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OBSERVATIONS ON AMINE OXIDASE IN CEPHALOPODS

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Amine oxidase occurs in all species of vertebrates which have been examined, but the enzyme has also been found in some echinoderms and molluscs (Blaschko, Richter & Schlossmann, 1937). The distribution of the enzyme in the tissues of *Sepia officinalis* has been studied in greater detail, and it was found that amine oxidase occurred in many organs; in *Sepia* liver the enzymic activity was higher than in any animal tissue previously examined (Blaschko, 1941).

The function of amine oxidase in the metabolism of mammals is not yet fully understood. The enzyme acts upon many sympathicomimetic amines, and from what is known about the substrate specificity of the enzyme, there can be little doubt that in the living organism it is the catalyst responsible for the inactivation of many amines of pharmacological interest which belong to this group. What is still under discussion is the part which the enzyme takes in the *in vivo* inactivation of adrenaline and noradrenaline, the two substances of this group which are most active in vertebrates, and which are known to be substrates of the enzyme *in vitro* (Blaschko *et al.* 1937).

In 1913, Henze reported the occurrence of tyramine in the posterior salivary glands of *Octopus macropus*. Tyramine is one of the amines most rapidly oxidized by amine oxidase. The amine has since been found in the posterior salivary glands of *O. vulgaris* (Erspamer & Boretti, 1951) where, in addition to tyramine, another monophenolic sympathicomimetic amine has been described; this compound is called octopamine (Erspamer, 1948); it has been identified with *p*-hydroxyphenylethanolamine by Erspamer & Boretti (1951).

In the autumns of 1950 and 1951, specimens of *O. vulgaris* became readily available in Plymouth, and we were interested to find out if amine oxidase occurred in this species which is known to contain tyramine. The present paper contains a study of the distribution of the enzyme in some of the organs

of *O. vulgaris* and of some of its properties; a few observations on the *Sepia* enzyme are also reported, as well as observations on some other species of molluscs.

METHODS

The specimens of *Octopus vulgaris* and *Sepia officinalis* were dissected in Plymouth. The organs were immediately frozen in solid CO_2 and taken to Oxford in a thermos flask; there they were stored in the refrigerator at $-10°$ C. Under these conditions the amine oxidase from cephalopods, like the mammalian enzyme, retains its activity for many months. With the exception of one experiment on the ink sac of *Sepia*, however, the experiments here described were carried out soon after the organs had been collected.

Enzymic activity was usually studied in freshly prepared homogenates in 0·067 M-sodium phosphate buffer of pH 7·4. In addition to the homogenates, each manometer flask contained 0·2 ml. of 0·1 M-semicarbazide, 0·4 ml. of either water or of 0·05 M-amine hydrochloride (either tyramine or *iso*amylamine), and enough of the phosphate buffer to bring the total volume to 2·0 ml. The equivalent of fresh weight of tissue per flask varied from 100 mg for liver to 466 mg for brain. The gas phase was O_2. The experiments were carried out at a temperature of either 25 or of 37° C. The enzymic activities were expressed in terms of q_{O_2}, i.e. the μl. O_2 consumed by 100 mg of wet tissue in 1 hr.

RESULTS

Amine oxidase in tissues of Octopus vulgaris

In most experiments the enzymic activity was tested at 25° as well as at 37° C, as it was thought that the preparations from invertebrate tissues might be impaired at the higher temperature; the results of our experiments make it unlikely that this precaution was necessary.

Amine oxidase activity was found to be widely distributed in the organs of *O. vulgaris*. A summary of our results with different tissues of *O. vulgaris*, compared with *Sepia* and guinea-pig liver, is given in Fig. 1. It gives, in terms of q_{O_2}, the enzymic activities with both tyramine and *iso*amylamine as substrates.

In the liver the activity was as high as in the *Sepia* liver and about twice that of the guinea-pig liver. Of the other organs examined, the posterior salivary glands come next, in order of decreasing activity, then the anterior salivary glands, and in brain the activity was low. This is the same sequence as that found previously in *Sepia*; in *Sepia* the enzymic activity of the brain was not tested, and in *Octopus* we did not include the 'kidneys' which were highly active in *Sepia* (Blaschko, 1941).

In the earlier work on *Sepia* liver it was found that the relative rate of oxidation of tyramine was high compared with that of other amines. This was considered to be a characteristic difference between the mammalian and the molluscan enzymes, but our present experiments show that this generalization is not valid. The relatively high rate of oxidation of tyramine by the enzyme from *Sepia* liver has been confirmed, but it is also evident that the *Octopus* enzyme shows a much less marked preference for tyramine.

