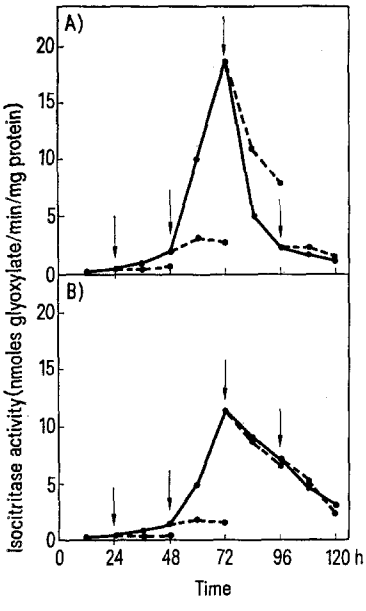
synthesized therein, on disappearence of enzyme activities from cotyledons. The aim of this investigation was to study the influence of embryo axis on isocitritase formation and subsequent disappearance from these cotyledons.

Flax seeds were surface sterilized before germination. The intact or embryo axis excised seeds were germinated at 30 °C in sterile petri dishes lined with filter paper and containing suitable quantities of water. At the time indicated, they were immersed in 10^{-3} *M* cycloheximide (Calbiochem; USA) for 8 h. Isocitritase activity in the cotyledons was determined by the combined procedures of CARPENTER and BEEVERS[7] and ROCHE et al.[8]. Protein concentration was determined as described by LOWRY et al.[9].

Flax cotyledons, either of the intact seedlings or excised from the embryo axis, develop isocitritase with activity peak on 3rd day of germination (Figure A and B). However, as compared with the intact cotyledons, the excised cotyledons develop only 50% activity, and loss of enzyme activity from these cotyledons is markedly slow. PENNER and ASHTON[3] have likewise demonstrated an inhibition in the formation and subsequent decline of protease activity in excised squash cotyledons and have attributed this to a lack of supply of a factor required for the synthesis of the protease inhibitor. As evident from the Figure, while cycloheximide prevented the development of isocitritase both in intact and excised cotyledons, suggesting de novo synthesis, decline of only the former was inhibited. Similar data has been interpreted to favour the existence of specific degrading enzyme[10] or inactivating protein[11]. Thus, the cycloheximide sensitive, rapidly turning over, specific inactivating system responsible for the accelerated decline of isocitritase, whether a degrading enzyme or inactivating protein, appears to be absent from excised cotyledons. Presumably, the slow decline of isocitritase in excised cotyledons is due to the lack of such inactivation mechanism.



Effect of cycloheximide on germination induced changes of isocitritase in intact (A) and excised flax cotyledons (B). The arrows indicate the time of cycloheximide treatment. Each value represents the mean of at least 3 separate determinations.

During germination of several oil-rich seeds, isocitritase is detectable only during conversion of fats to carbohydrates; and it is interesting to note that a specific inactivation mechanism exists for this enzyme in flax cotyledons, which seems to be regulated by embryo axis. Obviously in cells with long cell cycles, like those of the cotyledons, it is advantageous to have the ability specifically to destroy unwanted proteins in response to the environment. Hormonal regulation of protein levels via altered synthesis and inactivation has been demonstrated in animal systems[12,13], but no such data appears to be available in plants. The present study indicates that embryo axis, presumably by substances synthesized by it, influences not only the development but accelerates decline of isocitritase in cotyledons. We failed, however, to observe any effect of indoleacetic acid, gibberellic acid, benzyladenine and kinetin on decline of isocitritase, although the latter 2 compounds stimulate isocitritase development (data not given). Further studies to identify the factor(s) responsible for accelerated decline of isocitritase are in progress.

[7] W. D. CARPENTER and H. BEEVERS, Plant Physiol. *34*, 403 (1959).
[8] T. E. ROCHE, J. G. WILLIAM and B. A. MCFADDEN, Biochim. biophys. Acta *206*, 193 (1970).
[9] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[10] H. PRESSEY and R. SHAW, Plant Physiol. *41*, 1657 (1966).
[11] M. ZUCKER, Plant Physiol. *43*, 365 (1968).
[12] A. BOCTOR and A. GROSSMAN, J. biol. Chem. *245*, 6331 (1970).
[13] A. L. GOLDBERG, J. biol. Chem. *244*, 3223 (1969).

Octopamine, Dopamine and Noradrenaline Content of the Brain of the Locust, *Schistocerca gregaria*

H. A. ROBERTSON[1]

Psychiatric Research Unit, University Hospital, Saskatoon (Saskatchewan, Canada S7N OW8), 1 October 1975.

