ENZYMIC OXIDATION OF AMINES IN DECAPODS

BY H. BLASCHKO AND JEAN M. HIMMS

Department of Pharmacology, University of Oxford and the Marine Biological Laboratory, Plymouth

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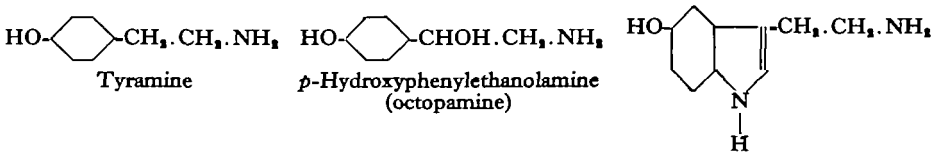
Some time ago it was shown that a high concentration of the enzyme amine oxidase was present in the liver of *Eusepia officinalis* (Blaschko, 1941); it has since been found that this is also true for *Octopus vulgaris* (Blaschko & Hawkins, 1952*a*). In both species the enzyme occurs in other tissues as well as in the liver.

The question arises: what is the functional significance of the enzyme in cephalopods? Tyramine, one of the chief substrates of amine oxidase, was first isolated from the posterior salivary glands of *Octopus macropus* (Henze, 1913), and it has since been shown that other substrates of amine oxidase are present in octopods. The existing knowledge has recently been reviewed by Erspamer (1952), to whom we owe much new work on this subject. He has found tyramine in the posterior salivary glands not only of *O. macropus*, but also of *O. vulgaris*; he did not find it in the gland of *Eledone moschata*. An amine closely related to tyramine was present in the glands of all three octopods; this he called octopamine and he identified it as (−)-*p*-hydroxyphenylethanolamine. The third amine is 5-hydroxytryptamine, a substance which also occurs in mammals; it has been found in the glands of *E. moschata* and *Octopus vulgaris*. Erspamer points out that the amines occur only in extracts of the posterior salivary glands, and not in other tissue; he has never found any of these amines in *Sepia* or *Loligo*.



Tyramine — *p*-Hydroxyphenylethanolamine (octopamine) — 5-Hydroxytryptamine

In *Sepia* amine oxidase had previously been found in the liver, the posterior salivary glands, the 'kidneys' and the wall of the ink sac (Blaschko, 1941). We have now made a more thorough survey of the occurrence of the enzyme in *Sepia*, and studies of amine oxidase activity in the organs of *Loligo forbesii* are also reported.

MATERIAL AND METHODS

Specimens of *Sepia* were sent from Plymouth and dissected in Oxford on the same day. The specimens were no longer alive but in reasonably good condition. *Loligo* was dissected fresh in Plymouth, and the tissues were brought to Oxford in solid CO_2. Enzyme preparations were made by grinding the tissues in an ice-cold

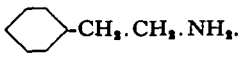
mortar and suspending them in 0·067 M-sodium phosphate buffer of pH 7·4. The extracts were used fresh, with the exception of the preparation of optic lobe from *Loligo* which served in the experiment of Fig. 1; this extract was dialysed overnight in order to reduce the 'enzyme blank'. In the experiments summarized on Table 1, semicarbazide was added in a final concentration of 0·01 M. Semicarbazide does not inhibit amine oxidase, but it prevents the further oxidation of the aldehyde formed in the enzymic reaction. The manometric determination of oxygen consumption was carried out in conical manometer flasks. Flasks of two different sizes were used, according to the amount of tissue available. The larger flasks contained in the main compartment: 1·4 ml. extract and 0·2 ml. 0·1 M-semicarbazide, in the side bulb: 0·4 ml. water or 0·05 M-substrate hydrochloride, and in the inner tube: 0·3 ml. N-KOH. In the smaller flasks the corresponding amounts were: 0·49 ml. extract, 0·07 ml. semicarbazide, 0·14 ml. water or substrate and 0·1 ml. KOH. Experiments were carried out in an atmosphere of oxygen at a temperature of either 25 or 37·5° C. Enzymic activity is expressed in terms of q_{O_2}, i.e. μl. O_2 consumed by 100 mg. of fresh weight of tissue per hour. These figures were calculated from the initial readings, usually those during the first 15 min.

We are grateful to Dr W. Feldberg, F.R.S., for the gift of 5-hydroxytryptamine (enteramine) picrate, and to Prof. A. Th. Knoppers for that of (±)-*p*-hydroxyphenylethanolamine hydrochloride.

In the nomenclature of the organs we have followed Tompsett (1939).

