Chapter 6

Metabolism of indolealkylamines

By

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With 3 Figures

A. Introduction

Biological inactivation by enzymes has been of interest to the pharmacologist for a long time. The classical study by OTTO LOEWI of the mechanism of action of eserine has led to the recognition that at many, if not all, cholinergic nerve endings destruction of the mediator by enzymic hydrolysis determines the time course of the response of the effector organ. More recently, ARUNLAKSHANA, MONGAR and SCHILD (1954) have found that inhibitors of the enzyme histaminase enhance the response of the excitable tissue to histamine (see also BLASCHKO and KURZEPA 1962).

However, there are also many suggestions that the time course of the response of the effector cell may be determined by other factors. OGSTON (1955) has pointed out that *diffusion* alone may rapidly lower the effective concentration of a compound locally released, e.g., of a mediator at a nerve ending. Such a lowering of the effective concentration by diffusion may be facilitated if the compound passes readily through cell membranes and enters the effector cell (see BLASCHKO 1954, 1956, 1958); this is somewhat analogous to the mechanism proposed by BROWN and GILLESPIE (1957) for the removal of the mediator at adrenergic nerve endings. For this substance it has also been made likely that, once released, it can again be taken up and stored by the nerve ending (BURN and RAND 1958; HERTTING, AXELROD, KOPIN and WHITBY 1961). Similar mechanisms may have to be discussed for the indolealkylamines.

Whatever the fate of the pharmacologically active compound at the effector site, it must, unless it is excreted unchanged, be ultimately destroyed by enzyme action. This may happen at a place far from the site of action. It is known that the blood platelets take up 5-hydroxytryptamine. Thus this amine may be biologically inactivated at or near the site where the blood platelets are destroyed.

B. Enzymes acting on indolealkylamines

I. Amine oxidases

1. The intracellular (and carbonyl-reagent-insensitive) amine oxidase

The enzyme amine oxidase (also called "mono" amine oxidase by some authors) is a general catalyst of the biological inactivation of many amines, including the catechol amines and the indolealkylamines. It was discovered as tyramine oxidase (HARE 1928), but in 1937 a number of observers showed that the enzyme had a much wider spectrum of substrate specificities and also acted

on tryptamine (BLASCHKO, RICHTER and SCHLOSSMANN 1937a, b, c; KOHN 1937; PUGH and QUASTEL 1937a, b). This was soon confirmed (see HOLTZ and BÜCHSEL 1942).

At this time, studies of two still unidentified naturally occurring products led to a renewed interest in amine oxidase. These substances were enteramine and serotonin. ERSPAMER, in a series of papers on "*enteraminase*" showed that this catalyst of the biological inactivation of enteramine in mammalian and cephalopod tissues had properties indistinguishable from amine oxidase. Likewise, REID and his colleagues found that serotonin was inactivated by an amine-oxidase-like catalyst. These observations, which have often been reviewed (BLASCHKO 1952b, 1958; ERSPAMER 1954, 1961), were extended to synthetic material soon after this had become available (FREYBURGER, GRAHAM, RAPPORT, SEAY, GOVIER, SWOAP and VANDER BROOK 1952; BLASCHKO 1952a).

Much work has been done on amine oxidase, but there are still important gaps in our knowledge of this enzyme. These gaps, some of which will be discussed in the following pages, are of relevance for our understanding of the biological inactivation of the indolealkylamines.

a) Preparation of amine oxidase

One of the main difficulties that stand in the way of the purification of the enzyme is its extreme insolubility. Lysolecithin, detergents and ultrasonic disintegration have all been used to solubilize the oxidase, but although by these means highly dispersed preparations have been obtained, the enzyme is always again rapidly rendered insoluble. In 1957, WEISSBACH, REDFIELD and UDENFRIEND described a water-soluble preparation of amine oxidase from guinea-pig's liver and this preparation, which proved very similar to the enzyme preparations commonly described, was used for kinetic studies. In particular, it served to establish beyond doubt that the *primary product* in the *oxidation* of *tryptamine* was *indolylacetaldehyde*. However, otherwise little is known about the properties of the preparation, except that the enzymic activity could be precipitated between 25 and 40 per cent saturation with ammonium sulphate; a further purification was achieved by precipitation at pH 5.0—5.1.

Acetone-dried powders of mammalian liver have also been found useful for kinetic studies, but here the activity is present in the insoluble residue.

b) Intracellular localization of amine oxidase

Our ignorance as to the mechanism of electron transport in the amine oxidase reaction is still as complete as it was in 1952 when this subject was last reviewed (BLASCHKO 1952b). The main location of amine oxidase is in the *mitochondria*. This was first shown for *rat liver* (COTZIAS and DOLE 1951; HAWKINS 1952), but has since been confirmed for the *duodenal mucosa of the dog* (BAKER 1959), for the *bovine adrenal medulla* (BLASCHKO, HAGEN and HAGEN 1957) and for *rat brain* (ARNAIZ and DE ROBERTIS 1962).

A tenfold purification of the *mitochondrial amine oxidase* of rat brain has recently been reported (SEIDEN and WESTLEY 1962) in work where the mitochondria were isolated, lysed and sonicated; kynuramine oxidation was used for enzyme assay.

HAWKINS (1952) always found some of the amine oxidase in the microsomal fraction. In 1952, the question as to whether or not this was a truly microsomal location had to be left open, but further analysis by DE DUVE, BEAUFAY, JACQUES, RAHMAN-LI, SELLINGER, WATTIAUX and DE CONINCK (1960) has confirmed that the distribution of the rat liver amine oxidase is truly bimodal.

About 70 per cent of the total enzymic activity was found to be associated with cytochrome oxidase, a further support for the idea that this is really a mitochondrial location, but there was also a microsomal location. This microsomal component appeared to be present in a special structural element, as the activity could be shown not to be associated with any of the other microsomal enzymes.

Too little is known of the *microsomal amine oxidase* to assess its functional significance. Earlier speculations on the function of amine oxidase were based on the mitochondrial location of the enzyme (BLASCHKO 1954); mitochondria are truly intracellular constituents and this portion of the oxidase can act only on amines which have entered the cell. On the other hand, of the microsomal enzyme we cannot say where it is situated in the intact cell. We must remember that the microsomal fraction is defined only by the technique of centrifugation; it may well include fragments of the cell surface. This has been fully discussed elsewhere (HOLTER 1962; BLASCHKO 1962 b).

It may be worthwhile to emphasize that the microsomal amine oxidase here discussed is to be distinguished from the microsomal enzymes that have been described by BRODIE (1956) and by AXELROD (1954); enzymes of this group are conveniently discussed later.

However, for the mitochondrial enzyme it remains true that it will probably only act on amine present within the cytoplasmic fluid.

The extracellular amine oxidases are discussed below.

Work on the chromaffin tissue of the adrenal medulla has shown that the enzyme is present in the tissue in which the catechol amines are made and stored (LANGEMANN 1951; BLASCHKO, HAGEN and HAGEN 1957). There is little analogous information for the enterochromaffin cells, generally believed to be the main seat of 5-hydroxytryptamine formation (VIALLI 1962). The cells that form this system are scattered throughout the epithelial lining of the digestive tract, and although homogenates of the intestinal mucous membrane contain amine oxidase, the tissue from which such homogenates are prepared contains many different types of cell.

LANGEMANN and KÄGI (1956) have examined the amine oxidase content of kidney and liver metastases of human enterochromaffinomata, and homogenates from these have been found to have amine oxidase activity. Thus it looks as if the enterochromaffin cell, like the chromaffin cell, contains both amine-forming and amine-destroying catalysts, but it is known that the elementary particle that stores the amines is not the particle that contains the oxidase (see article by HAGEN and COHEN).

HAGEN (1959) has found an *amine oxidase* in *mouse mastocytoma* tissue, but whereas this enzyme acted on dopamine, 5-hydroxytryptamine was not oxidized; mouse mastocytoma tissue is rich in 5-hydroxytryptamine. Similarly, no significant oxidation of 5-hydroxytryptamine was recently found by DAY and GREEN (1962) in *mouse mastocytoma cells in tissue culture.*

Observations on the *intracellular localization* of the oxidase in *invertebrate* tissues is even more incomplete. Some time ago (BLASCHKO, HAGEN and HAGEN, unpublished) homogenates of the "liver" of *Octopus vulgaris* were prepared in 0.7 M sucrose. The bulk of the amine oxidase and of the succinic dehydrogenase were both recovered in the "large granule" fraction; upon ultracentrifugation of this fraction over a sucrose density gradient, similar to that used by BLASCHKO et al. (1957), the two enzymic activities were very similarly distributed in the gradient tube. This result suggests that in cephalopods, as in mammals, the *main location of amine oxidase is mitochondrial.*

c) Distribution of amine oxidase

Much of our knowledge on the distribution of the enzyme has been reviewed before (BLASCHKO 1952b, 1958). In all vertebrates tested, amine oxidase has been found. Apart from skeletal muscle, all organs seem to contain the enzyme, but it is not always certain if the enzyme is present in the parenchymatous tissue, or in nervous tissue present in the organ. Until recently *liver* and *kidneys* were considered the richest sources of amine oxidase, with the exception of the rat kidney, which is poor in enzyme. However, a new rich source of amine oxidase has recently been found in the *human salivary glands, parotid* and *submaxillary* (STRÖMBLAD 1959). These two organs contain the highest amine oxidase activity yet encountered in mammalian tissue. Using 10^{-2} M tyramine as substrate, the rate of oxygen uptake, expressed in μl O_2/hr/g of fresh tissue, ranged from 700 to 2850. In one experiment with human submaxillary gland extract the rate of oxidation with 5-hydroxytryptamine was 70 p.c., and that with tryptamine 66 p.c., of the rate of oxidation with tyramine. Thus, the rates of oxidation of the two indolealkylamines tested in the human salivary gland preparation were extremely high. It is of interest that in earlier observations by STRÖMBLAD (1956) on the cat's salivary glands, much lower rates of oxidation of tyramine were found; the mean values for the cat's submaxillary glands were 206 μl O_2/hr/g fresh tissue, and for the parotids the corresponding figure was 105. These are activities of the order of magnitude usually found in mammalian liver. Thus, as far as is known, the high concentration of the oxidase in the human salivary glands is unique.

When we consider the wide distribution of *amine oxidase* in many mammalian tissues and in particular the high concentration of enzyme in many glands and in plain muscle, it appears ar first sight surprising that so much attention has been given to the presence of the enzyme in *nervous tissue*, and in particular in the *central nervous system*, where the concentration of enzyme in terms of tissue weight is only moderate. However, this interest is readily understood if we remember that the substrates of amine oxidase, catechol amines and 5-hydroxytryptamine, are present, and that the level of these amines in the brain can be modified by drug action.

