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Influence of Neurohormone on the Pattern of Nitrogen Excretion in the Freshwater Prosobranch, *Viviparus bengalensis*

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Excretory products such as ammonia, urea and uric acid in different body components like foot, mantle and hepatopancreas were analysed in the freshwater prosobranch, *Viviparus bengalensis* by injecting aqueous homogenate of pleuropedal and cerebral ganglia. Ammonia and urea levels in the foot increased only slightly, increased significantly ($p<0.005$) in the mantle and decreased significantly ($p<0.005$) in the hepatopancreas.

Uric acid level was found to be increased significantly ($p<0.005$) in the foot and hepatopancreas while in the mantle there was a slight decrease in the uric acid level.

Boiled ganglionic extract did not provoke any significant change in ammonia, urea and uric acid levels.

Key Words: **Neurohormones, Nitrogen excretion, Excretory products,** *Viviparus bengalensis*

Introduction

An extensive study on nitrogen excretion in pulmonates was made by Baldwin and Neeadham (1934). Baldwin (1935) confirmed the presence of enzyme arginase in *Helix pomatia*. Bricteux Gregoire and Florkin (1962), suggested that amino acid metabolism is directed towards the synthesis of uric acid in *Helix pomatia*. Jezewska et al. (1963) have shown that xanthine and guanine form a major part of nitrogenous excreta in *Helix pomatia*. Lee and Campbell (1965) suggested that the origin of uric acid in *Otala lactea* is the same as reported for uricotelic invertebrates and vertebrates. Duerr (1967) determined the content of uric acid in pulmonates and prosobranchs. Speeg and Campbell (1968, 1969) studied problems of nitrogen excretion in pulmonates in relation to enzymes involved in the metabolism of nitrogenous substances. The available information was reviewed by Florkin (1966), Potts (1968) and by Campbell and Bishop (1970).

Srinivasa Reddy and Swami (1975) studied the uric acid hynthesis during aestivation in *Pila globosa*. Nayeemunnisa (1972) and Shylaja and Alexander (1976) advocated the involvement of neuroendocrine factors in the control of nitrogen end-products in the

amphibious snails, *Pila globosa* and *Pila virens* respectively. Recently Hanumante and co-workers (1977) reported neurohormonal influence on urea and uric acid levels in the freshwater pulmonate, *Lymnaea acuminata.*

However, there is no much information on the mechanism of regulation (nervous or hormonal) in the freshwater prosobranch, *Viviparus bengalensis.*

Material and Methods

The common freshwater snails, *Viviparus bengalensis* were collected from Godavari river, Paithan near Aurangabad. The snails after being brought to the laboratory were maintained in an aquarium which was sufficiently big to provide natural conditions. The snails were fed on algae and *Hydrilla.* The laboratory temperature was 30±1°C. The healthy adults were chosen and maintained in the laboratory for one week before sacrificing them for experimentation. The feeding was stopped 24 hr before starting the experiment.

The snails were grouped into three batches of 10 each.

Batch I: each snail was injected 0.1 ml distilled water

Batch II: each snail was injected 0.1 ml ganglionic extract

Batch III: each snail was injected 0.1 ml boiled ganglionic extract.

Injections were given at 7.00 AM by syringe digited from 0.01 to 1 ml, with the help of 26 guage hypodermic needle in the foot sinus. The central nervous system was dissected; pleural, pedal and cerebral ganglia were pooled and homogenised in a clean ice-chilled mortar and pestle in distilled water. The supernatant after centrifugation was used for injecting into the second batch of animals. The ganglionic extract was boiled for 7–8 min and the supernatant after centrifugation was used for injection into the third batch of animals.

Foot, mantle and hepatopancreas were excised 2 hr after injection, and homogenised to a particular concentration in distilled water. The homogenate was first made protein-free by adding 10% sodium tungstate and 2/3N sulphuric acid. The supernatant after centrifugation was used for the analysis of ammonia, urea and uric acid.