EXPERIMENTS

In the earlier work on *Sepia* and *Octopus*, tyramine and *iso*amylamine had been used as the chief substrates in testing for amine oxidase activity. Both these amines have some disadvantages: *iso*amylamine is oxidized rather slowly by amine oxidase, and tyramine might also be oxidized by a phenolase. In the present experiments on *Sepia* we have therefore used β-phenylethylamine,



This compound is not oxidized by phenolases and the rate of its oxidation by amine oxidase is reasonably fast, although slightly less than that of tyramine. The observations on the tissues of *Loligo* were carried out earlier, and phenylethylamine was not used in every experiment.

Table 1 gives a list of all experiments on different tissues of *Sepia*, using 0·01 M-β-phenylethylamine hydrochloride as substrate. The incubation was carried out in the presence of 0·01 M-semicarbazide.

We have confirmed that the liver gives highly active preparations. High activity was also found in other parts of the digestive system, e.g. the pancreas, the stomach and the caecum. As in the earlier work, the extracts of the posterior salivary glands were active, but the amount of material from the anterior salivary glands did not suffice for a measurement of enzymic activity. Activity was also high in the renal appendages and in the pericardial gland. In order to obtain more material for the study of amine oxidase in the vascular system the venous and arterial hearts were

pooled; these also gave highly active extracts. Amine oxidase activity was also found in the ink sac and in the gills. Enzymic activity was also found in the gonads and various parts of the male and female genital systems.

Amine oxidase activity was found in all parts of the nervous system. The stellate, gastric, and cerebral ganglia and the optic lobes were dissected out separately, but the remaining head ganglia were pooled. These included the superior buccal, the brachial, pedal and visceral ganglia. In the eye the optic fibre layer was the only tissue with amine oxidase activity. The white body also gave active extracts.

Table 1. *Amine oxidase in organs of* Eusepia officinalis

(Substrate: 0·01 M-β-phenylethylamine hydrochloride; $t = 37{\cdot}5°$ C.; O_2. Enzymic activity is expressed in terms of q_{O_2}, i.e. in μl. O_2/100 mg. fresh tissue/hr. Semicarbazide concentration: 0·01 M.

Organ	Wt. of tissue per flask in mg.	q_{O_2}	Organ	Wt. of tissue per flask in mg.	q_{O_2}
Posterior salivary gland	245	38	Female gonads	245	19
Liver*	49	280	Nidamental gland	700	17
Liver*	140	282	Male gonads	245	26
Pancreas	245	108	Seminal vesicle	200	42
Stomach	125	103	Cerebral ganglion	245	38
Caecum	245	75	Optic lobe	245	48
Hearts	700	72	Other head ganglia	245	61
Renal appendages	245	109	Stellate ganglion	245	30
Pericardial gland	245	120	Gastric ganglion	185	35
Ink sac	125	52	Eye: lens	245	0
Skin	125	0	Eye: optic fibre layer	245	8
Muscle	125	0	Eye: outer coat	245	0
Gills	700	34	White body	245	17

* The same liver preparation was used in two experiments.

Earlier observations on the absence of enzymic activity from the muscle of the mantle were confirmed, and no activity was found in the skin.

In the experiments on *Loligo*, phenylethylamine has not been used in all experiments, and Table 2 gives data on the oxidation of the three amines used as substrates.

Table 2. *Amine oxidase in organs of* Loligo forbesii

(Substrate concentrations: 0·01 M; $t = 37{\cdot}5°$ C.; O_2.)

q_{O_2} with	Tyramine	*iso*amylamine	β-phenyl-ethylamine
Organ			
Anterior salivary gland	36	—	—
Posterior salivary gland	15	1	—
Liver (i)	435	—	—
(ii)	206	—	—
(iii)	77	9	30
Gills	13	0	8
Optic lobe (i)	39	7	—
(ii)	98	16	59

The liver, the anterior and posterior salivary glands, the optic lobes and the gills were examined. All these tissues gave enzymically active extracts. In addition, we have carried out one experiment at 25° C. with an extract of ink sac of *Loligo*. This experiment closely followed the arrangement used previously in the study of the ink sac enzyme in *Sepia* (Blaschko & Hawkins, 1952*a*) except that tyramine and phenylethylamine were used as substrates. The oxygen uptake during the first 10 min. with tyramine was 66 μl. O_2/100 mg. of tissue/hr.; with phenylethylamine the corresponding figure was 22 μl. O_2. This shows that both amines were oxidized.

