3,4-Dihydroxyphenylalanine (Dopa) Decarboxylase Activity in the Arthropod Nervous System

By L. L. MURDOCK, R. A. WIRTZ and G. KÖHLER
Fachbereich Biologie, Universität Konstanz, D 7750 Konstanz, Germany

(Received 31 August 1972)

1. When homogenates of brains from mature adult locusts (*Locusta migratoria*) were incubated with L-3-(3,4-dihydroxyphenyl)[3-¹⁴C]alanine the major radioactive metabolite was dopamine, suggesting the presence of a dopa (3,4-dihydroxyphenylalanine) decarboxylase. 2. Decarboxylation of L-dopa by this tissue, measured under optimum conditions by a radiochemical method, was 21 μmol of CO_2/h per g wet wt. Apparent decarboxylation of L-tyrosine proceeded at 0.34 μmol of CO_2/h per g wet wt. There was no detectable decarboxylation of L-tryptophan, L-histidine or L-phenylalanine. 3. Dopa decarboxylase activity was found in all major regions of the ventral nerve cord of the mature locust (range: 4–7 μmol of CO_2/h per g wet wt.) but was low or absent in thoracic peripheral nerve. 4. Marked decarboxylation of L-dopa was found in homogenates of brains of four other species of insects, and in brain and ventral nerve cord, but not in the claw nerve, of the crayfish. 5. The activity of the locust brain enzyme may be slightly lower at the time of imaginal ecdysis than during the mature period. By contrast, the dopa decarboxylase that produces dopamine as an intermediate in cuticle biosynthesis is known to be high in activity at the time of ecdysis and low in activity during the intermolt stages.

Catecholamines may have a physiological role in the nervous system of arthropods (see Murdock, 1971*b*). Catecholamine-containing neurons have been identified by the histochemical fluorescence method (Elofsson *et al.*, 1966; Frontali, 1968; Klemm, 1968, 1971). Quantitative analysis of insect nerve structures reveals the presence of dopamine [2-(3,4-dihydroxyphenyl)ethylamine] and, in lesser quantities, noradrenaline (Frontali & Häggendal, 1969; Klemm & Björklund, 1971). Catecholamines show pharmacological activity when tested on various arthropod preparations such as crayfish gut (Florey, 1961), the light organ of fireflies (Smalley, 1965) and the cockroach heart (Miller, 1968). Iontophoretic injection of dopamine into the neuropil of the ant brain causes inhibition of spontaneously firing neurons (Steiner & Pieri, 1969).

Studies on the nature and function of catecholamines in the arthropod nervous system are complicated by the fact that these compounds also have a major functional role outside the nervous system; they serve as material in the biosynthesis of the cuticle. To differentiate catecholamines and related enzymes in the nervous system from those involved in cuticular biosynthesis, it is necessary to study animals in a known moulting stage and to analyse defined neural structures.

A large gap in our knowledge of catecholamines in the nervous system of arthropods concerns the pathways of their biosynthesis and destruction.

Decarboxylation of aromatic amino acids in insects has occasionally been examined (Sekeris, 1963; Marmaras *et al.*, 1966; Colhoun, 1963), but quantitative values for given tissues have apparently not yet been determined. As a first step towards characterizing the enzymes involved in the biosynthesis and destruction of catecholamines in the arthropod nervous system, we have surveyed the dopa (3,4-dihydroxyphenylalanine) decarboxylase activity of major neural structures of the locust. Nerve tissues from four other insects and a crayfish were also examined. A related goal was to learn if the activity of neural dopa decarboxylase is different in freshly ecdysed and mature adult insects. If the primary function of the enzyme in the nervous system is biosynthesis of catecholamines with specific neural functions, its activity would not be expected to vary much with the moult cycle (Murdock, 1971*b*). In this respect the neural enzyme would differ from the dopa decarboxylase activity associated with cuticular biosynthesis, which increases at the time of ecdysis and is low at other times (Shaaya & Sekeris, 1965; Karlson & Herrlich, 1965).

