ROLE OF L-DOPA DECARBOXYLASE IN THE BIOSYNTHESIS OF CATECHOLAMINES IN NERVOUS TISSUE AND THE ADRENAL MEDULLA

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The existence of an enzyme which is known to catalyze the formation of catecholamines might tempt one to overestimate its importance in the biosynthesis of the *hormonal* catecholamines and to overlook other possible metabolic pathways. I shall therefore not confine my contribution to the role played by dopa decarboxylase. Attention will also be drawn to other reactions which may occur, at least under experimental conditions. We shall then have to decide upon the metabolic steps most likely to be followed physiologically, and determine whether there is only one way for the biosynthesis of sympathomimetic amines.

Twenty years ago, in 1938, we incubated an extract from guinea-pig kidney with 3,4-dihydroxyphenylalanine (dopa). When we injected the incubate intravenously into an anesthetized cat, we obtained a strong rise in blood pressure. From the incubate we could isolate hydroxytyramine (dopamine) in the form of its tribenzoyl derivative. Only L-dopa was decarboxylated (29, 42).

The L-dopa decarboxylase from guinea-pig kidney was so active that the decarboxylation could be measured manometrically in the Warburg apparatus. This was not possible when kidney or liver extracts were incubated with histidine (40, 41, 74) or tyrosine (25–28). The L-histidine and tyrosine decarboxylase showed so little activity that the decarboxylation could only be demonstrated by bioassay. Contrary to dopa, decarboxylation is therefore certainly not the main metabolic pathway for L-histidine and tyrosine in the body.

In biological tests, dopamine was found to be 50 to 100 times less active than adrenaline. Like adrenaline, its pressor activity is enhanced by cocaine and reversed by sympatholytic drugs. Contrary to tyramine, tryptamine and serotonin its action on the nictitating membrane of the cat is not enhanced by iproniazid, *viz.*, by an inhibition of the monoamine oxidase (2). It causes a fall in blood pressure in guinea-pigs and rabbits (35). That was one reason for assuming that the physiological role of the dopa decarboxylase was not so much to form a pharmacologically active amine from a pharmacologically inert amino acid, but rather to form the precursor for the biosynthesis of adrenaline (29, 42).

The transformation of dopa to dopamine by the dopa decarboxylase was also demonstrated *in vivo* (33, 35). We injected ourselves with 50 mg of L-dopa intravenously and found nearly 40% of the equivalent amount of dopamine in the urine (35). In cats, L-dopa, injected intravenously, causes a rise in blood pressure (35) which is enhanced by vitamin B_6 and by iproniazid (2). In rabbits, the subcutaneous or intravenous application of dopa produces hyperglycemia: 10 mg/kg L-dopa were equivalent to 20 mg/kg DL-dopa (39), *i.e.*, only the L-isomer is decarboxylated.

Dopamine is formed in the body also without administration of dopa, for it is

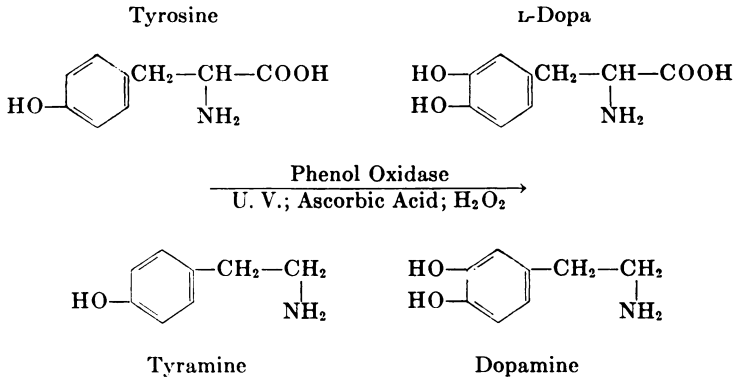
normally present in the urine (35). Expressed in μg/l, it makes up the major part of the catecholamines (18). However, the pressor activity of extracts from urine is due mainly to another, more potent catecholamine: noradrenaline or arterenol, as we could show in 1944 (36).