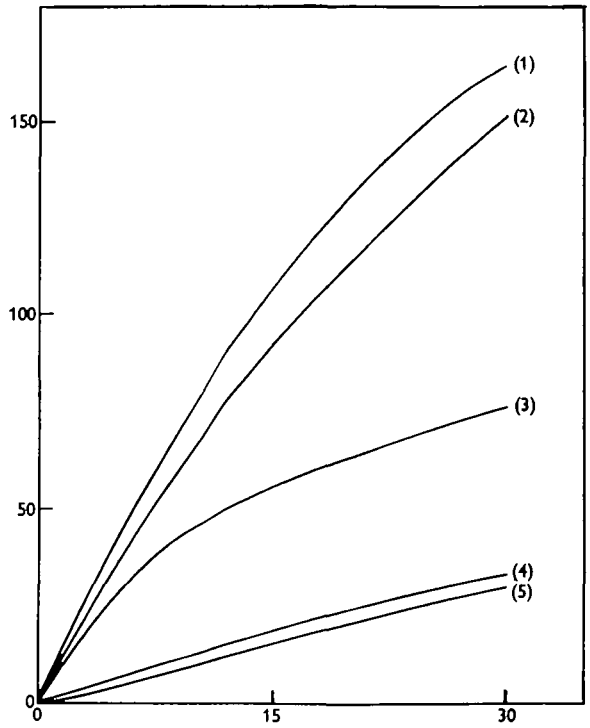


Fig. 1. Rate of oxidation of amines by a dialysed homogenate of optic lobe of *Loligo forbesii*. Initial substrate concentrations: 0·01 M. Semicarbazide concentration: 0·01 M. Gas phase: O_2; $t = 37·5°$ C. Abscissa: time in min. Ordinate: μl. O_2 consumed. (1) tyramine; (2) tryptamine; (3) β-phenylethylamine; (4) *iso*amylamine; (5) (±)-*p*-hydroxyphenylethanolamine.

The substrate specificity of the cephalopod enzyme resembles that of the mammalian enzyme. Fig. 1 is taken from an experiment in which an extract of optic lobe of *Loligo* was incubated with various substrates. In this experiment the oxygen uptake with tryptamine was almost as fast as with tyramine, and at the end of the experiment a brown colour had developed in the flask that contained tryptamine. This is similar to observations of Pugh & Quastel (1937) on the mammalian enzyme. In another experiment, with the same extract, 5-hydroxytryptamine was oxidized almost as rapidly as tryptamine.

The ability of the cephalopod enzyme to oxidize aliphatic amines is similar to that of the mammalian enzyme. Fig. 2 shows the relative rates of oxidation in the homologous series of aliphatic monoamines $CH_3(CH_2)_nNH_2$ by a preparation of *Sepia* liver. A similar experiment was carried out with a preparation of *Loligo* liver. It can be seen that the lower members of this series were not oxidized. Oxygen uptake was observed with *n*-butylamine and the rate was maximal with *n*-heptylamine; there was little oxygen uptake with *n*-decylamine; *n*-dodecylamine and

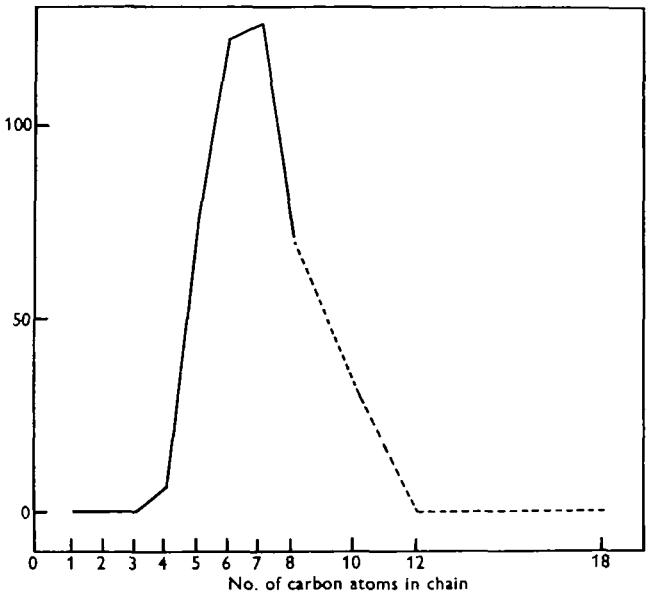


Fig. 2. Homogenate of *Sepia* liver; rate of oxidation in relation to chain length in the homologous series of aliphatic monoamines: $CH_3(CH_2)_nNH_2$. Abscissa: number of carbon atoms $(n+1)$. Ordinate: μl. of O_2 consumed by 100 mg. of tissue per hr. Gas phase: O_2; $t = 37{\cdot}5°$ C. Initial substrate concentrations: 0·01 M.

n-octadecylamine were not oxidized. The *Loligo* liver extract gave similar results with *n*-amylamine, *n*-hexylamine, *n*-heptylamine and *n*-octylamine, but it was inactive with *n*-butylamine and *n*-decylamine.

It is known that the mammalian enzyme does not act on histamine and the short-chain members of the series of aliphatic diamines $NH_2(CH_2)_nNH_2$, e.g. putrescine $(n = 4)$ and cadaverine $(n = 5)$, but that it does oxidize long-chain diamines (Blaschko & Duthie, 1945; Blaschko & Hawkins, 1950). The same is true for the cephalopod enzyme: *Sepia* liver did not act on histamine, putrescine and cadaverine, but tridecamethylene diamine was oxidized.

