reacts directly with molecular oxygen or with another hydrogen acceptor which is not yet identified.

The amine oxidase system also reduces anaerobically the ditetrazolium compound described by Rutenberg *et al.* (149).

Measurement of enzymic activity. Usually the rate of the amine oxidase reaction is measured manometrically. This method is very convenient, but not very accurate; it is difficult to know if the oxygen is consumed only in the amine oxidase reaction. The measurement of the ammonia formed is more reliable (see 47), but readings cannot be taken as easily as in the manometric method. A sensitive qualitative method for the detection of the enzyme has been described by Florence and Schapira (69): tissue extracts are incubated with β-phenylethylamine in a stoppered vessel; the delicate odor of the phenylacetaldehyde formed indicates the presence of amine oxidase.

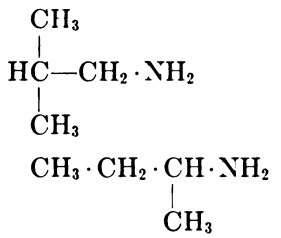
Amine oxidase and partial pressure of oxygen. The amine oxidase reaction is less rapid when the reaction is measured in an atmosphere of air instead of oxygen (129). Kohn (112) has found that in air the rate of oxidation of tyramine is about one third of that in oxygen, and in 5% oxygen it is about one third of the rate in air. The partial pressure at which amine oxidase is saturated with oxygen must therefore be very high.

This is a property in which the enzyme differs characteristically from most other respiratory enzymes which are saturated with oxygen at partial pressures which are likely to occur in the tissue. This property has been considered as an indication that the enzyme in the living tissue is very sensitive to changes of oxygen pressure (see 22). It seems more likely, however, that in the living cell amine oxidase does not transfer hydrogen to molecular oxygen, but to some other hydrogen acceptor. This hydrogen acceptor has not yet been identified.

Substrate specificity. Many members of the series $CH_3(CH_2)_nNH_2$ are substrates of the enzyme (134, 112, 34, 1). Rate of oxidation and affinity vary with the

number of carbon atoms in the carbon chain. The lowest homologue, methylamine, is not attacked at all. Ethylamine, however, is oxidised slowly by amine oxidase, and with increasing length of the chain the rate of oxidation increases; with 5 or 6 carbon atoms a maximum is reached and with a further increase in chain length the oxidation rate falls off. In a preparation from rabbit's liver, dodecylamine was slowly oxidised, but octadecylamine was neither oxidised nor did it act as an inhibitor of the enzyme (28). The decrease of affinity for amine oxidase with increasing chain length is almost certainly due to the low solubility of the higher members of the series and to micelle formation (111); the effective concentration of the long-chain amines is too low for oxidation to proceed at a measurable rate.

A few branched aliphatic monoamines have also been examined. Isoamylamine is rapidly oxidised. The guinea-pig liver enzyme oxidises not only n-butylamine, but also isobutylamine; sec-butylamine is not oxidised (151, 14).



isobutylamine — oxidised

sec-butylamine — not oxidised

Although the enzyme oxidises many monoamines which contain the grouping $—CH_2—NH_2$ *e.g.*, n-amylamine, it does not act on the corresponding diamines, *e.g.*, cadaverine.

oxidised by amine oxidase: $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$ (n-amylamine)

not oxidised by amine oxidase: $NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$ (cadaverine)

The presence of the second amino group interferes with substrate specificity by lowering the affinity: putrescine and cadaverine have little or no inhibitory action on amine oxidase (18).

The short-chain diamines like putrescine and cadaverine are substrates of another enzyme, histaminase (187). This enzyme is therefore also called diamine oxidase, whereas the term "monoamine" oxidase is proposed for amine oxidase. It has been shown, however, that amine oxidase acts on a great number of diamines (28, 29), and that the lack of affinity for amine oxidase is restricted to a small number of short-chain diamines.

When the members of the homologous series of diamines $NH_2(CH_2)_nNH_2$ are examined with a preparation of amine oxidase, *e.g.*, a washed suspension of acetone-dried powder of rabbit's liver, it is found that the lower members from n = 2 to n = 6 are not oxidised, but beginning with the member with 7 methylene

groups oxidation occurs; with increasing length of the polymethylene chain oxidation by amine oxidase becomes more rapid, until a maximum is reached. The compound with 13 methylene groups is most rapidly oxidised; beyond n = 13 the rate of oxidation decreases with increasing chain length (see Fig. 1).

Competition experiments have shown that not only the rate of oxidation of the diamines increases with the number of methylene groups, but also their affinity for amine oxidase: when a mixture of isoamylamine and nonamethylene diamine is oxidised by amine oxidase, the rate of oxidation is almost that with isoamylamine alone; if a mixture of the diamine with 13 —CH_2— groups and isoamylamine is oxidised the rate is the same as that with the C_{13}-diamine alone.

These experiments tell us that when two basic groups are present in the molecule the second basic group in some way interferes with the attachment of the

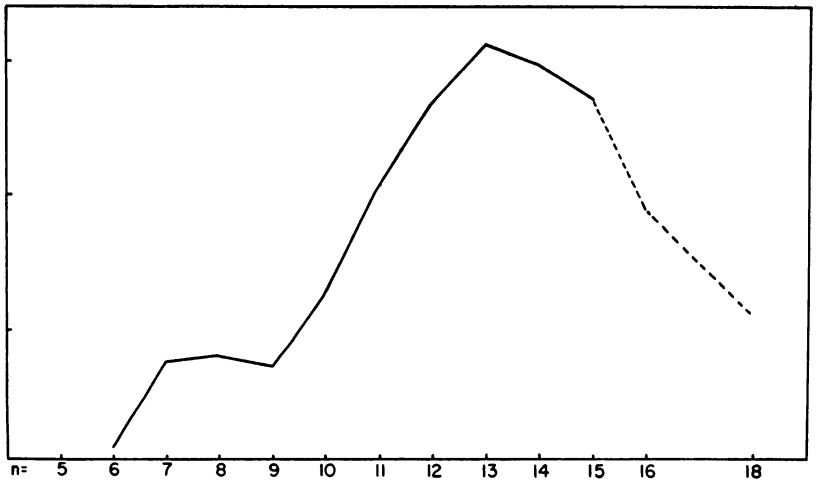


FIG. 1. Oxidation of aliphatic diamines of the homologous series $H_2N(CH_2)_nNH_2$ by amine oxidase (rabbit's liver). Abscissa: number (n) of carbon atoms in polymethylene chain; Ordinate: rate of oxidation (in arbitrary units).

first basic group to the enzyme; this interference decreases with increasing distance between the two basic groups: the diamine with 13 methylene groups has a higher affinity for the enzyme than the monoamine isoamylamine. The gradual decrease of the oxidation rate (and affinity) with even longer polymethylene chains may be due to decreasing solubility or to micelle formation, but no information on these properties is available for the diamines.

The reason for the disturbing influence of the second basic group is not known.

It is unlikely that it is due to changes in the dissociation constants, as it seems that there is little difference between the constants of diamines which are not oxidised and those which are (160).

In order to understand the different behaviour of short-chain and long-chain diamines, we have to consider their orientation on the active surface of the enzyme. We have seen that in the series of aliphatic monoamines the lowest members are relatively poor substrates of amine oxidase; an optimum configuration

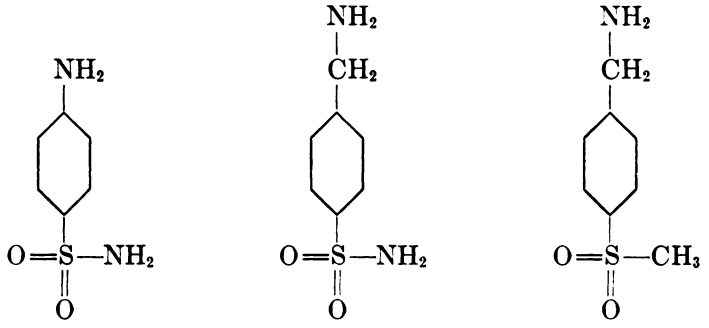
is reached with amylamine (C_5) or hexylamine (C_6). This suggests that the presence of the group $—CH_2 \cdot NH_2$ at which the reaction occurs is not a sufficient condition for affinity; the hydrocarbon moiety is also essential. This may ensure that the substrate molecule is lifted up and arranged at an angle to the active surface (28). Short-chain diamines cannot take up such a position; they would tend to arrange themselves parallel to the surface. With increasing number of methylene groups, the orientation of the diamines becomes more like that of the monoamines and a successful enzyme-substrate reaction can occur.