Fig. 1 shows that the experimental temperature affects the ratio:

$$\frac{\text{oxidation rate of tyramine}}{\text{oxidation rate of } \textit{iso}\text{amylamine}}$$

in *Sepia* and *Octopus* liver but not in guinea-pig liver.

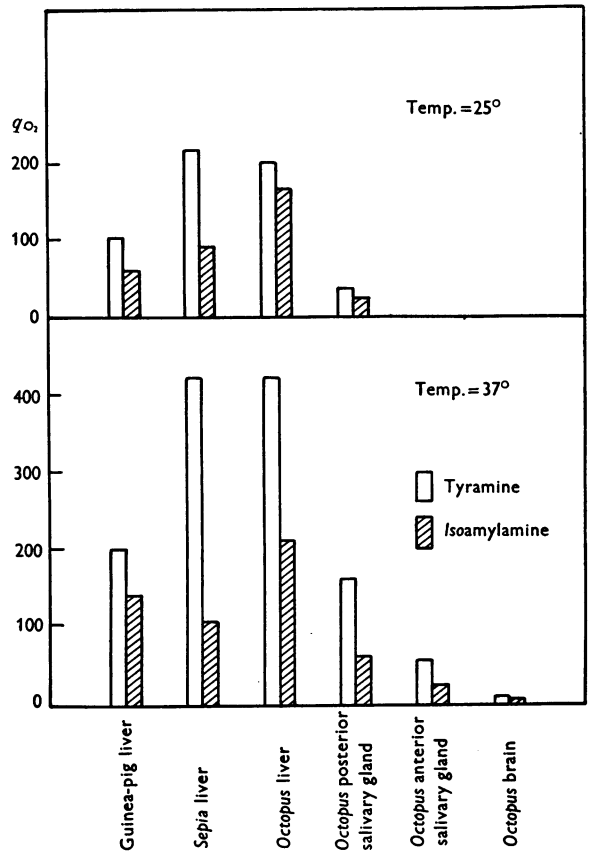

Fig. 1. Amine oxidase activity in tissue homogenates. The enzymic activities are expressed in terms of q_{O_2}, i.e. as μl. O_2 consumed by 100 mg of fresh weight of tissue per hr.

In *Sepia* and *Octopus* liver the ratio is higher at 37° than at 25° C. This is explained by the relatively small increase in the rate of oxidation of *iso*-amylamine with increasing temperature. In the guinea-pig's liver the increase in the oxidation rates of both amines is similar and the ratio remains approximately the same.

Properties of the Octopus *liver enzyme*

Like the mammalian enzyme the amine oxidase of *Octopus* liver is fully active after prolonged dialysis against distilled water. This is probably due to the insolubility of the enzyme.

In mammals amine oxidase activity is known to be associated with the particulate constituents of the liver cells; a recent study of the distribution of amine oxidase in the rat liver cell (Hawkins, 1952) makes it likely that about two-thirds of the total enzymic activity is localized in the mitochondria and one-third in the smaller particles of the microsome fraction. It appears that in the *Octopus* liver the enzyme is also present in the particles. A homogenate of *Octopus* liver was centrifuged until a clear supernatant fluid was obtained. The supernatant was without amine oxidase activity; all the activity of the homogenate was present in the sediment.

It has been shown that acetone-dried powders of mammalian liver can be obtained which have amine oxidase activity (Blaschko & Hawkins, 1950). We have tried to prepare similar powders from *Octopus* liver, but without success. The powders were without amine oxidase activity. It was thought possible that in *Octopus* the acetone treatment might split off a prosthetic group, and in order to test this possibility we collected the acetone filtrates during the preparation of the powders; the filtrates were then evaporated under reduced pressure and the dried residues taken up in a few ml. of water. The addition of this fluid to a suspension of the powders did not restore enzymic activity. The instability of the cephalopod enzyme under acetone treatment thus remains unexplained. In contrast to amine oxidase the D-amino-acid oxidase of *Octopus* liver is retained in acetone-dried powders (Blaschko & Hawkins, 1952).

Amine oxidase in the ink sac of Sepia

In 1941, it was found that tyramine was oxidized by a preparation of the wall of the ink sac, and this oxidation was attributed to amine oxidase, as no evidence for the oxidation of tyramine by the powerful phenol oxidase of the ink sac was obtained. Recently, however, Dr F. Ghiretti of Naples has told us that according to his experiments tyramine is a substrate of the phenol oxidase present in the organ. It was therefore thought desirable to repeat the earlier experiments under more stringent conditions. In these experiments, therefore, potassium cyanide was added. Phenol oxidase is sensitive to cyanide but amine oxidase is cyanide-insensitive. In addition both tyramine and *iso*amylamine were used as substrate, because the latter is not attacked by phenolases. Two experiments under slightly differing conditions were carried out; in both there was an immediate oxygen consumption in excess of the blank with both amines, more with tyramine, less with *iso*amylamine. One of these experiments is described in detail.