Studies of the distribution of amine oxidase in the mammalian central nervous system have been published by many authors. There are differences in the activity of extracts prepared from different parts of the brain. To quote only one series of observations, in which the enzymic activity was measured, using 5-hydroxytryptamine as substrate, in the dog's brain the hypothalamus has the highest enzymic activity (BOGDANSKI, WEISSBACH and UDENFRIEND 1957). However, compared with other enzymes, e.g. L-dopa decarboxylase, the differences between different parts of the brain are not very great.

A comparative study of the development of amine oxidase in the brain has recently been published by KARKI, KUNTZMAN and BRODIE (1962). They have found that the amine oxidase content of guinea-pig brain at birth is almost the same as that of the adult animal, but that in the rat's brain the amine oxidase content of the adult animal is about three times that of the newborn. However, by the use of a histochemical method, SHIMIZU and MORIKAVA (1959) report changes in the amine oxidase of the rat's brain: whereas at birth the region of the pons, the locus caeruleus, the habenula, the periventricular nuclei of the hypothalamus and the nucleus ambiguus showed moderate to strong action at birth, other parts showed activity or increase in activity only after birth.

The occurrence of *amine oxidase in invertebrates* has been known for a long time (BLASCHKO, RICHTER and SCHLOSSMANN 1937c). The phyla in which the

presence of the enzyme has been demonstrated, are the *Annelida*, the *Echinodermata*, the *Mollusca* and the *Arthropoda*.

The Mollusca are by far the best studied invertebrate phylum. Amine oxidase has not been found in all the molluscan species studied, but it should be borne in mind that the manometric technique, which has been used in this work, is relatively insensitive. With more sensitive methods of detection, the oxidase may well be found in species in which it has not hitherto been demonstrated.

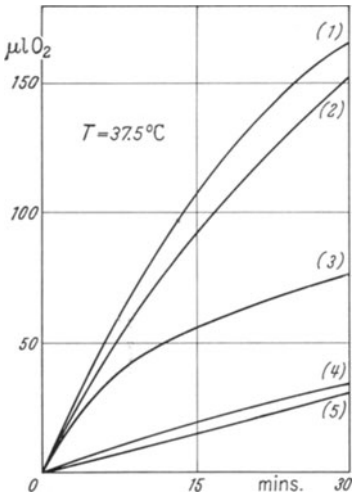


Fig. 1. Rate of oxidation of amines by a dialysed homogenate of optic lobe of *Loligo forbesii*. Initial substrate concentrations: 0.01 M. Semicarbazide concentration: 0.01 M. Gas phase: O_2; $t = 37.5°C$. Abscissa: time in min. Ordinate: µl O_2 consumed. (1) tyramine; (2) tryptamine; (3) β-phenylethylamine; (4) *iso*amylamine; (5) (±)-*p*-hydroxyphenylethanolamine

Eusepia officinalis was the first cephalopod species in which amine oxidase was found to be present in a number of tissues (BLASCHKO 1941). This was followed by a study of the enzyme in other *Cephalopods* (ERSPAMER 1948; BLASCHKO and HAWKINS 1952; BLASCHKO and HIMMS 1954). All members of this group that have been tested are rich in amine oxidase. With the exception of striated muscle, the enzyme seems to be almost ubiquitous. As in the Vertebrates the tissues of the digestive system are richest in enzyme, followed by excretory organs, and the gonads. The whole of the nervous system is moderately rich in enzyme. In Fig. 1 there is shown an experiment carried out by BLASCHKO and HIMMS (1954) on the *optic lobe* of *Loligo forbesii*; it can be seen that the enzymic oxidation of tryptamine was almost as rapid as that of tyramine; in another similar experiment 5-hydroxytryptamine was oxidized almost as rapidly as tryptamine. It may be mentioned here that both tryptamine and 5-hydroxytryptamine are also rapidly oxidized by the tissues of *Octopus vulgaris* (BLASCHKO 1952a; BLASCHKO and PHILPOT 1953). The occurrence of amine oxidase in the Octopods is of interest, because it is known that indolealkylamines occur in some of the tissues. A comparison of the amine oxidase activities of the *posterior salivary glands* of two species of *Octopus* is therefore of interest, *O. vulgaris*. where ERSPAMER has shown the occurrence of large amounts of 5-hydroxytryptamine in the gland, and *O. macropus*, where 5-hydroxytryptamine is not found in high concentration. The following were the O_2 uptakes (in µl/g fresh tissue/hr) in the two extracts (substrate concentration 1.2×10^{-2} M):

	Substrate			
	Tyramine	β-phenylethylamine	Tryptamine	5-hydroxytryptamine
O. vulgaris . . .	325	570	215	160
O. macropus . .	120	300	80	47.5

Thus, there were slight differences in the relative rates of oxidation, but all 4 substrates tested were oxidized by both species. One point of interest is the fact that in both the indolethylamine and the β-phenylethylamine series the introduction of a phenolic hydroxyl group lowered the rate of the enzymic reaction. This is different from what was found with the optic lobe extract of *L. for-*

besii. In mammals, tyramine is almost invariably oxidized more rapidly than β-phenylethylamine; the rabbit liver is the only known exception to this rule.

Other molluscan species tested include *Mytilus edulis*. Here no enzymic activity was at first found in the *chief digestive gland* (BLASCHKO and HAWKINS 1952), but after the extreme 5-hydroxytryptamine sensitivity of the anterior retractor muscle of the byssus in Mytilus had been discovered, this question was tackled again. It was now found (BLASCHKO and HOPE 1957) that both the digestive gland and the anterior retractor muscle itself contained small amounts of enzyme. It is interesting that this oxidase differs from enzymes from other sources: no oxygen uptake with *iso*amylamine could be detected. However, both tryptamine and 5-hydroxytryptamine are oxidized. In assessing these results it must be remembered that manometry is not a very sensitive method of amine oxidase assay; there is no doubt that the methods more recently introduced (see OZAKI, WEISSBACH, OZAKI, WITKOP and UDENFRIEND 1960) would readily detect the enzymic activity encountered in these two tissues. This limitation must be borne in mind when it is stated that amine oxidase has not been demonstrated in the gill plates of *Mytilus edulis* (BLASCHKO and HOPE 1957; BLASCHKO and MILTON 1960).

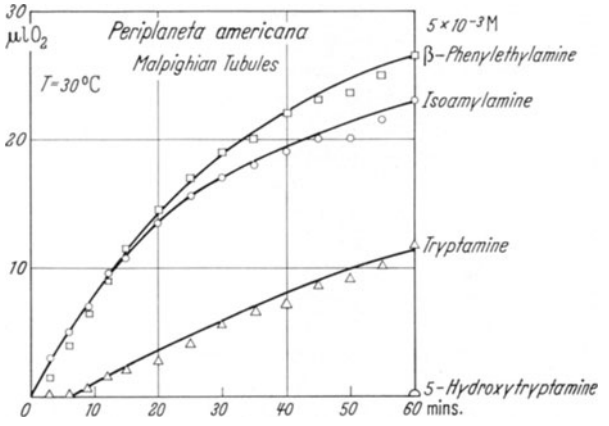


Fig. 2. Oxidation of amines by a homogenate of Malpighian tubules of *Periplaneta americana* (male specimens). Each flask contained the equivalent of about 0.11 gms of tissue

In the study of the amine oxidase of the *earthworm* gut (BLASCHKO and HIMMS 1953) no indolealkylamine was included, but in the polychaete *Annelid, Chaetopterus variopedatus*, a homogenate of the gut acted upon tryptamine, at about one-half of the rate of oxidation of β-phenylethylamine.

In the *Echinoderms*, three species of Asteroids, *Luidia ciliaris*, *Asterias rubens* and *Porania pulvillus* readily oxidized tryptamine, the only indolealkylamine tested with these homogenates. It has already been pointed out that nothing is known at present about biogenic amines in this phylum; here is a gap in our present knowledge of this group of substances.

Until recently, nothing was known of the occurence of *amine oxidase* in *Arthropods*. In 1937, no enzymic activity could be detected in the hepatopancreas and the gills of *Cancer pagurus*. At that time, no attention was given to the indolealkylamines. A more systematic attempt to settle the problem of the occurrence of amine oxidase has recently been made. The species selected was the *cockroach Periplaneta americana* (BLASCHKO, COLHOUN and FRONTALI 1961; BLASCHKO and COLHOUN, unpublished). Only one tissue contained enough enzymic activity for a satisfactory quantitative assay of enzymic activity, the *Malpighian tubules*; these are a collection of ducts surrounded by contractile tissue that open into the lumen of the gut at the boundary of mid-gut and hind-gut. They have an excretory function. Fig. 2 gives the result of an experiment with a preparation from the Malpighian tubules. It can be seen that tryptamine was readily oxidized. On the other hand, 5-hydroxytryptamine, tested in a similar experiment, was not attacked.

The Malpighian tubules, although the only tissue that gave extracts with enzymic activity high enough to be studied by manometry, is not the only organ of *P. americana* in which enzymic activity was present. All parts of the gut were enzymically active, but no activity could be detected in homogenates of the nerve cord.

The enzyme in *Periplaneta*, therefore, has two distinctive properties:

1. Absence of any significant action on 5-hydroxytryptamine.
2. Absence of significant enzymic activity in nervous tissue.

The absence of action on 5-hydroxytryptamine with the homogenates of insect tissue is of interest. In a recent study of the distribution of 5-hydroxytryptamine WELSH and MOORHEAD (1960) found a little 5-hydroxytryptamine-like material in some insects; in an extract of the nerve cords of a cockroach, *Blaberus gigantea*, small amounts of 5-hydroxytryptamine were detected. Similarly, COLHOUN (1963) has recently described the presence, in small amounts, of 5-hydroxytryptamine in glands, brain and ventral nerve cord of *P. americana*. He also noted formation of the amine from 5-hydroxytryptophan in *Periplaneta* homogenates; this interesting observation may be an expression of the dopa decarboxylase activity of the tissues (see article by HAGEN and COHEN in this volume).

WELSH and MOORHEAD (1960) did not make a particular study of amine oxidase, but it is of interest that they report that in the *green glands* of *Cancer borealis* and the *coxal glands* of *Limulus polyphemus* they obtained evidence of the presence of amine oxidase. These two glands, like the Malpighian tubules in insects, have an excretory function.

