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A COMPARATIVE STUDY OF HYDROXYINDOLE OXIDASES

BY

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A comparative study has been carried out of the oxidation of 5-hydroxytryptamine and related compounds by the oxidase present in the gill plates of *Mytilus edulis* and of caeruloplasmin, the copper containing oxidase of mammalian plasma. Both preparations oxidized indole derivatives carrying a hydroxyl group in the 4-, 5-, 6-, or 7- position. The oxidation of bufotenine was compared with that of its 4- and 6-hydroxy analogues; the 4-hydroxy analogue is psilocine, a naturally occurring hallucinogenic compound. Bufotenine and the 6-hydroxy analogue were oxidized by both preparations with the formation of brown pigments; psilocine was more rapidly oxidized with the appearance of a blue colour. 4-Hydroxytryptamine and 7-hydroxytryptamine were also oxidized, the former with the formation of a blue compound. The N-1-methyl derivatives of both bufotenine and psilocine were also oxidized. The *Mytilus* preparation acted also on 4-, 5-, and 7-hydroxytryptophan and on 5-hydroxyindole, none of which was oxidized by caeruloplasmin. The *Mytilus* enzyme also oxidized 5-hydroxyindoleacetic acid. Paraphenylenediamine, a very good substrate of caeruloplasmin, was much more slowly oxidized by the gill plate enzyme. The evidence suggests that the two enzymes catalyse the same reactions, but that the substrate specificity of the mammalian oxidase is somewhat more restricted. Both enzymes are " hydroxyindole oxidases," not specific for 5-hydroxyindoles alone. Inhibitors of the *Mytilus* oxidase included inhibitors of copper enzymes but not edetate or carbon monoxide. The action of pig serum on 5-hydroxytryptamine was due to caeruloplasmin and not to amine oxidase.

The oxidation of 5-hydroxytryptamine by amine oxidase and the subsequent formation of 5-hydroxyindoleacetic acid are reactions that play an important part in the inactivation of 5-hydroxytryptamine in mammals (see Udenfriend, 1958; Blaschko, 1958). However, it is also known that other pathways of breakdown of 5-hydroxytryptamine must exist. Erspamer (1955) has shown that in man and dog less than one-half of the amine administered is excreted as 5-hydroxyindoleacetic acid in the urine, and he has found that in a number of herbivorous species the urine contains only insignificant amounts of 5-hydroxyindoleacetic acid. It is not known to what extent this deficit is accounted for by known metabolites of 5-hydroxytryptamine, for example, the O-glucuronate and the O-sulphate, the N-acetyl derivative or 5-hydroxyindoleaceturic acid (Curzon, 1957; Chadwick and Wilkinson, 1958; McIsaac and Page, 1959).

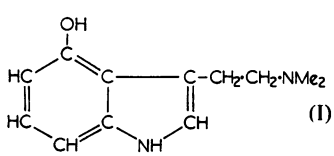
Little is known of the biological inactivation of 5-hydroxytryptamine in invertebrates. In molluscs, amine oxidase has been found in many species (Blaschko, Richter, and Schlossmann, 1937; Blaschko, 1941). Erspamer (1948) reported that enteramine was destroyed by the amine oxidase from Octopods, and after the identity of enteramine with 5-hydroxytryptamine had been established it was shown that the synthetic amine was oxidized by the molluscan enzyme (Blaschko, 1952a). In *Mytilus edulis* the digestive gland as well as the anterior retractor muscle of the byssus contain an amine oxidase that acts on 5-hydroxytryptamine (Blaschko and Hope, 1957).

In the gill plates of *Mytilus* there occurs another enzyme which oxidizes 5-hydroxytryptamine (Blaschko and Milton, 1959, 1960). This differs from amine oxidase. It acts also on other 5-hydroxyindoles, for example, 5-hydroxytryptophan and bufotenine. Unlike amine oxidase, this enzyme is cyanide-sensitive and it may be a kind of phenol oxidase, acting upon the indole ring with the formation of a quinone-imine, a reaction discussed some time ago (Blaschko and Philpot,

1953). Support for this formulation is seen in two observations: first, a brown pigment is formed in the enzymic reaction and, second, the gill plate preparation, even when somewhat purified, is still able to oxidize catechol derivatives, for example, dopa or dopamine.