We called the sympathomimetic pressor principle of the normal urine, with noradrenaline as the main constituent, "Urosympathin" to indicate that it had exerted sympathin functions, before it was excreted into the urine (36). The pressor (36) and hyperglycemic (46, 62, 63) actions of extracts from pig and cattle suprarenals were comparable to those of a mixture containing 75 % adrenaline and 25 % noradrenaline. It was shown by von Euler (14–16) that the pressor activity of extracts of sympathetic ganglia and nerves was due chiefly to noradrenaline.

When we found the high decarboxylase activity in the liver and in the kidney and, in guinea-pigs, also in the intestine (37, 45) and the pancreas (38), we were unable to detect the enzyme in extracts from the suprarenal glands of guinea-pigs. We therefore assumed that dopamine was not formed in the suprarenal gland, but that it was brought there via the blood stream from the liver or the kidney. We now know that the suprarenal medulla itself does contain dopa decarboxylase. Langemann (51) was the first to demonstrate the presence of dopa decarboxylase in the suprarenals of cattle. We confirmed this result with pig suprarenals (31) and later on with the suprarenals of all species investigated, including the guinea-pig (77). Our previous negative results with guinea-pig suprarenals were due to the low concentrations of our extracts, especially since the suprarenal cortex, which is free from the enzyme, is particularly well developed in the guinea-pig. Besides, the coenzyme pyridoxal-5-phosphate was not available 20 years ago.

It is pertinent in this context that the dopa decarboxylase of the suprarenal medulla is activated very strongly by pyridoxal-5-phosphate, and that the cortex contains a heat stable activator which resembles pyridoxal-5-phosphate as was shown by the author together with Bachmann in 1952 (31).

Dopamine therefore can be formed from L-dopa within the suprarenal medulla, provided the amino acid is offered.



In our first investigations we found it difficult to explain where dopa, the substrate of our highly active enzyme, comes from. This amino acid is no normal constituent of the proteins. There is, therefore, no dietary dopa. On the other hand, we know that dopa can be formed from tyrosine by the action of phenol oxidases (56, 57) and by ascorbic acid in the presence of oxygen (1, 59). Thus the high ascorbic acid content of the suprarenal cortex might be of importance for a non-enzymatic formation of dopa from tyrosine.

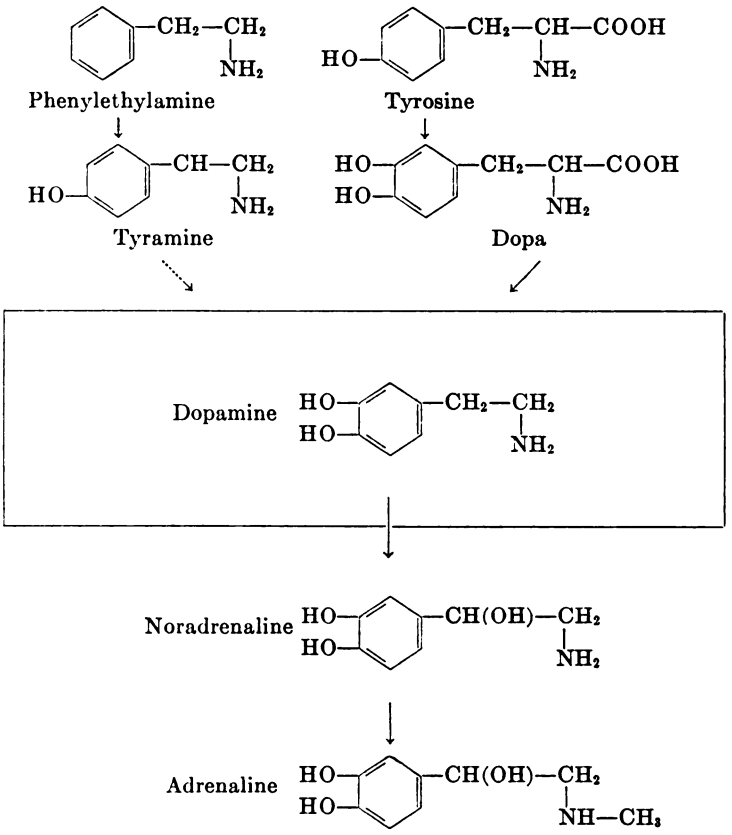
Nevertheless this does not necessarily mean that the dopamine found in the body and in the urine is due exclusively to decarboxylation of dopa. We could show that tyramine is transformed into dopamine by ultraviolet irradiation. This effect of irradiation is probably due to H_2O_2 being formed in the solvent, since we found that H_2O_2 and, again, ascorbic acid in the presence of oxygen transformed tyramine into dopamine (34). We did not investigate whether under these conditions noradrenaline also was formed.