Aromatic amines, like aniline, in which the amino group is directly attached to the ring, are not attacked by amine oxidase (34). If it is true that amine oxidase acts on the ionised amines, then it can easily be understood why aromatic amines are not oxidized: at pH 7.4 about 99.7% of ethylamine is present in the ionized form, but only about 0.4% of the aniline is present as ions.

Whereas aniline and similar compounds are weak bases, the strongly basic character is re-established in aromatic compounds in which the amino group is not directly attached to the ring. It is not surprising, therefore, to find that they are substrates of amine oxidase, like the aliphatic amines.

Benzylamine, the compound studied by Schmiedeberg and his colleagues, is oxidized by amine oxidase (34). There are species differences in the rate of oxidation of benzylamine: it is more rapidly oxidized in the rabbit liver than in the guinea-pig liver (16).

One derivative of benzylamine is of therapeutic interest; this is ambacide (Marfanil), the p-sulphamido derivative. This compound is a substrate of amine oxidase (11, 27). As would be expected, sulphanilamide is not a substrate of the enzyme; the amino group in sulphanilamide is directly attached to the ring and is not strongly ionized at pH 7.4.



Sulphanilamide Ambacide "V335"

The compound V335 is a substrate of amine oxidase (27).

With increasing length of the side chain the rate of oxidation increases: β-phenylethylamine is oxidised more rapidly than benzylamine and γ-phenylpropylamine is more rapidly oxidised than β-phenylethylamine (12). Amines with longer side chains appear not to have been examined.

The derivatives of β-phenylethylamine have been most carefully studied: this is because this group includes so many "biogenic" amines, *e.g.*, tyramine,

adrenaline, noradrenaline, hydroxytyramine, hordenine and mescaline; in addition, many synthetic derivatives of β-phenylethylamine have therapeutic interest.

This is not the place to discuss the pharmacological properties of this group of amines. Since the classical work of Barger and Dale (4) they have been most extensively studied (see 35). The metabolism of derivatives of phenylethylamine has also been reviewed in recent years (90, 84, 10, 12), and I shall in the following discuss chiefly those aspects which are connected with the question of the activity of amine oxidase in the living organism.

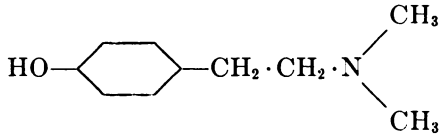
Amine oxidase was first described as "tyramine oxidase" (86), and tyramine is a typical substrate of the enzyme. Not only compounds with the phenolic group in para-position, as in tyramine, but also meta-phenolic amines like neosynephrine are oxidised (34); both the meta- and ortho-analogues of tyramine are substrates of amine oxidase (136).

Of the diphenolic derivatives of phenylethylamine the catechol amines have been most extensively studied. The biogenic amines which belong to the group are adrenaline, noradrenaline and hydroxytyramine; they are oxidised by amine oxidase and so are many synthetic catechol amines. The resorcinol derivative, 3:5-dihydroxyphenylethylamine, is also oxidised by amine oxidase (83). One derivative of hydroquinone has been studied; this is 2:5-dihydroxyphenylethylamine; although the compound was not very stable in aqueous solution, it appears to be oxidised by amine oxidase (31).

Substitution of the amino group. If one of the two hydrogens of an amino group is substituted by a methyl group, the rate of oxidation by amine oxidase is not very much affected. If the hydrogen is replaced by a more bulky group (*e.g.*, ethyl or iso-propyl) the oxidation rate is much more reduced.

If both hydrogen atoms in an amino group are replaced by methyl, the rate of oxidation is very much reduced. Other tertiary amines like tri-butylamine or tri-isoamylamine are not oxidised at a measurable rate (34; see also 1).

A tertiary amine which was shown to be oxidised by amine oxidase is hordenine, the N-dimethyl derivative of tyramine (112):



hordenine

The rate of oxidation of hordenine differs in different species: in the guinea-pig's liver it is much more slowly oxidized than in the pig's kidney (34). That the difference in the oxidation rate of tertiary amines is a species difference was confirmed by Randall (136) in a systematic study of the effect of progressive N-methylation on the rate of oxidation by amine oxidase. He examined a great number of phenylethylamine derivatives, always comparing the rate of oxidation of the primary, the secondary and the tertiary amine. Two enzyme preparations were used, one from guinea-pig's liver, the other from cat's liver. He did

not find marked differences in the rates of oxidation of the primary and secondary amines; sometimes the primary, sometimes the secondary amine was attacked a little more readily. There was a systematic difference in the rate of oxidation of the tertiary amines by the liver preparations of the two species: the rate of oxidation was always less than with the primary and secondary amines, but this reduction was much greater with the guinea-pig liver enzyme than with the cat liver enzyme.

Quaternary ammonium compounds are not oxidised by amine oxidase.

Adrenaline differs from noradrenaline by one N-methyl group. Both amines are oxidized by amine oxidase (34), and the rate of oxidation is similar. When an equimolecular mixture of the two amines is oxidized by an acetone-dried powder of rabbit's liver, the ratio: $\frac{\text{adrenaline}}{\text{noradrenaline}}$ increases as the reaction proceeds. In other words, in the mixture noradrenaline is more rapidly oxidised (25, 42). The affinity of noradrenaline for amine oxidase has not been determined. Figures for the affinity of adrenaline for the enzyme have been given (112, 34), but it should be remembered that the measurement of the oxidation rates of the catechol amines are made inaccurate by the fact that some oxygen is consumed in reactions other than that catalysed by amine oxidase.

The side chain hydroxyl group. Noradrenaline and adrenaline both contain the hydroxyl group on the carbon atom adjacent to the carbon atom which carries the amino or methyl-amino group. The presence of this hydroxyl group lowers the affinity of the amines for amine oxidase. It is probably due to this fact that the oxidation of adrenaline by amine oxidase was not at first noticed and only described in 1937 (33).

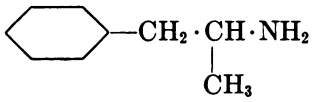
Competitive inhibitors of amine oxidase. It has already been mentioned that compounds like tri-isoamylamine, in which the nitrogen atom carries more than one aliphatic substituent, are not oxidized by amine oxidase. They inhibit the enzyme; this type of inhibition may well be a competitive one (see 1). A sharp line between competitive inhibitors and substrates cannot always be drawn. Any substrate of amine oxidase, because it has affinity for the enzyme, may interfere with the oxidation of another substrate. This is the basis of the phenomenon of substrate competition. If the affinity of a given substrate for amine oxidase is high, but its rate of oxidation slow, it will act as an inhibitor. A well-known example of this kind is the inhibition of the cholinesterase of serum by eserine, which is a substrate with a high affinity for the enzyme, but which is hydrolysed at a very slow rate (49).

There is one group of amines which are not oxidised by the enzyme at a measurable rate, but which act as competitive inhibitors of amine oxidase: these are compounds in which the amino group is not attached to a terminal carbon atom. Most of the substances studied have a methyl group attached to the carbon atom which is immediately adjacent to the basic group:

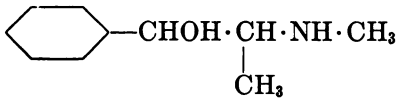
$$\begin{array}{l} \text{R—CH·NH}_2 \\ \quad\quad | \\ \quad\quad \text{CH}_3 \end{array}$$

Ephedrine was the first inhibitor of this group which was studied, but it was soon found that this property was shared by many other derivatives of β-phenylisopropylamine (17) and by β-phenylisopropylamine itself (125).

When the percentage inhibitions caused by equimolar concentrations of phenylisopropylamine derivatives are determined, it is found that these substances fall into two classes of different inhibitory strengths (20). The class which includes phenylisopropylamine itself contains more potent inhibitors than the class which includes ephedrine.



β-phenylisopropylamine (amphetamine)

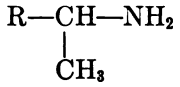


ephedrine

The second class differs from the first in the presence of the hydroxyl group in the side chain. This shows that the hydroxyl group in the side chain affects the affinity of competitive inhibitors in the same way as that of substrates, *e.g.*, of the adrenaline-noradrenaline type.