It seemed probable that catalysts analogous to the gill plate enzyme occur in mammals. Caeruloplasmin, the copper containing protein of mammalian plasma, has been reported to act on 5-hydroxytryptamine (Porter, Titus, Sanders and Smith, 1957; Martin, Eriksen and Benditt, 1958; Nakajima and Thuillier, 1958; Siva Sankar, 1959; Zarafonetis and Kalas, 1960; Curzon and Vallet, 1960). Since caeruloplasmin is also able to oxidize catechol compounds and, as a copper enzyme, is cyanide-sensitive, it was decided to compare the action of the oxidase from *Mytilus* gill plates with that of pig plasma caeruloplasmin.

In order to obtain more information on the reaction catalysed by the two enzymes, the study has been extended to indole derivatives other than those carrying a hydroxyl group in position 5. The compounds tested were all hydroxyindoles. They include the 4-hydroxy and the 6-hydroxy analogue of bufotenine (I and II) and two *N*-methyl derivatives, that of bufotenine (III) and the corresponding 4-hydroxy compound (IV). In addition, 4-hydroxytryptamine, 7-hydroxytryptamine, 4-hydroxytryptophan, and 7-hydroxytryptophan were tested. The formula of 7-hydroxytryptamine is shown in (V).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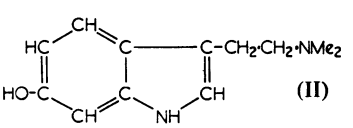
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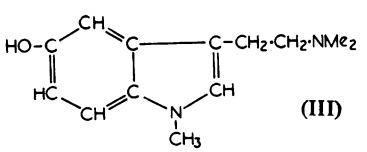
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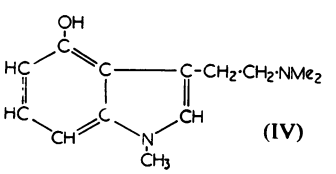
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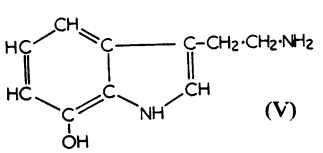

(V)

Of these compounds, substance (I) is known to be of pharmacological interest; it has been identified with psilocine, a hallucinogenic substance occurring in the fungus, *Psilocybe mexicana Heim* (Hofmann and Troxler, 1959; Hofmann, Heim, Brack, Kobel, Frey, Ott, Petrzilka and Troxler, 1959).

Methods

Enzyme Preparations.—The *Mytilus* gill plate preparations used were similar to those described by Blaschko and Milton (1960). Homogenates of the gill plates were prepared and the supernatant fluid, after centrifugation for 2 hr. at 5,000 *g*, was fractionated with ammonium sulphate, the material precipitating between 30 and 60% saturation being rich in oxidase activity. The precipitate was dissolved and dialysed first against running tap water and then in the cold room against distilled water. The dialysed preparation was freed from a slight precipitate by centrifugation for 30 min., at 100,000 *g*, and the supernatant fluid was used.

Caeruloplasmin was prepared from pig serum according to Holmberg and Laurell (1948). Material precipitating between 35 and 50% saturation with ammonium sulphate was collected and dialysed against running tap water overnight. Impurities were removed by two successive centrifugations, the first after adjusting the *p*H to 6.2 with 5% acetic acid, the second after adjustment to *p*H 5.5. The caeruloplasmin was then precipitated in 15% ethanol at 0°. The precipitate was resuspended in 0.9% sodium chloride, and after dialysis overnight the *p*H was adjusted to 6.5 and an equal volume of an ethanol-chloroform (9:1, v/v) mixture was added. The resulting precipitate was extracted with saline until the washings no longer had the blue colour characteristic of caeruloplasmin. The second ethanol-chloroform treatment used by Holmberg and Laurell (1948) was omitted. The extracts were combined and concentrated by precipitation with ammonium sulphate at 65% saturation. Dialysis against tap water overnight was followed by centrifugation for 30 min. at 5,000 *g*, in order to remove a turbid contaminant.