In the earlier work it was found that the enzymes of both *Sepia* and *Octopus* were inactivated when the tissues were acetone-dried: this was in contrast to the mammalian enzyme which is fully active in acetone-dried preparations. We have therefore tested the effect of acetone drying on the enzyme from *Loligo* liver and have

found that some oxidase activity is retained in acetone-dried powders. In one experiment, one *Loligo* liver, fresh weight 19·3 g., was used for the preparation of a 1 in 10 homogenate. This gave a q_{O_2} with tyramine of 170 (first 15 min.). Eight livers, fresh weight 117·75 g., were converted into 23·75 g. of acetone-dried powder. The q_{O_2} with tyramine was 165. This result indicates that about 20% of the enzymic activity was retained.

Like *Sepia* and *Octopus*, *Loligo* liver also has *D*-amino-acid oxidase activity. Since the pattern of substrate specificity of the *Sepia* and *Octopus* enzyme has already been described (Blaschko & Hawkins, 1952*b*), we have not made a thorough study of this enzyme, but we have found that the ability to oxidize *D*-methionine is retained in acetone-dried preparations of *Loligo* liver.

DISCUSSION

The experiments reported show that amine oxidase is widely distributed in the tissues of the two decapod species studied.

Very high activity was found in the alimentary canal (stomach and caecum) and its glandular appendages (salivary glands, liver and pancreas). This is analogous to what is found in mammals. It is possible that the enzyme is active in preventing the uptake of amines harmful to the animal from the lumen of the gastro-intestinal tract, but this would not explain its presence in the salivary glands. The digestive tract in *Loligo* has recently been studied by Bidder (1950); she discusses the question whether in cephalopods food particles reach liver and pancreas from the intestinal canal before absorption; with most other observers she considers this unlikely. This is probably also true for octopods (Falloise, 1906). Since amine oxidase is an intracellular enzyme, it is therefore unlikely that it has a digestive function.

As far as the salivary glands are concerned no digestive enzymes have been found in them (Romijn, 1935). The posterior salivary glands are characterized by their toxic secretion; this has been chiefly studied in octopods, but the experiments of Romijn show that the gland of *Sepia* contains a substance with a similar toxic action. It seems likely that this action of the salivary secretion is not due to tyramine and similar amines or to 5-hydroxytryptamine, and it is interesting in this connexion that these amines have never been found in the posterior salivary glands of decapods. However, the glands have amine oxidase activity and the possibility cannot be ruled out that the glands—or some other parts of the animals—contain some amines that have not yet been identified. The fact that in all cephalopods hitherto examined, octopods as well as decapods, liver and pancreas are so particularly rich in enzyme is interesting, because in these organs amines have never been found. This might be understood if we assume that in octopods the posterior salivary glands are the organs where the amines are synthesized, but that the organs particularly rich in amine oxidase are those where they exert their biological effect and where they are destroyed. In these organs one would expect to find little amine.

A special functional significance of amines is also made likely by the finding that organs not connected with the digestive tract are rich in enzyme. These are tissues

like the pericardial gland, the renal appendages, the hearts and the gills, which are connected with the vascular system.

Enzymic activity was also present in all parts of the nervous system examined. In *Loligo*, we have only examined the optic lobe, but in *Sepia* all the other large ganglia were also studied. The finding of amine oxidase in nervous tissue establishes another parallelism with the mammalian enzyme. Here again we know little about the biological significance of the enzyme.

The substrate specificity of the *Sepia* and *Loligo* enzymes is very similar to that of the mammalian enzyme. Straight-chain aliphatic monoamines were oxidized, and the optimal substrate configuration was that of *n*-amylamine and *n*-hexylamine, and the straight-chain diamine with 13 carbon atoms is also oxidized.

In conclusion, it can be said that, unless we consider amine oxidase in decapods as an interesting atavism without any biological significance, the wide distribution of the enzyme in the tissues of *Sepia* and *Loligo* suggests some function for amines in the metabolism of these species which is not yet recognized.

SUMMARY

1. The enzyme amine oxidase has been found in many organs of *Sepia officinalis* and of *Loligo forbesii*.

2. The enzyme oxidizes not only tyramine and related compounds, e.g. *p*-hydroxyphenylethanolamine ('octopamine') and β-phenylethylamine, but also aliphatic monoamines and long-chain diamines as well as tryptamine and 5-hydroxytryptamine.

3. The liver of *Loligo* contains *D*-amino-acid oxidase.

We wish to record our thanks to the Director and Staff of the Marine Biological Laboratory at Plymouth whose help has made these observations possible.

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