Limulus belongs to another class of the Arthropods, the *Arachnoidea*. Another set of observations on amine oxidase in spiders by WITT, BRETTSCHNEIDER and BORIS (1961) is of interest: these authors, working with homogenates of these three species: *Araneus diadematus*, *Araneus sericatus* and *Neoscona vertebrata*, have found that the enzymic oxidation of tyramine is inhibited by iproniazid. In these experiments (+)-amphetamine also disappeared when incubated with the homogenates. The mammalian enzyme is known not to act on amphetamine.

d) Histochemistry of amine oxidase

All histochemical studies of the distribution of amine oxidase in the tissues are based on the use of tryptamine or 5-hydroxytryptamine as substrates. BLASCHKO and HELLMANN (1953) exploited the observation that both these amines, when oxidized by preparations containing amine oxidase, give rise to pigments. The chemistry of the pigment formation is unknown. It was shown that this simple procedure gave a good picture of the distribution of amine oxidase in *renal tissue*. The main localization of the enzyme was in the proximal convoluted tubules; less was found in the distal convoluted tubules; no enzyme was present in the collecting tubes of the medulla. HELLMANN (1955) also demonstrated amine oxidase in the *apocrine sweat glands* of the horse by the same procedure. ARIOKA and TANIMUKAI (1957) using a similar method, demonstrated the presence of the enzyme in the *brain*, particularly in the peri-vascular spaces. However, it is not certain if these results demonstrate the presence of enzyme at the sites of pigment deposition.

Another procedure, also based upon the oxidation of tryptamine, has been adopted by KOELLE and VALK (1954); here the sections are preincubated with hydrazine, then incubated in presence of both tryptamine and 2-hydroxy-3-naphthoic acid hydrazide; the pigment is then developed with tetrazotized

O-dianisidine. The pictures obtained have confirmed the observations of BLASCHKO and HELLMANN (1953) on the distribution of the enzyme in the kidneys. The method, in the hands of the authors, was unsuitable for use in the central nervous system.

The third method for the histochemical demonstration of amine oxidase is based also on the use of either tryptamine or 5-hydroxytryptamine as substrates (GLENNER, BURTNER and BROWN 1957; GLENNER, WEISSBACH and REDFIELD 1960). It is based on a novel use of tetrazolium salts: the reduction of tetrazolium by indolyl-3-acetaldehyde (or its 5-hydroxylated derivative), to form the corresponding formazan. This method, which is easy in application, gives satisfactory pictures; in the renal tissue they confirm the earlier findings. GLENNER et al. (1957) also give interesting pictures of the localization of the oxidase in the mucosal epithelium of the guinea-pig's duodenum.

SHIMIZU, MORIKAWA and OKADA (1959) have used these methods for the study of the distribution of amine oxidase in the brain of rodents. They could not find any deposition of formazan in the neocortex, but there was a deposition of pigment in the neuropil of the central gray matter. The most conspicuous deposition of formazan was seen in the habenular nuclei and in the midline gray matter of the thalamus; otherwise the thalamic nuclei were negative. Other conspicuously positive areas were: the locus coeruleus, the dorsal nucleus of the vagus nerve, and the area postrema.

Further observations on the distribution of the enzyme in mammalian brain would clearly be desirable (see also SMITH 1963).

e) Indolealkylamines as substrates of amine oxidase

α) Tryptamine and 5-hydroxytryptamine. Both *tryptamine* and *5-hydroxytryptamine* are *oxidized by amine oxidase.* Tryptamine is usually a substrate that is fairly rapidly oxidized in the manometric experiment, but with 5-hydroxytryptamine the rates of oxygen uptake greatly vary from species to species. In mammals, the guinea-pig (liver and kidney) is a species where the two amines are oxidized at relatively rapid rates, the cat (liver and kidney) one in which they are both slowly oxidized in comparison with tyramine. There are other species, e.g. the rabbit, where tryptamine is relatively rapidly oxidized, but where the oxidation of 5-hydroxytryptamine is very slow.

In addition to species differences, differences in the relative rate of oxidation of 5-hydroxytryptamine have been described (HAGEN and WEINER 1959; HOPE and SMITH 1960). For instance, HAGEN and WEINER reported that mouse mastocytoma tissue readily acts on tryptamine and β-phenylethylamine, but has no detectable action on 5-hydroxytryptamine. HOPE and SMITH also studied the rate of enzymic oxidation of tryptamine and 5-hydroxytryptamine in extracts of various mouse tissues. Taking the rate of oxidation of 5-hydroxytryptamine as 100, the rate with *tryptamine* was:

in brain	49
in liver	147
in kidney	47
in spleen	61
in lung	35

In mouse liver, benzylamine was oxidized at approximately the same rate as *5-hydroxytryptamine*; in mouse kidney benzylamine was not oxidized.

These observations raise the question as to the homogeneity of amine oxidase. It is impossible at present to decide whether the observations just described

find their explanation in the presence of more than one enzyme in one and the same tissue extract or whether they are due to the presence of a different organ-specific oxidase in each tissue.

β) N-substituted amines. The di-N-methylated derivative of 5-hydroxytryptamine, bufotenine, is oxidized by amine oxidase at a scarcely measurable rate (BLASCHKO and PHILPOT 1953; GOVIER, HOWES and GIBBONS 1953). In the β-phenylethylamine series, there are marked species differences in the rate of oxidation of the N-substituted amines; this is confirmed for the tryptamine derivatives by the study of GOVIER et al. (1953). Using a preparation of guinea-pig's liver these authors found the following rates of oxygen uptake:

with tryptamine	165 µl
with N-methyltryptamine	160 µl
with N-*iso*propyltryptamine	0 µl
with N-dimethyltryptamine	0 µl
with 5-hydroxytryptamine	152 µl
with N-methyl-5-hydroxytryptamine	158 µl
with N-dimethyl-5-hydroxytryptamine (bufotenine)	28 µl

γ) Other indolealkylamines. ERSPAMER, FERRINI and GLÄSSER (1960) have compared the oxidation of the following compounds by guinea-pig kidney and liver homogenates; the oxygen uptake with 5-hydroxytryptamine was taken as 100:

	With liver	With kidney
4-hydroxytryptamine	49	48
6-hydroxytryptamine	49	54
7-hydroxytryptamine	71	74
5-methoxytryptamine	90	94
Iso-5-hydroxytryptamine	10	26
Tryptamine	118	130
Psilocybine	0	5

VANE (1959) has found that 5-methyltryptamine is readily oxidized by the amine oxidase present in rat gastric fundus, this preparation also acted on tyramine, tryptamine, 5-hydroxytryptamine, but not on 5-hydroxy-α-methyltryptamine.

FELLMAN, FUJITA and BELBER (1962) have recently shown that the 2:3-dihydro-derivative of 5-hydroxytryptamine is also oxidized by amine oxidase; the rate of oxidation is similar to that of 5-hydroxytryptamine but the affinity of the dihydro derivative is much less.

2. Amine oxidases inhibited by carbonyl reagents

It is now known that the classical intracellular amine oxidase is not the only enzyme that removes amino groups from monoamines. The enzyme that has been discussed in the preceding paragraphs is characterized by two properties:

a) Immunity from inhibition by carbonyl reagents, e.g. *semicarbazide*, and by cyanide;

b) Ability to oxidize *secondary* as well as *primary amines* (BLASCHKO 1960).

The amine oxidases to be discussed in what follows differ from the classical enzyme in two points. They all are members of a group characterized by sensitivity to both cyanide and carbonyl reagents, and by their *inability to act on secondary amines.* Enzymes of this group active on indolealkylamines are:

1. The *amine oxidase* of pea seedlings. This enzyme is probably present also in other *plants*.

2. The *benzylamine oxidase* of *mammalian blood plasma*.

Plant amine oxidase was first described by WERLE and v. PECHMANN (1949). It has been purified both in WERLE's laboratory and by KENTEN and MANN (1952) (see also MANN 1955, 1961). KENTEN and MANN (1952) compared the relative rates of oxidation of a variety of substrates; they found the following rates of oxidation: with putrescine 342 μl O_2; with cadaverine 378 μl O_2; with β-phenylethylamine 134 μl O_2; with tryptamine 49 μl O_2. In other words, the rate with tryptamine was much lower than with the two diamines. Other indole derivatives were not tested. WERLE, TRAUTSCHOLD and AURES (1961), using a highly purified preparation of *pea* oxidase, report these relative rates of oxidation:

with cadaverine	1000
with spermidine	570
with β-phenylethylamine	81
with tryptamine	27
with 5-hydroxytryptamine	8
with 5:6-dihydroxytryptamine	1

A comparison of the results of these two groups of workers makes one suspect that there may be a second catalyst present in peas that is responsible for the oxidation of the indolealkylamines; this second catalyst appears to be removed in the progressive purification of the diamine oxidase.

As to the plasma oxidases, there are two of these, spermine oxidase and benzylamine oxidase. It has been shown that spermine oxidase occurs mainly in the blood plasma of ruminants (BLASCHKO 1962a; BLASCHKO and BONNEY 1962). The bovine plasma enzyme has recently been obtained in crystalline form by YAMADA and YASUNOBU (1962); the purified enzyme did not act on tryptamine and 5-hydroxytryptamine. With a crude benzylamine oxidase preparation, consisting of 1.6 ml each of the crude dialysed sera rates of oxidation of benzylamine and tryptamine were (BLASCHKO, FRIEDMAN, HAWES and NILSSON 1959):

Substrate	Serum		
	Horse	*Pig*	*Dog*
Benzylamine	29 μl O_2	47 μl O_2	70 μl O_2
Tryptamine	11 μl O_2	15 μl O_2	24 μl O_2

These experiments show that the rate of oxidation of tryptamine was by no means negligible.

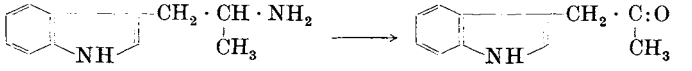
The purification and crystallization of the benzylamine oxydase of pig plasma has been recently achieved by BUFFONI and BLASCHKO (1963).

As far as the oxidation of 5-hydroxytryptamine is concerned, the crude sera all acted on it, although at differing rates. However, it was shown that the slow oxidation of 5-hydroxytryptamine by *pig serum* was not due to the amine oxidase present in the plasma; the ability to act on 5-hydroxytryptamine could be separated from the benzylamine oxidase activity, and it was made likely that the catalyst responsible for the *oxidation* of *5-hydroxytryptamine* was *caeruloplasmin* (BLASCHKO and LEVINE 1960b). Whether the more rapid oxidation of *5-hydroxytryptamine* by *horse serum* is due also to caeruloplasmin or whether it is at least in part due to the benzylamine oxidase has not been investigated.

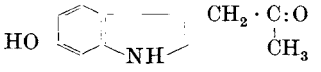
The presence of the plasma amine oxidases is of interest, because of their variable substrate specificity. It seems quite possible that in those species in which this activity occurs, the blood plasma takes an active part in the biological inactivation of indolealkylamines.

3. Microsomal deaminating enzymes

We owe much of our knowledge of the microsomal "detoxicating" enzymes to the work of B. B. BRODIE (1956) and his colleagues. It was AXELROD, who in 1954 showed that (—)-amphetamine was deaminated by a suspension of rabbit liver microsomes. A similar pathway has recently been described for *α-methyltryptamine:* rat liver *microsomes* act on α-methyltryptamine, to give *3-indolylacetone:*



In addition, a 6-hydroxylation occurs (see below); thus 6-hydroxy-3-indolylacetone is also formed (SZARA 1961):



It must remain open whether or not the enzyme described by AXELROD (1954) in the rabbit liver is identical with the rat liver enzyme; however AXELROD found little enzymic activity on amphetamine in the rat liver. Both preparations require the presence of the reduced form of nicotinamide-adenine dinucleotide phosphate.