Measurement of Enzyme Activity. — Oxygen uptake was measured manometrically. The temperature of the manometer bath was 37.5°, with both the molluscan and mammalian preparations. The gas phase was air. The substrate concentrations were 5×10^{-3} M for the indole derivatives and 10^{-2} M for paraphenylenediamine. In the experiments with the *Mytilus* oxidase, 0.067 M sodium phosphate buffer of *p*H 7.4 was used. With caeruloplasmin, the *p*H of the buffer was 6.0; in the experiments with the indole derivatives 0.067 M sodium phosphate buffer was used, in experiments with paraphenylenediamine

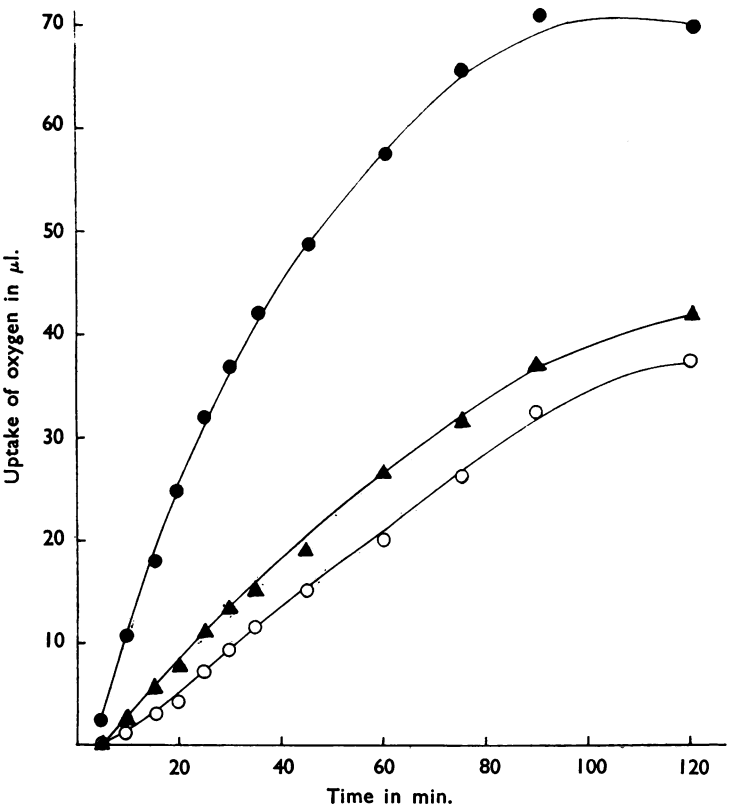


FIG. 1.—The rate of oxidation of bufotenine (O—O), psilocine (●—●), and 6-hydroxy-*N*-*N*-dimethyltryptamine (▲—▲) by *Mytilus* gill plate oxidase. The enzyme was incubated at 37.5° with 5×10^{-3} M substrate in phosphate buffer at *p*H 7.4. The gas phase was air.

0.01 M acetate buffer was used. The substrates were tipped in from the side arms of the conical manometer flasks.

Substances Used.—The 5-hydroxyindole was prepared by Dr. E. W. Gill, of this department, by catalytically debenzylating 5-benzyloxyindole (Ek and Witkop, 1954); it was purified by sublimation and recrystallization. The bufotenine was prepared by Dr. R. B. Barlow, of Edinburgh University; this was the sample used by Blaschko and Milton (1960). Compounds I, II, III and IV were given to us by Dr. A. Hofmann, of Sandoz A.-G., Basel.

Professor V. Erspamer, Parma, kindly gave us samples of 4-hydroxytryptamine creatinine sulphate, 7-hydroxytryptamine hydrochloride, 4-hydroxytryptophan, and 7-hydroxytryptophan; all these substances were prepared in the laboratories of Farmitalia, Milan.

We should like to record our gratitude for these valuable gifts.

RESULTS

Observations on the Mytilus Gill Plate Oxidase: Substrate Specificity

The observation that bufotenine is a substrate of the gill plate enzyme was readily confirmed. Bufotenine was therefore used together with the other indole derivatives. Fig. 1 shows an experiment in which the oxidation of bufotenine was compared with that of its 4-hydroxy and 6-hydroxy analogues. The first of these is psilocine. It can be seen that the rate of oxidation of the 6-hydroxy analogue was similar to that of bufotenine; psilocine was oxidized more rapidly. The experiment was terminated after 2 hr. when the uptake of oxygen with psilocine had ceased; with the 5- and 6-hydroxy analogues it still continued at a slow rate. The total uptake with psilocine was 70 μl. O_2; this

