Uttar Pradesh J. Zool. 22(1) : 73 - 79, 2002 ISSN 0256 - 971X

GROWTH AND MORPHODIFFERENCIATION ON THE TISSUES PROTEIN PROFILE AND PHOSPHATASES ACTIVITY IN *ACHATINA FULICA* (FERUSSAC) (GASTROPODA : PULMONATA)

D. INDRA AND K. RAMALINGAM
PG & RESEARCH DEPTT. OF ZOOLOGY, GOVT. ARTS COLLEGE,
NANDANAM, CHENNAI - 600 035, INDIA.

Achatina fulica (Ferussac), a stylommatophoran molluscan pest of agriculture and horticultural crops, has been found to be distributed ubiquitously in different parts of India (Singh, 1986). The protein characterization on its developmental profile, revealed its heterogeneous distribution in the albumin gland, digestive gland, body mass muscle, foot muscle and hemolymph. The increased concentration of protein in these tissues during reproductive and postreproductive stages revealed its synthesis. The enhanced activity of phosphatases in the reproductive and postreproductive phases attribute their role in differentiation and development. The electropherograms of immature, reproductive and postreproductive stages revealed the specific increase of high molecular weight fractions in all tissues, while an increase of both low and high molecular weight fractions in albumin gland. The heterogeneity of protein content in the tissues and the temporal changes in enzyme activity may infer the phasic expression of genetic induction and protein synthesis during growth and morphodifferentiation of the snails.

INTRODUCTION

Studies that have been made on the pulmonate land snail, *Achatina fulica* (Ferussac), pertained to their growth, synchronized with the reproductive gland maturation. Such studies on growth with reference to the shell, peristomium lip formation and the reproductive system have been carried out by several investigations (Berry & Chan, 1968; Sakae, 1968). The sequence of development and sexual maturation have also been studied under laboratory conditions (Pawson & Chase, 1984). However the tissue biochemical correlates to the development and differentiation are lacking. In the present study the tissue proteins were analysed both quantitatively and qualitatively pertaining to the development of *A. fulica* alongside with the phosphatases activity and water content.

MATERIALS AND METHODS

Specimens of *A. fulica* were obtained from the local fields and gardens during rainy months (September - December). Snails which emerged from the ground after rain were collected and kept in glass tanks (75l x 30b x 30h cm) and acclimatized to room temperature in the laboratory. The stock animals were fed with cabbage and carrot *ad libitum*, during acclimatory period. The residues of food and excreta were removed daily from the tank, so as to avoid contamination, and fresh food was given every day. The snails were categorized into three groups *viz.* Immature, mature reproductive and post reproductive (Wolda, 1970). The immature group was further divided into two categories *viz.* Immature I and Immature II on the basis of the presence or absence of protein gland (albumin gland), in the individuals. These two categories correspond to the juveniles and young adult groups defined by Wolda (1970) and Tomiyama (1991). From each category, six animals were taken and dissected. The hemolymph was collected using butterfly vein needle. The hemolymph protein was precipitated with 1N NaOH. The animals were dissected and tissues, footmuscle, body mass muscle, digestive gland and protein gland were taken for protein analysis. The foot muscle was ground in a mortar using acid washed sand and homogenized in 1N NaOH. Other tisues (100 mg) were homogenized in 1N NaOH using glass - glass homogenizer.

For the electrophoretic separation of the protein, the tissues were homogenized in double distilled water and extract was used for the electrophoresis (SDS - PAGE) (Davis, 1964). The protein fractions were read densitometrically after the PAGE - Electrophoresis in Molecular Dynamics 300A computing densitometer. The total protein in the tissues was determined by following the procedure of Lowry *et al.* (1951). Water content in the tissues was estimated by Gravimetic method (Ramalingam, 1989). The values between the different developmental stages were analyzed using one way ANOVA and the difference between the means was separated using Krushkawallis - non parametric test. A P-value of less than 0.05 was considered significant (Zar, 1974). Acid and alkaline phosphatase were determined following the procedure of Tenniswood *et al.* (1976). The phosphatase activity was expressed in µg of PNPP hydrolysed to PNP/h/100 mg net weight.

RESULTS AND DISCUSSION

Protein content : The tissue protein level in various tissues at different phases of development are given in Fig. 1. In the digestive gland the total protein content in the immature stages showed between 1923.08 µg to 2884.62 µg/100 mg wet wt. In the reproductive and postreproductive stages its level decreased insignificantly. It decreased to 2070.57 µg/100 mg wet wt. in the former and 2025.64 µg/100 mg wet wt. in the latter. In the Immature stage I, the animals showed no development of protein gland. In the Immature stage II, the mean protein concentration was found to be 798 µg/100 mg wet wt. But reproductive and postreproductive stages showed increased levels. About three fold increase was noticed in both stages. The above increases were found to be significant ($p<0.05$).