Apparently, the possibility must be considered that dopamine as a precursor of the suprarenal and sympathetic hormones may also be formed by mechanisms other than the decarboxylation of dopa.

A. Suprarenal medulla

After the discovery and synthesis of adrenaline, repeated attempts were made to demonstrate a biosynthesis of the hormone by incubating suprarenal tissue or by perfusing the isolated gland with simple aromatic amines like phenylethylamine and tyramine. In 1924, Nikolaeff (54) perfused the suprarenals of cattle with Ringer solution containing tyramine; in 1935, Schuler and Wiedemann (68) incubated guinea-pig suprarenals with tyramine and in 1940 Devine (12) incubated the suprarenals of cattle with phenylethylamine. These authors claimed to have found a transformation of the amines into adrenaline. On incubating tissue slices of suprarenals from several animal species aerobically with phenylethylamine and tyramine, we found in 1949 a formation of more active pressor substances, although not as regularly and not as fast as with dopamine (43). These experiments constituted no proof for a synthesis of adrenaline from the amines used. We did, however, conclude from our results that the suprarenals were able to oxydize phenyl- to phenol-derivatives. This was proven by Kirshner and Goodall (21, 50) and by Rosenfeld, Leeper and Udenfriend (58) who found that isotopically labeled tyrosine was transformed into dopamine and noradrenaline in the suprarenal medulla. So far as I can see, no experiments have been carried out incubating suprarenal tissue with labeled phenylethylamine and tyramine. Udenfriend and Wyngaarden (71) injected these two amines intraperitoneally into rats; their results suggest that these compounds do not act as precursors of the hormones in the suprarenal medulla. However, this may not be quite conclusive, since, with this route of application, the amines may have been destroyed by the monoamine oxidase in the liver before they reached the suprarenal glands. On the other hand, there was a formation of noradrenaline in the suprarenals from dopamine injected intraperitoneally. No matter whether only the amino acid,

tyrosine, or the amine, tyramine, may act as precursor of the hormones; in both cases dopamine would be the intermediary product, either with or without participation of dopa decarboxylase. The dopa decarboxylase activity of the suprarenal medulla suggests that it is obtained by decarboxylation of dopa. Dopa itself has never been found in experiments with radioactive precursors. This is explained by the high rate of the conversion of dopa to dopamine. Experiments with specific inhibitors of the dopadecarboxylase should make possible the isolation of dopa.

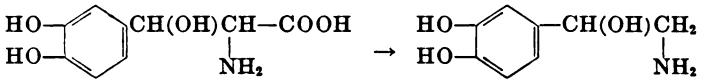


Experiments with isotopically labeled compounds have shown conclusively that in the suprarenal medulla dopa and dopamine are converted into noradrenaline and the latter into adrenaline. This metabolic pathway has been well established by the investigations of Demis *et al.* (9), Hagen (23, 24), Kirshner and Goodall (21, 50), Rosenfeld *et al.* (58), and Masuoka *et al.* (52), and has repeatedly been pointed out (5) so that I need not discuss it here in detail.

In the perfusion experiments of Rosenfeld *et al.* (58) with calf adrenals the incorporation of radioactivity into noradrenaline was greater when dopamine-C^{14} was perfused than in the case of labeled tyrosine. This indicates that in the sequence of reactions from tyrosine to noradrenaline the hydroxylation essential

for the conversion of tyrosine to dopa is the rate-limiting reaction. The fastest reaction obviously is the decarboxylation of dopa to dopamine. Aerobic conditions were required as well for the conversion of dopamine to noradrenaline, *i.e.*, the introduction of a hydroxyl group into the side chain, as for the N-methylation of noradrenaline to adrenaline.