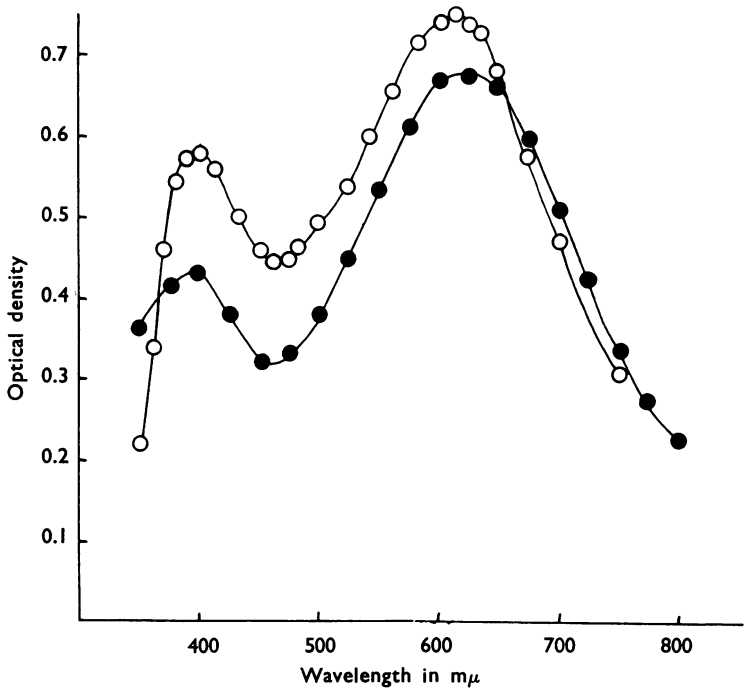


FIG. 2.—Absorption spectra of pigments formed in the oxidation of psilocine by the *Mytilus* enzyme (●—●) and by ferric sulphate (O—O).

represents 1.25 atoms of oxygen/mole of psilocine added. For 5-hydroxytryptamine, the total oxygen uptake is 0.9 atoms/mole of substrate (Blaschko and Milton, 1960).

During the oxidation of these three compounds, coloured products were formed. As observed by Blaschko and Milton (1960), the flask containing bufotenine acquired a brownish colour. With the 6-hydroxy analogue, a similar yellowish-brown colour developed. In the flask containing psilocine a deep blue colour became apparent. After centrifugation the blue pigment from psilocine remained in the supernatant fluid; any colour deposited with the sediment could be brought into solution by adding a few drops of N hydrochloric acid. The brown pigments formed from the other two isomers, on the other hand, remained entirely in the sediment and could not be similarly eluted.

The absorption spectrum of the blue pigment obtained from psilocine is shown in Fig. 2. The curve shows absorption maxima at 620 and at 400 mμ. That the blue material was an oxidation product was made likely by the observation that the colour was bleached upon adding sodium bisulphite, a reducing agent. Also, a blue compound was formed when a solution of psilocine in sodium phosphate buffer of *p*H 7.4 was oxidized by adding ferric sulphate. The time course of the development of the blue colour was followed spectrophotometrically by measuring the increase in absorption at 625 mμ. The reaction was complete within 20 min. The absorption was then measured throughout the visible range (see Fig. 2); the spectrum obtained was similar to that of the material formed when psilocine was oxidized by the gill plate enzyme, except for a shift of the absorption maximum at 620 mμ to 610 mμ and a relative increase of the peak at 400 mμ.

The *N*-methyl derivatives of both bufotenine and psilocine (III and IV) were also tested. They were also oxidized by the *Mytilus* oxidase. A comparison of the oxidation of the two *N*-methyl derivatives with that of the two parent compounds is shown in Fig. 3. Methylation of the ring nitrogen of psilocine depressed the initial rate of oxidation by 40%, but the rate of oxidation of *N*-methylbufotenine was about 30% higher than that of bufotenine. The oxidation product of *N*-methylpsilocine had a distinctly more greenish-blue hue, as compared to the deep