In the Immature stages I and II, the protein concentration remained at a higher level. But in reproductive and postreproductive stages it decreased significantly, when compared to immature stages ($p<0.05$).

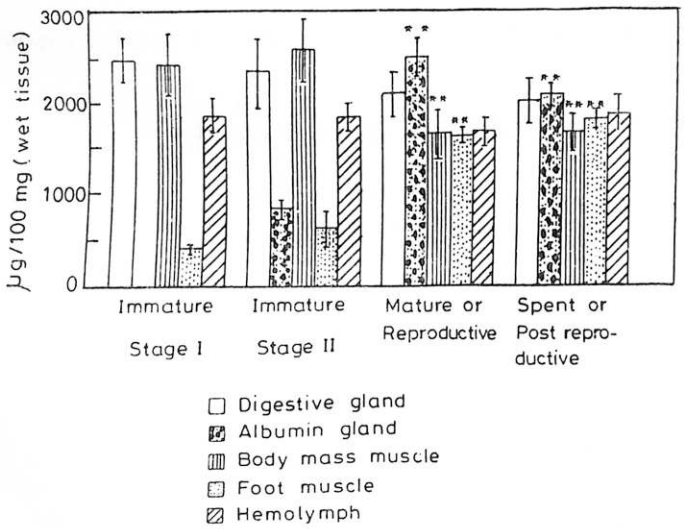

Fig. 1 : Tissue protein content in different developmental stages in *Achatina fulica.*

In the foot muscle, the total protein level was noticed in all the four stages. In the Immature stage I and II, the protein concentration remained at a lower level. But in reproductive and postreproductive stages, it showed significant increase, when compared to previous stages ($p<0.05$). The total protein level in the hemolymph remained uniform in the Immature I and II and postreproductive stages. It showed an insignificant decrease in the reproductive stage, when compared to immature stages.

Water content : The results on tissue content are given in Table I. The percentage of water content on the digestive gland, remained more or less similar in stages I, III and IV. It decreased in stage II. The percentage of water content in the albumin gland increased in stage II (60.25%) when compared to stage III and stage IV. In the latter it decreased markedly to 27.5% and 21.75% respectively. The percentage water content levels in all the stages, remained constant in the body mass muscle. Increased water content was present in foot muscle at stages I, III and IV. In stage II, it showed decrease. In stage I, the hemolymph water content remained at 51.4%. It was more or less similar in stage II and III. However it increased to 93.6%% in the stage IV after egg laying.

Table I : Tissue water content (%) in different developmental stages of *Achatina fulica.*

Tissues	Stage I	Stage II	Stage III	Stage IV
Digestive gland	56.9 ± 3.56*	44.3 ± 8.99*	56.8 ± 3.12	54 ± 5.561*
Albumin gland	Nil	63.3 ± 8.01*	27.5 ± 2.22*	21.8 ± 4.81*
Body mass muscle	70.5 ± 9.04	68.3 ± 2.61	66.1 ± 4.14	68.2 ±7.99
Foot muscle	77.5 ± 2.57*	59.3 ± 10.99*	74.04 ± 4.34*	69.8 ±2.61*
Hemolymph	51.4 ± 11.76*	48.9 ± 11.18*	45.3 ±3.26*	93.6 ±2.68*

Significant at 0.05; Mean ± S.D.

Electrophoresis : The Electropherograms of tissue proteins in different stages of *A. fulica* are given in Fig. 2. In digestive gland, ten fractions in Immature I and eleven fractions in Immature II were discernible. In reproductive stage, the extract fractionated into fourteen fractions and in the postreproductivie stage the fractions increased to nineteen. The protein gland (albumin gland) twelve fractions in Immature stage II and thirteen fractions in reproductive and postreproductive stages were discernible. In the body mass muscle, eight protein fractions in Immature stage I and seven fractions in the Immature stage II were discernible. In the reproductive stage the extract fractionated into ten and increased to thirteen in the postreproductive stage. In the foot muscle, nine fractions in the Immature stage I and eleven fractions in the Immature stage II were discernible. On the other hand thirteen and twelve fractions were discernible in the reproductive and postreproductive stages respectively. The protein in the hemolymph showed eleven fractions in the Immature stage I and thirteen fractions in the Immature stage II and reproductive stage (III). In the postreproductive stage (IV) twelve fractions were discernible.