Materials and Methods

Animals

Specimens of *Locusta migratoria* were reared in our laboratory as recommended by Hunter-Jones (1966). 'Mature' locusts were those that had passed the

imaginal (to adult) ecdysis 3 or more weeks before the experiment. The *Schistocerca gregaria* were a gift of Dr. N. Klemm, Institut für Angewandte Zoologie Würzburg, Germany. The cricket, *Gryllus campestris*, the honey bee, *Apis mellifera*, and the bumble bee, *Bombus lapidarius*, were collected in the field. Specimens of the crayfish, *Astacus astacus*, were purchased from commercial suppliers and maintained as described by Murdock (1971a).

Reagents

L-3-(3,4-Dihydroxyphenyl)[1-¹⁴C]alanine (L-[1-¹⁴C]dopa), L-3-(3,4-dihydroxyphenyl)[3-¹⁴C]alanine (L-[3-¹⁴C]dopa) and L-3-phenyl[1-¹⁴C]alanine were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. L-[1-¹⁴C]Tryptophan (side-chain label), L-[carboxy-¹⁴C]histidine, L-[1-¹⁴C]tyrosine (side-chain label) and NaH¹⁴CO₃ were supplied by New England Nuclear G.m.b.H., Frankfurt/Main, Germany. Stock solutions of L-[1-¹⁴C]dopa in double-distilled water containing 2% (v/v) ethanol were prepared in 10 μ Ci portions, which were used within 1 week. No obvious radiochemical impurity was detected by chromatography and scanning during this time, nor were there usually any increases in spontaneous release of ¹⁴CO₂. L-[1-¹⁴C]Tyrosine is known to contain L-[1-¹⁴C]dopa as an impurity (Nagatsu *et al.*, 1964), so it was necessary to purify the stock material. This was conveniently done by subjecting 10 μ Ci portions to one-dimensional chromatography on Whatman no. 3MM paper with propan-1-ol-formic acid-water (20:1:5, by vol.), a solvent system that clearly separates dopa from tyrosine. The paper was first repeatedly washed in the solvent system and dried in air. After separation, the tyrosine peak was located by scanning, eluted off with 0.1% (v/v) formic acid, dried *in vacuo* and then dissolved in a 1 mM solution of non-radioactive tyrosine. The L-phenyl[1-¹⁴C]-alanine was purified in the same way. L-[1-¹⁴C]-Tryptophan and L-[1-¹⁴C]histidine were used without further purification. Working stock solutions were diluted with the non-radioactive amino acid to the desired specific radioactivity, which was checked by ninhydrin assay (Blackburn, 1968) and liquid-scintillation counting (Packard Tri-Carb, Zürich, Switzerland) at known counting efficiency (measured with internal standards).

Identification of dopamine as a metabolite of dopa

A portion (50 μ l) of a *Locusta* brain homogenate (five brains homogenized in 100 μ l of 0.32M-sucrose) was incubated for 1 h at 37°C together with 20 μ l of L-[3-¹⁴C]dopa (48 nmol, 1 μ Ci) and 30 μ l of 200 mM-Tris-HCl buffer, pH 6.8 (containing 0.01%, w/v, reduced ascorbic acid). Control experiments were carried out with heated homogenates (5 min at 95°C).

The reaction was stopped by adding 400 μ l of 95% (v/v) ethanol containing 0.1% (w/v) reduced ascorbic acid. After the mixture had been left for 30 min in ice, it was centrifuged (4000g, 10 min, room temperature) and the supernatant taken for chromatography. Portions (each 2 μ g) of non-radioactive dopa and dopamine were spotted on thin layers of cellulose (Avicel, 250 μ m thickness; Merck, Darmstadt, Germany) together with 10 μ l of the supernatant. The solvent systems were: (1) phenol-formic acid-water (75:1:25, w/v/v), saturated with CO₂; (2) methanol-butan-1-ol-benzene-water (4:3:2:1, by vol.) (Schneider & Gillis, 1965); (3) acetone-2-methylpropan-2-ol-formic acid-water (180:180:1:39, by vol.) (Schneider & Gillis, 1965). One- and two-dimensional (system 1 and then system 2; or system 2 and then system 3) runs were made. After separation, the positions of the standards were located by spraying with the potassium ferrocyanide-formaldehyde reagent (Dawson *et al.*, 1969) and heating at 100°C for 5 min. One- or two-dimensional scans were made with the Dünnschicht-Scanner (Berthold, Waldbad, Germany) to determine the distribution of radioactivity.