Summary. The octopamine, dopamine and noradrenaline content of the brain of the locust, *Schistocerca gregaria* has been determined using sensitive radiochemical-enzymatic assays. Octopamine and dopamine are present in high concentration but the noradrenaline content is only 1/25 that of octopamine. Both reserpine and fusaric acid (a dopamine-β-hydroxylase inhibitor) produce a significant depletion of the octopamine stores.

Dopamine has long been recognized as the major catecholamine in insects; noradrenaline is present only in small amounts[2-5]. Histochemical studies using the technique of FALCK and HILLARP have demonstrated that catecholamines and 5-hydroxytryptamine (5-HT) occur intraneuronally in the insect central nervous system[5,6]. The ultrastructural localization of catecholamines in small granular vesicles in insects has also been described[7-9]. Dopamine has been applied iontophoretically to single neurons in the insect brain where it inhibits spontaneously active neurons[10].

Octopamine, the phenolic analogue of noradrenaline, is also a major amine in the insect central nervous system[11]. It is also found in the nervous systems of various

Table I. Octopamine, dopamine and noradrenaline in the locust brain

Region	Octopamine (μg/g)	Dopamine (μg/g)	Noradrenaline (μg/g)
Brain	2.43 ± 0.45 (8) [a]	0.87 ± 0.36 (5)	0.11 ± 0.03 (3)
Optic lobes	3.91 ± 0.40 (3)	0.66 ± 0.13 (4)	—
Brain minus optic lobes	0.86 ± 0.12 (3)	0.77 ± 0.16 (4)	—

[a] Mean ± SEM. Number of determinations in parentheses.

other invertebrates including molluscs, crustaceans and annelids and, in small amounts, in mammalian nervous systems[12]. In the mollusc, *Aplysia*, single neurons with a high endogenous octopamine content have been identified[13] as well as neurons with specific octopamine receptors[14]. In the cockroach ventral nerve cord octopamine has a stimulatory effect on glycogenolysis, mediated via a cyclic AMP-induced phosphorylase activation[15,16]. Cockroach ventral nerve cord contains specific octopamine-sensitive, dopamine-sensitive and 5-HT-sensitive adenylate cyclases[17]. The function of octopamine is still unknown although it is possible that is has some role in neuromuscular transmission[18–20]. There is also good evidence that octopamine is the transmitter at the firefly lantern[21].

In this communication I report the distribution of octopamine and the catecholamines in the brain of the locust, *Schistocerca gregaria* together with the effects of reserpine and the dopamine-β-hydroxylase inhibitor, fusaric acid[22] on the levels of octopamine. The purpose of this investigation was to determine the levels of both the catecholic and phenolic amines in the brain of a single species as a basis for further work on monoamine metabolism in insects.

Adult desert locusts (*Schistocerca gregaria*) were obtained from Dr. J. E. PHILLIPS, University of British Columbia. Brains (supraoesophageal ganglia) were removed and frozen on dry ice. For octopamine determinations, individual brains were homogenized in 20 volumes of 0.02 *M* *Tris*-HCl buffer, pH 8.6 containing 1 m*M* pargyline (Abbott Laboratories), heated to 95 °C for 3 min and centrifuged 10,000 × *g* for 5 min. 50 μl aliquots were then assayed for octopamine and phenylethanolamine simultaneously using a radiochemical-enzymatic assay described elsewhere[20]. In the assay, partially purified phenylethanolamine-N-methyl transferase (Miles Laboratories) catalyzes the transfer of ^{3}H-methyl groups from ^{3}H-methyl-S-adenosylmethionine (^{3}H-SAM, New England Nuclear, S.A. 12.6 Ci/mmole) to octopamine and phenylethanolamine. 1 ng of DL-P-octopamine (Sigma) and 1 ng of phenylethanolamine were added to brain homogenates as internal standards. 50 μl of the *Tris*-HCl buffer solution was processed in parallel as a blank. The authenticity of the N-^{3}H-methyl-octopamine (^{3}H-synephrine) formed in the assay was checked by chromatographic separation in 5 different solvent systems on thin layers of silica gel (Eastman Kodak, No.6061) as described previously[20]. Phenylethanolamine was not detected, thus confirming earlier observations in which dansyl derivatives of phenylethanolamine were purified by TLC followed by identification in a high resolution mass spectrometer (ROBERTSON, PHILIPS, WU and DYCK, unpublished observations). The sensitivity of the enzyme assay (twice blank) as used was 2.5 ng/g tissue for octopamine and 10 ng/g for phenylethanolamine.

Dopamine and noradrenaline were assayed using the catechol-*o*-methyl transferase method described by CUELLO, HILEY and IVERSEN[23]. Brain tissue from 4 insects was homogenized in 5 or 10 volumes of cold 0.1 *N* perchloric acid and centrifuged at 10,000 × *g* for 5 min. 10 μl aliquots of the supernatant were then assayed for dopamine and noradrenaline using catechol-*o*-methyl transferase (Miles Laboratories) to catalyze the transfer of ^{3}H-labeled methyl groups from ^{3}H-SAM to dopamine and noradrenaline. The 3-^{3}H-methoxytyramine and ^{3}H-normetanephrine formed were separated by paper chromatography. The sensitivity of the assay was 30 ng/g for dopamine and 50 ng/g for noradrenaline.