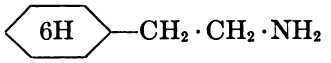
The competitive character of the amphetamine inhibition was studied by Mann and Quastel (125) who found that the competition of amphetamine with tyramine for amine oxidase was strictly reversible. They confirmed the weaker inhibitory action of compounds of the ephedrine type.

The inhibitory action of amines of the general structure

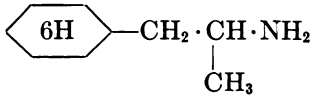


has often been confirmed (128, 94, 95, 68). Heegaard and Alles have shown that also in the aliphatic series the amines in which the amino group is not attached to a terminal carbon atom act as inhibitors.

Similarly, β-cyclohexylethylamine is a substrate of amine oxidase, but β-cyclohexylisopropylamine is not oxidised (167):

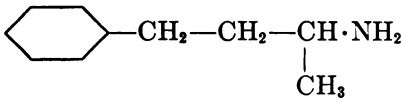


oxidised



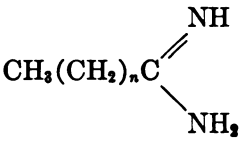
not oxidised

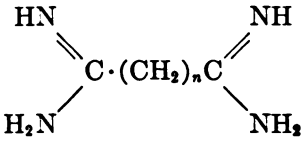
Fellows and Bernheim have recently shown that 2-amino-4-phenyl-n-butane and some of its derivatives also inhibit amine oxidase (68).

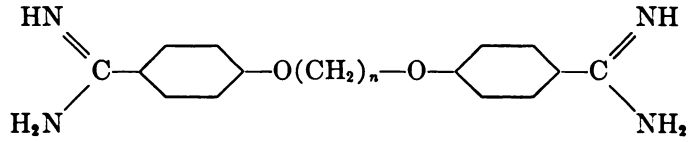


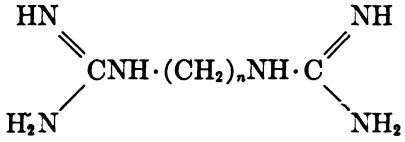
Amidines as inhibitors of amine oxidase. Many amidines are inhibitors of amine oxidase (27, 67; see also 6). It is likely that these compounds inhibit the enzyme because their basic group combines with the receptor for the amino group.

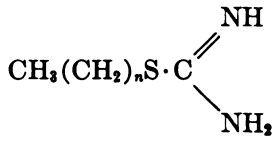
A study of the relation between the chemical constitution of amidine compounds and inhibitory strength has been made in several homologous series of amidine derivatives. These include:

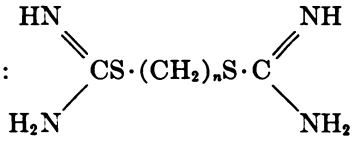
(1) Monoamidines: 

(2) Diamidines: 

(3) Propamidine-Pentamidine series: 

(4) Diguanidines: 

(5) Mono*iso*thioureas: 

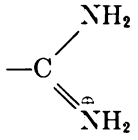
(6) Di*iso*thioureas: 

Certain general rules emerge from the study of the inhibitory action of these compounds. Firstly, in the monoamidine series ((1) and (5)), the first members have little inhibitory action; the inhibition rises at first with increasing chain length, but falls off again after a maximum is reached. Secondly, the series of diamidines ((2), (3), (4) and (6)) contain stronger inhibitors of amine oxidase than the mono-substituted compounds; propamidine and pentamidine are the most active inhibitors of amine oxidase so far described. Thirdly, in the di-substituted series the maximum inhibitions are reached with longer chains than in the mono-substituted series.

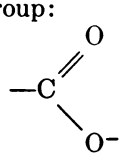
It can be seen that the affinity of amine oxidase for the amidine compounds is analogous in many ways to the affinity of the enzyme for the amines, except that the latter are oxidised whereas the former inhibit. In the monoamine series the short-chain compounds are slowly oxidised and, after a maximum is reached, the rate of oxidation and affinity fall off with increasing chain length, and in the

diamine series, the long-chain members are oxidised with an optimum chain length which is greater than in the monoamine series.

These analogies suggest that the amidine group combines with the active centre in the enzyme which normally reacts with the amino group. The amidines are even stronger bases than the amines and they are fully ionised at the pH of physiological interest. It has been suggested by Walker (176) that the ionised amidinium group:



reacts with the ionised carboxyl group:



to form a ring which is stabilised by resonance. Possibly the enzyme protein reacts with the amidines in a similar way.

Pentamidine and some other amidine compounds are very firmly held by the enzyme and not easily removed by washing (27, 67). A more detailed study of the reversibility of the inhibition has been made for some of the mono*iso*thioureas (series (5)); in this series the removal of the inhibitor by washing becomes less complete as chain length increases. The n-butyl derivative was completely removed after three washings; the n-hexyl derivative was almost fully removed after six washings; the n-octyl derivative was incompletely removed after six washings, and there was very little indication of removal of the n-decyl derivative after six washings. In these experiments the inhibitor concentrations were chosen so as to produce about equal percentage inhibitions of the enzyme (about 60–80%); the concentrations were:

of S-*n*-butyl *iso*thiourea: 25.0×10^{-3} M
of S-*n*-hexyl *iso*thiourea: 6×10^{-3} M
of S-*n*-octyl *iso*thiourea: 1.2×10^{-3} M
of S-*n*-decyl *iso*thiourea: 0.2×10^{-3} M

The most firmly held compound is the strongest inhibitor; the feeble inhibitor is easily removed by washing.

These observations suggest that the reaction between amidine group and active group of the enzyme does not determine the force by which the amidine is held on the enzyme, but this depends upon the nonspecific forces between the non-polar moiety of the amidine molecules and the enzyme surface. The reaction between amidines and enzyme can thus be considered as a model of the reaction between the enzyme and amines.

Other inhibitors

Methylene blue. The strong inhibitory action of methylene blue has already been mentioned. Not all redox indicators are inhibitors of the enzyme (129, 131).

The mechanism of the inhibition by methylene blue is not known. Although it is a strong inhibitor of the enzyme, this seems to depend upon experimental conditions. Some authors report that, with low partial pressures of oxygen, methylene blue causes an increase of the rate of oxygen uptake (112, 81).

Sulphydryl reagents. Amine oxidase is inhibited by —SH reagents (70, 163). The concentration of inhibitor must be fairly high. The inhibition is partly reversed by glutathione.

Metal-complex formers. Cyanide, carbon monoxide and azide are not inhibitors of the enzyme, and there is at present no evidence that a heavy metal is part of amine oxidase.

Carbonyl reagents do not inhibit the enzyme. Semicarbazide is often used to ensure that the aldehyde formed in the enzymic reaction is not further oxidised, *e.g.*, by aldehyde oxidase; it is without any action upon the oxygen uptake due to amine oxidase.

Alcohols. Octyl alcohol is an inhibitor of the enzyme. Heim (96) has recently studied the action of the homologous series of aliphatic alcohols on the enzyme: the lower members increase the oxygen consumption, but the higher members inhibit. This increase of the oxygen uptake is probably not due to any activating effect of the lower alcohols on the enzyme, but to the oxidation of the alcohols by the hydrogen peroxide formed.

The action of local anaesthetics. The inhibitory action of cocaine and many related substances on amine oxidase was first described by Mrs. Philpot (130); this effect has often been confirmed (128, 97, 98). Percaine is a very strong inhibitor of the amine oxidase from guinea-pig liver; then there follow in decreasing order of inhibitory activity, cocaine, stovaine, butyn and procaine (128). In liver extracts, some anaesthetics which contain ester linkages are hydrolysed by enzymes present in the tissue; they lose their inhibitory action as hydrolysis proceeds (98). In kidney extracts, where esterases are absent, no hydrolysis occurs and the inhibitory action on amine oxidase is retained.

Antihistamines. Schuler (159) showed that antistin was an inhibitor of amine oxidase. This was confirmed by Tickner (174) who found that not only antistin but a number of other antihistaminics were inhibitors of amine oxidase. He used a rabbit's liver homogenate as source of enzyme and tyramine in a concentration of about 10^{-3} M as substrate. Under these conditions 10^{-3} M diphenhydramine hydrochloride (benadryl) caused an inhibition of about 50%; the weakest inhibitor was mepyramine with 32% inhibition.