Dopa decarboxylase activity

The dopa decarboxylase assay was essentially the radioassay method of Tait (1970), but with a smaller incubation volume of 100 μ l. The use of such a relatively small incubation volume carried with it the danger that significant evaporation would occur during the incubation, leading to concentration of the reaction mixture. This was evaluated by incubating 100 μ l samples of the L-[1-¹⁴C]dopa stock in closed vials, removing 5 μ l samples at 10 or 20 min and comparing the counts obtained with those in equal portions of the unincubated stock solution. In this way the error introduced after 20 min incubation was found to be only about 4%.

Nerve tissues were dissected out, weighed, and homogenized in all-glass homogenizers with 50 μ l of 0.32M-sucrose/mg wet wt. of tissue. Because of the limited quantity of tissue available, this homogenate was used for all assays. Care was taken to disperse the suspension before removing samples for assay. The standard assay mixture was composed of 20 μ l each of 200 mM-Tris-HCl, pH 6.9, 1 mM-pyridoxal 5'-phosphate, 5 mM-L-[1-¹⁴C]dopa and water. After 10 min incubation of the assay mixture at 37°C, 20 μ l of the homogenate was added to start the reaction. It was stopped after 10 min by adding 100 μ l of 1M-H₂SO₄. The vial was immediately capped with a rubber stopper, from which hung a 1.5 cm $\times$ 1.0 cm glass-fibre paper that had been wetted with 20 μ l of 10% (w/v) KOH. The ¹⁴CO₂ was absorbed for 30 min, and then the KOH-soaked paper was removed, dried in a warm air stream and added to a scintillation vial

containing 5 ml of scintillation mixture (Bray, 1960). After standing for at least 10h in the cold (3°C), the samples were counted for 10min in a Packard Tri-Carb liquid-scintillation spectrometer (Packard, Zürich, Switzerland). Control $^{14}\text{CO}_2$ values were determined by substituting sucrose for the nerve-tissue homogenate. The overall efficiency of $^{14}\text{CO}_2$ trapping and counting was checked by taking samples of $\text{NaH}^{14}\text{CO}_3$ through the procedure; it was $49.6 \pm 1.4\%$ (S.D.; $n = 15$).

High and erratic 'spontaneous' $^{14}\text{CO}_2$ production from L-[1- ^{14}C]dopa was found when the pH was above 8. For this reason no assays with L-dopa as substrate could be made above pH8. Near pH7 the background radioactivity was usually approx. 100c.p.m.

In initial experiments we found that boiled enzyme preparations gave slightly lower blanks than did blanks with no enzyme (sucrose only). The reason for this was unclear. Sucrose blanks were always used.

Decarboxylation of other aromatic amino acids

Decarboxylation of the other aromatic amino acids was assayed at several pH values in the range pH 6–9 at a final substrate concentration of 1 mM, with the exception of L-tyrosine, which was assayed at 0.2 or 0.4 mM. We observed no pH- or pyridoxal phosphate-dependent spontaneous decarboxylation with these substrates.

Results

Biosynthesis of dopamine from L-dopa by locust brain homogenates

When L-[3- ^{14}C]dopa was incubated with homogenates of brains from mature adult *Locusta migratoria* for 1h, a major radioactive metabolite was produced, which was separable from dopa by one- or two-dimensional t.l.c. In all chromatographic systems the radioactivity in this metabolite coincided with the pink spot given by dopamine after spraying with ferrocyanide-formaldehyde. This indicates that the metabolite is dopamine. Only traces of other metabolites were observed. When L-[3- ^{14}C]dopa was incubated with boiled brain homogenates, the only radioactive substance recovered was the unchanged precursor. It is noteworthy that dopamine accumulated in the absence of an inhibitor of monoamine oxidase.

The optimum pH for dopa decarboxylase (3,4-dihydroxy-L-phenylalanine carboxy-lyase; EC 4.1.1.26) activity of the mature locust brain in both the Tris-HCl and potassium phosphate buffers was in the range pH 6.5–7.0 (Fig. 1). Measurements could not be made above pH 8 because of the instability of L-dopa above this pH. Preliminary experiments (Murdock, 1971b) had indicated that the optimum is

Fig. 1. Effect of pH on *Locusta* brain dopa decarboxylase activity

Details of the assay are given in the Materials and Methods section. Buffers: ●, Tris-HCl; ○, potassium phosphate.

approx. pH 8.0. This high value, and the lower values for dopa decarboxylase activity in locust brain reported in that paper, have subsequently been found to be due to sub-optimum assay conditions. In the range pH 6.5–7.0 the activity is approximately equal in both buffers.

