# METABOLISM OF EPINEPHRINE AND NOREPINEPHRINE

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Our knowledge of the inactivation and synthesis of catechol amines is still incomplete. A number of enzymic reactions have been studied in vitro, but we do not know how many of these occur in the living organism. Some information on this point is now accumulating and it seems certain that in the near future we shall learn much about the biological significance of the enzymes already known and of some still to be discovered.

Adrenaline and noradrenaline are biologically highly active substances. Little of them is present in the body at any one time. It appears also that little is formed and needs to be destroyed; therefore the enzymes required for synthesis and for inactivation need not be very plentiful. There are indications that the rate of turnover of adrenaline is in fact very low (38); nothing is known about noradrenaline, but its turnover rate is probably higher. Until quantitative data for all catechol amines are available, the activities required of enzymes concerned in formation and breakdown cannot be estimated.

*Inactivation mechanisms.* One important gap in our knowledge is that we do not know what contribution is made by those enzymes which catalyse what we call the phenolase type of oxidation, enzymes which contain either iron or copper. They attack the catechol moiety, and adrenochrome (or noradrenochrome) is one of the oxidation products. Experiments with methyl-labelled adrenochrome have not, so far, produced any evidence that adrenochrome is a normally occurring metabolite of adrenaline (34). And yet, it is difficult to believe that the phenolase type of oxidation does not occur. Adrenaline, noradrenaline and Corbasil ($\alpha$-methylnoradrenaline) all have a similar short-lived type of effect and their action is similarly potentiated by ephedrine although Corbasil is not a substrate of amine oxidase. These are strong arguments in favour of assuming that this type of response is linked with the presence of the catechol group. It would be particularly interesting to know more about the oxidation of noradrenaline, the chief mediator of adrenergic nerves. I mention here the observations of Angenent and Koelle (1) who discuss the possibility that DOPA oxidase is the enzyme responsible for the inactivation of adrenaline in the pigmented rabbit iris.

Our knowledge of amine oxidase has recently been reviewed (5, 7). That in the living organism the inactivation of some of the adrenaline is due to the action of amine oxidase seems now to be generally accepted. I recall the evidence furnished by Schayer and his colleagues who have shown that in the inactivation of adrenaline the $\beta$ carbon and the methyl carbon atoms suffer different metabolic fates (32, 33). The metabolites believed to arise through the action of amine oxidase disappear from the urine when amine oxidase is inhibited (34).

The oxidation of adrenaline, noradrenaline and dopamine by amine oxidase

was only discovered when we learnt to distinguish the breakdown due to enzyme action from autoxidation (15). Active breakdown of adrenaline by liver was also demonstrated by Bain, Gaunt and Suffolk (2). They used blood as the medium of incubation: the blood caused maximal protection from autoxidation; however, active destruction of adrenaline in the presence of added liver slices could clearly be demonstrated. This method is of interest, since very little is known of the inactivation of sympathomimetic amines by intact cells.

Amine oxidase, as we now know, occurs in many tissues that contain these amines. The suprarenal gland, the uterus, the blood vessels, the iris and the muscle of the nictitating membrane may be cited as examples. Chromaffine tissue has been found in all vertebrates, and all vertebrates contain amine oxidase. Chromaffine cells are said to be absent in Amphioxus, and Miss Himms and I have not been able so far to demonstrate any amine oxidase in this species.

Chromaffine cells also occur in invertebrates, *e.g.*, in annelid worms. We have recently demonstrated the presence of amine oxidase in the gut of the earthworm (12); this is interesting, since in this species the gut is the only tissue known to receive adrenergic fibres (29). We have already discussed the occurrence of both amine oxidase and sympathomimetic amines in molluscs, *e.g.*, in Octopus and Sepia (7, 10, 13).

In the last few years another substrate of amine oxidase has been found in vertebrates and molluscs: 5-hydroxytryptamine. Last year when I studied the oxidation of this amine by amine oxidase I noticed that a brown pigment was formed in this reaction (6). It is known that tryptamine is also a substrate of amine oxidase (16, 30) and Pugh and Quastel noted already the formation of a brown pigment when tryptamine is oxidised by amine oxidase. Mrs. Philpot and I have recently found that in this oxidation reaction not only a pigment but also a strongly fluorescent compound is formed from either tryptamine or 5-hydroxytryptamine. Both fluorescent material and pigment probably arise in a secondary reaction of the aldehyde formed as the direct result of the amine oxidase reaction.