4. Microsomal demethylating enzymes

These enzymes can also be included under the general heading of "amine oxidases", since the reaction catalysed involves the oxidative fission of a C—N bond; the methyl group gives rise to formaldehyde:

$$R_1CHR_2N \cdot CH_3 + O_2 + H_2O \rightarrow R_1CHR_2NH + HCHO + H_2O_2$$

This was first shown by AXELROD (1953, 1955) for (—)-ephedrine; in these experiments rabbit liver microsomes were used. With rat and dog liver microsomes demethylation of ephedrine was much less, but this may have been due to the presence of an inhibitor, as addition of rat liver microsomes depressed the rate of demethylation of ephedrine by the rabbit liver preparation.

These findings are of interest in view of the observation that the *rabbit liver microsomal* preparation, in the presence of supernatant fraction, nicotinamide-adenine dinucleotide phosphate and semicarbazide, demethylated N,N-dimethyltryptamine, with the formation of N-methyltryptamine and formaldehyde (SZARA and AXELROD 1959). Evidence for the formation of dimethyltryptamine-N-oxide was also obtained.

Experiments in rats showed that *N-methyltryptamine* was also formed in this species. These observations are of interest, since they raise the question as to the identity of the demethylating enzymes for ephedrine and the indolealkylamines.

II. Reactions of the phenolic hydroxyl groups

In addition to oxidative deamination of the indolealkylamines, there are enzymic reactions of the phenolic hydroxyl groups which have been studied. The physiological significance of some of these reactions is still unknown.

1. Hydroxyindole oxidases

An oxidation of *5-hydroxytryptamine*, possibly with formation of a quinone-imine, was first discussed by BLASCHKO and PHILPOT (1953), but no experimental evidence in favour of such a reaction was brought forward until PORTER, TITUS, SANDERS and SMITH (1957) reported an oxidation of 5-hydroxytryptamine by *caeruloplasmin*, the *copper*-containing oxidase of mammalian blood plasma. Although GELLER, EIDUSON and YUWILER (1959) were unable to find such an oxidation, the observation by PORTER et al. (1957) has since been confirmed by many authors (NAKAJIMA and THUILLIER 1958; MARTIN, ERIKSEN and BENDITT 1958; SANKAR 1959; ZARAFONETIS and KALAS 1960; LEVINE 1960; BLASCHKO and LEVINE 1960a; CURZON and VALLET 1960).

Another line of enquiry opened up with the finding by BLASCHKO and MILTON (1960), that 5-hydroxytryptamine was oxidized by an extract of the *gill plates* of *Mytilus edulis*. Some purification of the oxidase responsible was achieved later (BLASCHKO and LEVINE 1960a).

Caeruloplasmin is a copper-containing catalyst, and what is known of the *Mytilus* gill plate enzyme, e.g. its *inhibition by cyanide*, is compatible with the view that it also is a copper protein. That iron-containing proteins are also able to attack the phenolic hydroxyl group of 5-hydroxytryptamine, was shown by BLUM and LING (1959), who observed oxidation of the amine, with pigment formation, when it was incubated with denatured oxyhaemoglobin. The authors considered that the oxidation was brought about by a peroxidatic action, a suggestion supported by the observation of RODNIGHT (1958) that carbon monoxide inhibits this type of destruction of 5-hydroxytryptamine. Similarly, HORITA (1962) has demonstrated the oxidation of 5-hydroxytryptamine by the cytochrome-cytochrome oxidase system.

Table 1. *Relative rates of oxidation of different substrates by the hydroxyindole oxidase of Mytilus gill plates and by pig plasma caeruloplasmin* (BLASCHKO and LEVINE 1960a)

Substrate	Hydroxyindole oxidase from *Mytilus* gill plates	Pig plasma caerulo-plasmin
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

It can thus be stated that both vertebrate and invertebrate enzymes are able to oxidize 5-hydroxytryptamine. It was already shown by BLASCHKO and MILTON (1960) that both *bufotenine* and *5-hydroxytryptophan* were also readly oxidized by the *Mytilus* gill plate enzyme, thus excluding an amine oxidase type of reaction. This led to a systematic study of the substrate specificites of both the *Mytilus* enzyme and pig plasma caeruloplasmin (BLASCHKO and LEVINE 1960a); in the course of this work it was found that indole derivatives substituted with a hydroxyl group in positions 4, 6 or 7 were also attacked. A table showing the rates of oxidation of various substrates, taken from the paper by BLASCHKO and LEVINE (1960a) is appended (Table 1).

The table shows that the substrate specificities are similar, but that there are also some differences. All the hydroxytryptamines tested were oxidized by both preparations, but the mammalian preparation did not act on any of the hydroxytryptophans tested (4-, 5- and 7-hydroxytryptophan), although these were

oxidized by the *Mytilus* preparation at rates similar to the rates of oxidation of the corresponding amines.

Thus, we can say that this type of oxidation is widespread in animal preparations. The oxidation reaction, starting from 5-, 6-, or 7-hydroxy compounds, leads to melanin-like pigments. The oxidation product of 5-hydroxytryptamine by caeruloplasmin has been studied by BENDITT and his colleagues (ERIKSEN, MARTIN and BENDITT 1960; MARTIN, BENDITT and ERIKSEN 1960). It was postulated that one of the compounds, probably a precursor of the melanin-like pigment formed, was a dimer formed by dehydrogenative coupling of the benzene ring moieties of two molecules of 5-hydroxytryptamine.

The observations on the enzymic oxidation of the *4-hydroxyindole* compounds are of interest, since these substances do not form melanin-like compounds, but bright-blue substances some of which are water-soluble (BLASCHKO and LEVINE 1960a; HORITA and WEBER 1961; HORITA 1962).

It may be mentioned here that hydroxyindole oxidases may have a wider distribution in Molluscs; one of us has recently found an active enzyme of this type in the *crystalline style of Pinna nobilis*. It is of interest that the crystalline style enzyme will not only act on 5-hydroxytryptamine and on psilocine (on the latter also with the appearance of a blue pigment) but also on catecholamines. In other words, hydroxyindole oxidase activity may be a property of certain phenol oxidases. This agrees with the finding that hydroxyindole oxidase is cyanide-sensitive.

However, the ability to oxidize hydroxyindoles is by no means a property of all phenol oxidases. In Dr. E. BUEDING'S laboratory, MANSOUR (1958) has found that the phenol oxidase of *Fasciola hepatica (the liver fluke)* will readily act on catecholamines and on tyramine, but is without action on *5-hydroxytryptamine*. Yet, the latter is not entirely without effect: in 10^{-2} M concentration, 5-hydroxytryptamine almost completely *inhibits* the oxidation of 5×10^{-3} M tyramine. This inhibition is competitive.

We have confirmed MANSOUR'S statement that the Harding-Passey mouse melanoma oxidase hardly acts on 5-hydroxytryptamine; the preparation is also inactive with psilocine. Here too 5-hydroxytryptamine has an initial inhibitory effect on the oxidation of substrate, (L-dopa), but later the curves of oxygen consumption in the presence and absence of 5-hydroxytryptamine cross, suggesting that in the presence of substrate 5-hydroxytryptamine is oxidized. This is clearly demonstrated in the presence of both substrate (L-dopa) and psilocine; no initial depression of oxygen uptake was seen in the presence of psilocine, but there was an appearance of the typical blue colour, indicative of oxidation of the 4-hydroxyindole derivative (unpublished observation).

The phenol oxidase of fungi is of particular interest in view of the occurrence of 4-hydroxyindoles *(psilocine and psilocybine)* in these plants. We have tested the oxidation of both 5-hydroxytryptamine and psilocine by an extract of an acetone-dried powder of cultivated mushrooms. This preparation did not act on the hydroxyindoles, but the oxygen uptake in the presence of 2×10^{-3} M L-dopa was greatly enhanced when either of the two indolealkylamines was present. In the presence of psilocine, the typical blue colour appeared.

This observation is not without interest for the significance of psilocine in those species of *Psilocybe* in which psilocine or psilocybine occurs. We have, through the courtesy of Dr. A. HOFMANN, of Messrs. SANDOZ, Bâle, tested a dried preparation of *Psilocybe semperviva*. However, this was inactive with both L-dopa and psilocine. Since the condition of the preparation is not known, it must remain an open question whether or not these fungi contain an oxidase

that will act on psilocine, either directly or indirectly, in the presence of a catechol substrate. It seems interesting that one of the members of this group is called *Psilocybe caerulescens* (HEIM 1959). One is tempted to speculate if these hydroxyindoles serve as regulators of oxygen uptake in the species in which they occur. Such a view would be in conformity with the theories of certain plant biochemists, who consider the phenolases as "terminal oxidases".

α) 5:6-Dihydroxytryptamine. The possibility that this compound may serve as a hormone in *Arthropods* (CARLISLE 1956) appears to have found confirmation in the work by CARLISLE and TIWARI (1961). This compound, prepared by SCHLOSSBERGER and KUCH (1960), was kindly given to us by Dr. H. G. SCHLOSSBERGER; we have found that it is oxidized by pig plasma caeruloplasmin and the Mytilus gill plate preparation (unpublished observation). In each case there was appearance of a dark colour. In assessing this result, we must remember that the action on 5:6-dihydroxytryptamine is probably a manifestation of the general catechol oxidase activity of these preparations.

2. Conjugases and dephosphorylase

Observations on intact animals, to be discussed below, make it certain that enzymic *conjugations* with *glucuronate* and *sulphate* occur in animals. The presence of *psilocybine* in fungi (discussed in other sections of this volume) also shows that *phosphorylation* reactions do occur. Nothing, however is known about the enzymes responsible for these reactions.

HORITA and WEBER (1961) in their study of the oxidation of psilocine have shown that rat kidney homogenates, by the action of alkaline phosphatase, rapidly convert psilocybine to psilocine. They discuss the possibility that psilocine is the pharmacologically active compound. A study of the enzymic hydrolysis in various animals shows that in the rat and the mouse the kidney was enzymically most active; in guinea-pig and rabbit the small intestine was the most active tissue. Heart and brain tissue also dephosphorylated psilocybine (see also HORITA and WEBER 1962).