Locust brain dopa decarboxylase activity with L-[1- ^{14}C]dopa as substrate decreased with time. L-Dopa can form a complex with pyridoxal 5'-phosphate (Buzard & Nytech, 1959; Black & Axelrod, 1969). The decrease in rate with time may be accounted for by assuming that the L-dopa-pyridoxal 5'-phosphate complex accumulates during the course of the assay, and that it is inhibitory toward dopa decarboxylase. The formation of the inhibitory complex during the routine assays would cause an underestimation of the true dopa decarboxylase activity. However, this effect was minimized by using short incubation times. With L-tyrosine as substrate, yields of $^{14}\text{CO}_2$ increased linearly over a 60 min period.

With L-[1- ^{14}C]dopa as substrate there was a linear relationship between the reaction rate and the quantity of added brain homogenate. Values obtained

with less than $5\mu\text{l}$ of homogenate were irregular because of the inhomogeneity of the tissue brei and the low counting rates compared to background.

Effect of substrate concentration on dopa decarboxylase activity

Examination of the substrate dependence of locust brain dopa decarboxylase activity reveals typical saturation kinetics (Fig. 2). There is no apparent substrate inhibition. The Lineweaver-Burk plot shows the apparent K_m for dopa to be approx. 0.1 mM , a value somewhat lower than that found for the partially purified vertebrate enzyme (approx. 0.4 mM ;

Lovenberg *et al.*, 1962) but similar to that found in crude preparations of *Aplysia* nerve tissue (0.12 mM ; Weinreich *et al.*, 1972). The formation of a L-dopa-pyridoxal 5'-phosphate complex in the assay mixture, and the use of crude tissue homogenates, which may contain enzyme sequestered in synaptosomes or other particles, makes it difficult to know the actual concentrations of L-dopa and cofactor in the vicinity of the enzyme during the assay. For this reason the estimate of the apparent K_m is only approximate.

Effects of inhibitors and pyridoxal phosphate

It is possible that the apparent 'decarboxylase' activity could have arisen via the action of a tyrosinase, which could oxidize dopa to dopa-quinone, part of which could be spontaneously decarboxylated to form melanin. During the course of these studies several non-nervous tissues were examined. The only ones that showed a marked capability to convert L-[1- ^{14}C]dopa into $^{14}\text{CO}_2$ were the testes and the hindgut. The hindgut homogenate 'decarboxylase' activity increased severalfold on standing at 0°C , reaching maximal values after approx. 10h. Decarboxylase activity in homogenates of brains of mature locusts, by contrast, decreased very slightly ($<5\%$) under the same conditions. Homogenates frozen for 2 months at -30°C retained 90% of their original activity. It is characteristic of insect tyrosinases that full activity is attained only after an incubation period, and that activation proceeds more rapidly at low temperatures (Gilmour, 1961). A second difference between brain and hindgut was that whereas brain decarboxylase activity was completely inhibited by 1 mM -hydroxylamine hydrochloride, this inhibitor did not affect the fully developed hindgut activity. Further, 2 mM -thiourea, a known tyrosinase inhibitor, failed to inhibit brain dopa decarboxylase activity but decreased hindgut dopa 'decarboxylation' by 60–80%. Another difference between brain and hindgut 'decarboxylase' activity was that black particles, presumably melanin, were found to accumulate in assay vials for the hindgut enzyme, but were not observed during assays of the brain enzyme.

When O_2 was excluded by carrying out the reaction under N_2 , the dopa decarboxylase activity in brain homogenates was the same as in the presence of air (Table 1). This also indicates a decarboxylase rather than a tyrosinase activity, which requires oxygen.

Omission of pyridoxal phosphate from the standard reaction mixture resulted in a slight but significant decrease in activity ($P < 0.02$; Student's t test). Apparent coenzyme-independence is not unusual for dopa decarboxylase; it was found with rat liver extracts (Awapara *et al.*, 1962) and with pig kidney preparations (Clark *et al.*, 1954). Presumably there is sufficient pyridoxal present in the crude homogenate to support nearly full activity.