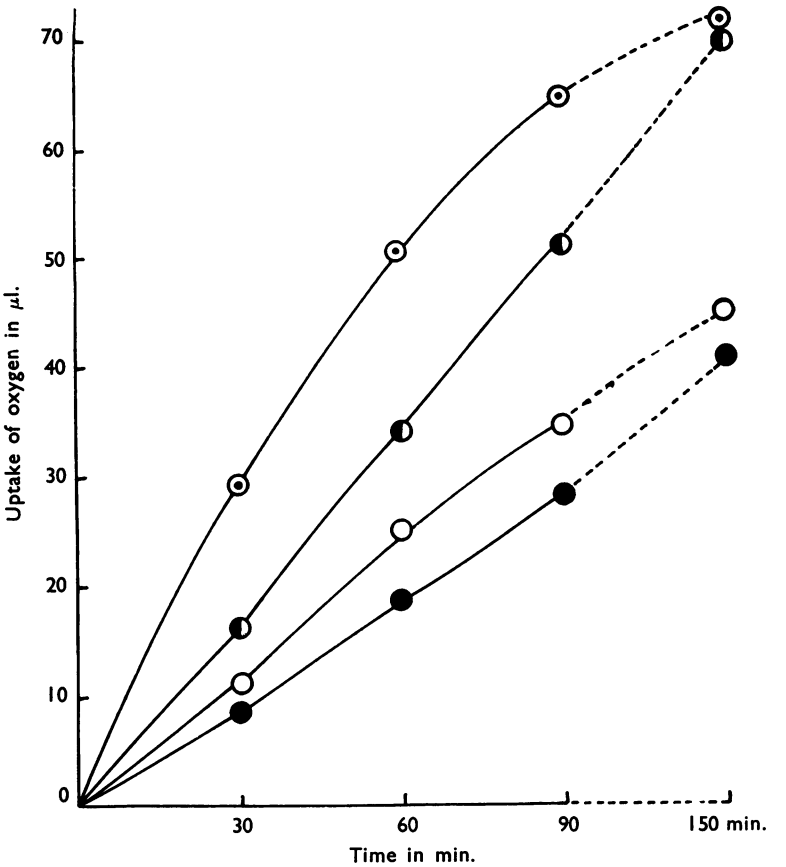


FIG. 3.—The rate of oxidation of bufotenine (●—●—●), psilocine (⊙—⊙), N-methylbufotenine (O—O), and N-methylpsilocine (◐—◐) by the *Mytilus* gill plate preparation. The enzyme was incubated at 37.5° with 5×10^{-3} M. Substrate in phosphate buffer at *p*H 7.4. The gas phase was air.

blue colour when psilocine was used as the substrate. This difference in colour is reflected in a difference in the absorption spectrum. The ratio, absorption at 400 mμ: absorption at 620 mμ, was increased from 0.685 for the oxidized psilocine to 1.14 for the oxidized N-1-methylpsilocine.

The gill plate preparation also oxidized 4- and 7-hydroxytryptamine as well as 4- and 7-hydroxytryptophan. In presence of one of the two 4-hydroxy compounds, oxygen uptake was rapid and was accompanied by appearance of a blue colour, similar to that of the oxidation product of psilocine. The oxidation of the two 7-hydroxy compounds was less rapid, being about equal to the rate of oxidation of 5-hydroxytryptamine; a brown pigment was formed when the 7-hydroxy compounds were oxidized.

In order to establish the role of the side chain in the enzymic oxidation, 5-hydroxyindole was also tested. It was oxidized, and its rate of oxidation was very similar to that of 5-hydroxytryptamine. However, in contrast to the brown pigment formed during the oxidation of 5-hydroxytryptamine, the product of oxidation of 5-hydroxyindole had a yellow-green colour.

Another hydroxyindole tested was 5-hydroxyindoleacetic acid. It was also rapidly oxidized by the gill plate enzyme, at about the same rate as 5-hydroxytryptamine. It is known that 5-hydroxyindoleacetic acid is oxidized by human caeruloplasmin (Curzon and Vallet, 1960).

Paraphenylenediamine was also oxidized by the gill plate preparation but at a rate only slightly greater than 5-hydroxytryptamine. This was in

sharp contrast to the more rapid rate of oxidation of paraphenylenediamine by caeruloplasmin.

Inhibitors of the Mytilus Oxidase

It has already been reported that the gill plate enzyme is cyanide-sensitive (Blaschko and Milton, 1960). Other substances that were tested as inhibitors of the oxidation of 5-hydroxytryptamine are listed in Table I. No inhibition was seen with

TABLE I

INHIBITORS OF THE HYDROXYINDOLE OXIDASE OF *MYTILUS* GILL PLATES

Substrate 5×10^{-3} M 5-hydroxytryptamine.