3. Hydroxyindole-O-methyl transferase

The elucidation, by LERNER, of the chemical structure of *melatonin* led to a study of the O-methylation mechanism by AXELROD and WEISSBACH (1960, 1961). The method of enzyme assay employed was the study of the enzymic transfer of the methyl group from S-adenosylmethionine, labelled with C14 in the methyl group, to N-acetyl-5-hydroxytryptamine. A preparation from *bovine pineal glands* was prepared, purified by ammonium sulphate precipitation (45 to 68% saturation), followed by adsorption to, and subsequent elution from, alumina gel Cγ, until a final preparation was obtained that was 25 times as active as the crude homogenate. The pineal gland is the only organ in which this enzyme has been found; homogenates of pineal glands of *Macaca mulatta* had more than twice the activity, those of the cat about one-half, of that of the bovine glands. The N-acetyl derivative of 5-hydroxytryptamine was the substrate tested that was most rapidly attacked; if its rate of methylation be taken as 100, the corresponding rates were:

with bufotenine	14
with 5-hydroxyindoleacetic acid	12
with N-methyl-5-hydroxytryptamine	9
with 5-hydroxytryptamine	7
with 4-hydroxy-N-acetyltryptamine	8

No 5-hydroxyindole-O-methyl transferase was found in the "parotid" gland of *Bufo marinus*, the South American toad (MÄRKI, AXELROD and WITKOP 1962).

III. Introduction of the phenolic hydroxyl groups

A very unsatisfactory feature of our knowledge of the formation of 5-hydroxyindole derivatives in the animal body is our ignorance of the enzymic mechanism of the introduction of the phenolic hydroxyl group; this is fully discussed in the contribution of HAGEN and COHEN in this volume.

The introduction of the 4-hydroxyl group, as in psilocine, is also achieved by enzymes hitherto unidentified.

More is known about the mechanism of *6-hydroxylation*. JEPSON, UDENFRIEND and ZALTZMANN (1959) demonstrated the enzymic conversion of *tryptamine* to 6-hydroxytryptamine by rabbit liver microsomes. Other lipid-soluble indoles were also hydroxylated. Similarly, SZARA and AXELROD (1959) described a preparation of rabbit liver microsomes which in the presence of nicotinamide-adenine dinucleotide phosphate, soluble supernatant fraction from liver and semicarbazide, produced a phenolic amine that was, after some initial uncertainty over the exact position of the hydroxyl group, identified as the 6-hydroxy derivative of *dimethyltryptamine*.

In subsequent publications these findings have been extended (SZARA 1961; SZARA and PUTNEY 1961; SZARA, HEARST and PUTNEY 1960): *N,N-diethyltryptamine* was shown to be similarly 6-hydroxylated; rat liver microsomes were shown to convert both α-methyltryptamine and 3-indolylacetone to the corresponding 6-hydroxy compounds (SZARA 1961). Rat liver microsomes incubated with nicotinamide-adenine dinucleotide phosphate and magnesium ions were able to bring about this reaction. SZARA and PUTNEY (1961) found that brain microsomes were not able to catalyse this reaction, but that many lipid-soluble indole derivatives reacted with the rat or rabbit liver microsome system. The hydroxylation could be inhibited by either chlorpromazine or reserpine.

IV. Introduction of N-methyl groups

An *enzyme* capable of *N-methylating 5-hydroxytryptamine* has recently been described by AXELROD (1961, 1962a). This enzyme has a number of remarkable properties. First of all, it occurs in high concentration in *rabbit lungs*; apart from the rabbit it has only been found, and in small amounts, in one human lung, but not in others. No activity has been detected in similar preparations from lungs of rat, mouse, guinea-pig, cat and monkey (*Macaca mulatta*?). In the rabbit, there was also some activity in these tissues (in decreasing order of activity): adrenal gland, kidney, spleen, heart and liver; there was no activity in extracts of brain, hypothalamus, intestines and salivary gland. Secondly, there is a wide spectrum of substrate specificities; some of the substances tested are shown here:

5-hydroxytryptamine	100	(—)-metanephrine	38
N-methyl-5-hydroxytryptamine	70	Dopamine	16
Tryptamine	81	N-methyldopamine (epinine)	43
4-hydroxytryptamine	22	(—)-noradrenaline	23
6-hydroxytryptamine	65	(—)-adrenaline	13
Tyramine	54	Aniline	85
N-methyltyramine	54	Indole	0
Octopamine	12	Mescaline	37
(±)-ephedrine	21		

Bufotenine appears not to have been investigated as a possible substrate.

The rabbit lung enzyme is a soluble enzyme; it resembles all the other methyl transferases in that it transfers the methyl group of S-adenosylmethionine. The enzyme was inhibited by *p*-chloromercuribenzoic acid, a reagent for sulphydryl groups. Chlorpromazine was a strong inhibitor, causing 45% inhibition in a concentration of 2.5×10^{-5} M. Imipramine, in 10^{-4} M concentration, halved the rate of methylation of 5-hydroxytryptamine.

The enzyme responsible for the N-methylation of noradrenaline, phenylethanolamine-N-methyl transferase, does not act on tryptamine or its 5-hydroxy derivative (AXELROD 1962b). This enzyme, which occurs mainly in the adrenal gland, will not N-methylate some sympathomimetic amines that are substrates of the rabbit lung transferase, e.g., dopamine, amphetamine or tyramine.

The only other source of an "unspecific" *N-methyl transferase* system described occurs in the *South American toad (Bufo marinus)*. The "*parotid*" gland of this animal yields a preparation that will N-methylate 5-hydroxytryptamine, its mono-N-methyl derivative, tryptamine, as well as a number of sympathomimetic amines (MÄRKI et al. 1962).

V. N-acetylase

WEISSBACH, REDFIELD and AXELROD (1960) were able to obtain from homogenates of *rat liver* and of *bovine pineal glands* supernatant fractions that were able to convert 5-hydroxytryptamine to *N-acetyl-5-hydroxytryptamine*. The latter was identified not only by paper chromatography but also enzymically, by demonstrating its conversion to melatonin. The active fractions required the presence of ethylene diamine tetra-acetate and an acetyl-coenzyme-A generating system (coenzyme A and phosphotransacetylase). Rat brain was also active, but the enzyme system appeared more labile.

VI. Oxidative deamination and transamination involving 5-hydroxytryptophan

Reactions involving the formation of 5-hydroxyindolepyruvic acid are of interest because of the possibility that pathways involving this oxo-acid give rise to 5-hydroxyindoleacetic acid (see BLASCHKO and HOPE 1955; UDENFRIEND, TITUS, WEISSBACH and PETERSON 1956).

1. Amino-acid oxidases

BLASCHKO and HOPE (1955, 1956) tested two preparations using DL-5-hydroxytryptophan as substrate; with a preparation from the digestive gland of *Mytilus edulis*, a source of a typical L-amino-acid oxidase, they found these rates of oxygen uptake:

with L-*tryptophan*	60 μl O_2
with DL-*5-hydroxytryptophan* . .	19 μl O_2
with L-phenylalanine	108 μl O_2
with L-tyrosine	110 μl O_2

In another experiment (BLASCHKO and HOPE, unpublished) the L-amino-acid oxidase of *cobra venom* was tested. It may be mentioned that the most active sample used was one collected in India in 1899. Of this sample, 5 mg consumed in the first 15 minutes:

with 10^{-2} M L-tryptophan	195 μl O_2
with 2×10^{-2} M DL-5-hydroxytryptophan .	68 μl O_2

Thus, L-amino-acid oxidase of both vertebrate and invertebrate origin acted on 5-hydroxytryptophan.

2. Transaminases

SANDLER, SPECTOR, RUTHVEN and DAVISON (1960) first demonstrated that *5-hydroxytryptophan* was transaminated in a reaction in which the oxo-acid accepting the amino group was α-oxoglutarate. They suggested that this enzyme, that they found in *rat liver*, was possibly identical with the transaminase for L-*tryptophan*. This was confirmed by SPENCER and ZAMCHEK (1960). There is reason to believe that the rat liver enzyme is less specific than was originally believed: JACOBY and LA DU (1962) have presented evidence that suggests that one and the same enzyme in rat liver acts on tyrosine, phenylalanine and tryptophan.

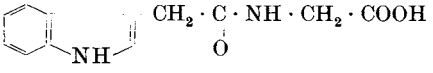
HAAVALDSEN (1962) has found that liver is not the only tissue that will transaminate 5-hydroxytryptophan; he has described a transaminase for this amino acid in *rat brain* that will use either α-oxoglutarate or pyruvate as acceptors.

C. Metabolism of indolealkylamines

I. Metabolites arising from deamination reactions

1. Metabolites of tryptamine and of its N-methylated derivatives

Although the occurrence of tryptamine derivatives in mammals was securely established only when 5-hydroxytryptamine was identified a little over ten years ago, it had been known for some time that tryptamine is metabolized. In 1913, EWINS and LAIDLAW fed tryptamine to a *dog* and isolated from the *urine* a compound that they identified as *indoleaceturic acid*, the product of condensation of indoleacetic acid and glycine (see also GUGGENHEIM and LÖFFLER 1916):



Indoleaceturic acid

WEISSBACH, KING, SJOERDSMA and UDENFRIEND (1959) have since described observations indicating that *indole-3-acetic* acid is a normal metabolite. They estimate that in *normal human urine* about 5—18 mg of the acid are excreted in 24 hrs, but that in certain abnormal conditions the excretion of the acid may rise to as much as ten times this amount. The acid is present partly free, partly in a form from which it is set free by acid hydrolysis. Pathological conditions in which indole-3-acetic acid excretion was high were: *diabetes*, FRIEDREICH'S *ataxia*, *progressive muscular dystrophy*, *amyotrophic lateral sclerosis and idiopathic sprue*. In a case of *sprue* 53—67 mg of the free acid, and 175—220 mg of total acid, as determined after acid hydrolysis, were excreted in 24 hrs. *Loading* the *diet* with L-*tryptophan* (20 mg per kg of body weight) led to an increase mainly of free *indole-3-acetic acid*. It may be mentioned that there was no concomitant increase in the excretion of 5-hydroxyindoleacetic acid (5-HIAA) in this experiment. The authors believe that both bacterial and mammalian enzymes are involved. Most of the acid is believed to arise, by transamination in the presence of both α-oxoglutarate and pyridoxal-5-phosphate, from indolyl-3-pyruvic acid, bypassing the amine stage. However, WEISSBACH et al. (1959) found that mammalian tissues (kidney and liver) as well as faecal bacteria formed tryptamine from L-tryptophan. Some of the acid, therefore, was probably derived from tryptamine

by oxidation. We can summarize these findings by saying that the ultimate source of indole-3-acetic acid is L-tryptophan, but that it remains open how much of this had first been converted to tryptamine.

The work just quoted was preceded by a study of the urinary excretion of metabolites after administration of tryptamine and its N-methylated derivatives in the *rat*. ERSPAMER (1955) identified both *indoleacetic* and *indoleaceturic acids* as metabolites of *tryptamine*, *N-methyltryptamine* and *N,N-dimethyltryptamine*. With increasing methyl substitution the percentage recoveries of the acids decreased: 85 p.c. recovery from tryptamine, 36 p.c. from N-methyltryptamine and 17 p.c. from N,N-dimethyltryptamine. The low recovery of the acid from the tertiary amine is what would be expected from what is known of the metabolic fate of the compound *in vitro*.