Fig. 2. Dependence of dopa decarboxylase activity of brain homogenates from mature *Locusta* on substrate concentration

(a) Velocity versus substrate concentration; (b) Lineweaver-Burk plot of the same results (v is expressed in c.p.m.). The apparent K_m for L-dopa is approx. 0.1 mM . Standard conditions were used, as described in the Materials and Methods section.

Table 1. *Effect of DL- α -methyldopa and pyridoxal 5'-phosphate on dopa decarboxylation by homogenates of mature adult Locusta brain*

The standard assay conditions, the preparation of nerve-tissue homogenates and the age of mature adult locusts were as described in the Materials and Methods section. DL- α -Methyldopa or hydroxylamine hydrochloride was added to make a final concentration of 1 mM. Rates are expressed in μmol of CO_2/h per g wet wt. of tissue as means $\pm$ S.D. with the number of observations in parentheses.

Assay conditions	Dopa decarboxylase activity (μmol of CO_2/h per g wet wt.)
Standard (air)	20.5 ± 1.9 (7)
Standard (N_2)	21.7 ± 1.7 (5)
Standard minus pyridoxal 5'-phosphate	17.3 ± 1.8 (5)
Standard plus 1 mM-DL- α -methyldopa	12.4 ± 1.1 (6)
Standard plus 1 mM-DL- α -methyldopa minus pyridoxal 5'-phosphate	1.8 ± 0.6 (5)
Standard plus 1 mM-hydroxylamine hydrochloride	0.0

Table 2. *Decarboxylation of dopa by nerve-tissue homogenates from adult Locusta migratoria*

The assay conditions, the preparation of nerve-tissue homogenates and the age of mature locusts were as described in the Materials and Methods section. 'Thoracic chain' refers to pooled ganglia and connectives of the thoracic ventral nerve cord. 'Abdominal chain' refers to pooled ganglia and connectives of the abdominal nerve cord, excluding the last abdominal ganglion. 'Peripheral nerve' refers to nerves leaving the pro-, meso- and meta-thoracic ganglia and serving muscle or sensory units in the thorax. 'Freshly ecdysed adults' were animals that had passed the imaginal (to adult) ecdysis within 6–72 h before being taken for assay. Rates are expressed in μmol of CO_2/h per g wet wt. of tissue as means $\pm$ S.D. with the numbers of observations in parentheses. The value for peripheral nerve is the average of two experiments with pooled tissues from five locusts.

Tissue	Dopa decarboxylase activity (μmol of CO_2/h per g wet wt.)
Mature adults	
Brain (minus optic lobes)	20.9 ± 2.4 (26)
Optic lobes of brain	19.4 ± 1.1 (5)
Suboesophageal ganglion	6.2 ± 0.8 (4)
Thoracic chain	5.1 ± 1.5 (4)
Abdominal chain	3.6 ± 2.1 (4)
Last abdominal ganglion	6.8 ± 1.3 (3)
Peripheral nerve	0.9
Freshly ecdysed adults	
Brain (minus optic lobes)	15.7 ± 1.3 (12)
Last abdominal ganglion	7.4 ± 0.3 (3)

DL- α -Methyldopa (1 mM) caused a marked inhibition of dopa decarboxylase activity (approx. 40%); this is similar to the inhibition caused by this substance against crude enzyme preparations from whole *Calliphora* larvae (Sekeris, 1963) and from pig kidney cortex (Sourkes, 1954). In the absence of added pyridoxal phosphate, DL- α -methyldopa causes a potent (>90%) inhibition of locust brain dopa decarboxylase (Table 1). The dopa decarboxylase in pig kidney-cortex preparations shows similar co-enzyme-dependent inhibition by DL- α -methyldopa (Sourkes, 1954). Also, the inhibition of guinea-pig kidney dopa decarboxylase by D-dopa was significantly decreased in the presence of added pyridoxal phosphate (Werle & Aures, 1959).

Distribution of dopa decarboxylase in the locust nervous system

Dopa decarboxylase activity was found in all regions of the central nervous system of the mature locust (Table 2). Activity was highest in the brain and its optic lobes. About one-fourth as much activity was found in the different regions of the ventral nerve cord. The dopa decarboxylase activity in the last abdominal ganglion was significantly higher ($P < 0.05$) than in the remainder of the abdominal chain. These observations establish that the locust central nervous system, particularly the brain and optic lobes, has a rather high concentration of dopa decarboxylase.