Inhibitor	Concentration	% Inhibition
Sodium diethyldithiocarbamate ..	10^{-3} M	47
Sodium azide ..	10^{-2} M	88
Salicylaldoxime ..	10^{-2} M	27
Diquinolyl ..	10^{-3} M	91
Edetate	10^{-2} M	0
Carbon monoxide	80% CO + 20% O_2	0

carbon monoxide and with 10^{-2} M edetate. It is of interest that not all copper containing enzymes are inhibited by carbon monoxide. Keilin and Mann (1939) found no inhibition of laccase; caeruloplasmin is also not inhibited (Holmberg and Laurell, 1951a).

Observations on Caeruloplasmin of Pig Plasma

Under the conditions of our experiments, 5-hydroxytryptamine was oxidized by the preparations of caeruloplasmin. Geller, Eiduson and Yuwiler (1959) did not see any oxidation of 5-hydroxytryptamine by caeruloplasmin, but observations of Porter *et al.* (1957) and other authors already quoted are in agreement with our findings. Using 10^{-2} M paraphenylenediamine as standard, the relative initial rate of oxidation of 5-hydroxytryptamine was one-sixtieth of that of paraphenylenediamine. It may be mentioned that this refers to experiments in which the 5-hydroxytryptamine was oxidized in phosphate buffer of *p*H 6.0; its rate of oxidation was less in acetate buffer of the same *p*H. The reverse is true for paraphenylenediamine: its oxidation is far more rapid in acetate than in phosphate buffer (Holmberg and Laurell, 1951b).

Oxidation of 5-hydroxytryptamine was accompanied by the appearance of a brown pigment, similar to what was seen when the amine was oxidized by the preparation from *Mytilus* gill plates.

The pig plasma preparation also oxidized bufotenine and its 6-hydroxy and 4-hydroxy analogues. Psilocine was oxidized more rapidly, bufotenine more slowly, than 5-hydroxytryptamine. The 6-hydroxy analogue was oxidized at about twice the rate of 5-hydroxytryptamine; this is different from what was observed with the *Mytilus* enzyme.

Brown pigments were formed from bufotenine and the 6-hydroxy analogue, but with psilocine a deep blue colour appeared during incubation; this is analogous to the observations with the *Mytilus* enzyme. Measurement of the absorption spectrum of the blue oxidation product showed the same two maxima seen with the *Mytilus* enzyme (see Fig. 2).

Neither 5-hydroxyindole nor 4-, 5- or 7-hydroxytryptamine was oxidized by caeruloplasmin. In order to ensure that the lack of oxidation of these amino-acids was not due to a difference in *p*H optimum, measurements with 5-hydroxytryptophan as substrate were carried out at *p*H 5.0, 6.0 and 7.4. The absence of oxidation of the amino-acids is in contrast to what is seen with the *Mytilus* enzyme. Thus, the nature of the side chain is of importance in determining whether or not an indole derivative is oxidized by caeruloplasmin.

Like the gill plate enzyme, caeruloplasmin readily attacked both 4- and 7-hydroxytryptamine. Again, a deep blue colour appeared during oxidation of the 4-hydroxy compound, and the reaction proceeded at a rate considerably more rapid than with 5-hydroxytryptamine. In contrast to the *Mytilus* preparation, caeruloplasmin oxidized 7-hydroxytryptamine at nearly 10 times the rate seen with 5-hydroxytryptamine; the pigment formed again had a dark brown colour.

Table II lists the initial rates of oxidation of all substances tested, in % of that of 5-hydroxytryptamine. The table contains the results obtained with the *Mytilus* enzyme as well as with the pig plasma preparation.

In the blood plasma of a number of mammalian species there occurs an amine oxidase that acts on benzylamine and other amines (Blaschko, Friedman, Hawes, and Nilsson, 1959); such an enzyme is found in pig plasma. Dialysed pig serum slowly oxidizes 5-hydroxytryptamine. In the work of Blaschko *et al.* (1959) it was assumed that the oxidation of 5-hydroxytryptamine was catalysed by the amine oxidase present. However, it has now been found that in the purification of

TABLE II

RELATIVE RATES OF OXIDATION OF DIFFERENT SUBSTRATES BY THE HYDROXYINDOLE OXIDASE OF *MYTILUS* GILL PLATES AND BY PIG PLASMA CAERULOPLASMIN

The initial rate of oxidation of 5-hydroxytryptamine is taken as 100.