This may also be an appropriate place to refer to the work of ARMSTRONG, SHAW, GORTAKOWSKI and SINGER (1958), who have made a careful paper-chromatographic study of over a hundred *normal human urines*. They identified and characterized numerous compounds, all *indole derivatives*. However, the question as to the origin of these substances, listed in Table 2, was left open.

Table 2.
List of some indole derivatives identified in normal human urine (ARMSTRONG et al. 1958)

Indole-3-carboxaldehyde	β-(indole-3)-propionic acid
Indole-3-carboxylic acid	β-(indole-3)-acrylic acid
Indole-3-glyoxylic acid	Tryptophan
Indole-3-glyoxylamide	N-acetyl tryptophan
Indole-3-glycolic acid Na	5-hydroxyindole
Indole-3-acetic acid	5-hydroxyindole-2-carboxylic acid
Indole-3-acetamide	5-hydroxyindole-3-carboxylic acid
Indoleaceturic acid	5-hydroxyindole-3-acetic acid
N-(indole-3-acetyl)-α-alanine	β-(5-hydroxyindole-3)-lactic acid
N-(indole-3-acetyl)-β-alanine	5-hydroxytryptophan
N-(indole-3-acetyl)-β-amino*iso*butyric acid	5-methoxyindole
N-(indole-3-acetyl)-γ-aminobutyric acid	5-methoxyindole-2-carboxylic acid
N-(indole-3-acetyl)-aspartic acid	5-methoxyindole-3-carboxylic acid
N-(indole-3-acetyl)-glutamic acid	5-methoxyindole-3-acetic acid
N-(indole-3-acetyl)-glutamine	β-(5-methoxyindole-3)-lactic acid
β-(indole-3)-pyruvic acid	5-methoxytryptophan
β-(indole-3)-pyruvic acid oxime	2-pyrrolecarboxylic acid
β-(indole-3)-lactic acid	

ARMSTRONG et al. (1958) give these quantitative data on the excretion in *normal human urine*:

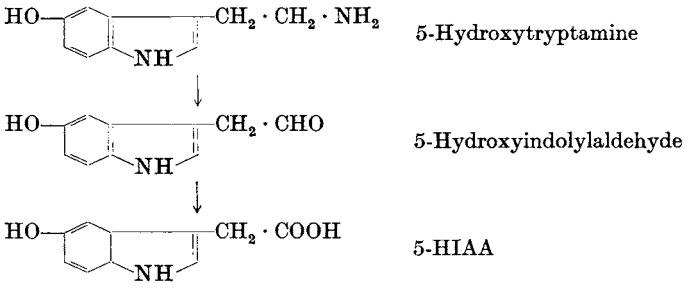
Indole-3-acetic acid	1—10 μg per mg of creatinine excreted
Indolelactic acid	0.2—2 μg per mg of creatinine excreted
Indoleacetylglutamine	1—3 μg per mg of creatinine excreted
5-hydroxyindoleacetic acid . .	1—5 μg per mg of creatinine excreted

2. Metabolites of 5-hydroxytryptamine and related amines

a) 5-Hydroxytryptamine

The role of amine oxidase in the catabolism of 5-hydroxytryptamine was independently and almost simultaneously established in work from UDENFRIEND's and ERSPAMER's laboratories (TITUS and UDENFRIEND 1954; UDENFRIEND, TITUS and WEISSBACH 1955; UDENFRIEND, TITUS, WEISSBACH and PETERSON 1956; ERSPAMER 1954, 1955, 1956). This work elucidated what is now known to be the main pathway of 5-hydroxytryptamine breakdown, the oxidative deamina-

tion of the amine by amine oxidase, followed by a further oxidation of the aldehyde formed, to give the corresponding carboxylic acid, *5-hydroxyindoleacetic acid* (5-HIAA):



This is the general pathway of biological inactivation of amines in the animal body (see BLASCHKO 1952b).

The work of UDENFRIEND and of ERSPAMER has given rise to a great volume of observations on the excretion of 5-HIAA in man and in animals. In these observations it has been established that 5-HIAA could be derived both from endogenous and from exogenous 5-hydroxytryptamine (and 5-hydroxytryptophan). To quote only one example of amine from an exogenous source: ANDERSON, ZIEGLER and DOEDEN (1958) discovered the high 5-hydroxytryptamine content of *bananas* in their study of the urinary excretion of 5-HIAA in monkeys and in children. ERSPAMER (1961) gives a number of references to papers in which an increased output of 5-HIAA was reported after administration of the amine in animals and in man; since then, ERSPAMER and BERTACCINI (1962) have published new observations on the fate of *administered — orally* and *parenterally* — LD-*5-hydroxytryptophan* in rats. They found that administration of large doses of the racemic amino acid led to an increase in the excretion not only of 5-HIAA but also of its glucuronide.

The discovery by LEMBECK (1953) of *5-hydroxytryptamine* in a *carcinoid* tumour *(enterochromaffinoma)* was followed by that of a great increase in the *urinary* excretion of *5-HIAA* in this condition (PAGE, CORCORAN, UDENFRIEND, SJOERDSMA and WEISSBACH 1955; SJOERDSMA, WEISSBACH and UDENFRIEND 1955; UDENFRIEND, WEISSBACH and SJOERDSMA 1956).

Conversely, after *removal of the large intestine in man* the *urinary* excretion of *5-HIAA* is markedly *reduced* (HAVERBACK and DAVIDSON 1958; BERTACCINI and CHIEPPA 1960). The latter authors report a decrease in the 24 hr interval from 3.04 ± 1.24 mg in normal control subjects to 1.44 ± 0.39 mg in operated subjects (see also BERTACCINI 1958).

The *excretion of 5-HIAA*, its basic value and its increase, due either to an increased turnover of endogenous amine or in response to an increased intake of the amine or its precursor, leave no doubt that the determination of the level of 5-HIAA can serve as a useful index of the metabolism of 5-hydroxytryptamine. The most important clinical application is in the diagnosis of enterochromaffinoma.

However, it is still uncertain to whait extent we are permitted to use the urinary excretion of 5-HIAA as a quant tative indicator of the breakdown of 5-hydroxytryptamine *in vivo*.

The considerations that limit the use of the measurement of the urinary output of 5-HIAA can be conveniently discussed under these headings:

α) 5-HIAA may arise from 5-hydroxytryptophan without the intermediate formation of 5-hydroxytryptamine.

β) Oxidative deamination of the amine may occur, but the aldehyde formed as the first reaction product may not be further oxidized to give 5-HIAA.

γ) Rupture of the indole ring may occur.

δ) 5-Hydroxytryptamine may be metabolized by other routes: N-acetylation, O-methylation or O-conjugation, and

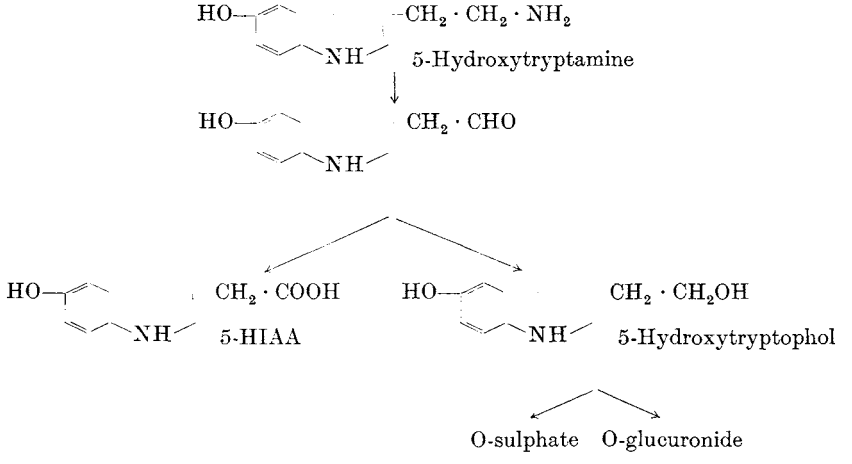
ε) 5-Hydroxytryptamine may be excreted unchanged.

ad α) 5-HIAA may be formed by reactions that bypass the amine, by way of 5-hydroxyindolepyruvic acid, in transamination or oxidative deamination. However, although such reactions are known to occur *in vitro* it is at present not known how important they are in metabolism. One suspects that they are more likely to occur with ingested 5-hydroxytryptophan than when the amino acid is formed *in vivo*.

ad β) The possibility of alternate reactions of the aldehyde was first discussed when the appearance of a dark pigment in the *oxidative deamination of 5-hydroxytryptamine* was described (see BLASCHKO 1958); it was believed that *pigment formation* was due to a condensation reaction of the aldehyde formed in the oxidative deamination catalysed by amine oxidase.

The possibility that the *low* excretion of 5-HIAA in *herbivores* (rabbit and guinea-pig) is due to reactions of the aldehyde other than oxidation to the carboxylic acid is also discussed by NAKAI (1958).

A possibly more important source of error under this heading has recently come to light in the work of KVEDER, ISKRIĆ and KEGLEVIĆ (1962). These authors administered C14-labelled 5-hydroxytryptamine to rats and showed that the *O-glucuronide of 5-hydroxytryptophol* was a major *metabolite;* some of the *O-sulphate* of *5-hydroxytryptophol* was also excreted. These observations indicate the following two pathways of breakdown of 5-hydroxytryptamine, both involving oxidative deamination:



KVEDER et al. (1962) *question* the importance of *N-acetyl-5-hydroxytryptamine* as a major metabolite of 5-hydroxytryptamine (see MCISAAC and PAGE 1959). It may be mentioned here that the *N-acetyl* derivative was found in *rat urine* after the administration of 5-hydroxytryptamine by WEISSBACH, LOVENBERG, REDFIELD and UDENFRIEND (1961), but in amounts smaller than those reported by MCISAAC and PAGE (1959). Both WEISSBACH et al. (1961) and KVEDER et al. (1962) discuss the possibility of an "amine oxidation" insensitive to the known

inhibitors of "mono" amine oxidase. The possibility that amine oxidases akin to the mammalian plasma oxidases discussed above may act, must be borne in mind but no evidence for the presence of such enzymes in the tissues of the rat has hitherto been obtained.