There are two other points I would like to mention: 1) According to the results of Gurin and Delluva (22), phenylalanine injected intraperitoneally acts as a precursor of adrenaline and noradrenaline in the suprarenals of rats. It has been known for a long time that phenylalanine is oxidized to tyrosine in the perfused dog liver (13) and by liver tissue (70). Apparently it has not been investigated whether the suprarenal gland is also able to convert phenylalanine to tyrosine and thus use it for the synthesis of the hormones. 2) Although dopa is attacked particularly efficiently by dopa decarboxylase, it is not the only substrate for this enzyme. Blaschko *et al.* (7) could show in 1949 that *o*- and *m*-tyrosine are also decarboxylated. What is more important with regard to the biosynthesis of adrenaline, he also found that dihydroxyphenylserine is decarboxylated by guinea-pig kidney (6) (see also 75, 76). We find that all organ extracts which decarboxylate dopa, including those from the suprarenal medulla, also attack the phenylserines (47). However, the activity is much weaker and is much less enhanced by pyridoxal-5-phosphate than in experiments with dopa as substrate. Nevertheless, Schmiterlöw (60) found an increased excretion of noradrenaline in the urine of rabbits injected with dihydroxyphenylserine subcutaneously. There can be no doubt that dihydroxyphenylserine is decarboxylated *in vitro* and *in vivo*, though much more slowly than dopa, and thereby is transformed into noradrenaline.



3,4-Dihydroxyphenylserine Noradrenaline

Dihydroxyphenylserine has never been found in the organism, but this is no proof against its possible function as a precursor for noradrenaline. Dopa, too, has been isolated only in very few investigations: It was found by Goodall (20) in the suprarenal medulla of thyroidectomized sheep, and by Weil-Malherbe (73) once in a chromaffin tumor. Substrates of a very active enzyme cannot be expected to accumulate and will therefore be difficult to detect. Nevertheless the rather slow decarboxylation of dihydroxyphenylserine should favour the detection of this hydroxy-amino acid.

On the other hand, dopamine is regularly found in the suprarenal medulla of cattle and sheep, as shown by Goodall (20) and by Shepherd and West (69). Dengler (10, 11) in my laboratory could show that in the suprarenals of sheep about 2% of the total catecholamine content was dopamine. This suggests that noradrenaline is formed by decarboxylation of dopa and subsequent oxidation of the dopamine rather than by decarboxylation of dihydroxyphenylserine. Dihydroxyphenylserine may be an example for substances which, under experimental conditions, can perhaps be utilized by the suprarenal medulla as precursors for

the sympathetic hormones, but are probably no physiological precursors, because, under normal conditions, they are not offered to the suprarenal gland.

From these results we can draw the following conclusions. Under experimental conditions there may be several ways for the formation of noradrenaline and adrenaline. Physiologically the biosynthesis of the hormones seems to proceed along the following steps: tyrosine—dopa—dopamine—noradrenaline—adrenaline. This is the classical sequence of reactions suggested as early as 1939 by Blaschko (3; see also Holtz 29, 42) and based on his excellent investigations on the substrate specificity of dopa decarboxylase. Dopamine apparently has a key position in the biosynthesis of the sympathetic hormones. Under certain experimental conditions it may perhaps originate from phenylethylamine and tyramine in the suprarenals. However, the dopa decarboxylase activity of the suprarenal medulla suggests that dopamine is normally formed by decarboxylation of dopa which in turn is probably obtained by oxidation of tyrosine in the suprarenal glands. The presence of dopamine in the suprarenal medulla makes it unlikely that dihydroxyphenylserine is a physiological precursor of noradrenaline, although the phenylserine can be decarboxylated by extracts from suprarenal glands and is thereby transformed into noradrenaline.

B. Nervous tissue

The same is true for the other organs where the catecholamines have a physiological function: peripheral nervous tissue and brain. The highest dopa decarboxylase activity is found in peripheral sympathetic ganglia, in postganglionic adrenergic neurones and in the sympathetic trunk. In the brain and in the spinal cord the activity is about 20 times weaker. Hypothalamus, thalamus and caudate nucleus as well as the cerebellum contain more dopa decarboxylase than the cerebral cortex. The white matter of the brain is free of the enzyme. We did not find any dopa decarboxylase activity in the phrenic nerve; the low activity encountered in the vagus nerve may be due to an admixture of adrenergic fibres (48). The distribution of L-glutaminic acid decarboxylase is quite different: it is highest in the cerebellum and entirely absent from sympathetic nerves and ganglia (49).