Substrate	Hydroxyindole Oxidase from *Mytilus* Gill Plates	Pig Plasma Caeruloplasmin
4-Hydroxytryptamine	520	700
5-Hydroxytryptamine	100	100
7-Hydroxytryptamine	100	1,100
Psilocine	145	230
Bufotenine	60	44
6-Hydroxy analogue of bufotenine	55	170
N-Methyl psilocine	120	—
N-Methyl bufotenine	78	—
4-Hydroxytryptophan	200	0
5-Hydroxytryptophan	50	0
7-Hydroxytryptophan	100	0
5-Hydroxyindoleacetic acid	82	—
5-Hydroxyindole ..	100	0
Paraphenylenediamine	150	6,000

caeruloplasmin the ability to oxidize 5-hydroxytryptamine could be separated from the benzylamine oxidase action; activity with 5-hydroxytryptamine always ran parallel to the ability of the fractions to oxidize paraphenylenediamine. In other words, the experimental evidence, to be described in detail elsewhere, suggests that the oxidation of 5-hydroxytryptamine by pig serum is brought about by the caeruloplasmin present, not by the amine oxidase.

Attempts were also made to study the effect of 5-hydroxytryptamine on the oxidation of paraphenylenediamine by caeruloplasmin. However, it was noted that a rapid oxygen uptake occurred when 5-hydroxytryptamine was added to paraphenylenediamine in the absence of caeruloplasmin; at the same time a deep purple colour appeared in the flask. This observation made it impossible to find out if the enzymic oxidation of paraphenylenediamine was affected by 5-hydroxytryptamine.

DISCUSSION

The experiments described have shown many similarities between the two enzyme preparations studied, but there are also minor differences. In a preliminary note, in which the action of *Mytilus* oxidase on the derivatives of 4-hydroxy and 6-hydroxyindole was described, the name "hydroxyindole oxidase" was proposed for the gill plate enzyme (Blaschko and Levine, 1960), because oxidation was not restricted to indole derivatives carrying a hydroxyl group in the 5-position. We can now say that both the *Mytilus* enzyme and caeruloplasmin will act on derivatives of 4-, 5-, 6- and 7-hydroxyindoles. There are some differences in substrate specificity: the *Mytilus* oxidase acted also on 4-, 5- and 7-hydroxytryptophan and on 5-hydroxyindole, none of which was attacked by the pig plasma enzyme. One is left with the impression that the range of substrates of the mammalian oxidase is narrower, a difference from the *Mytilus* oxidase probably due to specific differences in the two enzyme proteins.

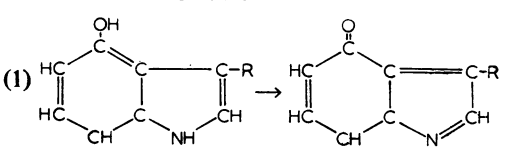
The reactions catalysed by the two enzymes lead to the formation of the same coloured products. The chemical composition of these compounds has not been studied, but the fact that a similar blue colour is obtained when psilocine is oxidized by ferric ions suggests that the reaction involves an oxidation at the phenolic hydroxyl group. The colour reaction of psilocine with Keller's reagent (Hofmann *et al.*, 1959) may be similarly explained.

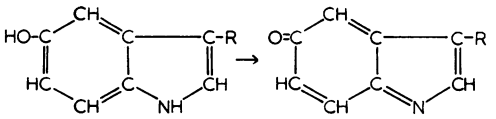
The possibility has already been discussed that the first attack of the *Mytilus* enzyme on the 5-hydroxyindoles results in the formation of a quinone-imine (Blaschko and Milton, 1960). It was therefore of interest to study the hydroxyindole derivatives which contain a phenolic hydroxyl in different positions of the indole nucleus. We have now found that 4-, 6- and 7-hydroxyindole derivatives are oxidized by the two oxidases.

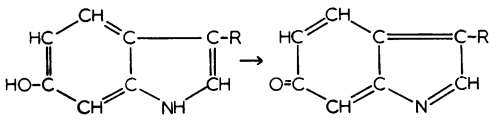
A quinone-imine could also be formed from 7-hydroxyindoles, since in these compounds the phenolic hydroxyl group is in position ortho to the indole nitrogen, but the question arises whether the oxidation of substances carrying a hydroxyl group in positions 4- or 6- can be reconciled with the formulation previously given.