The sympathetic blocking agents 933F (174), ergotoxine (16), ergotamine and ergometrine (128), also are weak inhibitors of amine oxidase.

An inhibition of the enzyme by xanthine derivatives, *e.g.*, by theophylline, caffeine and theobromine, has also been described (99).

PART III

Amine Oxidase and Amine Metabolism in the Living Organism

It is often difficult to derive a clear picture of the mode of action of an enzyme in the living animal from the study of its properties *in vitro*. This is true also for

amine oxidase. Two main questions which we are unable at present to answer may be briefly stated at the outset:

(a) We do not know if in the living cell amine oxidase reacts directly with molecular oxygen as it does in the manometer vessel or whether a chain of oxido-reduction catalysts take part in the reaction. I have already discussed some observations which suggest that the enzyme does not normally react with molecular oxygen. If this is so, then measurements of enzymic activity made so far do not represent the true rate of the enzymic reaction *in vivo*. Data obtained in manometric experiments give relative rates only of enzyme activity, but the rate at which amines are broken down in the tissues may be different.

(b) We do not know if in the body the deamination of amines is the only reaction catalysed by the enzyme; it seems possible that in the organism the amine oxidase reaction, coupled with an energy-yielding reaction, runs in the reverse direction, *i.e.*, in the direction of amine formation. Here we have no experimental data which can help us. We can only say that we do not know of any reactions in which the aldehydes could be formed which might be the precursors of the biogenic amines found in the animal body.

In the following, I shall discuss the role of amine oxidase, first in the metabolism of substances which are not normally found in the body, and secondly, in the metabolism of amines which might normally be substrates of the enzyme.

A. Metabolism of amines not normally found in the body

The fate of the simple aliphatic monoamines is breakdown by deamination. No characteristic products of the metabolism of straight-chain monoamines have been described, but it is known that they increase the output of urinary urea (122). We can ask: is there an agreement between the deamination of these substances *in vivo* and their oxidation by amine oxidase?

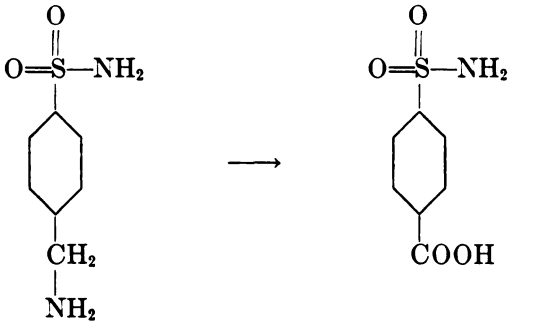
Methylamine, the simplest of the aliphatic amines, presents an anomaly. In the living organism, it is broken down completely (142) and its nitrogen is excreted as urea. There is no evidence to suggest that amine oxidase acts on methylamine. We must conclude that amine oxidase is not the enzyme responsible for its breakdown.

In man, dimethylamine is not metabolised, but recovered in almost theoretical amounts from the urine (142). Dimethylamine is also not oxidized by amine oxidase.

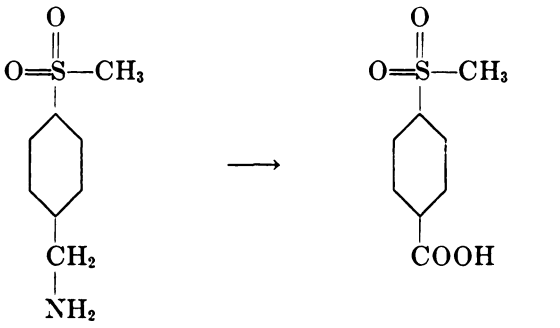
The excretion of some other aliphatic amines in man has been studied by Rechenberger (142). He found that 32% of ethylamine was recovered from urine, but only about 10% of propylamine and 2% of *n*-butylamine. Of diethylamine, 86% was recovered from urine, and about 15% of *iso*butylamine. These data are easily interpreted on the assumption that amine oxidase is the catalyst responsible for deamination in the living organism of aliphatic monoamines. We have seen that for the short-chain monoamines the rate of oxidation by amine oxidase rises with increasing chain length. It is therefore not difficult to understand that about one third of the ethylamine escapes destruction, whereas of propylamine more is destroyed and of *n*-butylamine even more. *Iso*butylamine is oxidised much less rapidly by amine oxidase than *n*-butylamine (142), and

diethylamine is scarcely oxidised by the enzyme. In the dog, 1 g. doses of propylamine, given on 10 consecutive days, completely disappeared (5). *Iso*amylamine, a good substrate of amine oxidase, is also completely metabolised in the dog; in the perfused liver, the metabolic product isolated is *iso*valeric acid (122). This is the product we would expect to find if amine oxidase was active; the intermediate product, *iso*valeraldehyde, would be expected to be oxidised by the aldehyde oxidase of liver to the corresponding carboxylic acid.

Benzylamine and benzylamine derivatives are excreted as the corresponding carboxylic acids. We have already discussed the work of Schmiedeberg and his school on benzylamine which is excreted as benzoic acid (or hippuric acid); it is interesting that this amine is fully metabolised as we would expect it to be if amine oxidase were the catalyst of the primary breakdown reaction. Similarly, 84% of the theoretical amount of ambacide appears in rabbits urine as p-carboxybenzenesulphonamide, and 87% of "V 335" appears as p-methylsulphonylbenzoic acid (88):



Ambacide — p-carboxybenzenesulphonamide



V 335 — p-methylsulphonylbenzoic acid

Hartles and Williams have also studied the fate of ingested p-hydroxybenzylamine in the rabbit; 40% was excreted as free p-hydroxybenzoic acid, 39% as the glucuronide of p-hydroxybenzoic acid and 19% as its sulphate (89). Here again, it seems probable that amine oxidase was the catalyst; it is not known if oxidation to the free hydroxy acid preceded esterification or if the esterified amines were oxidised by amine oxidase.

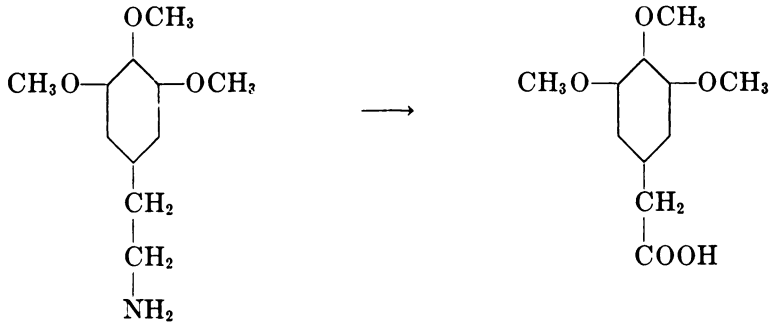
It seems particularly interesting that the benzylamine derivatives are converted to the carboxylic acid so quantitatively. Like benzylamine itself, they are not very rapidly attacked by amine oxidase. We can therefore conclude that in the living body the enzyme is able to attack amines which are only slowly oxidised *in vitro*.

The fate of phenylethylamine and of tyramine has already been mentioned in the introduction when the work of Ewins and Laidlaw and of Guggenheim and Löffler was discussed (65, 85). Here again, the carboxylic acids are recovered; this indicates that amine oxidase has been the catalyst. There is one phenolic amine which is an exception to the general rule: 2:5-dihydroxyphenylethylamine is oxidized in the body without giving rise to characteristic breakdown products (120). This can be easily understood: amine oxidase would be expected to oxidize the amine to the corresponding aldehyde (31); the aldehyde would be further oxidized to the corresponding carboxylic acid, homogentisic acid. The metabolic pathway would from then on be the same as for tyrosine which is also broken down via homogentisic acid and fully oxidised.

Conversely, many amines which are not attacked by amine oxidase are excreted unchanged. This is true for amphetamine and ephedrine (144). The resistance to amine oxidase is responsible for the characteristic long-lasting effect of ephedrine and related compounds which was first clearly recognised by Chen and his colleagues (45).

Amphetamine is not quantitatively excreted in the urine (105, 105a, 118, 13). Some of it is metabolised. In the rabbit this breakdown of amphetamine is more marked than in man, where about one half is excreted (87). This inactivation may also be by oxidation, but not catalysed by amine oxidase; it is possible that ascorbic acid acts as hydrogen acceptor in a dehydrogenation reaction which precedes deamination (10).