Ink sac tissue of a weight of 2·2 g was cut finely with scissors and ground in a cooled mortar; 4·4 ml. of 0·067 M-sodium phosphate buffer were added. Further grinding resulted in a fine suspension which was used in the manometric experiments. In the experiment described, the main compartment of each flask contained 0·8 ml. of this suspension, 0·4 ml. of the phosphate

buffer, 0·2 ml. of 0·1 M-semicarbazide and 0·2 ml. of 0·1 M-KCN (neutralized); the side bulb contained:

in flask 1 0·4 ml. water;
in flask 2 0·4 ml. 0·05 M-tyramine hydrochloride;
in flask 3 0·4 ml. 0·05 M-*iso*amylamine hydrochloride.

The potash tube contained 0·3 ml. of the cyanide-potash mixture recommended by Umbreit (1945). Oxygen consumption in μl. in the three flasks was:

	Flask 1 (μl.)	Flask 2 (μl.)	Flask 3 (μl.)
After 10 min	− 7	−17·5	−10·5
After 20 min	−10·5	−33·5	−18

This shows that with both amines the oxygen consumption was greater than in the enzyme blank (flask 1). With tyramine the oxygen consumption began at once and showed no marked increase with time; with *iso*amylamine the oxygen consumption was less. These observations, together with the fact that the reaction occurred in 10^{-2} M-HCN, make it likely that the oxygen uptake was due to amine oxidase. This experiment was carried out on tissue which had been stored in the refrigerator for 9 months after the dissection of the animals, so it is possible that the enzymic activity was impaired and does not represent the activity of fresh tissue.

Observations on Mytilus *and* Helix

In a few experiments on *Mytilus edulis* and a species of *Helix* collected in Oxford no evidence for the presence of amine oxidase was obtained. In some of the experiments with *Helix*, oxygen began to be taken up slowly after a delay of about half an hour when the homogenates were incubated with tyramine. The slow onset of oxygen uptake, combined with the fact that no oxygen was consumed in the presence of *iso*amylamine, suggests that this oxidation was due to a phenolase type of enzyme; this is supported by the observation that the contents of the flasks incubated with tyramine showed a slight darkening when compared with the enzyme blank.

The results with *Helix* are in agreement with earlier observations of Blaschko *et al.* (1937).

DISCUSSION

The observations reported show that the tissues of *Octopus*, like those of *Sepia*, contain a very active enzyme of the amine oxidase type. Not only tyramine, but also *iso*amylamine were oxidized. That the oxidation of tyramine was brought about mainly by amine oxidase and not by a phenolase is supported by the observation that the onset of oxygen consumption was immediate and that no increase in the rate of oxidation was observed in the short periods of incubation used. The enzymic activities found in *Sepia* liver and guinea-pig's liver are much higher than those previously described (Blaschko, 1941); this is explained by the use of homogenates in the present work; formerly, the organs were ground in a mortar with sand and then centrifuged. We now know

that a considerable amount of active material is lost on the centrifuge when intact cells and large cell fragments are spun down (Hawkins, 1952).

Octopus vulgaris contains tyramine, and we have now shown that this species is very rich in amine oxidase. Not only tyramine, but also octopamine (Erspamer, 1948) are substrates of the enzyme. It is not known if these amines occur in the liver which is so rich in amine oxidase, but they occur in the posterior salivary glands and these also give enzymically highly active homogenates.

Our findings raise again the question as to the biological significance of amine oxidase. If it is true, as Sereni (1930) believed, that the amines found in the salivary gland function as hormones in the animal, it seems very possible that the oxidase is present in the different tissues of *O. vulgaris* as the mechanism, or one of the mechanisms, by which the amines are destroyed. Another possibility is that the enzyme in the living animal works in the reverse, i.e. that it is active in the biosynthesis of these amines. Of these two alternatives the first seems the more likely one, as from the observations on amine metabolism in mammals it seems very likely that also in the living animal the enzyme works in the direction of deamination.

SUMMARY

1. Amine oxidase has been found in tissue homogenates of *Octopus vulgaris*. Very high enzymic activity is present in the liver. Enzymic activity of the posterior salivary gland is just under one-half of that of the liver; there is less activity in the anterior salivary glands and much less in the brain.

2. *Iso*amylamine is more actively oxidized by the *Octopus* enzyme than by the *Sepia* enzyme.

3. No amine oxidase was found in *Helix* and *Mytilus*.

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