Like extracts from the suprarenal medulla, those from sympathetic ganglia and nerves decarboxylate not only dopa, but also dihydroxyphenylserine. It was therefore important that my colleague Schümann (64) showed the sympathetic ganglia and nerves to contain dopamine, which can only originate from the decarboxylation of dopa. The dopamine of the nerves is localized in the cytoplasma, the noradrenaline in granular elements of the nerves (65). Recently, Carlsson *et al.* (8) found dopamine also in the brain of rabbits.

There are two facts I should like to emphasize: 1) In the suprarenal medulla dopamine makes up only about 2 % of the total catecholamine content. In nervous tissue nearly 50 % of the catecholamines are dopamine. 2) In concentrated extracts from splenic nerves and stellate ganglia containing up to 500 μg of noradrenaline equivalents, Schümann (64) obtained no evidence for the presence of adrenaline, either by paper chromatography or by biological assay of the eluates from the chromatograms. In the brain too, Carlsson (8) found, so far as I see,

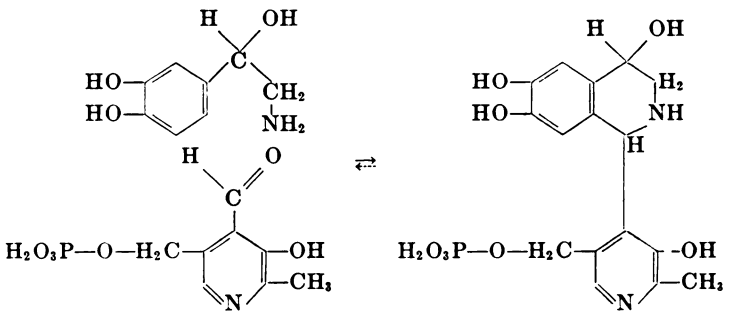
only dopamine and noradrenaline, no adrenaline. Goodall and Kirshner (16) incubated sympathetic ganglia and nerves with radioactive tyrosine and dopa. They were able to detect radioactive dopamine and noradrenaline, but not with certainty adrenaline.

I think it possible that the small admixtures of adrenaline found by other workers in splenic nerves, stellate ganglia (17) and brain (53, 72), were due to the relatively high dopamine content of nervous tissue interfering with the assay. This would lead to the important conclusion that in nervous tissue the biosynthesis of catecholamines proceeds only up to noradrenaline. Apparently, nervous tissue is unable to carry out the methylation to adrenaline, in contrast to the chromaffin cells of the suprarenal medulla, where the methylation seems to occur in specific granula. The real sympathetic transmitter would not be a mixture of noradrenaline and adrenaline; the postganglionic neurones of sympathetic nerves would be "noradrenergic" (30, 64, 65).

There exists an approximate correlation between the L-dopa decarboxylase activities and the noradrenaline content in the sympathetic nervous tissue and the brain. Both enzyme activity and noradrenaline content are highest in sympathetic ganglia and nerves; in the central nervous system they are highest in the hypothalamus. On the other hand, M. Vogt (72) finds that the noradrenaline content for instance of the caudate nucleus (0.06 μg/g) is far lower than that of the hypothalamus (1 μg/g) and of the medial nuclei of the thalamus (0.24 μg/g), while we measured an approximately equal decarboxylase activity in these three areas. For several reasons we cannot expect a precise agreement between dopa decarboxylase activity as estimated in organ extracts and noradrenaline content.

1. One reason is of methodical nature. We have to incubate 2 to 3 ml of an extract with 1 to 2 mg of dopa in order to obtain a decarboxylation strong enough to be measured manometrically. In these unphysiologically high concentrations, dopa can inhibit its own decarboxylation by combining irreversibly with the coenzyme, pyridoxal-5-phosphate. Dopa, dopamine and noradrenaline form Schiff bases with pyridoxal-5-phosphate which are easily transformed into tetrahydroisoquinoline derivatives, as shown some years ago by Schott and Clark (61). We have been able to confirm that this leads to an inactivation of the coenzyme as well as, in the case of dopamine and noradrenaline, to a pharmacological inactivation of the amines (49).