The metabolism of mescaline presents a special case. In man, about 50% of the amine is excreted unchanged in the urine (144), but in the rabbit it is almost quantitatively excreted as the corresponding carboxylic acid, 3,4,5-trimethoxyphenylacetic acid (165):



Mescaline — 3,4,5-trimethoxyphenylacetic acid

That mescaline is destroyed in the rabbit is in agreement with observations on tissue preparations: the liver of the rabbit differs from that of most other mammals in that it contains an enzyme that actively deaminates mescaline (7).

We can sum up the results of all these observations by saying that catalysts of the amine oxidase type must be assumed to be the chief means of inactivation of amines in the organism, but that in special instances other mechanisms are at work. It seems remarkable that even substances which are very slowly oxidised *in vitro* are metabolised in the living animal as one would expect them to be metabolised if amine oxidase were the catalyst responsible for the first step of oxidation.

B. The biological significance of amine oxidase

As more is learned about "detoxication reactions", it becomes evident that they represent metabolic pathways of chemically related substances which normally occur in the body. These normal metabolites are usually broken down completely, often to water, carbon dioxide and ammonia (or urea), end products which do not tell us much about the metabolic pathways followed. Until the introduction of isotopic tracer techniques, most of our knowledge of these metabolic pathways came from the study of foreign substances which were not broken down completely, but which led to characteristic end products that gave some information about the steps by which the breakdown had occurred.

We have to ask: is amine oxidase an enzyme which catalyses the breakdown of substances normally found in the body or does it chiefly deal with substances introduced from outside?

Amine oxidase as "detoxicating" agent. It seems quite possible that amine oxidase does serve some truly "detoxicating" functions. As has been pointed out some time ago, the high concentration of the enzyme in liver and intestine may prevent amines taken up from the alimentary canal from reaching the general circulation (14). But we have to ask if the uptake of amines, such as tyramine, from the intestine is an event that is likely to occur on a large scale and often enough to require the presence of a specific catalyst in so many different tissues.

Bacterial formation of amines takes place in an acid milieu and is completely suppressed in an alkaline environment. The enzymic formation of tyramine from tyrosine by *Streptococcus faecalis* is at an optimum at pH 5.0–5.5 (75). Moreover, the bacterial amino-acid decarboxylases are adaptive enzymes which are formed in the bacterial cell only when growth occurs in an acid environment (74). These are conditions not very often associated in the intestinal lumen with a high bacterial activity. Thus it seems that only under exceptional conditions would amine oxidase be necessary to destroy amines formed in the gut.

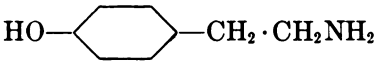
It may, however, happen that the food has been exposed to putrefaction and that the amines are already present in it when it is eaten. This may occur more often in animals which normally feed on putrefying meat. It would be interesting to know if the tissues of animals with such feeding habits are particularly rich in amine oxidase.

Amine oxidase is present in so many tissues other than liver and intestine that it seems unlikely that the enzyme serves only the function of removing amines taken up from the intestine. We have to enquire if amines which can be substrates of amine oxidase *in vivo* are normally found in the body.

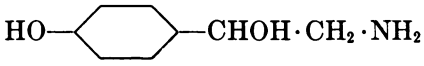
Sympathicomimetic amines

Invertebrates. Tyramine was first found by Henze (100) in the posterior salivary

glands of *Octopus macropus*. This has since been confirmed by Erspamer who found tyramine also in the posterior salivary glands of *Octopus vulgaris*, a closely related species, where it occurs together with another monophenolic amine, octopamine (56). From a study of extracts of posterior salivary glands of *O. vulgaris* by paper chromatography it seems likely that octopamine is l-p-hydroxyphenylethanolamine (60, 58), an amine closely related to tyramine:



tyramine



p-hydroxyphenylethanolamine

Final proof of the identity of octopamine with hydroxyphenylethanolamine will have to await isolation and chemical identification of the amine.

The function of these amines in *Octopods* is not yet known. Originally it was believed that tyramine was the poisonous principle of the saliva, but this is contested. Sereni (161) believed that tyramine acted as a hormone in these animals.

The tissues of *Octopus vulgaris* are rich in amine oxidase. The posterior salivary glands where these amines are found is second only to the "liver" in enzymic activity (30). The *Octopus* enzyme, like the mammalian enzyme, oxidizes both tyramine and p-hydroxyphenylethanolamine (16). Data summarized in the following table will show that the rate of oxidation of the ethanolamine derivative is much slower than that of tyramine. (In the table, the initial oxygen uptake is expressed in μl. O_2 consumed per 100 mg. of fresh weight per hour.)

TISSUE	WITH TYRAMINE	WITH dl-p-HYDROXYPHENYL-ETHANOLAMINE	WITH BOTH AMINES
rabbit liver	200	38	182
Octopus "liver"	254	74	198
Octopus posterior salivary glands	44	8.5	26.5

The slow oxidation of the ethanolamine derivative is in agreement with what is known about the introduction of a hydroxyl group in β-position to the amino group.

It has recently been found by Ghiretti that the tyrosinase of *Cephalopods* is able to oxidize tyramine (78); this suggests that these animals may also form hydroxytyramine (from tyramine) and noradrenaline (from "octopamine"). The diphenolic amines would be the primary oxidation products of the corresponding monophenolic amines in the tyrosinase reaction.

In tissue extracts the diphenolic amines would be oxidised further to compounds of the adrenochrome type.

The occurrence of p-hydroxyphenylethanolamine is also of interest in con-

nexion with the problem of biosynthesis of adrenaline and sympathin; possible pathways involving hydroxyphenylethanolamine have been discussed elsewhere (26).

The presence in *Octopods* of both sympathicomimetic amines and amine oxidase makes it possible that the function of the enzyme is to be found in the removal of these amines. It would clearly be desirable to have more precise information about the part played by these amines in *Octopods* before the significance of amine oxidase can be discussed. If they act as hormones, the enzyme may well serve a local inactivation mechanism. As in vertebrates, the chief digestive gland ("liver") is very rich in enzyme; this may be useful in the "detoxication" of amines taken up in the food. It seems difficult to believe that the enzyme serves a similar purpose in the other organs in which it has been found.

Other invertebrate phyla have not been nearly as fully studied. In echinoderms, where amine oxidase has been found (34), nothing is known about the occurrence of sympathicomimetic amines. On the other hand, annelids like the leech and the earthworm contain adrenaline (77) and probably also noradrenaline (62), but no amine oxidase has hitherto been found in these species. It must be admitted, however, that the method of demonstrating the presence of the enzyme was very crude: the entire animal was ground up in a mortar and examined; since it is known that only a few structures in the vascular and digestive system have adrenergic innervation, it would be interesting to examine these for the presence of the enzyme.

Vertebrates. The occurrence of sympathicomimetic amines in vertebrates has recently been discussed in these Reviews by Euler (63). Three sympathicomimetic amines have so far been found: adrenaline, noradrenaline and hydroxytyramine.* Only the first two are known to act as hormone and mediator; for hydroxytyramine no similar function has hitherto been established. Both hydroxytyramine and noradrenaline are associated with adrenaline not only in nervous and endocrine tissue, but also in exocrine tissue: noradrenaline occurs in small amounts in the secretion of the Chinese toad (*Bufo gargarizans*) (121), and hydroxytyramine occurs in the parotoid gland of *Bufo marinus* (82). In mammals, hydroxytyramine has been found in the heart and the adrenal medulla (80).

That the three amines are so often found together supports the idea of a genetic relationship: hydroxytyramine may be considered as a precursor of noradrenaline, and noradrenaline in its turn as a precursor of adrenaline (24). This does not mean that the three amines do not have their own specific regulatory functions.

Hydroxytyramine is formed by the enzyme DOPA decarboxylase which was discovered by Holtz, Heise and Ludtke in the mammalian kidney (103), but it has since been found in the liver (19), in the pancreas (102), in the guinea-pig's intestine (101) and in the adrenal medulla of the ox (116).