Table II. Effect of reserpine and fusaric acid on octopamine levels in the locust central nervous system

Octopamine (μg/g ± SEM, N)		
Control	Reserpine [a]	Fusaric acid [b]
1.82 ± 0.02 (3)	0.26 ± 0.07 (4)	0.44 ± 0.07 (4)

[a] After 7 days, with injections of 30 μg/g Serpasil (Ciba) on days 1 and 3. Controls received 30 μl of distilled water. [b] 18 h after injection of 100 μg/g fusaric acid in 30 μl of distilled water. Injections were made into the ventral abdominal haemocoel using a micrometer syringe (Gilmont Instruments Inc., Great Neck, N.Y.).

[1] The author is a M.R.C. of Canada Fellow. Thanks are due to Drs. A. A. BOULTON, A. V. JUORIO and P. H. WU for helpful discussion.

[2] E. OSTLUND, Acta physiol. scand. *31*, Suppl. 112, 1 (1954).
[3] U. S. VON EULER, Nature, Lond. *190*, 170 (1961).
[4] N. FRONTALI and J. HAGGENDAL, Brain Res. *14*, 540 (1969).
[5] N. KLEMM and S. AXELSSON, Brain Res. *57*, 389 (1973).
[6] N. FRONTALI, J. Insect Physiol. *14*, 881 (1968).
[7] G. MANCINI and N. FRONTALI, Z. Zellforsch. *103*, 341 (1970).
[8] H. A. ROBERTSON, Cell Tissue Res. *148*, 237 (1974).
[9] H. A. ROBERTSON, J. exp. Biol. *63*, 413 (1975).
[10] F. A. STEINER and L. PIERI, Progr. Brain Res. *31*, 191 (1969).
[11] H. A. ROBERTSON and J. E. STEELE, J. Physiol., Lond. *237*, 34P (1974).
[12] H. A. ROBERTSON and A. V. JUORIO, Int. Rev. Neurobiol. *19*, in press (1977).
[13] J. M. SAAVEDRA, M. J. BROWNSTEIN, D. O. CARPENTER and J. AXELROD, Science *185*, 364 (1974).
[14] D. O. CARPENTER and G. L. GAUBATZ, Nature, Lond. *252*, 483 (1974).
[15] H. A. ROBERTSON and J. E. STEELE, J. Neurochem. *19*, 1603 (1972).
[16] H. A. ROBERTSON and J. E. STEELE, Insect Biochem. *3*, 53 (1973).
[17] J. A. NATHANSON and P. GREENGARD, Science *180*, 308 (1973).
[18] P. D. EVANS, B. R. TALAMO and E. A. KRAVITZ, Brain Res. *90*, 340 (1975).
[19] G. HOYLE, J. exp. Zool. *193*, 425 (1975).
[20] H. A. ROBERTSON, Experientia *31*, 1006 (1975).
[21] H. A. ROBERTSON and A. D. CARLSON, J. exp. Zool., *195*, 159 (1976).
[22] T. NAGATSU, H. HIDAKA, H. KUZUYA, K. TAKEYA, H. UMEZAWA, T. TAKENDRI and H. SUDA, Biochem. Pharmac. *19*, 35 (1970).
[23] A. C. CUELLO, R. HILEY and L. L. IVERSEN, J. Neurochem. *21*, 1337 (1973).

The octopamine, dopamine and noradrenaline content of locust brain and regions is shown in Table I. Octopamine and dopamine are present in substantial quantities but noradrenaline is barely detectable. The octopamine values are similar to those reported for cockroach brain[11] and the dopamine and noradrenaline values compare favourably with those obtained for locust brain using fluorimetric methods[5]. Octopamine is concentrated in the optic lobes of the brain while dopamine is more uniformly distributed between the optic lobes and the remainder of the brain. The optic lobes constitute about half the weight of a 4 mg locust brain. Table II shows the effect of reserpine (60 μg/g) and fusaric acid (100 μg/g) on the octopamine levels in the locust central nervous system (brain and ventral nerve cord). Both reserpine and the dopamine β-hydroxylase inhibitor cause a significant decrease in the level of octopamine in the nervous system. These treatments also produce a large decrease in locomotory activity. Reserpine is known to reduce dopamine content in the cockroach brain[6]. The fact that inhibition of dopamine β-hydroxylase leads to a decrease in octopamine levels adds weight to the suggestion that octopamine is synthesized from tyramine which has also been demonstrated in the locust central nervous system[24] (and ROBERTSON, PHILIPS, WU and DYCK, unpublished observations). Locust brain homogenates can decarboxylate tyrosine and DOPA (3,4-dihydroxyphenylalanine)[25] and they also exhibit tyrosine hydroxylase and dopamine β-hydroxylase activity[26]. In view of the high levels of octopamine and dopamine and the low noradrenaline content in the insect brain, it would appear that distinct catecholaminergic and octopaminergic systems are present. The octopaminergic system would be characterized by the enzymes tyrosine decarboxylase and dopamine β-hydroxylase and the dopaminergic system by the enzymes tyrosine hydroxylase and DOPA decarboxylase. Similar octopaminergic and dopaminergic systems have been proposed and largely substantiated for another arthropod, the lobster[18].

