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I. BIOSYNTHESIS OF CATECHOLAMINES IN INSECTS

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INTRODUCTION

Catecholamines were first detected in insects by Oestlund (10). By paper chromatography and by biological tests he identified dopamine, noradrenaline (NE) and adrenaline (E) in the mealworm, *Tenebrio mollitor*. He also established a correlation between amine concentration and developmental stage, larvae and adult insects showing higher concentrations than the pupae. No explanation was given to this finding. The function of the catecholamines in the insect organism was discussed by Oestlund; they were considered as serving the same function as in mammals, *i.e.*, that of specific adrenergic neutrotransmitters of the vegetative nervous system. Difficult to explain was the great concentration of dopamine, which could not be accounted for only as a precursor of NE and E.

During our studies of puparium formation of the blowfly, *Calliphora erythrocephala*, we identified a catecholamine derivative, N-acetyl-dopamine (7), as the substance responsible for the tanning (darkening and hardening) of the insect cuticle. Tanning of the insect cuticle is a result of the interaction of *o*-quinones with the cuticular proteins (11), the quinone being formed from the corresponding diphenol by oxidation with a phenoloxidase present in the cuticle. Since the *o*-diphenols hitherto isolated from different insect cuticles had a carboxylic-acid side chain, it was surprising to find a catecholamine derivative serving as a tanning agent. Thus an explanation was given for the high amount of dopamine found by Oestlund in *Tenebrio*: dopamine serves as precursor for the biosynthesis of N-acetyl-dopamine.

DISTRIBUTION OF CATECHOLAMINES IN INSECTS

Catecholamines and catecholamine derivatives have been detected in different insect species, holometabolic as well as hemimetabolic ones. They have been found in *Calliphora erythrocephala*, *Tenebrio mollitor*, *Drosophila melanogaster*, *Schistocerca gregaria* and others. Tyramine, N-acetyltyramine and very probably the O-glucoside of N-acetyltyramine have also been detected.

BIOSYNTHESIS OF DOPAMINE

As in mammals, tryosine is the precursor of dopamine. The biosynthesis was followed by injecting C^{14}-labelled tyrosine into the larvae of *Calliphora* (and other insects) and isolating the labeled metabolites after different intervals (22). In addition, potential precursors were incubated with insect homogenates and from the incubation mixtures the metabolites were isolated. It became evident from these studies that the fate of tyrosine depends on the age of the larvae. In larvae of earlier developmental stage tyrosine is catabolised through a pathway involving transamination to *p*-hydroxyphenylpyruvic acid and reduction to the