Hydroxytyramine is rapidly oxidised by amine oxidase (34), and its affinity for amine oxidase is high (1, 10). It is therefore to be considered as an important

*The term "dopamine" has recently been proposed by Dale for what is here called hydroxytyramine; this new name is preferable because it is less ambiguous and because it stresses the relationship between amino-acid and amine.

substrate of amine oxidase in the living animal. It seems doubtful if hydroxytyramine is quantitatively transformed to noradrenaline.

The finding of DOPA decarboxylase in the suprarenal medulla of the ox makes it likely that, at least in this species, hydroxytyramine is formed for the production of adrenaline and noradrenaline in the gland. It is not known whether this is the same in other species; the large amounts of enzyme, especially in liver and kidneys, may not only serve the function of producing hydroxytyramine for synthesis of noradrenaline and adrenaline, but we must consider the possibility that hydroxytyramine itself has some important regulatory function which is not yet recognized. If this is so, amine oxidase may be a mechanism which removes surplus hydroxytyramine not required for conversion into noradrenaline or adrenaline.

Amine oxidase occurs wherever the DOPA decarboxylase has been found, but the oxidase appears to have a much wider distribution. This may indicate that amine oxidase acts also in tissues other than those in which hydroxytyramine is formed, but possibly DOPA decarboxylase is more widely distributed than is known at present. The decarboxylase is a pyridoxalphosphate enzyme, and it may be that small amounts escape detection in tissue extracts in the absence of added codecarboxylase, as it is known that the enzyme tends to become dissociated when it is highly diluted.

A functional relationship between DOPA decarboxylase and amine oxidase is also discussed by Polonovski and his colleagues (150, 133, 79). This is based on an earlier observation (22) that hydroxytyramine inhibits the decarboxylase. They showed that adrenaline and noradrenaline and many other amines with a high affinity for amine oxidase inhibited the decarboxylase. In the adrenal medulla of the ox, Langemann (116) considers that the accumulation of amines might cause an inhibition of amine formation.

Oxidation by amine oxidase is probably an important mechanism of biological inactivation of hydroxytyramine, because the amine has a high affinity for amine oxidase. Since it is a catechol amine, two other mechanisms of inactivation have to be considered: oxidation by phenolase type of enzymes (including the cytochrome system) and conjugation of the phenolic groups with sulphuric or glucuronic acid.

These same three mechanisms of inactivation are of importance in the inactivation of adrenaline and noradrenaline: oxidative deamination, oxidation to adrenochrome (or noradrenochrome) and conjugation.

We do not know to what extent these three modes of enzymic attack contribute to the inactivation of the two amines *in vivo*. The existing evidence is most satisfactory for conjugation, and it is likely that conjugation will contribute greatly to the inactivation of adrenaline and other catechol amines when they are ingested orally or when they circulate in the blood for a sufficient length of time, so that conjugation can occur in intestine and liver. This is supported by experiments in which β-labelled radioactive dl-adrenaline was administered (for structure, see below) (153). When 0.04 μg. of adrenaline per g. body weight was injected intravenously in rats, no adrenaline, free or conjugated, was recovered from the urine. When 0.3 μg./g. was injected, about 6% was excreted

as free, and 3% as conjugated, adrenaline. When 45 μg./g. of adrenaline were given by mouth, about 18% of the dose was recovered as conjugated adrenaline.

The action of phenolases and related enzymes on adrenaline results in the formation of adrenochrome. The same type of reaction also occurs in what is generally called the "autoxidation" of adrenaline.

Bacq (3) has recently pointed out that autoxidation of adrenaline is not a powerful factor in the inactivation of adrenaline, as the cells contain many substances which prevent autoxidation. On the other hand, he is inclined to attribute to the "phenolase" type of oxidation a major importance in the biological inactivation.

Substances which counteract the autoxidation of adrenaline also prevent the enzymic oxidation by phenolases. This can be easily demonstrated. When a solution of adrenaline is incubated in a manometric flask and a preparation of mushroom oxidase is added, oxygen consumption begins at once and a red colour develops. This indicates that oxidation products of adrenaline accumulate. If the same solution is incubated with mushroom phenolase in the presence of ascorbic acid, oxygen consumption again begins at once, but little colour develops. This observation is easily interpreted: the enzyme acts on adrenaline in the presence and absence of ascorbic acid; when ascorbic acid is present, the first oxidation product of adrenaline does not accumulate, but is immediately reduced to adrenaline by the ascorbic acid which is oxidized. Adrenaline is thus continuously regenerated until all ascorbic acid added has been oxidised to dehydro-ascorbic acid; only then will coloured oxidation products of adrenaline begin to accumulate. Similarly sulphydryl compounds protect adrenaline, and it is likely that all these substances exert their action also *in vivo*.

It is not necessary here to discuss the question of the chemical structure of the first oxidation product of adrenaline; it is sufficient to say that experimental evidence tells us that a reversible oxidation precedes the irreversible oxidation to adrenochrome. This may explain observations of Ruiz-Gijon who has described a compound, adrenoerythrine (127a, 148), which is approximately as active as adrenaline itself when tested for pressor or hypoglycemic action. If this compound is identical with the first oxidation product of the phenolase oxidation, we would not be surprised to find that it is reduced to adrenaline in the organism. It must be emphasized, however, that this interpretation is not accepted by Ruiz-Gijon.

Since the phenolase type of oxidation is catalysed by the cytochrome system which is almost ubiquitous in animal tissues it is surprising that there exists no evidence for formation of adrenochrome and related substances. From what has been said, the formation of adrenochrome in the living organism will require conditions in which substances like ascorbic acid and sulphydryl compounds fail to protect adrenaline from irreversible oxidation. Such a "disinhibition" of the oxidizing mechanism will only occur when the protecting substances are absent (or fully oxidized). It can be hoped that experiments with "labelled" adrenochrome (153) may eventually tell us something about this type of inactivation of adrenaline-like compounds.

It may be added that the reversible oxidation of adrenaline to the first oxida-

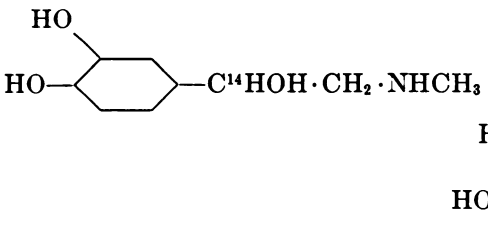
tion product is likely to occur much more readily in the tissues than the irreversible oxidation to the adrenochrome stage.

The oxidation of adrenaline and noradrenaline by amine oxidase was discovered when it became possible to exclude the "phenolase" type of oxidation by the use of cyanide (33). Since then the contribution of amine oxidase to the inactivation of adrenaline and sympathin has been under discussion.

Until recently, chemical methods were not sufficiently sensitive to allow studies of the metabolic fate of adrenaline and noradrenaline. Two recent investigations will be discussed which suggest that amine oxidase is active on these two amines.

Lund (123) has followed the rate of disappearance of adrenaline and noradrenaline from the circulating blood in the rabbit and from the perfusion fluid in isolated rabbit's organs, the liver and the hind legs. In the liver the rate of disappearance of both amines was much higher than in the hind legs. The concentration of amines in these experiments was very high; it would be interesting to study the disappearance of adrenaline in lower concentration with an even more sensitive method (see, *e.g.*, 178a). Lund discusses the possibility that deamination and conjugation are responsible for the high rate of inactivation in the liver.

The second type of experiment makes use of radioactive tracer technique. Schayer (152, 153) synthesized two preparations of adrenaline which contain the radioactive isotope C^{14}. One of these has a "labelled" carbon atom in position β to the methylamino group; in the other the N-methyl carbon is labelled:



β-labelled adrenaline methyl-labelled adrenaline

If these preparations are given intravenously or by mouth, the radioactive carbon atoms suffer different fates. About 90% of the β-carbon atom is excreted in the urine, but in the same time, only about 50–60% of the methyl carbon atom is excreted. This indicates that at least 30–40% of the adrenaline molecules are split between the β carbon and the methyl carbon atoms. Two reactions could bring this about: demethylation or oxidative deamination. The β-labelled adrenaline gives rise to a radioactive fraction in the urine which can be extracted with ether after acidification. No radioactive fraction of this kind is present when methyl-labelled adrenaline is given. These observations are compatible with the idea that some of the adrenaline had been metabolised by amine oxidase. It seems that with the help of this method we may learn to what extent the various pathways contribute to the breakdown of adrenaline and noradrenaline administered in small amounts.