The enzyme activity in the last abdominal ganglion from freshly ecdysed locusts is not significantly different from that of such ganglia from mature animals. The activity in homogenates of brains from freshly ecdysed animals, however, is less than in homogenates of brains from mature locusts ($P < 0.01$).

Low dopa decarboxylase activity was found in the thoracic peripheral nerves (Table 2). Similar apparent decarboxylase activity was found in homogenates of several other tissues (Malpighian tubules, fat-body, leg muscle, foregut). Since it was not determined whether this activity was due to a true decarboxylase or to tyrosinase, the question of tissue distribution of the enzyme outside the central nervous system remains open. If there is dopa decarboxylase activity in thoracic peripheral nerve tissue, its activity is low compared with that in the central nervous system. The relatively long dissection time (approx. 1 h) needed to collect sufficient tissue for analysis would have favoured the development of tyrosinase activity.

Decarboxylation of dopa by nerve tissue from other arthropods

Homogenates of tissues of the central nervous system from a variety of insects and the crayfish, *Astacus astacus*, contain considerable dopa decarboxylase activity (Table 3). It would appear that the enzyme is particularly concentrated in brain, and that less, though considerable, activity is found in elements of the ventral nerve cord (compare *Astacus* brain with abdominal chain; also see comparable values for locusts in Table 2). The variability in activity in brain from different species may or may not reflect true differences in the concentration of the enzyme. The arbitrary use of one set of assay conditions for tissues from a variety of species is not entirely

satisfactory for comparative studies. No dopa decarboxylation by homogenates of crayfish claw nerves was detected.

Decarboxylation of other aromatic amino acids

With the exception of tyrosine, the other aromatic amino acids were not decarboxylated at measurable rates by locust brain homogenates. Under the reaction conditions used we estimate the following limits of sensitivity: L-tryptophan, $<0.70 \mu\text{mol}$ of CO_2/h per g wet wt.; L-phenylalanine, $<0.16 \mu\text{mol}$ of CO_2/h per g wet wt.; L-histidine, $<0.13 \mu\text{mol}$ of CO_2/h per g wet wt. By using longer incubation times and larger amounts of tissue per assay the sensitivity of the method could undoubtedly be increased.

With 40 min incubations and a 1:25 (w/v) locust brain homogenate at pH 8.0, the production of $^{14}\text{CO}_2$ from L-[1- ^{14}C]tyrosine proceeded at 0.34 ± 0.06 ($n = 10$) μmol of CO_2/h per g wet wt. Studies with lower tyrosine concentrations showed this to be a maximal rate. A broad pH optimum between 7.5 and 8.5 was observed with this substrate.

Discussion

A number of observations point to the presence of dopa decarboxylase in the nerve tissue of *Locusta* and other arthropods. (1) Dopamine is almost the exclusive metabolite of L-dopa in locust brain homogenates. (2) An enzyme is present that produces $^{14}\text{CO}_2$ from L-[1- ^{14}C]dopa. (3) This enzyme has a pH optimum for dopa near 7, which is usual for dopa decarboxylases from a variety of sources. (4) The reaction rate declines with time, which is commonly observed with various dopa decarboxylases. (5) A pyridoxal phosphate requirement is indicated by the

Table 3. *Decarboxylation of dopa by nerve tissues from some arthropods*

The standard assay conditions and the preparation of nerve-tissue homogenates were as described in the Materials and Methods section. 'Brain' refers to the supraoesophageal ganglion exclusive of the optic lobes. The *Astacus* 'brain' included considerable portions of the circumoesophageal connectives. 'Peripheral nerve' refers to the nerve trunk serving the claw. 'Abdominal chain' refers to the pooled ganglia and connectives of the abdominal nerve cord. All values are means of duplicate or more analyses on pooled tissues from two or more animals.