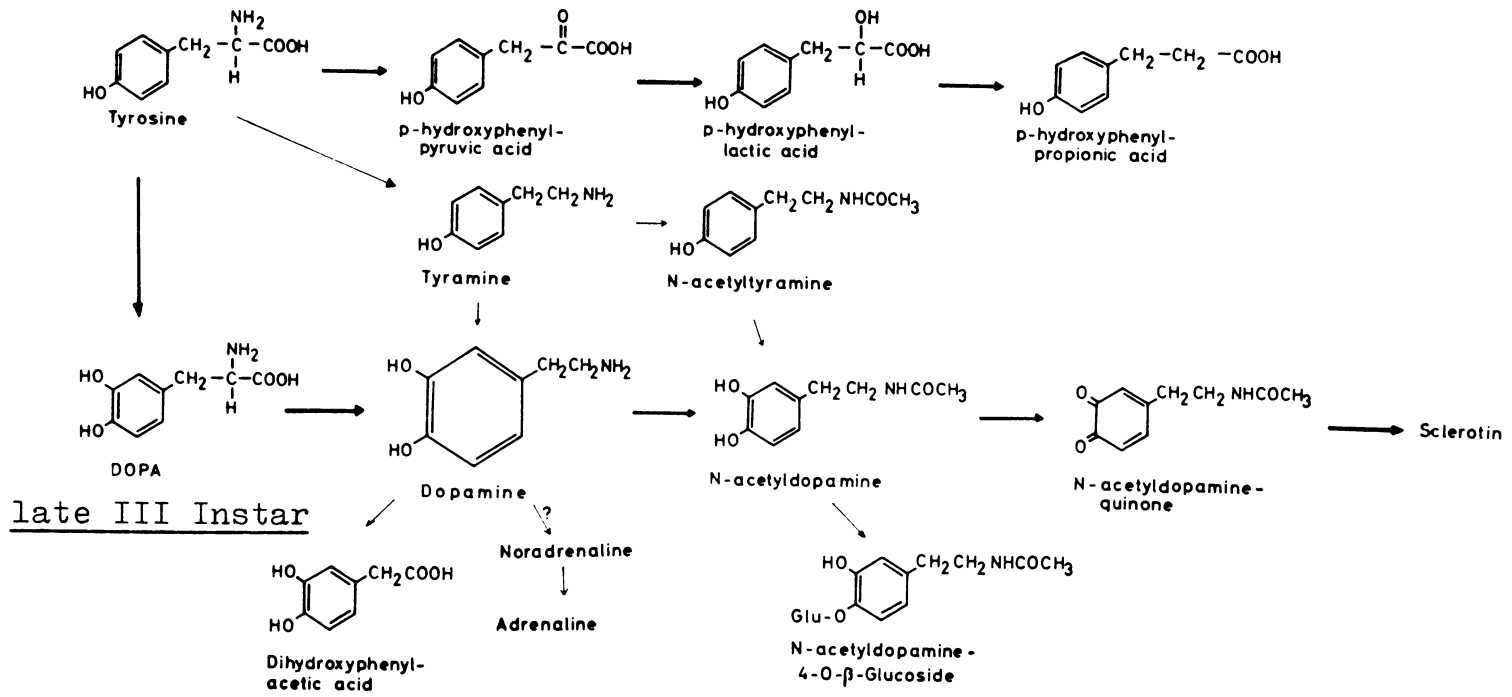
early III Instar
Tyrosine
p-hydroxyphenyl-pyruvic acid
p-hydroxyphenyl-lactic acid
p-hydroxyphenyl-propionic acid
Tyramine
N-acetyltyramine
DOPA
Dopamine
N-acetyldopamine
N-acetyldopamine-quinone
Sclerotin
late III Instar
Noradrenaline
Adrenaline
Dihydroxyphenyl-acetic acid
N-acetyldopamine-4-O-β-Glucoside

FIG. 1

corresponding lactic and propionic acid derivatives (fig. 1). Part of the tyrosine is decarboxylated to tyramine by a tyrosine decarboxylase and then acetylated to N-acetyltyramine. Tyramine can also be hydroxylated to dopamine as the larvae enter their last, third, stage. In larvae of the late third instar, tyrosine is mainly hydroxylated to dopa, which is further decarboxylated by a dopa-decarboxylase to dopamine. So there are two pathways of dopamine formation, a minor one from tyramine and the major one through dopa.

FATE OF DOPAMINE

Figure 1 shows the further transformation of dopamine. Most of the dopamine is acetylated to N-acetyldopamine, which is either incorporated into the cuticle and used in the sclerotization process or stored as N-acetyldopamine-4-O-glucoside; the latter is used at the time of the imaginal eclosion for the sclerotization of the adult insect then taking place (15). A small part of the dopamine can be oxidatively deaminated to dihydroxyphenylacetic acid, which was isolated from incubation mixtures of dopamine with *Calliphora homogenates* (17). Part of the dopamine serves as precursor to NE and E although these reactions have not received due attention in insects.