It may be mentioned that *5-hydroxytryptophol* as a metabolite of the amine in mammals has its counterpart in the catecholamine series; these are observations that originated with the isolation of what was first described as "substance Z" and eventually identified as ω-hydroxy-acetovanillone (see V. EUW, NEHER, REICHSTEIN, TAIT, TAIT and WETTSTEIN 1959; AXELROD, KOPIN and MANN 1959).

ad γ) There is no positive evidence in favour of the suggestion that hydroxyindole oxidase might be active *in vivo* and initiate a pathway of breakdown that would lead to a rupture of the indole ring. Conditions under which such oxidases might operate in the living animal have been discussed elsewhere (BLASCHKO and LEVINE 1960c); it was pointed out that the situation for 5-hydroxytryptamine is somewhat analogous to that for adrenaline, where the possibility of the formation of adrenochrome has been discussed for a long time, without satisfactory evidence forthcoming so far.

ad δ) There can be no doubt that both O-methylation and N-acetylation of 5-hydroxytryptamine can occur; this has been discussed already. As already mentioned, the work of KVEDER et al. (1962) throws some doubt on the extent of N-acetylation.

ad ε) The *urinary* excretion of *5-hydroxytryptamine*, first suggested by TWAROG and PAGE (1953) and supported by the observations of RODNIGHT (1956) and others (see ERSPAMER 1961), is well-established. However, the contribution of the excretion of the free amine to the total catabolism of the 5-hydroxyindoles is small.

Since ERSPAMER'S (1961) review, ERSPAMER and BERTACCINI (1962) have reported observations on rats that had received the racemic form of *5-hydroxytryptophan* by various routes. Whereas after the *administration* of the amine the amounts of free amine excreted in the urine were small, amounting to about 1.5 p.c. or less of the dose administered (see also GESSNER, KHAIRALLAH, MCISAAC and PAGE 1960), after the intraperitoneal injection of the amino acid, the amine excreted could amount to as much as one-quarter of the L-form of the amino acid administered.

In man, free 5-hydroxytryptamine was first found in patients with carcinoid tumours (PERNOW and WALDENSTRÖM 1954). For a recent classification of carcinoid tumours excreting both 5-hydroxytryptamine and 5-hydroxytryptophan see WILLIAMS and SANDLER 1963; PEART, PORTER, ROBERTSON, SANDLER and BALDOCK 1963.

Summing up the findings discussed so far we can say that the importance of *amine oxidase* as a major instrument for the *biological inactivation of 5-hydroxytryptamine* is firmly established. In contrast to the urinary indoleacetic acid, which seems mainly, although not exclusively, derived from tryptophan via the oxo-acid, most of the 5-HIAA excreted in the urine appears to have passed through the stage of free 5-hydroxytryptamine, and provided that known exogenous sources of 5-hydroxytryptophan or of 5-hydroxytryptamine are excluded, most of the acid seems to be "endogenous", that is to say, material that has been 5-hydroxylated *in vivo*, probably with the amine contributing the major portion. This still leaves an uncertainty, the fate of much of the 5-hydroxytryptamine that is not excreted as 5-HIAA. ERSPAMER (1955) has given several instances of the recovery of 5-HIAA after administration of 5-hydroxytryptamine. The

recovery *varied* from *species* to species; in the guinea-pig it was 0.5—1 p.c., in the rabbit 1—1.5 p.c., in the rat 5.5—33 p.c., in the dog 25 p.c. and in man 20—52 p.c. It is particularly interesting that *5-HIAA* was readily detected in the *urine* of *young ruminants*, but absent from that of *adult* animals. It is known that in newborn ruminants the plasma oxidases are absent or poorly developed (BLASCHKO and HAWES 1959; BLASCHKO and BONNEY 1962), but it is unknown if these observations can account for the differences in the 5-HIAA excretion. It may be mentioned that caeruloplasmin, a mammalian hydroxyindole oxidase, is also absent from the blood plasma or poorly developed at birth (ELSNER, HORNYKIEWICZ, LINDNER and NIEBAUER 1953; SCHEINBERG and STERNLIEB 1960).

b) Other 5-hydroxyindolealkylamines

According to ERSPAMER (1955), *excretion* of *5-HIAA* after administration to rats of *bufotenidine*, the fully N-methylated quaternary onium compound derived from 5-hydroxytryptamine, was doubtful, but with the tertiary amine, *bufotenine*, *5-HIAA* was definitely identified as a *metabolite*. In addition, unchanged bufotenine was also recovered from the urine. This observation is in keeping with the slow oxidation of bufotenine by amine oxidase; this has already been discussed.

GESSNER, KHAIRALLAH, MCISAAC and PAGE (1960) have since reported that after subcutaneous or intraperitoneal injection of bufotenine in rats an average of 22 p.c. of unchanged *bufotenine* and of 7 p.c. of *5-HIAA* was found in the urine in the first 48 hrs; conjugation with glucuronic acid was doubtful. The authors suggest that the longlasting effects of the tertiary amines, *bufotenine* and *psilocybine*, are related to their *relative immunity* from the action of *amine oxidase*.

c) Derivatives of 5-hydroxyindoles

ERSPAMER, GLÄSSER and NOBILI (1961) have found that both *5-hydroxy-N-acetyltryptophan* and *5-acetoxy-N-acetyltryptophan*, administered intraperitoneally to rats, are *hydrolysed*, the latter first by removal of the O-acetyl group; then further hydrolysis removes the N-acetyl group. A surprising result was that of the two substances administered a relatively large portion is excreted as free 5-hydroxytryptamine; the authors take this as an indication that the hydrolytic removal of the N-acetyl group occurs mainly in the kidneys; removal of the N-acetyl group is immediately followed by decarboxylation of the 5-hydroxytryptophan, also in the kidneys.

3. 4-Hydroxyindoles

Work on these compounds has been stimulated by the discovery of the two hallucinogenic amines *psilocybine* and *psilocine* and by the elucidation of their chemical constitution (HOFMANN, HEIM, BRACK, KOBEL, FREY, OTT, PETRZILKA and TROXLER 1959). On finding that the L-form of 4-hydroxytryptophan was a substrate of the mammalian decarboxylase (ERSPAMER, GLÄSSER, PASINI and STOPPANI 1961), the metabolic fate of both *4-hydroxytryptophan* and *4-hydroxytryptamine* was investigated (ERSPAMER, GLÄSSER, NOBILI and PASINI 1960; ERSPAMER and NOBILI 1961, 1962). 4-DL-Hydroxytryptophan was administered to rats, orally, subcutaneously or intraperitoneally, and to human subjects by mouth. Six urinary metabolites were definitely identified: *4-hydroxytryptophan*, *4-hydroxytryptamine* and *4-HIAA*, together with their *glucuronides* (the enteric juice of *Helix* served as source of β-glucuronidase for the chromatographic identification). Of these metabolites, the glucuronides were demonstrated only in rat's urine; they were not found in human urine.

In assessing these results, it must be remembered that of the racemic amino acid probably the L-form only is metabolized by decarboxylation. The traces of free amine, therefore, can with a high degree of certainty be considered as derived from the L-form.

It is of interest that in the experiments in *man*, with doses of 200—1000 mg of the DL-amino acid ingested, only about 10—12 p.c. appeared as 4-HIAA in the urine; the amine represented about 3—5 p.c. of the dose given. In the rat, 25—40 p.c. of the dose could be accounted for as the 4-hydroxyindoles and their O-glucuronides, with the latter contributing an important fraction. No evidence of excretion of an O-sulphate or of N-acetyl-4-hydroxytryptamine was obtained. In view of the ease of oxidation of 4-hydroxytryptamine by hydroxyindole oxidases one is wondering if such an oxidation might have contributed to the breakdown of the 4-hydroxyindoles. However, this possibility has not hitherto been explored.

GESSNER et al. (1960) report that in rat urine about 11 p.c. of unchanged psilocybine appears after 48 hrs; there also is some evidence fort he excretion of a glucuronide.

II. N-Acetylated metabolites

Although the work, already quoted (WEISSBACH et al. 1961; ERSPAMER and BERTACCINI 1962; KVEDER et al. 1962) throws some doubt on the quantitative importance of N-acetylation as a metabolic route for the breakdown of 5-hydroxytryptamine, it seems unsafe at present to rule it out altogether. We have to remember the observations, also quoted already, on the occurrence of an N-acetylase acting on 5-hydroxytryptamine in rat and bovine tissues (WEISSBACH, REDFIELD and AXELROD 1960). Although such an enzyme, at least in the pineal gland, is probably mainly concerned with the formation of melatonin, it seems interesting that the rat liver also contains the enzyme. It seems therefore at present advisable to keep the possibility in mind that in some species at least N-acetylation may contribute to the biological inactivation of 5-hydroxytryptamine, although it may be less important than originally believed by MCISAAC and PAGE (1959).

III. Excretion of O-sulphates

There is some doubt as to the extent to which O-sulphate formation contributes to the breakdown of 5-hydroxytryptamine. CHADWICK and WILKINSON (1960) incubated rat liver homogenates with the amine, and they isolated a compound that they tentatively identified as the O-sulphate of 5-hydroxytryptamine. They also reported that of the amine administered, about 30 p.c. was excreted as 5-HIAA, and that of this amount again 70 p.c. was excreted as the O-sulphate of 5-HIAA. This statement sill lacks confirmation by other observers. ERSPAMER and BERTACCINI (1962) have tentatively identified one of the substances of which they obtained evidence by paper chromatography as the *O-sulphate* of *5-hydroxytryptamine*, and KVEDER et al. (1962) discuss the possibility that one of the metabolites of 5-hydroxytryptamine is the O-sulphate of 5-hydroxytryptophol.

However, there is good evidence, to be discussed below, that the *O-sulphate* of *6-hydroxymelatonin* is a principal metabolite in the catabolism of melatonin (KOPIN, PARE, AXELROD and WEISSBACH 1961). It is of interest that *etryptamine*, 3-(2-aminobutyl) indole, is reported to be *excreted* as the *O-sulphate* and the *O-glucuronide* (EBERTS and DANIELS 1962).

IV. Excretion of O-glucuronides

There is little doubt that in some species of mammals the *O-glucuronide of 5-hydroxytryptamine* is an important *metabolite* of the amine. This was first studied by WEISSBACH, REDFIELD and UDENFRIEND (1958) and by MCISAAC and PAGE (1959); the latter authors found the O-glucuronide of 5-hydroxytryptamine as a major metabolite in the rabbit.

WEISSBACH et al. (1961) studied the presence of radioactive metabolites in mice after administering C14-labelled 5-hydroxytryptamine. The mice were killed $1\frac{1}{2}$ hrs after receiving 1 mg of the amine. In normal mice 30 p.c. of the total radioactivity was recovered as the O-glucuronide; in mice that had previously received a dose of iproniazid the percentage rose to 70. In *mouse urine*, collected for 12 hrs after giving 250 mg/kg of 5-hydroxytryptamine, 30 p.c. was present as the *O-glucuronide*; this value was 73 p.c. in urine from iproniazid-treated mice. The *O-glucuronide of 5-HIAA* was also present, mainly in the animals not treated with the inhibitor of amine oxidase; in the former it amounted to about 10 p.c. of the total radioactivity recovered. The authors mention that in urine from patients suffering from malignant *carcinoid (enterochromaffinoma)* the *O-glucuronide* was *a minor metabolite*, together with a little N-acetyl-5-hydroxytryptamine.