Species	Tissue	Dopa decarboxylase activity (μmol of CO_2/h per g wet wt.)
<i>Locusta migratoria</i>	Brain	21.0
<i>Schistocerca gregaria</i>	Brain	8.8
<i>Gryllus campestris</i>	Brain	4.0
<i>Apis mellifera</i>	Brain	6.3
<i>Bombus lapidarius</i>	Brain	18.0
<i>Astacus astacus</i>	Brain	11.5
	Abdominal chain	5.0
	Peripheral nerve	<0.25

experiments with hydroxylamine hydrochloride. (6) DL- α -Methyldopa inhibits the enzyme, particularly so in the absence of added pyridoxal phosphate. (7) The observed decarboxylation has no need for O₂ and is not blocked by thiourea, both observations arguing against a significant involvement of tyrosinase. (8) Of the common aromatic amino acids only L-dopa was decarboxylated at a rapid rate, although a slight decarboxylation of tyrosine may have occurred.

The observation that L-dopa is decarboxylated by an enzyme in homogenates of brains from mature adult locusts firmly establishes that there is decarboxylase activity in insects long after the last moult. This fact alone indicates that the enzyme responsible for the decarboxylation has a different function from cuticular biosynthesis. Colhoun (1967) has reported decarboxylation of L-dopa and 5-hydroxytryptophan in inter-moult housefly larvae, but these studies were done with homogenates of whole animals, so conclusions about the function of the enzyme have to be regarded as tentative. In the locust, *Schistocerca gregaria*, decarboxylase activity measured in whole insect homogenates is higher by a factor of 20 or more during the first days after the imaginal ecdysis compared with values from animals that are nearly mature (Karlson & Herrlich, 1965). This decarboxylase(s) is believed to function in the biosynthesis of dopamine and tyramine during the course of synthesis of the cuticular tanning agent, N-acetyldopamine. By contrast, we found that, compared with the mature period, during the first days after ecdysis the brain dopa decarboxylase activity decreases slightly. This relative independence of the moulting cycle indicates that, unlike the cuticle-synthesizing enzyme, the brain dopa decarboxylase may not be profoundly influenced by the moulting hormone ecdysone. A question of considerable interest is raised by these observations: are there two distinct and separate species of dopa decarboxylase in the arthropods, or is there only one enzyme, which is strongly induced by ecdysone in certain tissues such as epidermis or blood, but is largely or wholly unaffected by the hormone in tissues such as nerve?

The dopa decarboxylase activity in crude homogenates of locust brain is approx. 30-fold that in crude homogenates of pig brain, approx. 10-fold that in crude preparations of rat brain, and approx. one-tenth of that in crude homogenates of rabbit pineal (Christensen *et al.*, 1972). Locust brain can thus be considered a comparatively rich source of the enzyme.

Comparative studies with the locust and the crayfish indicate that the dopa decarboxylase is in highest concentration in brain, present in lower but significant amounts in various regions of the ventral nerve cord, and low or absent in peripheral (sensory and motor) nerve structures serving skeletal muscles and exo-

skeleton. Histochemical fluorescence studies of neural structures of arthropods have revealed catecholamine-containing structures, certain regions of brain and optic lobes being particularly rich in the compounds (Frontali, 1968; Klemm, 1968, 1971; Elofsson *et al.*, 1966; Elofsson & Klemm, 1972). Fluorescent cell bodies and neuropile have also been observed in the ventral nerve cord (Elofsson *et al.*, 1966; Plotnikova, 1967; Čech & Knoz, 1970; Klemm, 1971), although they may be less abundant there than in the brain. Catecholamine-containing cells in peripheral motor or sensory axons serving skeletal muscles and exoskeleton have not yet been reported. Thus the distribution of dopa decarboxylase in locust and crayfish neural structures parallels the distribution of catecholamines as revealed by the histochemical fluorescence method.

The dopa decarboxylase activity observed here is probably responsible for the biosynthesis of neural dopamine, which may serve as a neurotransmitter. The present results do not permit a decision about the pathway of dopamine biosynthesis. L-Dopa decarboxylation is rapid compared with the rate at which CO₂ is formed from the carboxyl group of L-tyrosine. The possibility exists that the observed 'decarboxylation' of tyrosine is actually a result of prior hydroxylation of tyrosine to dopa, either via tyrosine hydroxylase or due to a low tyrosinase activity in the brain homogenates. The alternative possibility, that tyrosine decarboxylation represents one step in the production of monophenolic amine precursors, which are themselves important in the nervous system, must also be considered.