This pigment formation from tryptamine and 5-hydroxytryptamine is of interest, since both the enzyme and the substrate (5-hydroxytryptamine) normally occur in close proximity in the tissues. The reaction has therefore been used for a histochemical study. Dr. K. Hellmann and I have incubated fresh tissue sections, cut on the freezing microtome, with either tryptamine or 5-hydroxytryptamine (11). In tissues which contain amine oxidase a brown pigment soon begins to accumulate. The color seems to follow the distribution of amine oxidase: in the liver, the pigment is uniformly distributed in the parenchyma cells; in the kidneys, where amine oxidase is known to be localized chiefly in the cortex, it is the cortex which is most deeply pigmented, whereas the medulla remains almost uncolored. In the renal cortex, the pigment is not uniformly distributed: the glomeruli remain colorless, whereas the epithelial cells of the proximal convoluted tubules are most deeply pigmented. Does this observation indicate that amine oxidase is mainly located in these tubules? This is an interesting problem that awaits further analysis.

Dr. Hellmann has also used the tryptamine reaction in frozen sections of skin (24). In the human skin the sweat glands give a negative tryptamine test; the

sweat glands of the cat give a very feebly positive reaction. In the adrenergically innervated sweat glands of the horse the tryptamine reaction is very strongly positive. In one of these sections, Dr. Hellmann has also found pigment in the media of an arteriole; this is of interest in view of the demonstration of amine oxidase in blood vessels (31, 37).

If we accept that at least some of the adrenaline and noradrenaline is destroyed by amine oxidase, we are led to a conclusion as to the site of destruction which seems worth discussing. We have already pointed out some time ago that amine oxidase is located in the cytoplasmic granules of the liver cell (14); further analysis has shown that most of the enzyme is present in the mitochondria (20, 23). Therefore we must conclude that that part at least of the catechol amines that is destroyed by amine oxidase penetrates the cell wall and passes into the cytoplasm. Thus from the metabolic studies we gain important information about the movement of biologically active substances. Adrenaline and the related sympathomimetic amines contain a non-quaternary nitrogen atom; they are able to pass through the cell membrane. It seems to me worthwhile to ask if here we have a clear difference between sympathin and acetylcholine, the quaternary compound which exerts effects on the cell membrane and which has a much simpler mechanism of inactivation.

I think the metabolic studies make it likely that for adrenaline and related compounds we shall have to distinguish between first-phase effects, exerted upon the cell membrane from the outside, and second-phase effects, which depend upon the presence of the amine inside the cell. It may be that in the first phase, we see the sympathomimetic effects *sensu strictiori*, while in the second phase we observe the effects upon carbohydrate metabolism. Many of the differences between compounds of this group may become clearer when we learn to take into account individual differences in the rates of penetration. Tachyphylaxis, characteristic of the amphetamine-ephedrine group, may be due to retention at the cell membrane. Potentiation of adrenaline-like actions is often explained by interference with enzymic destruction; we should, however, consider the possibility that it can also be brought about by a competitive interference with entry across the cell membrane. A potentiating substance acting by such a mechanism would cause an amine to be present in an effective concentration for a longer time and thus mimic the picture of an inhibition of an intracellular inactivating enzyme. May I quote as an example the potentiation of the response to adrenaline by cocaine, or by certain quaternary compounds, *e.g.*, by some of the substituted phenyl ethers of choline recently studied by Brown and Hey (18). This concept could easily be extended to explain the absence of a clearcut line of division between potentiating and blocking agents.

*Biosynthesis of adrenaline and noradrenaline.* Let us point out from the outset that in addition to the two catechol amines named in this heading, a third amine of this group occurs in vertebrates: dopamine (22, 35). In 1937 we showed that dopamine is rapidly destroyed by amine oxidase (16), and in 1938 Holtz, Heise and Lüdtke discovered the enzyme L-DOPA decarboxylase (27), an enzyme that forms dopamine. Holtz and his colleagues suggested that decarboxylation, followed by oxidation by amine oxidase, was a normal pathway of L-amino acid

breakdown. In the following year, I proposed that DOPA decarboxylase was the catalyst of one of the steps in the biosynthesis of adrenaline and sympathin (3). This suggestion is not yet proved. I should like to summarise some of the facts by which it is supported.