Ammonia was analysed by Formol titration method of Malfatti as described by Hawk et al. (1976), urea was determined by urease nesselerization method as described by Varley (1967) and uric acid was estimated by Cyanide free technique of Caraway (1963) as described by Varley (1967).

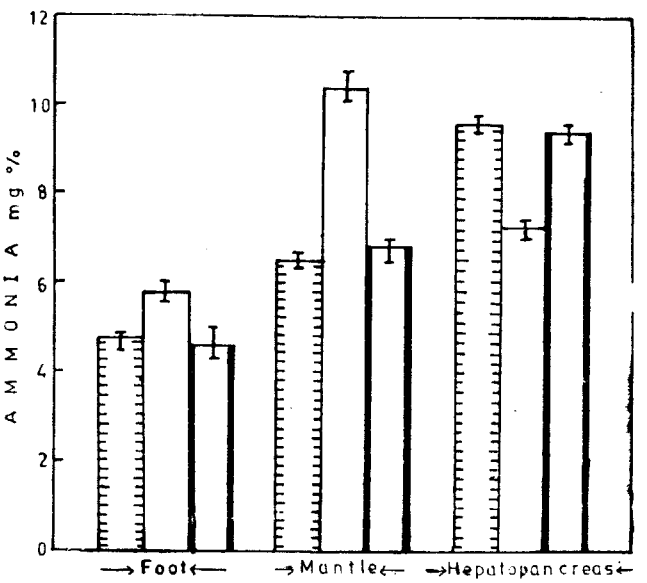

Figure 1 Ammonia content in foot, mantle and hepatopancreas of *V. bengalensis* under different experimental conditions

Control, 0.1 ml dist. water/snail

Ganglion extract 0.1 ml/snail

Boiled ganglion extract 0.1 ml/snail

Results and Discussion

The ganglionic extract injection had a pronounced influence on nitrogen excretion in *Viviparus bengalensis.* (figures 1 to 3). Pleuropedal cerebral complex provoked notable changes—2 hrs after injection—in ammonia, urea and uric acid concentrations in the tissues examined, whereas the boiled ganglionic extract produced no changes in these nitrogen end-products.

Nitrogen excretion is an adaptive character related to available water resources. All freshwater gastropods are ammonotelic (Campbell & Bishop 1970). *Viviparus bengalensis* is also ammonotelic, as the concentration of ammonia in the tissues examined is more as compared to urea and uric acid. Ammonia and urea levels in the foot were elevated only slightly but the levels of these two end-products in the mantle increased significantly. ($P<0.005$), on the contrary in the hepatopancreas the ganglionic extract injection showed significant decline in ammonia and urea ($P<0.005$). Nayeemunnisa (1972) in *Pila globosa* reported that the injection of ganglionic extract from the active animals is capable of raising the level of ammonia and lowering the level of uric acid in the mantle of acstivated animals. Normally the mantle is known to store urea and uric acid. On injecting the ganglionic extract, urea increased significantly, only uric acid showed a decrease, contrary to the expected increase.

In the hepatopancreas of the snails receiving the ganglionic extract, ammonia and urea decreased, while uric acid increased ($P<0.005$). Decrease in urea level in the hepatopancreas may be due to its mobilization to blood stream caused by neurohormonal principle affecting the urea cycle in the snails. Hanumante et al. (1978) showed in *Lymnaea acuminata* that blood urea increased following the ganglionic treatment.

Decrease in urea may also be due to low

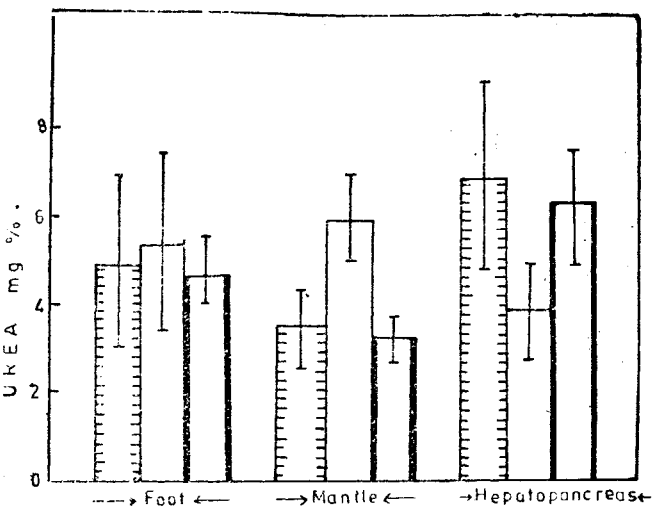

Figure 2 Urea content in foot, mantle and hepatopancreas of *V. bengalensis* under different experimental conditions.