Gaddum and Kwiatkowski first interpreted the potentiating action of ephedrine as due to the inhibitory action on amine oxidase (73). Schapira (150) and Thompson and Tickner (173) have since found amine oxidase in the rabbit's ear, and the possibility that amine oxidase might be active in removing adrenaline or sympathin in peripheral tissues has to be considered.

Many amines which have an affinity for amine oxidase are more or less perfect sympathicomimetics. This is true for inhibitors and substrates, not only of the phenylethylamine or phenyl*iso*propylamine series, but also for aliphatic amines like amylamine, even for long-chain diamines such as hexadecamethylene diamine $H_2N(CH_2)_{16}NH_2$. Yet, it seems by no means certain that all amines with affinity for amine oxidase are sympathicomimetic; dodecamethylene diamine is oxidized even more rapidly by amine oxidase than the C_{16} diamine, but no sympathicomimetic properties have been reported for this compound.

In order to account for the connexion between affinity for amine oxidase and sympathicomimetic action one can either assume that the sympathicomimetic effect is produced by an increase in sympathin concentration in the neighbourhood of the nerve ending; this could be brought about by inhibition of an enzyme which destroys sympathin; or the parallelism can be explained by a similarity in structure between the tissue receptors which are sensitive to sympathin, and the part of the enzyme which specifically combines with the substrate.

It is not known if an enzyme is responsible for the inactivation of sympathin at adrenergic nerve endings. The strict temporal and spatial limitation of the stimulus which is required in cholinergic nerves may not be necessary in the adrenergic system. However, the wide distribution of amine oxidase in structures innervated by adrenergic nerves makes it worth while to discuss some of the facts which point to a connexion between sympathicomimetic effect and affinity for amine oxidase.

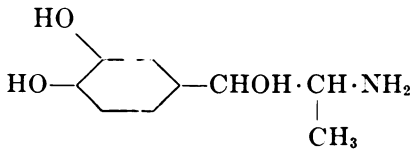
We may distinguish "direct" sympathicomimetic effects from "indirect" ones. The former are effects exerted upon the tissue receptors for sympathin; the latter are effects exerted by preserving sympathin in the neighbourhood of the excitable structure.

An "indirect" sympathicomimetic effect can only occur when there is sympathin present in the neighbourhood of the nerve endings. There are indications that the amount of sympathin present after denervation is greatly reduced (63, 64); this indicates that less or no sympathin is released. The tissue receptors for sympathin remain excitable after denervation. It should therefore be possible to use the postganglionically-denervated organ in order to distinguish the direct action of a substance on the tissue receptors from its indirect action. If no sympathin is released, the indirect action would no longer be present.

Some time ago, Burn and Tainter (44) showed that after the section of the postganglionic sympathetic nerves the cat's pupil became very insensitive to both tyramine and ephedrine, whereas the sensitivity to adrenaline was increased. Later it was shown that tyramine and ephedrine had very little action in the sympathetically-denervated fore limb of the cat. In the perfused hind limb of the dog the two amines gradually lost much of their vasoconstrictor action;

this could be restored when adrenaline was added to the perfusion fluid (40). In another set of experiments, Bülbring and Burn (38) found that the denervated nictitating membrane of the cat, although more sensitive to small doses of tyramine, responded with less powerful contractions than the normal nictitating membrane as the dose of tyramine was increased; with high doses the contractions of the normal nictitating membrane were much stronger than those of the denervated membrane.

Observations like these show that the denervated organ may prove useful in the study of "direct" actions of sympathicomimetic substances on the receptor protein; they do not allow us to conclude that the indirect actions are brought about by an inhibition of amine oxidase. Other inactivating mechanisms may be present. von Euler (63) reports that solutions of adrenaline and noradrenaline acquire a red colour when incubated with nervous tissue or spleen. He discusses the possibility that a phenolase type of enzyme may occur in these tissues. The inhibition of phenolase might explain observations on corbasil.



Corbasil

This amine has a vasoconstrictor action on the blood vessels of the rabbit's ear, which resembles that of adrenaline. Ephedrine sensitizes the vessels of the rabbit's ear to corbasil (106), yet the amine is not oxidised by amine oxidase. Corbasil is a substrate of phenolases, and an inhibition of phenolase by ephedrine would explain this observation. Nothing is known to suggest that ephedrine has such an inhibitory action.

The normally innervated nictitating membrane of the cat is more sensitive to adrenaline than to noradrenaline. After denervation the membrane becomes equally sensitive to both substances, and this is brought about by a larger increase in sensitivity to noradrenaline (39). Burn interprets this phenomenon as caused by a reduction in amine oxidase in the neighbourhood of the nerve endings: the membrane is normally less sensitive to injected noradrenaline, because the amine oxidase acts more rapidly on noradrenaline, the sympathetic mediator. The evidence which supports this interpretation has recently been reviewed by Burn (41). Sympathetic denervation is followed by a reduction in the amine oxidase of the membrane; after 10–12 days the total enzymic activity had fallen to about 66 per cent of that of the normal membrane (43). A correlation was found between reduction of enzyme and sensitization to noradrenaline: the sensitization was most marked in the membranes in which the enzymic activity had been most strongly reduced.

An observation by West (184) may be quoted in support of this interpretation: methylene blue renders the nictitating membrane more sensitive to noradrenaline, so that it resembles the denervated organ in its sensitivity.

Amine oxidase as an enzyme designed to destroy noradrenaline at sympathetic nerve endings would account for these results. But some facts, already discussed, are not easily reconciled with this picture. One is the very high concentration of enzyme in organs like the liver and kidneys. We must assume that in these tissues the enzyme has some other function. Another difficulty is in the specificity pattern of the enzyme: substances like hydroxytyramine or tyramine are more rapidly oxidised by amine oxidase than is noradrenaline and they probably have a higher affinity than amines with a hydroxyl group in β-position. We have no indication that in tissues with adrenergic innervation the enzyme may be specifically adapted to sympathin, although such a possibility has been discussed by Bernheim and Bernheim (8).

Cocaine has long been known to sensitise the tissues to the actions of adrenaline (71), and this has been explained by the inhibiting action of cocaine on amine oxidase (130). In the frog's heart, the sensitisation by cocaine to sympathetic stimulation was shown not to be due to an increased release of sympathin (46). It has been reported that in the rabbit's ear cocaine increases the amount of sympathin which comes out in the perfusate, and that it increases the amount of adrenaline recovered from the perfusate (119, 175).

In the normally innervated nictitating membrane cocaine has a stronger sensitising effect on the response to noradrenaline than on that to adrenaline; as a result, the nictitating membrane after cocaine resembles the denervated membrane (41; see also 104). All these actions of cocaine could be interpreted by assuming that cocaine acts by inhibiting the mechanism which inactivates sympathin.

However, cocaine has also some actions which are not so easily interpreted on the basis of enzyme inhibition. It antagonises the action of tyramine (171). If the action of tyramine is mainly an indirect one, it is not surprising that cocaine has no potentiating effect on tyramine, but it is surprising that the action of tyramine should be abolished.

Many other substances have affinity for amine oxidase and have also either sympathicomimetic or sympathicolytic actions. Jang (107) finds that the action of ergotoxine upon the response to adrenaline is similar in principle to that of cocaine. Tickner has recently discussed the possibility that certain antihistaminics owe their sympathicomimetic side actions to their inhibitory effects on amine oxidase (174). Schuler has examined the inhibition of amine oxidase by a number of imidazoline derivatives; he finds that the compounds with marked sympathicomimetic or sympathicolytic action are the stronger inhibitors of the enzyme. More observations could be quoted which might be considered to support the idea that inhibition of amine oxidase may play a part in the pharmacological action of sympathicomimetic compounds. However, the alternative explanation, based on a similarity of receptor protein and enzyme protein, has not satisfactorily been excluded.

In conclusion, we can say that the organ deprived of sympathin seems suited for the study of the direct sympathicomimetic actions of a compounds. Secondly, an indirect action of a compound may be due to an inhibition of a mechanism

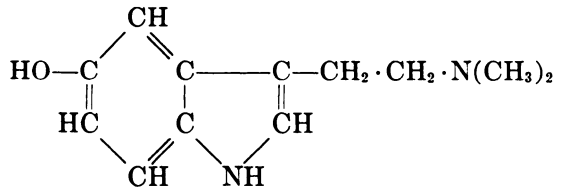
other than amine oxidase; the phenolase type of enzyme and its inhibitors have not been systematically studied. Thirdly, a compound may have an affinity for more than one structure in the tissues; Bacq (3) considers it as certain that ephedrine acts directly on the receptor protein and Gaddum (72) has pointed out that this is not necessarily in disagreement with the assumption that some actions of ephedrine are indirect.