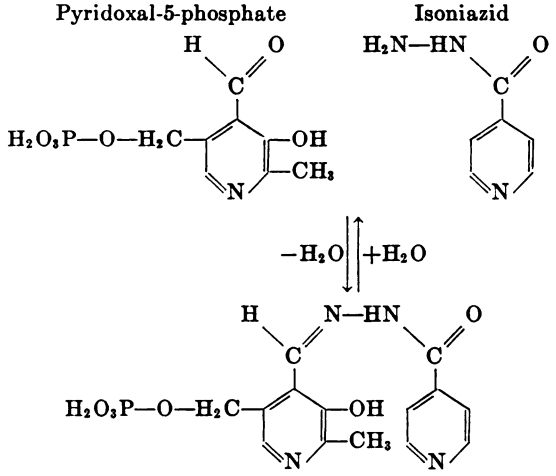
Noradrenaline



Pyridoxal-5-phosphate

If dopa is added to a brain homogenate, there is a decrease instead of an increase in the liberation of CO_2 (49). This inhibition by dopa was even more marked when glutaminic acid had been added to the incubates: glutaminic acid is decarboxylated by the glutaminic acid decarboxylase of the brain; the addition of glutaminic acid increased the liberation of CO_2 in a representative experiment from 29 to 63 mm^3. Dopa depressed the decarboxylation from 63 to 26 mm^3 CO_2, *i.e.*, under the control values. This is due to the inactivation of the pyridoxal-5-phosphate present in the homogenate which is also the coenzyme of the glutaminic acid decarboxylase. Addition of pyridoxal-5-phosphate prevented the inhibition by dopa; in this case both dopa and glutaminic acid were decarboxylated, and the amounts of CO_2 split off from each substrate were added. For the assay of L-dopa decarboxylase activity of the brain, we therefore always added pyridoxal-5-phosphate to the homogenates. Thereby of course we leveled out differences in decarboxylase activity which may be due to different concentrations of the coenzyme in the various areas of the brain.

In other organs, the substrate inhibition caused by the combination of dopa with the coenzyme may interfere with the assay of dopa decarboxylase activity in spite of the addition of pyridoxal-5-phosphate. In a dialyzed kidney extract the decarboxylation of L-dopa was greatly enhanced by small amounts of pyridoxal-5-phosphate, but the activation lasted only 20 to 30 minutes. Apparently, the added coenzyme was rapidly inactivated. This was prevented, when we previously incubated the same amount of pyridoxal-5-phosphate with isoniazid (isonicotinylhydrazide) to form an isonicotinylhydrazone. At first this complex did not activate the decarboxylation as strongly as the free pyridoxal-5-phosphate. However, the reaction progressed steadily and ended with a quantitative decarboxylation of the substrate (55). It is remarkable that in the form of its isonicotinylhydrazone, the coenzyme seems to be protected against the inactivation by dopa and dopamine, but, on the other hand, retains its coenzyme activity.



A similar protection of the coenzyme was obtained with other carbonyl reagents like semicarbazide, simple amino acids, particularly the diamino-mono-

carbonic acid lysine, also with kephaline, the colamine moiety of which has a free NH_2-group able to form a Schiff base (44). This may explain why in brain homogenates there is a nearly linear activation also with free pyridoxal-5-phosphate, and why the addition of a brain extract activates the decarboxylation of dopa by extracts from kidneys or suprarenals which otherwise stops after a relatively short time.

2. There is a second reason why no close correlation can be expected between dopa decarboxylase activity and noradrenaline content of the brain. The conversion of dopamine to noradrenaline may be quite different in the various areas of the brain, and we do not know the quantitative relation of the two amines. Still another amine is known to be present in the brain, *i.e.*, serotonin, and we think that serotonin is also formed by dopa decarboxylase. We find that all organs which decarboxylate dopa also decarboxylate 5-hydroxytryptophan (5-HTP), although they may not contain serotonin, for example splenic nerves, sympathetic ganglia, the suprarenal medulla and pheochromocytoma (78). On the other hand, carcinoid tumors of the intestine containing large amounts of serotonin also decarboxylate dopa. Lastly, α-methyl-dopa is a competitive inhibitor not only for the decarboxylation of dopa, but also for that of 5-HTP (78). The different time course of the decarboxylation of dopa and 5-HTP *in vitro* (49, 78) does not necessarily speak against the identity of dopa decarboxylase and 5-HTP decarboxylase. In contrast to dopa, 5-HTP combines only very slowly with the coenzyme and therefore produces no appreciable substrate inhibition.