Control, 0.1 ml dist. water/snail

Ganglion extract 0.1 ml/snail

Boiled ganglion extract 0.1 ml/snail

activity of enzyme arginase which brings about the formation of ornithine and urea from arginine. A systemic study of distribution of arginase activity was made by Gaston and Campbell (1966). The results of survey indicate that the level of arginase activity in different species and in different tissues may be extremely variable. The activity is usually higher in the hepatopancreas but not restricted to this organ only. According to Speeg and Campbell (1969), urea formed by arginase is converted to ammonia and CO_2 by urease which is present in the hepatopancreas and kidney. Nayeemunnisa (1975) demonstrated in *Pila globosa* that the level of enzyme urease may be influenced by neuroendocrine factor. In *Viviparus bengalensis* the level of enzyme arginase may be influenced by the said factor.

The increase in uric acid nitrogen is probably due to contribution from free amino

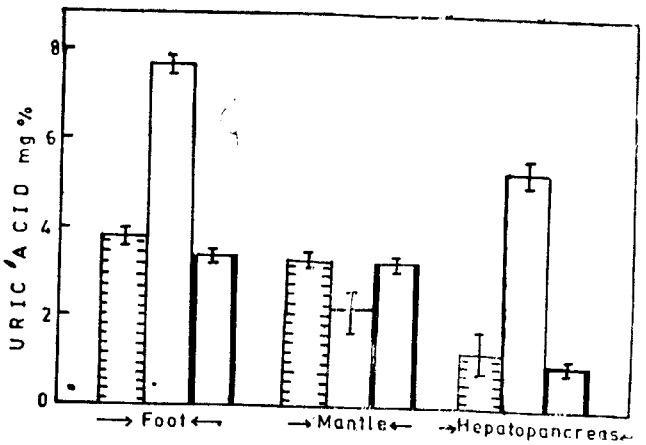

Figure 3 Uric acid content in foot, mantle and hepatopancreas of *V. bengalensis* under different experimental conditions.

Control, 0.1 ml dist. water/snail

Ganglion extract 0.1 ml/snail

Boiled ganglion extract 0.1 ml/snail

acid nitrogen, suggesting that uric acid, may be synthesised from free amino acid pool. This is in agreement with the findings of Duerr (1967) in which, several prosobranchs and pulmonates have been shown to store uric acid, which increased after injection in *Viviparus bengalensis.*

Multifold elevation in uric acid level in the hepatopancreas and foot caused by ganglionic extract injection may also be due to rapid metabolism of nucleoprotein which gives rise to purine bodies, of which uric acid is an end-product.

It is obvious that all gastropods accumulate uric acid and purines and that the amounts of uric acid in the gastropod kidney and tissues are far in excess of those expected, if the uric acid is simply reflected to general nucleic acid metabolism. Uric acid found in the freshwater gastropods may presumably be due to an incomplete breakdown of this product derived from purine metabolism. Likewise the urea reported in active forms may quite likely be formed by the action of arginase on exogenous arginine (Potts 1968). Low levels of urea and uric acid may be due to elimination of these end-products mostly by the way of ammonia (Reddy & Swami 1974). Low levels of ammonia in foot may be due to its prevalence in carbohydrate metabolism (Swami & Reddy 1975).

Boiled pleuropedal cerebral ganglionic extract failed to affect the nitrogen excretion, since the polypeptidic nature of the ganglionic extract is destroyed by heating. So it does not provoke any considerable change in the nitrogen end-products.