INDIVIDUAL ENZYMES

Tyrosine hydroxylation

The hydroxylation reaction was studied in more detail in the blowfly, *Calliphora erythrocephala*. It is catalyzed by a phenoloxidase showing mono- as well as diphenoloxidase activity (4, 25). It is thus distinct from the mammalian hydroxylase which catalyzes only the step leading from tyrosine to dopa. The phenoloxidase exists as an inactive soluble enzyme precursor. It is transformed to the active enzyme by an activator protein (5). Two methods have been developed to obtain the active phenoloxidase. One starts with whole *Calliphora* larvae homogenates, in which the enzyme is partly soluble but mostly bound to subcellular structures (mitochondria and microsomes). It shows high mono- and diphenoloxidase activity. Another method of obtaining the enzyme is to activate a highly purified preenzyme with a highly purified activator preparation *in vitro*. The enzyme so prepared is soluble and shows only diphenoloxidase activity, monophenols being hardly hydroxylated by it at all. If the activation process observed *in vitro* takes place in the presence of fat-body mitochondria, part of the activated enzyme is adsorbed on the mitochondria. Such preparations show both activities. It seems that the tertiary structure of the enzyme as well as some mitochondrial metabolites play a role in the substrate specificity. Lysis of the mitochondria with deoxycholate or by freezing and thawing reduces both mono- and diphenoloxidase activities, the hydroxylation reaction being much more sensitive.

The enzyme acts only on amines, amides and amino-acids (3). Acidic compounds are not oxidized by the enzyme. The highest activity is shown toward dopamine and N-acetyldopamine, which are the natural substrates. The enzyme (both mono- and diphenoloxidase activities) is inhibited by cyanide, phenyl-

thiourea, diethyldithiocarbamate and semicarbazide. Phenolcarboxylic acids inhibit the reaction of monophenol to melanin at certain steps, with accumulation of intermediary metabolites (18).

Dopa-decarboxylase

The dopa-decarboxylase from blowfly larvae was purified about 350-fold (17). It is a pyridoxal phosphate dependent enzyme. Dialysis reduces its activity to 50 % and Sephadex chromatography to 0 to 30 %. Addition of pyridoxalphosphate partially restores the activity. Enzyme activity is fully restored by addition of Fe^{++} ions. Mn, Mg or Zn ions have no effect. Dopa and to a lesser extent 5-hydroxytryptophan as well as dihydroxytryptophan are decarboxylated. Tyrosine, phenylalanine, histidine and tryptophan are not transformed at all. The enzyme is inhibited by a variety of metabolites as shown in table 1. Some amines show slight inhibition; a potent inhibitor is N-acetyldopamine, which in *Calliphora* is an end-product of the metabolic chain tyrosine to N-acetyldopamine. Perhaps we are dealing with a feed-back inhibition. This inhibition has also been observed *in vivo* in *Calliphora* (12).

Dopamine-β-oxidase and catechol-methyltransferase

Incubation of dopamine with *Tenebrio* extracts showed the presence of a substance having the same chromatographic properties as NE. No studies have been made on these enzymes.

TABLE 1

Inhibition of dopa-decarboxylase

Inhibitor	Concentration	Inhibition
	Mol/l	%
KCN	1.66×10^{-3}	37
p-Chloromercuribenzoate	3.3×10^{-3}	75
EDTA	3.3×10^{-3}	20
Semicarbazide	3.3×10^{-3}	40
Thiosemicarbazide	3.3×10^{-3}	50
Hydroxylamine	3.3×10^{-3}	100
Dopamine	2.08×10^{-3}	5
N-acetyldopamine	1.7×10^{-3}	35
Adrenaline	1.82×10^{-3}	6
Noradrenaline	1.69×10^{-3}	19
Tryptamine	2.1×10^{-3}	25
Serotonin	1.91×10^{-3}	28.5
Histamine	3.3×10^{-3}	19
N-Acetyl-dopa	1.7×10^{-3}	24
α-Methyl-dopa	1.7×10^{-3}	24

From Hoppe Seyl. Z. 332: 70, 1965.

Experimental conditions: 66 mU whole *Calliphora* homogenate, substrate: dopa, radiochemical test.

Transacetylase

This enzyme has a wide distribution in different insect species. It has been detected in *Calliphora*, *Tenebrio*, *Drosophila* and *Schistocerca* (2, 21). It catalyses the transfer of acetyl from acetyl-CoA to dopamine as well as to tyramine and histamine.

CONTROL OF ENZYMES INVOLVED IN CATECHOLAMINE METABOLISM

A direct hormonal control of two enzymes involved in catecholamine metabolism in *Calliphora* has been demonstrated. The molting hormone of the insects, ecdysone, has been shown to induce the *de novo* synthesis of dopa-decarboxylase in the epidermis of the larvae which is about to pupate (6, 23). The hormone stimulates nuclear RNA synthesis and the production of a specific messenger RNA controlling the formation of the dopa-decarboxylase (24). The system ecdysone-epidermis has proved a valuable tool for the study of the mechanisms of hormone action (16).