A close correlation between dopa decarboxylase activity and amine content of the brain could therefore perhaps only be expected if for each area we knew the exact content of dopamine, noradrenaline and serotonin.

The role and action of L-dopa decarboxylase in the brain can be strikingly demonstrated when L-dopa and 5-HTP are injected after inactivation of the monoamine oxidase by iproniazid. The amino acids easily penetrate the blood-brain barrier and are decarboxylated to the corresponding amines which accumulate in the brain because they can no longer be oxidized. It seems safe to assume that this accumulation occurs particularly at the specific localizations of the dopa decarboxylase, for instance in the hypothalamus, the brain stem and in the ascending reticular system. In mice we could show that pretreatment with iproniazid alone shortened hexobarbital or avertin anesthesia. The intravenous injection of dopa as well as of 5-HTP in animals which had been given iproniazid caused such a strong central excitation that anesthesia was prevented altogether (32). 5-HTP was inactive when the decarboxylase was inhibited competitively by α-methyl-dopa (78).

Summarizing the results just discussed under B, we may say that in nervous tissue the L-dopa decarboxylase is as important for the formation of the so-called "sympathin" as it is in the suprarenal medulla for the synthesis of the hormones. The "sympathin" is probably not a mixture of noradrenaline and adrenaline, but perhaps rather of noradrenaline and dopamine. The fact that brain and nervous tissue contain nearly as much dopamine as noradrenaline might suggest that in these organs dopamine is not merely a precursor of noradrenaline, but that in addition it might be an effector substance itself. An exact knowledge of the dis-

tribution of both amines in the brain might provide a clue to their different specific functions. The central excitation seen in animals pretreated with iproniazid after the injection of dopa sets in very promptly. It must therefore be attributed at least in part to the dopamine which is quickly formed by decarboxylation, and not to noradrenaline which is probably formed only slowly from dopamine. In fact, during the central excitation caused by an injection of L-dopa in rabbits Carlsson *et al.* (8) found an increase in the dopamine, but not in the noradrenaline content of the brain.

C. Other tissues

In a few organs, dopamine is practically the only catecholamine present. This is true for the lung, as shown by von Euler *et al.* (19) and by Schümann (66), and for intestine and liver as recently demonstrated by Schümann (67). In the intestine it is mainly localized in the mucosa. Up to 99 % of the catecholamine content of these organs consists of dopamine (Table 1).

It seems reasonable to assume that the minute amounts of noradrenaline come from the vessels and the nerves supplying them and that the dopamine found in the parenchyma is not the biochemical precursor of this noradrenaline. This suggests that dopamine, the primary product of the action of L-dopa decarboxylase, may be an effector substance also in non-nervous tissues. I think that it may be a local hormone.

In the Warburg apparatus Schümann found no measurable dopa decarboxylase activity in extracts from ox lung and intestinal mucosa. However, preliminary experiments have indicated the presence of the enzyme also in the intestine. By bioassay it was possible to show the enzymatic formation of small amounts of dopamine.

TABLE 1

Dopamine content of various tissues

	Dopamine µg/g		% of Catecholamines	
	Ox	Sheep	Ox	Sheep
Adrenal medulla	80	28	2	2
Splenic nerves	4.5	—	50	—
Stellate ganglion	1.5	—	50	—
Brain	—	0.1	—	50
Lung	1.1	25.0	98.6	99.5
Small intestine	17.0	3.5	98	99
a) Mucosa	37.0	7.6	99	99.5
b) Muscularis	7.0	2.8	99	99
Colon	22.0	4.8	99	99.5
Liver	1.5	0.4	95	97

Summary

The role played by L-dopa decarboxylase in the biosynthesis of catecholamines depends on the organ in which the primary product of its action, *i.e.*, dopamine, is formed.

In the suprarenal medulla the biosynthesis proceeds via noradrenaline to the methylated end-product, adrenaline. Dopamine is the precursor of the hormones.

In nervous tissue, the biosynthesis stops at the noradrenaline level. Here dopamine is the precursor of the transmitter, and besides this probably an effector substance of its own.

In lungs, intestine and liver dopamine itself is the end-product of the biosynthesis. Here it may act as a local hormone.

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