That the O-glucuronide of 5-hydroxytryptophol is excreted in rat urine after administration of the amine, has already been discussed (KVEDER et al. 1962).

It may be worthwhile to mention that the method of identification of the O-glucuronide employed by WEISSBACH et al. (1961) was an enzymic method, the fluorometric assay of the free amine formed after incubation with bacterial β-glucuronidase.

V. Formation and excretion of phenolic compounds

Experiments on tissue preparations in which hydroxylation reactions occur, have already been described. There is good evidence to show that these reactions also take place in the intact organism. SZARA and AXELROD (1959) demonstrated that N,N-dimethyltryptamine, administered intraperitoneally to rats led to the appearance of these excretion products in the urine: unaltered *dimethyltryptamine*, *N-methyltryptamine*, *indole-3-acetic acid*, *6-HIAA*, *tryptamine*, *the N-oxides* of both dimethyltryptamine and its 6-hydroxy derivative.

Similarly, after the intraperitoneal injection of α-methyltryptamine in *rats* both 6-hydroxy-α-methyltryptamine, its O-glucuronide as well as the *O-glucuronide* of *6-hydroxy-3-indolylacetone* were *excreted*, indicating that not only substances that are substrates of the classical amine oxidase, but also those that are substrates of microsomal deaminating enzymes undergo this type of hydroxylation (SZARA 1961). Incidentally, the excretion of the acetone derivative clearly shows that the microsomal deaminating enzyme also operates *in vivo*.

Similarly EBERTS and DANIELS (1962) have obtained evidence that sulphate or glucuronide conjugates of its 6-hydroxylated derivative are the principal metabolites of *etryptamine*, 3-(2-aminobutyl)-1-indole. Here also evidence for the occurrence of the microsomal type of oxidative deamination was obtained.

In order to understand the biological significance of these 6-hydroxylation reactions, we must remember that there exists one compound that is present in the body and is normally metabolized by 6-hydroxylation; this is melatonin (see below). An enzyme of this kind is therefore operative not exclusively on substances foreign to the body.

Whether indole compounds with a free 5-hydroxyl group are able to be thus hydroxylated in position 6, is unknown. In view of the instability of the

5,6-dihydroxyindole compound that would arise in such a reaction it might be difficult to demonstrate the occurrence of this type of reaction.

VI. Metabolism of melatonin

Our knowledge of the pathway of synthesis of this hormone is due to the work of AXELROD and his colleagues (WEISSBACH, REDFIELD and AXELROD 1960; AXELROD and WEISSBACH 1961). Beginning from 5-hydroxytryptamine, the work suggested that this amine was N-acetylated, and that *N-acetyl-5-hydroxytryptamine* was *O-methylated*. The enzymes involved in these conversions have already been discussed.

The metabolic fate of melatonin was studied by injecting melatonin tritiated in the acetyl group intravenously into rats. After 30 minutes, melatonin itself

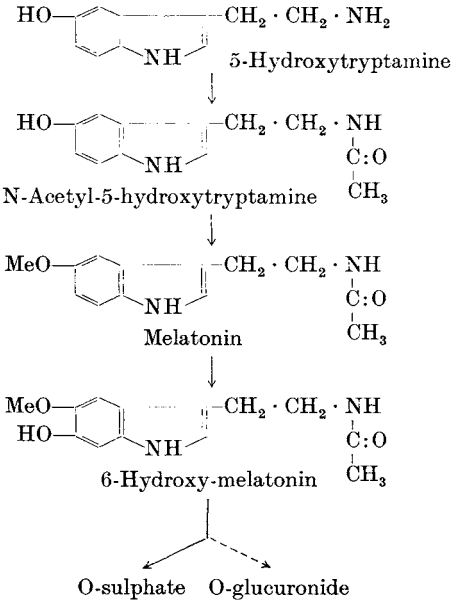


Fig. 3. Main pathway of formation and breakdown of melatonin (after KOPIN et al. 1961)

accounted for only 35—50 p.c. of the total radioactivity in the tissues (KOPIN, PARE, AXELROD and WEISSBACH 1960, 1961). In other words, it was rapidly either destroyed or eliminated. An analysis of the urinary metabolites was carried out, using methoxy-C14-labelled melatonin and acetyl-H3-labelled melatonin. Two compounds were identified as the *O-sulphate* and the *O-glucuronide* respectively of *6-hydroxy-melatonin* (N-acetyl-5-methoxy-6-hydroxytryptamine); a third metabolite was not fully characterized. The O-sulphate accounted for 70—80 p.c. of the total radioactivity of the urine; it thus represented the major metabolite of melatonin. The O-glucuronide accounted for about 5 p.c. of the radioactivity, and the unidentified compound for about 12 p.c.

These experiments have been supplemented by work on *liver microsomes*, suspended with a system that provides reduced nicotinamide-adenine dinucleotide phosphate; under these conditions a substance with the chromatographic properties of *6-hydroxy-melatonin* was formed upon incubation (KOPIN et al. 1961).

From the work just described a sequence of metabolic steps has been proposed which is considered as the main pathway of biosynthesis and breakdown of melatonin, at least in mammals (see Fig. 3). This pathway does not account for the *presence* in the *bovine pineal gland* of *5-methoxyindoleacetic acid* (LERNER and CASE 1960). Several possibilities may account for the presence of this substance: it may represent a stage of the breakdown of that portion of 5-hydroxytryptamine in the gland that is not required for melatonin synthesis, formed either by O-methylation from 5-HIAA, or by oxidative deamination of 5-methoxytryptamine, or it is an indication of an alternative mode of breakdown of melatonin in the bovine pineal gland: hydrolytic removal of the N-acetyl group, followed by oxidative deamination of the 5-methoxytryptamine formed.

That 5-methoxytryptamine is almost quantitatively excreted as 5-methoxyindoleacetic acid, after administration of amine labelled with C14 in the β-carbon atom, has been established by MCISAAC, KVEDER and PAGE (1960). It may be recalled that in the earlier work of ERSPAMER (1955) the recovery of 5-methoxyindoleacetic acid, after the administration of larger amounts of 5-methoxytryptamine, was 40—42 p.c. when the amine was given by mouth, and 71 p.c. after subcutaneous injection.

D. Summary

The study of the breakdown of the indolealkylamines described on the preceding pages is of interest for several reasons. We have already learnt to value the determination of the urinary 5-HIAA as a diagnostic tool. In addition we may hope, as our knowledge of the urinary metabolites of these amines gets more complete, to obtain a reliable estimate of the turnover of the naturally occurring compounds of this group.

For the pharmacologist the fate of a biologically active compound is of interest as an aid for the understanding of the time course of its action. Some of the limitations of this approach have already been discussed in the opening paragraphs of this review.

It has been established in the past decade that the inactivation of the sympathomimetic amines and histamine may proceed by more than one pathway. One of these is always initiated by oxidative deamination, catalysed by one or several of the amine oxidases, but there is also O-methylation with the catechol amines and N-methylation (and, in some species, N-acetylation) with histamine.

For 5-hydroxytryptamine oxidative deamination is also an important pathway. However, there are observations suggesting that amine oxidase is not a factor that determines intensity or time course of the response of the excitable tissue. VANE (1959) has found that the contraction of the plain muscle of the *rat's gastric fundus* preparation in response to a number of *indolealkylamines* is affected by *amine oxidase inhibitors* (iproniazid or phenyl*iso*propylhydrazine). The response of the preparation was potentiated when one of these amines was tested: tryptamine, N-methyltryptamine, 5-methoxytryptamine, 5-chlorotryptamine and 5-methyltryptamine. With these compounds Vane saw a 8.9- to 20-fold *potentiation of the response*. With N'N'-dimethyltryptamine, the response in the presence of an amine oxidase inhibitor was not even doubled; this is in keeping with the feeble action of amine oxidase on tertiary amines. With α-methyltryptamine, a substance not attacked by amine oxidase, there was no potentiation by an amine oxidase inhibitor. These observations can be understood on the assumption that destruction by amine oxidase is a factor that determines the intensity of the response of the excitable tissue, just as histaminase

inhibitors modify the response of the guinea-pig's ileum to histamine (ARUNLAKSHANA et al. 1954; BLASCHKO and KURZEPA 1962).

However, when the hydroxytryptamines, 4-hydroxytryptamine, 5-hydroxytryptamine or 6-hydroxytryptamine, were tested on the rat stomach preparation, no similar potentiation was seen. The two compounds carrying hydroxyl groups in position 4 or 5 were the most potent substances tested, but their effects were the same in the presence and in the absence of an inhibitor of amine oxidase. And yet, it is known that both amines are readily oxidized by amine oxidase.

In order to account for this paradox, VANE (1959) suggested that the two hydroxylated compounds were not attacked by amine oxidase, because they could not penetrate the membrane of the muscle cell and thus gain access to the inactivating enzyme, situated in the mitochondria.

This interpretation may be adequate to account for the observations just described, but it presents us with the paradoxical situation that compounds known to be metabolized *in vivo* by oxidative deamination, are not attacked in the isolated rat fundus. We would then have to postulate that the gastric enzyme acts on synthetic amines as would be expected but is without action on the only naturally occurring substance of this group, 5-hydroxytryptamine.

From what has been said before, we are not in doubt that amine oxidase is an important catalyst of the breakdown of 5-hydroxytryptamine in mammals. If we deny to the oxidase in the plain muscle cell a share in the task of biological inactivation of this amine, we have to postulate that in other sites, e.g. in the liver, the *permeability* of the *cell membrane* is such as to allow the intracellular enzyme to act. We also remember that a microsomal localization of liver amine oxidase has been established and that for all we know this fraction of the enzyme may be more closely linked to the cell surface.

Another interpretation of the observations by VANE is implicit in the finding by WEISSBACH et al. (1961), already discussed, which suggests that an amine oxidase inhibitor may not make inactivation impossible, but shift the catabolism of the amine into a different route; this is a possibility that has been discussed for other amines (see DAVISON 1958). We cannot at present assess the importance of such a shift, as the metabolic abilities of the muscle cell, apart from the established presence of amine oxidase, are too imperfectly known.

One fact that has emerged from the study of the metabolism of the indolealkylamines, are the many analogies that exist between the naturally occurring members of this group and the other amines for which GUGGENHEIM has coined the term "biogenic amines". In particular, we find many biochemical relationships between 5-hydroxytryptamine and the catechol amines and we suspect that there exists a cytogenetic counterpart to this relationship, between chromaffin cells and adrenergic neurones on the one side and 5-hydroxytryptamine, enterochromaffin cells and "tryptaminergic" neurones on the other. The enzymic mechanisms that act on the synthetic substances of this group can be seen as instruments of the catabolism of naturally occurring compounds.

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