The second enzyme controlled by ecdysone is the activator protein (13). Although there is some evidence pointing to a similar type of control as for the decarboxylase, due to the complexity of the phenoloxidase system the exact nature of ecdysone action in this case (4) is still unsolved.

Another type of control is metabolite control of some enzymes, especially of the phenoloxidase system and the decarboxylase. As mentioned, phenolcarboxylic acids inhibit the phenoloxidase. Such acids, products of the catabolic pathway of tyrosine, are found mainly in early third stage larvae at a time when the phenoloxidase activity is low. Switching off tyrosine metabolism from catabolism to decarboxylation by ecdysone leads to a decrease in the concentration of carboxylic acids. As a consequence mono- and diphenoloxidase activity of the phenyloxidase system are enhanced. Control of the decarboxylase by N-acetyldopamine has been discussed above.

COMPARISON OF CATECHOLAMINE BIOSYNTHESIS IN INSECTS AND IN MAMMALS

Although the general scheme of metabolism of catecholamines is the same in insects as in mammals there are some striking differences in individual enzymatic reactions. The hydroxylation of tyrosine to dopa is catalysed by an enzyme having different properties: the insect enzyme is a phenoloxidase which further transforms the formed dopa to melanin. In contrast to the insect enzyme, the tyrosine hydroxylase of the mammalian brain shows no diphenoloxidase activity and is insensitive to thiourea and diethyldithiocarbamate (9). The second main enzyme, dopa-decarboxylase, shows a greater similarity to its mammalian counterpart. It is a pyridoxal phosphate dependent enzyme although the degree of binding of the coenzyme to the apo-enzyme is somewhat different. The mammalian enzyme does not need Fe^{++} ions in contrast to the insect decarboxylase. Another difference is the substrate specificity. For the insect enzyme this is confined only to dopa and mono- and dihydroxytryptophan. On the other hand, the mammalian enzyme can decarboxylate, among other substances, tyrosine and phenylalanine (8).

Another enzyme, which, comparatively speaking, may prove to be important is the transacetylase, which, as mentioned, has a wide distribution in insects. This enzyme has been detected also in mammalian liver and adrenals (20). It is responsible for the formation of N-acetyldopamine and N-acetylnoradrenaline, which have lately been detected in human urine (11, 16). These metabolites are pharmacologically much less active than dopamine and NE so that the transacetylase, parallel to the transmethylase, should play an important role in the inactivation of the catecholamines. The presence of the transacetylase in the adrenals and the inhibitory action of N-acetyldopamine on dopa-decarboxylase point to a possible regulatory role of N-acetyldopamine on the biosynthesis of catecholamines in the adrenals.