In the present study we injected the extract of pleuropedal and cerebral ganglia together. This ganglionic extract provoked significant alterations in ammonia, urea and uric acid levels in *Viviparus bengalensis.* This study points out that one or more ganglia of the pleuropedal cerebral complex contain hormonal principle (s) which is (are) released upon homogenisation and upon administration into the intact snails produce (s) significant alterations in the nitrogen end-products.

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References

Baldwin E 1935 Problems of nitrogen catabolism in invertebrates. IV. The synthesis of uric acid in *Helix pomatia*; *Biochem. J.* **29** 1538–1546

_____and Needhanm J 1934 Problems of nitrogen catabolism in invertebrates. I. The snail (*Helix pomatia*); *Biochem. J.* **28** 1372–1392

Bricteux-Gregoires and Florkin N 1962 Les excreta del'escargot *Helix pomatia* et leur origine metabolique; *Arch int. Physiol Biochem.* **70** 496–506

Campbell J W and Bishop S H 1970 Nitrogen metabolism in molluscs; in *Comparative Biochemistry of Nitrogen Metabolism* Vol. 1 *The Invertebrates* pp 103–206 ed. J W Campbell (New York: Academic Press)

David N, Emerson and Duerr F G 1967 Some physiological effects of starvation in the intertidal prosobranch *Littorina*; *Comp. Biochem. Physiol.* **20** 45–53

Duerr F G 1967 The uric acid content in several species of prosobranch and pulmonate snail as related to nitrogen excretion; *Comp. Biochem. Physiol.* **22** 333–340

Florkin M 1966 Nitrogen metabolism; in *Physiology of Molluscs* Vol. 2 pp 309–351 ed. J Wilbur and C Young (New York: Academic Press)

Gastrons S and Campbell J W 1966 Distribution of arginase activity in molluscs; *Comp. Biochem. Physiol.* **17** 259–270

Hawk P B, Oser B L and Summerson W H 1976 *Practical Physiological Chemistry* (New York: McGraw Hill Book Co.)

Hanumante M M, Deshpande U D and Nagabhushanam R 1978 Susceptibility of urea and uric acid levels to neurohormone influence in the freshwater pulmonate, *Lymnaea accuminata*; *Hydrobiologia* **60** 125–128

Jezewska M M, Gorzkowski Heller J 1963 Nitrogen compounds in snail, *Helix pomatia*, excretion; *Acta biochem. Polon.* **10** 55–65

Lee T W and Campbell J W 1965 Uric acid synthesis in the terrestrial snails *Otala lactea*; *Comp. Biochem. Physiol.* **15** 457–468

Nayeemunnisa 1972 Aspects of nitrogen excretion during aestivation in the amphibious snail, *Pila globosa*. I. Evidence for neuroendocrine involvement in the pattern of nitrogen excretion; *Comp. Biochem. Physiol.* **42** 1049–1052

_____1975 Aspects of nitrogen excretion during aestivation in the amphibious snail, *Pila globosa* (Swainson). Evidence for enzyme urease susceptibility to neuroendocrine influence; *J. exp. Biol.* **13** 309–311

Potts M T W 1968 Aspects of excretion in molluscs; *Symp. Zool. Soc. land.* **22** 187–192

Shylaja R and Alexander K M 1976 Physiology of excretion in the freshwater gastropod, *Pila virens*, III. Role of neurohormone in excretion; *Indian J. exp. Biol.* **14** 239–241

Speeg K V and Campbell J W 1968 Formation and reutilization of ammonia gas by terrestrial snails; *Ann. Physiol.* **214** 1390–1402

_____and Campbell J W 1969 Arginine and urea metabolism in terrestrial snails; *Ann. Physiol.* **216** 1003–1012

Srinivasa Reddy and Swami K S 1975 Uric acid synthesis during aestivation in *Pila globosa*; *Curr. Sci.* **44** 235–236

Varley H 1967 *Practical Clinical Biochemistry* 4th edn (New York: William Heinemun Medical Book Ltd. and Interscience Books Incorporation) **158** pp