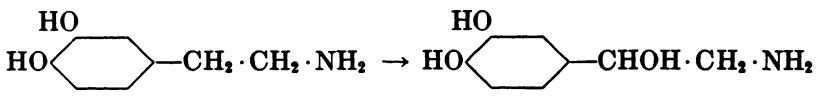
In 1950 my colleague, Dr. H. Langemann, found DOPA decarboxylase in the adrenal medulla of the ox (28); this has since been confirmed (36), and the enzyme has also been found in the pig adrenal medulla (26). Thus we know now that in some species the adrenal medulla contains a catalyst which forms a catechol amine. The amine most readily formed is dopamine, and it seems significant that this amine has been found not only in the urine (see 21) and in the heart of sheep (22), but also in the adrenal medulla of sheep and ox (22, 35). However, neither the decarboxylase nor the amine has been found in the adrenal medulla of all species.

Last year at Paris, Professor von Euler considered it difficult to regard dopamine as a serious candidate for an intermediary rôle in the synthesis of noradrenaline (21). I should prefer to consider this still an open question. We must remember that in many species there occur large amounts of extra-adrenal decarboxylase, not only in the kidneys (27), but also in the liver (3) and in other organs. This implies the possibility of extra-medullary amine formation. Some of this may be connected with the biosynthesis of sympathin, but amine formation for adrenaline synthesis may also take place outside the medulla. That dopamine does not accumulate in all species may mean that it is further metabolized as fast as it is formed. Dopamine may differ from adrenaline and noradrenaline in that it is chiefly an intermediate and not released either as a mediator or a hormone.

In 1939, it was found that DOPA decarboxylase does not act on methylamino acids, e.g., on N-methyl-DOPA. It was this fact which led me to suggest that sympathin might be one of the primary amines on the pathway of biosynthesis of adrenaline (3). You know that the conversion of noradrenaline to adrenaline has been demonstrated by Miss Bülbring (19).

The inability of the organism to form the secondary amine by direct decarboxylation of the methylamino acid can be understood if we assume that DOPA decarboxylase or a related enzyme is the catalyst of the decarboxylation reaction. DOPA decarboxylase activity disappears from the liver extracts of rats deficient in pyridoxine (8) but, provided the deficiency is not too severe, one can reconstitute the enzyme in vitro by adding synthetic pyridoxal phosphate (4). This shows that the enzyme depends on pyridoxal. The methylamino acids are not able to form the intermediate substrate-enzyme complex in which the carbonyl group of pyridoxal reacts with the primary amino group of the substrate.

The observations described show that although the steps occurring in adrenaline biosynthesis are not yet clear, we know some facts about the enzymic reactions which serve as useful pointers for further research. At present our ignorance is greatest in regard to the reaction in which the $\beta$-hydroxyl group is introduced, *e.g.*:



However, we can say that this is a reaction analogous to enzymic reactions already known.

Recent work has shown that in the adrenal medullary cell the bulk of the pressor amines is not present in freely diffusible form in the cytoplasm, but in granules. Dr. A. D. Welch, Dr. P. Hagen and I have already described some of their properties (9, 17, 39; see also 25). We have recently obtained fractions of granules which have a pressor amine content of as much as 15 to 20% of their dry weight. They represent the chief store of adrenaline, and it is tempting to believe that they are also the site of synthetic processes. Dr. Hagen and I have not been able to separate granules which carry adrenaline from those which carry the respiratory activity of the adrenal medullary cell. It looks, therefore, as if the same granules carry both the amines and the respiratory enzymes. We could understand this relationship if we assume that synthesis occurs in the granules. To take an example: the methylation of noradrenaline is ATP-dependent (19) and may require the vicinity of a system of oxidative phosphorylation. However, synthesis of adrenaline occurs in steps, and when we talk about synthetic reactions, we have to define what reaction we want to discuss. In the homogenates of the ox medulla, the DOPA decarboxylase activity is not found in the granules, but in the supernatant fluid. If this indicates that in the living cell the enzyme is present in the cytoplasmic sap, we must assume a two-way traffic of amines across the granular surface: the freshly-made amine—possibly dopamine—passes on to or into the granule, and the finished product passes in the outward direction. Thus, even in the ox adrenal medulla the sites of amine formation and of further chemical change, although they occur in the same cell, may be spatially distinct.

*Summary.* Although many details of the chemical reactions in which catechol amines are formed or destroyed remain to be elucidated, new lines of enquiry have come to the fore: these deal with the interrelations between chemical changes and spatial distribution of active substances. The adrenal medullary cell has been an object of histochemical study since the days of Vulpian and Henle; it may be hoped that the new findings will lead to an increased understanding of the mechanism by which the release of mediator is translated into secretory activity. Similarly, increased knowledge of the sites of enzymic inactivation of adrenaline and noradrenaline may pave the way for a more precise location of the sites of their biological action in the effector cell.

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