REFERENCES

1. Herrlich, P. und Sekeris, C. E.: Identifizierung von N-Acetyl-noradrenalin im Urin eines Patienten mit Neuroblastom. Hoppe-Seyl. Z. **339**: 249–250, 1964.
2. Karlson, P. und Ammon, H.: Zum Tyrosinstoffwechsel der Insekten. XI. Biogenese und Schicksal der Acetylgruppe des N-Acetyl-dopamins. Hoppe-Seyl. Z. **330**: 161–168, 1963.
3. Karlson, P. und Liebau, H.: Zum Tyrosinstoffwechsel der Insekten. V. Reindarstellung, Kristallisation und Substratspezifität der o-Diphenoloxydase aus *Calliphora erythrocephala*. Hoppe-Seyl. Z. **326**: 135–143, 1961.
4. Karlson, P., Mergenhagen, D. und Sekeris, C. E.: Zum Tyrosinstoffwechsel der Insekten. XV. Weitere Untersuchungen über das o-Diphenoloxydase System von *Calliphora erythrocephala*. Hoppe-Seyl. Z. **338**: 42–49, 1964.
5. Karlson, P. und Schweiger, A.: Zum Tyrosinstoffwechsel der Insekten. IV. Das Phenoloxydase System von *Calliphora erythrocephala* und seine Beeinflussung durch das Hormon Ecdyson. Hoppe-Seyl. Z. **323**: 199–210, 1961.
6. Karlson, P. und Sekeris, C. E.: Zum Tyrosinstoffwechsel der Insekten. IX. Kontrolle des Tyrosinstoffwechsels durch Ecdyson. Biochim. biophys. Acta **63**: 489–495, 1962.
7. Karlson, P., Sekeris, C. E. und Sekeri, K.: Zum Tyrosinstoffwechsel der Insekten. VI. Identifizierung von N-Acetyl-3,4-dihydroxy-β-phenäthylamin (N-Acetyl-dopamin) als Tyrosinmetabolit. Hoppe-Seyl. Z. **327**: 86–94, 1962.
8. Lovenberg, W., Weissbach, H. and Udenfriend, S.: Aromatic L-amino-acid-decarboxylase. J. biol. Chem. **237**: 89–93, 1962.
9. Nagatsu, T., Levitt, M. and Udenfriend, S.: Conversion of L-tyrosine to 3,4-dihydroxyphenylalanine by cell-free preparations of brain and sympathetically innervated tissues. Biochem. biophys. Res. Comm. **14**: 543–549, 1964.
10. Oestlund, E.: The distribution of catecholamine in lower animals and their effects on the heart. Acta physiol. scand. **112** (suppl. 31): 1–67, 1954.
11. Pryor, M. G. M.: Sclerotization. In: Comparative Biochemistry, ed. by M. Florkin and H. S. Mason, Vol. IV, pp. 371–394, Academic Press, Inc., New York, 1964.
12. Schlörer, J.: Unpublished experiments.
13. Schweiger, A. und Karlson, P.: Zum Tyrosinstoffwechsel der Insekten. X. Die Aktivierung der Präphenoloxydase und das Aktivator-Enzym. Hoppe-Seyl. Z. **329**: 210–221, 1962.
14. Sekeris, C. E.: Zum Tyrosinstoffwechsel der Insekten. XII. Reinigung, Eigenschaften und Substratspezifität der Dopa-decarboxylase. Hoppe-Seyl. Z. **332**: 70–78, 1963.
15. Sekeris, C. E.: Sclerotization in the blowfly imago. Science **144**: 419–420, 1964.
16. Sekeris, C. E.: Action of ecdysone on the RNA and protein metabolism of the *Calliphora erythrocephala*. In: Mechanisms of Hormone Action, ed. by P. Karlson, pp. 149–167, Academic Press, Inc., New York and Springer Verlag, Berlin, 1965.
17. Sekeris, C. E.: Unpublished experiments.
18. Sekeris, C. E.: In preparation.
19. Sekeris, C. E. und Herrlich, P.: Identifizierung von N-Acetyldopamin im Urin eines Patienten mit malignem Phaeochromocytom. Hoppe-Seyl. Z. **331**: 289–291, 1963.
20. Sekeris, C. E. und Herrlich, P.: Vorkommen von Arylamin-Transacetylase im Säugetierorganismus. Hoppe-Seyl. Z. **336**: 130–131, 1964.
21. Sekeris, C. E. and Herrlich, P.: In preparation.
22. Sekeris, C. E. und Karlson, P.: Zum Tyrosinstoffwechsel der Insekten. VII. Der katabolische Abbau des Tyrosins und die Biogenese der Sklerotisierungssubstanz, N-Acetyl-dopamin. Biochim. biophys. Acta **62**: 103–113, 1962.
23. Sekeris, C. E. and Karlson, P.: On the mechanisms of hormone action. II. Ecdysone and protein biosynthesis. Arch. Biochem. Biophys. **105**: 483–487, 1964.
24. Sekeris, C. E., Lang, N. und Karlson, P.: Zum Wirkungsmechanismus der Hormone. V. Einfluß von Ecdyson auf den RNA-Stoffwechsel der Epidermis der Schmeißfliege, *Calliphora erythrocephala*. Hoppe-Seyl. Z. In print.
25. Sekeris, C. E. and Mergenhagen, D.: Phenoloxidase system of the blowfly, *Calliphora erythrocephala*. Science **145**: 68–69, 1964.