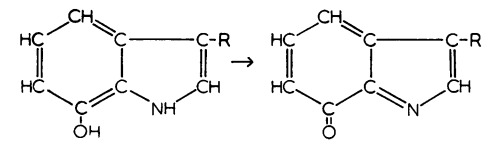
It is possible that the conjugated systems formed in the primary attack of the oxidases on 4- and 6-hydroxyindoles are different from those formed in the oxidation of the 5- and 7-hydroxyindoles. The reactions believed to occur are shown below:

(1)

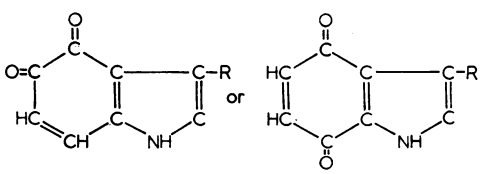


(2) 

(3) 

(4) 

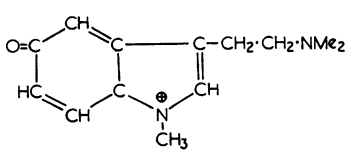
The formulation given for the 5-hydroxy compounds is that already discussed (Blaschko and Philpot, 1953; Blaschko and Milton, 1960). The oxidation of the 6-hydroxyindole derivative would also lead to a paraquinonoid structure, but the oxidation of the 4-hydroxyindoles would lead to an orthoquinonoid oxidation product. However, the reaction proposed under (1) is not the only one that has been considered. The blue oxidation product of psilocine may have a structure like this:



In this connexion it is of interest to note that Teuber and Staiger (1956) have prepared blue 4:5-diquinones by the action of potassium nitrosodisulphonate (Frémy's reagent) on hydroxyindoles.

The oxidation of 7-hydroxytryptamine (or of 7-hydroxytryptophan) would lead to the formation of an orthoquinone-imine.

The oxidation of the *N*-methyl derivatives of bufotenine and psilocine is of interest. If the formulation given under (2) is correct for bufotenine, the oxidation of the *N*-methyl derivative of bufotenine would be expected to lead to this product:



The structure of the oxidation product of *N*-methylpsilocine would probably be similar to the product of the enzymic oxidation of psilocine.

The evidence suggests that the mammalian and the molluscan enzymes catalyse the same reactions. Holmberg and Laurell (1951a) did not see any action of caeruloplasmin on monophenolic compounds. However, it has previously been pointed out that 5-hydroxytryptamine cannot be expected to behave like a typical monophenolic compound (Blaschko and Philpot, 1953; Blaschko and Milton, 1960); the presence of the indole nitrogen in position para to the phenolic hydroxyl group makes it comparable to a paradiphenol.

Caeruloplasmin is a copper-protein compound, and it is of interest that the properties of the molluscan oxidase are such that it too could be a metal-protein. This is an aspect that will have to await further purification of the *Mytilus* enzyme.

In *Psilocybe mexicana Heim* and related species of fungi, psilocine contributes only a small fraction of the total indole derivatives present (Hofmann and Troxler, 1959). Most of it is present as psilocybine, the phosphate ester of psilocine. Both psilocine and psilocybine are said to be hallucinogenic, but it seems worth while to consider the possibility that the phosphate ester exerts its central activity after being hydrolysed to psilocine and inorganic phosphate. At any rate, the biological inactivation of both substances may proceed *via* the free phenol.

This raises the question as to the physiological significance of the oxidation reactions here described. No precise answer to this question can at present be given. The catalytic action of caeruloplasmin on the catechol amines can easily be demonstrated *in vitro*, but it is still uncertain if such a reaction plays a part in their inactivation in the living animal. This has been fully discussed elsewhere (Blaschko, 1952b). The uncertainty arises from the fact that reducing agents, for example, ascorbic acid or sulphydryl compounds, immediately reduce the primary product of oxidation with regeneration of the free phenol. A similar mechanism might be operative in the oxidation of the hydroxyindoles. However, we do know that in both vertebrates and invertebrates oxidases of the type here studied are active on phenolic substances, in the formation of melanin. Enzymic processes of this kind may therefore occur in the living animal. It has been suggested that the indole derivatives might be possible pigment precursors in invertebrates (Blaschko and Milton, 1960), and similar considerations can be applied to the mammalian enzyme.

The oxidation of psilocine is so rapid that it is tempting to assume that the reaction studied is of importance in the biological inactivation of this compound. Whether the reactions here studied constitute an alternative pathway of catabolism of the 5-hydroxyindoles remains to be elucidated.

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