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AMINE OXIDASE IN *SEPIA OFFICINALIS*

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LITTLE is known of the amine metabolism in cephalopods. The experiments described in this paper are concerned with the occurrence in *Sepia* of amine oxidase, the system responsible for the inactivation of tyramine and other mono-amines. A few observations on the metabolism of diamines and on decarboxylases are also included.

The enzyme amine oxidase has chiefly been studied in vertebrates, but it also occurs in certain groups of invertebrates such as molluscs (*Mytilus*, *Patella*) and echinoderms [Blaschko, Richter & Schlossmann, 1937 *b*; Blaschko, unpublished]. Its function in the mammalian body is not known, as no clear evidence for the occurrence of its substrates such as tyramine and *iso*amylamine, exists. When adrenaline was found to be a substrate of the enzyme *in vitro* [Blaschko *et al.* 1937 *a*], it was suggested that it might be the chief substrate of the enzyme *in vivo* [see also Gaddum & Kwiatkowski, 1938], but it seems doubtful whether this view can be maintained. In the invertebrate it is obvious that the enzyme is not associated with the metabolism of adrenaline, since the oxidase does not occur in annelids (e.g. *Lumbricus*), which contain chromaffine tissue, and adrenaline has never been detected in molluscs and echinoderms.

The present study was undertaken because tyramine is said to occur in cephalopods, and to play a role as a hormone in their regulative processes. Tyramine was isolated from the posterior salivary glands in *Octopus* [Henze, 1913], and appears to be one of the poisonous substances present in the saliva of cephalopods [Bottazzi & Valentini, 1924]. According to Sereni [1930], these glands may be considered as endocrine organs which not only excrete tyramine but also release it into the circulation.

Methods and material

The experiments were carried out on tissue extracts of *Sepia officinalis*, which is caught in great numbers at Arcachon. The animals were dissected shortly after being caught. The organs were ground with sand and $M/15$ sodium phosphate buffer solution (*p*H 7·4) was added. Usually, equal amounts of organ and of phosphate solutions were taken, but in a few cases less tissue was used. The suspensions were centrifuged for 5 min. and the supernatant fluid was used for the experiment. The chief digestive gland, the liver, is easily dissected. The so-called "kidneys" represent a loose glandular structure, mixed up with blood vessels; no attempt at separation of these tissues was made. The muscle was taken from the mantle. The wall of the ink sac was used after removing the contents and repeated washing; but even then the tissue did not lose its black colour, and the extracts looked intensely dark. The ink gland forms part of the wall of the sac; it was used together with the rest of the wall.

The amine oxidase activity was determined in the presence of semicarbazide, in order to fix the aldehyde formed in the reaction, and to prevent uptake of oxygen from its further oxidation. The activity was measured by determining the extra amount of oxygen used after the addition of substrate. The activity is expressed in c.mm. O_2/g. fresh tissue/hr. Usually tyramine was the substrate; sometimes *iso*amylamine and (−)-*p*-sympatol ($C_6H_4OH.CHOH.CH_2NHCH_3$) were also tested. The oxygen uptake of the extracts was determined manometrically, as previously described [Bhagvat, Blaschko & Richter, 1939]. The flasks—conical flasks of the Barcroft-Warburg type—were prepared as follows:

Main flask: 1·6 c.c. enzyme preparation + 0·2 c.c. $M/2$ semicarbazide hydrochloride.

Side bulb: 0·2 c.c. $M/4$ amine hydrochloride.

Inner tube: 0·3 c.c. N KOH.

In a few experiments in which extracts were tested for the presence of histaminase (or diamine oxidase) histamine and putrescine were used as substrates; but no semicarbazide was added since it inhibits the enzyme responsible for the oxidation of these substrates.

Results

In Table I the oxygen uptake of various tissues is given with tyramine as substrate. Apart from muscle, all the tissues examined showed a considerable oxygen uptake. The highest activity was observed with extracts of liver, which exceeded even that of the mammalian liver. In

TABLE I. Amine oxidase activity of various organs of *Sepia* with $M/40$ tyramine, measured as c.mm. O_2 taken up/g. fresh tissue/hr.

Animal	Organ	Oxygen uptake	Temperature
Sepia	Liver	657	19·0
,,	"Kidneys"	246	21·0
,,	Posterior salivary gland	246	19·5
,,	Ink sac	173	18·5
,,	Muscle	0	19·5
Guinea-pig	Liver	486	37·0

the last row of the table the corresponding figure for the amine oxidase activity of the guinea-pig's liver, as determined at 37°, is given for comparison.

The conclusion that the oxygen uptake in these experiments really resulted from the action of amine oxidase is supported by the fact that the oxidation of tyramine by extracts of *Sepia* liver was abolished in the presence of *sec.*-octyl alcohol, known to be a strong inhibitor of amine oxidase. On the other hand, neither cyanide nor semicarbazide inhibited the oxidation, which again is in agreement with the observations on the mammalian enzyme.