The role of octopamine in the insect central nervous system, as in other nervous systems, remains obscure. This report demonstrates that insect nervous tissue contains large amounts of both octopamine and dopamine and, at least on the basis of the very low content, relegates noradrenaline to a lesser role in the functioning of insect neuronal systems. It has been proposed that the effects of certain neurotransmitters such as octopamine, dopamine, noradrenaline and 5-HT may be mediated by cyclic AMP which is formed intraneuronally by the membrane-bound enzyme, adenylate cyclase[27]. The presence of octopamine and dopamine, together with specific octopamine- and dopamine-sensitive adenylate cyclases in the insect central nervous system suggests that these amines may fulfill a neurotransmitter role.

[24] H. A. ROBERTSON, Studies on the distribution and biosynthesis of octopamine in the insect nervous system, Abstr. Commun. 5th Int. Meet. Int. Soc. Neurochem., Barcelona (1975).
[25] L. L. MURDOCK, R. A. WIRTZ and G. KOHLER, Biochem. J. *132*, 681 (1973).
[26] C. E. SEKERIS and P. KARLSON, Pharmac. Rev. *18*, 89 (1966).
[27] P. GREENGARD, J. A. NATHANSON and J. W. KEBABIAN, in *Frontiers in Catecholamines and Research* (Eds. E. USDIN and S. SNYDER; Pergamon, New York and London 1973), p. 377.

Isolation of Sodium-Complexing Polypeptides from Mammalian Blood and Cardiac Muscle

M. J. HICKLING[1], J. A. BARCLAY[2], S. J. WHITE[3] and K. WHITE[4]

Department of Science, College of Education, Henwick Grove, Worcester WR2 6AJ (England); Department of Physiology, The University, Birmingham (England); and The Computer Centre, The University, Birmingham (England),
21 November 1975.

Summary. Two distinct polypeptides have been isolated from rat heart and ox blood. They are both found to be effective in forming complexes with sodium ions, and it is suggested that they may have a function in stabilizing sodium ion activity.

In connection with the observations of LAICO et al.[5] that tissues contain considerable amounts of small molecular weight polypeptides, the following experimental findings may be of interest.

Following the demonstration of osmotically inactive sodium ions in the perfused rat heart[6], we instituted a search for those constituents responsible for depressing the activity of sodium ions in cardiac muscle. On the basis of previous work[7,8], it was evident that it would be necessary to look beyond simple metabolic intermediates for the agent required, and we therefore sought other constituents of cardiac muscle which had the required property.

Extracts of rat heart were fractionated by passage through Sephadex columns, using a dilute aqueous solution of sodium chloride as eluant. By the use of a flame photometer in conjunction with a sodium-responsive glass electrode, fractions were located which had the property of depressing the activity of sodium ions below the level to be expected for that ionic strength.

The investigation by these techniques was extended to mammalian blood, which was found to be a more convenient source of larger quantities of sodium-complexing agent. The substances isolated from these active fractions

[1] Acknowledgments. We are indebted to Professor G. A. GILBERT, F.R.S. and to Dr. C. J. LOTE for helpful discussion, and to the Royal Society for a Grant-in-aid which in part supported the investigation.
[2] J. A. BARCLAY, present address: 302, Quinton Road, Birmingham 17, England.
[3] S. J. WHITE, Dept. of Physiology, University of Birmingham, England.
[4] K. WHITE, Computer Centre, University of Birmingham, England.
[5] M. T. LAICO, E. I. RUOSLAHTI, D. S. PAPERMASTER and W. J. DREYER, Proc. natn. Acad. Sci. *67*, 120 (1970).
[6] J. A. BARCLAY, E. H. HAMLEY and K. WHITE, Biochem. J. *88*, 14P (1963).
[7] M. J. HICKLING and K. WHITE, Biochem. J. *96*, 52P (1965).
[8] J. A. BARCLAY, M. J. HICKLING and K. WHITE, Biochem. J. *99*, 11P (1966).