We thank Professor Ernst Florey for support and encouragement, and Miss Shizu Hayashi for providing us with an abundant supply of locusts. The work was supported, in part, by funds from the Deutsche Forschungsgemeinschaft (Forschergruppe 138: Biologische Grenzflächen und Spezifität).

References

- Awapara, J., Sandman, R. P. & Hanly, C. (1962) *Arch. Biochem. Biophys.* **98**, 520–525
- Black, I. B. & Axelrod, J. (1969) *J. Biol. Chem.* **244**, 6124–6129
- Blackburn, S. (1968) *Amino Acid Determinations*, pp. 77–78, Marcel Dekker, New York
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Buzard, J. A. & Nytych, P. D. (1959) *J. Biol. Chem.* **234**, 884–888
- Čech, S. & Knoz, J. (1970) *Experientia* **26**, 1125–1126
- Christensen, J. G., Dairman, W. & Udenfriend, S. (1972) *Proc. Nat. Acad. Sci. U.S.A.* **69**, 343–347
- Clark, C. T., Weissbach, H. & Udenfriend, S. (1954) *J. Biol. Chem.* **210**, 139–148
- Colhoun, E. H. (1963) *Experientia* **19**, 9–10
- Colhoun, E. H. (1967) in *Insects and Physiology* (Beament, J. W. L. & Treherne, J. E., eds.), pp. 207–213, Oliver and Boyd, Edinburgh

- Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research*, 2nd edn., p. 524, Oxford University Press, London
- Elofsson, R. & Klemm, N. (1972) *Z. Zellforsch.* **133**, 475–499
- Elofsson, R., Kauri, T., Nielsen, S.-O. & Strömberg, J. O. (1966) *Z. Zellforsch.* **74**, 464–473
- Florey, E. (1961) *J. Physiol. (London)* **156**, 1–7
- Frontali, N. (1968) *J. Insect Physiol.* **14**, 881–886
- Frontali, N. & Häggendal, J. (1969) *Brain Res.* **14**, 540–542
- Gilmour, D. (1961) *Biochemistry of Insects*, p. 249, Academic Press, New York
- Hunter-Jones, P. (1966) *Anti-Locust Res. Cent. Publ.* **1**–12
- Karlson, P. & Herrlich, P. (1965) *J. Insect Physiol.* **11**, 79–89
- Klemm, N. (1968) *Z. Zellforsch.* **92**, 487–502
- Klemm, N. (1971) *Z. Zellforsch.* **117**, 537–558
- Klemm, N. & Björklund, A. (1971) *Brain Res.* **26**, 459–464
- Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962) *J. Biol. Chem.* **237**, 89–93
- Marmaras, V. J., Sekeris, C. E. & Karlson, P. (1966) *Acta Biochim. Pol.* **13**, 305–309
- Miller, T. (1968) *J. Insect Physiol.* **14**, 1713–1717
- Murdock, L. L. (1971a) *Comp. Gen. Pharmacol.* **2**, 93–98
- Murdock, L. L. (1971b) *Comp. Gen. Pharmacol.* **2**, 254–274
- Nagatsu, T., Levitt, M. & Udenfriend, S. (1964) *J. Biol. Chem.* **239**, 2910–2917
- Plotnikova, S. I. (1967) in *Symp. Neurobiology of Invertebrates* (Salanki, J., ed.), pp. 59–68, Akademiai Kiado, Budapest
- Schneider, F. H. & Gillis, C. N. (1965) *Biochem. Pharmacol.* **14**, 623–626
- Sekeris, C. E. (1963) *Hoppe-Seyler's Z. Physiol. Chem.* **332**, 70–78
- Shaaya, E. & Sekeris, C. E. (1965) *Gen. Comp. Endocrinol.* **5**, 35–39
- Smalley, K. (1965) *Comp. Biochem. Physiol.* **16**, 467–477
- Sourkes, T. L. (1954) *Arch. Biochem. Biophys.* **51**, 444–456
- Steiner, F. A. & Pieri, L. (1969) *Progr. Brain Res.* **31**, 191–199
- Tait, G. H. (1970) *Biochem. J.* **118**, 819–830
- Weinreich, D., Dewhurst, S. A. & McCaman, R. E. (1972) *J. Neurochem.* **19**, 1125–1130
- Werle, E. & Aures, D. (1959) *Hoppe-Seyler's Z. Physiol. Chem.* **316**, 45–60