Derivatives of tryptamine

It has long been known that tryptamine is a substrate of amine oxidase (34, 135). The amine had never been found in the body, but an enzyme that decarboxylates the amino-acid tryptophan is said to occur in mammalian liver (180). Observations on tryptamine metabolism in animals reviewed in the historical introduction show that the amine is metabolised by oxidative deamination. The possibility that tryptamine may be converted to indoleacetic acid in plants has already been discussed.

Derivatives of tryptamine have been isolated from animal tissues and the possibility arises that amine oxidase is active in the metabolism of these substances.

Bufotenine. Bufotenine was found by Phisalix and Bertrand in the secretion of the skin glands of the toad. Jensen and Chen (108) suggested that it was an indole derivative, and this was proved by Wieland, Konz and Mittasch (185) who showed that bufotenine was N-dimethyl-5-hydroxytryptamine:



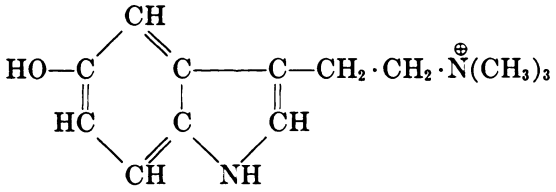
Bufotenine

The pharmacological properties of the pure substance have been studied by Raymond-Hamet (139, 140, 141) and by Erspamer (53). Bufotenine has pressor action which is enhanced by cocaine and antagonized by yohimbine. The pressor action is believed to be nicotine-like rather than adrenaline-like, because very little vasoconstrictor action can be demonstrated on the isolated perfused paw. The isolated rat's uterus, especially after treatment with oestrogens, is sensitive to bufotenine: contraction was observed in concentrations down to 2×10^{-8}.

Bufotenine is destroyed when it is incubated in an atmosphere of oxygen with extracts from tissues rich in amine oxidase: guinea-pig's liver, kidneys and small intestine. The inactivation is not affected by cyanide, but it is inhibited by β-phenyl*iso*propylamine and by methylene blue. Preparations of histaminase do not act on bufotenine.

These observations strongly suggest that the inactivation of bufotenine is brought about by an amine oxidase type of enzyme. The data given do not allow a comparison with the rate of oxidation of other amines. Bufotenine is a tertiary amine, and these are usually oxidised rather slowly by the guinea-pig enzyme.

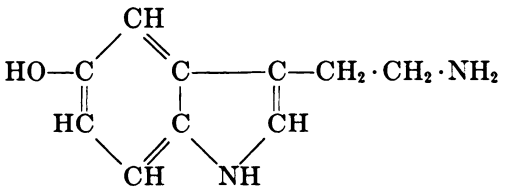
According to Erspamer (53), the quaternary base, bufotenidine:



Bufotenidine

is not inactivated by amine oxidase. This is in agreement with what is known of the properties of the enzyme.

5-Hydroxytryptamine. It now appears established that the primary amine corresponding to bufotenine is also present in extracts from animal tissues.



5-hydroxytryptamine
(Enteramine, Serotonin)

The vasoconstrictor principle present in cattle serum was first obtained as a crystalline substance in 1948 (137). It was then shown that this material contained creatinine sulphate together with an indole derivative probably identical with 5-hydroxytryptamine (138).

It has been shown that preparations from sheep lung which oxidised both tyramine and tryptamine also inactivated the serum vasoconstrictor substance (36).

The rate of oxidation of a sample of serotonin creatinine sulphate by preparations containing amine oxidase has recently been measured and compared with the rate of oxidation of tyramine and tryptamine (16). All three amines were rapidly oxidised; there was not very much difference in the rate of oxidation. Two preparations were used: a homogenate of guinea-pig's liver and one of the posterior salivary glands from *Octopus vulgaris*. It is known that tryptamine gives rise to a dark pigment when it is incubated with amine oxidase (135), and it was found that serotonin also forms a pigment.

In 1937, Vialli and Erspamer described a substance which they named enteramine. The substance was first characterized by a number of colour reactions, and has recently been identified as 5-hydroxytryptamine (59). Enteramine has been found in vertebrates and in invertebrates. In vertebrates, it occurs in the intestine, where it is believed to be the secretion product of the enterochromaffin cells, and also in the skin of some amphibians (61). In invertebrates, the posterior salivary glands of *Octopus* are a particularly rich source (55); it also occurs in the hypobranchial organ of *Murex* (57) and in the gastrointestinal tract of *Ascidia, e.g., Ciona intestinalis* (54).

Like bufotenine, enteramine has a marked excitatory effect upon the rat's uterus in oestrus. It also has an antidiuretic action on the hydrated rat; this is due to a constrictor action on the afferent glomerular vessels.

There are indications that enteramine from natural sources is a substrate of amine oxidase (51, 52). The "enteraminase" described by Erspamer is present in many animal tissues; it is inhibited by β-phenyl*iso*propylamine, by methylene blue and octyl alcohol, but not by cyanide. Enteramine from the posterior salivary glands of *Octopus vulgaris* and of *Eledone moschata* was also destroyed by preparations from guinea-pig's liver (55).

A sample of synthetic 5-hydroxytryptamine picrate has recently been tested as a substrate of amine oxidase. As expected, the amine was found to be rapidly oxidised by preparations of guinea-pig's liver and of the posterior salivary glands of *Octopus vulgaris* (16). In other words, the synthetic amine was oxidised like serotonin.

These observations raise the question whether the inactivation of 5-hydroxytryptamine is a normal function of amine oxidase. It would be necessary to show that 5-hydroxytryptamine is a normal tissue constituent. Erspamer considers enteramine as the secretion product of the enterochromaffine system and he stresses the hormone character of the amine. If this is so, then the removal of the amine may be a normal function of amine oxidase in the tissues. The strong action of enteramine on the afferent glomerular vessels might explain why the kidneys are so rich in amine oxidase.

The occurrence of 5-hydroxytryptamine raises the question: how is it formed? The very weak action on tryptophan of the decarboxylase described by Werle and Menniken (180) might be explained if the natural substrate of this enzyme in the body were not tryptophan but 5-hydroxytryptophan.

SUMMARY

Amine oxidase is an enzyme system that catalyses the oxidative deamination of many amines.

The enzyme is widely distributed in the animal kingdom and possibly in plants. In vertebrates, it occurs particularly in glandular tissue, in plain muscle and in the nervous system.

The enzyme is bound to the cytoplasmatic particles, mitochondria and microsomes.

Although amine oxidase reacts with oxygen *in vitro*, the possibility is discussed that in the living tissue the enzyme does not directly react with molecular oxygen.

The amines react with the enzyme when they are in the ionised state; amino compounds which are feebly ionised at the pH of the tissues are not oxidised.

The study of the substrate specificity of the enzyme for aliphatic amines shows that the reaction between amino group and the enzyme takes place only if there is also some hydrophobic group present in the substrate molecule. This is believed to be necessary for the attachment and orientation of the substrate molecule on the enzyme surface.

A review of the metabolism of amines not normally present in the body shows

that many of these are metabolised as would be expected if the amine oxidase reaction occurs in the body. Many amines which are not substrates of the enzyme are not destroyed in the body. The substrate specificity for amine oxidase is therefore an important factor in determining the duration of action of many amino compounds.

Possible substrates of the enzyme in the living organism are discussed. Of the sympathicomimetic amines which occur in mammals, hydroxytyramine may be an important substrate; the question of the oxidation of noradrenaline and adrenaline is connected with the problem of sympathicomimetic action of substances with affinity for amine oxidase (*e.g.*, tyramine, β-phenyl*iso*propylamine and its derivatives, cocaine). Reasons for and against the role of the enzyme as an agent that inactivates sympathin in the neighbourhood of adrenergic nerve endings are discussed.

Amine oxidase acts rapidly on 5-hydroxytryptamine; if it can be shown that this amine occurs regularly in the body, its inactivation may well be an important function of amine oxidase.

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