*iso*Amylamine and (−)-*p*-sympatol were used as substrates for the extracts from liver, and were found to be oxidized. These experiments revealed a characteristic difference in the activities of preparations from *Sepia* and from mammals. Whereas the mammalian enzyme showed no marked preference for tyramine as compared with *iso*amylamine, in *Sepia* the rates were greatly in favour of tyramine. A similar observation has been reported for the amine oxidase of the gastropod *Patella* [Blaschko *et al.* 1937 *b*]. In Table II the relative rates of oxidation of

TABLE II. Comparison of the relative rates of oxidation of tyramine, *iso*amylamine, and (−)-*p*-sympatol

		Relative rate of oxidation of		
Animal	Organ	Tyramine	*iso*Amylamine	(−)-*p*-Sympatol
Guinea-pig	Liver	100	105	48
Sepia	Liver	100	24	12
Patella	Viscera	100	20	13

*iso*amylamine, (−)-*p*-sympatol, and tyramine are given, that of the latter being taken arbitrarily as 100. In the table are included figures for *Patella*, similarly calculated, taken from the paper of Blaschko *et al.* The relative oxidation rates for the three substrates in the extracts from the two molluscs agree with each other, but they differ from those found in the guinea-pig. The enzymes from both *Sepia* and *Patella* oxidized

tyramine much more rapidly than the other two amines, whereas the preparation from the mammalian liver oxidized *iso*amylamine and tyramine at about the same rate. (–)-*p*-Sympatol was less rapidly oxidized in all extracts, but again the figures for *Patella* and *Sepia* agree with each other, and differ from those for the guinea-pig.

Oxidation of diamines. No evidence for the presence of histaminase (diamine oxidase) in *Sepia* has been obtained. The experiments were carried out with preparations of liver, histamine and putrescine being used as substrates, and with extracts of the "kidneys" and putrescine as substrate. There was no extra uptake of oxygen on the addition of the amines.

Experiments on decarboxylases. The claim that tyramine occurs in cephalopods led us to inquire whether there exists in *Sepia* an enzymatic mechanism for decarboxylating *l*(–)-tyrosine. As the enzyme which decarboxylates the closely related amino acid *l*(–)-dopa [Holtz, Heise & Lüdtke, 1938] is very specific for this substrate, and does not decarboxylate *l*(–)-tyrosine [Blaschko, 1939], we have also searched for the presence of this enzyme in *Sepia*. Extracts from liver, "kidneys", and the posterior salivary glands were used. They were found incapable of decarboxylation, no CO_2 being formed when *l*(–)-tyrosine or *l*(–)-dopa were added.

Discussion

Extracts of the tissues of *Sepia* show an uptake of oxygen when incubated with tyramine and other amines. There can be little doubt that the oxygen uptake observed is due to amine oxidase. All the characteristic properties of the enzyme, such as the inhibition by octyl alcohol, the lack of sensitivity to both cyanide and semicarbazide are present in the preparations from *Sepia* as well as in those from the guinea-pig. The difference in the relative rates of oxidation of different substrates may be explained as follows. The affinity of an enzyme to its substrate is determined not by the prosthetic group of the enzyme molecule, but by the specific protein to which this group is attached. In the case of amine oxidase, the two preparations from the more closely related animals show the same relative oxidation rates, but they differ from the vertebrate preparation. The protein constituents of the enzymes from the two molluscs are probably more closely related to each other than they are to the protein in the vertebrate enzyme. That such differences in affinity are due to the proteins, is in agreement with conceptions of substrate specificity [see Warburg & Christian, 1938].

Sepia is known to contain a tyrosinase [Przibram, 1902], but the oxidation of tyramine cannot be attributed to this enzyme, as the tyrosinase from *Sepia* oxidizes tyrosine, but not tyramine [Neuberg, 1908]. This was confirmed in manometric experiments. Moreover, the action of inhibitors on the oxidation—sensitivity to octyl alcohol, insensitivity to cyanide and semicarbazide—seems to rule out the possibility that a phenolase contributed to the oxidation of tyramine.

It seems doubtful whether Henze's [1913] observations can really be accepted as satisfactory evidence for the occurrence of tyramine in cephalopods. However, in connexion with his findings and Sereni's [1930] experiments, it is interesting that *Sepia* contains a powerful mechanism for the inactivation of tyramine, and the marked preference for tyramine makes it likely that this amine is the main substrate for the enzyme in the living animal. But the evidence available must be considered as insufficient. Furthermore, our experiments with decarboxylases have given no evidence for the presence of a tyrosine decarboxylase. It would be desirable to repeat these experiments on octopods, the animals used by Henze, but they were not at my disposal.

The inability of the extracts from *Sepia* to oxidize histamine and putrescine, in spite of their strong amine oxidase activity, demonstrates again the different role of the mono-amines and di-amines in metabolism. It may be mentioned that histaminase has up to the present not been found in any invertebrate tissue.

Summary

1. Extracts from various tissues of *Sepia officinalis* show that amine oxidase is present in the liver, the posterior salivary glands, the "kidneys", and the wall of the ink sac, but not in muscle. The highest activity was found in the liver, the activity being greater than in mammalian liver.

2. Unlike the mammalian enzyme, the amine oxidase of *Sepia* shows a marked preference for tyramine over other substrates. This may perhaps be taken as evidence in favour of the belief that tyramine plays some role as a hormone in cephalopods.

3. No evidence for the presence in *Sepia* of histaminase, or of a decarboxylase for either $l(-)$-tyrosine or $l(-)$-dopa was obtained.

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