Acid and alkaline phosphatase : The results on tissue phosphatase activity are given in Fig. 3. In the developmental stages of acid phosphatase activity in the digestive gland, albumin gland, body mass muscle, foot muscle and hemolymph showed a significant increase in both the mature and spent phases compared to that of immature phase I and II. In the body mass muscle, the acid phosphatase activity showed an insignificant decrease in both the mature and spent phases, compared to that of immature phase I and II. The alkaline phosphatase activity in the digestive gland decreased in phase II and mature phase but a significant increase in the spent phase compared to that of immature phase I. In the albumin gland, body mass muscle and hemolymph alkaline phosphate activity increased significantly in both the mature and spent phases from

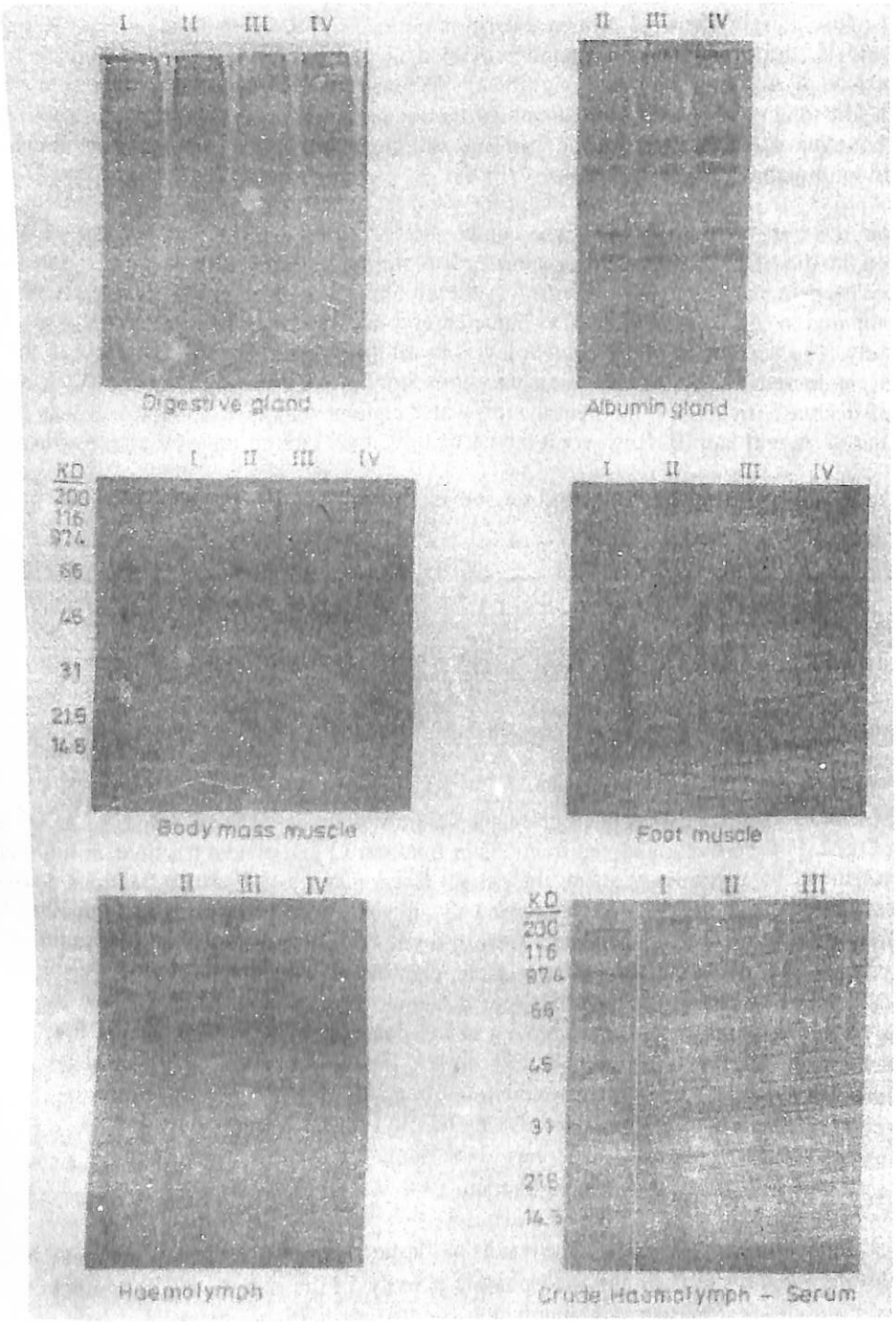

Fig. 2 : The electropherograms of protein in different developmental stages in *Achatina fulica.*

that of immature phases.

The total protein content increased significantly in the albumin gland consequent to growth and differentiation of the snails at the reproductive phase. The digestive gland showed higher

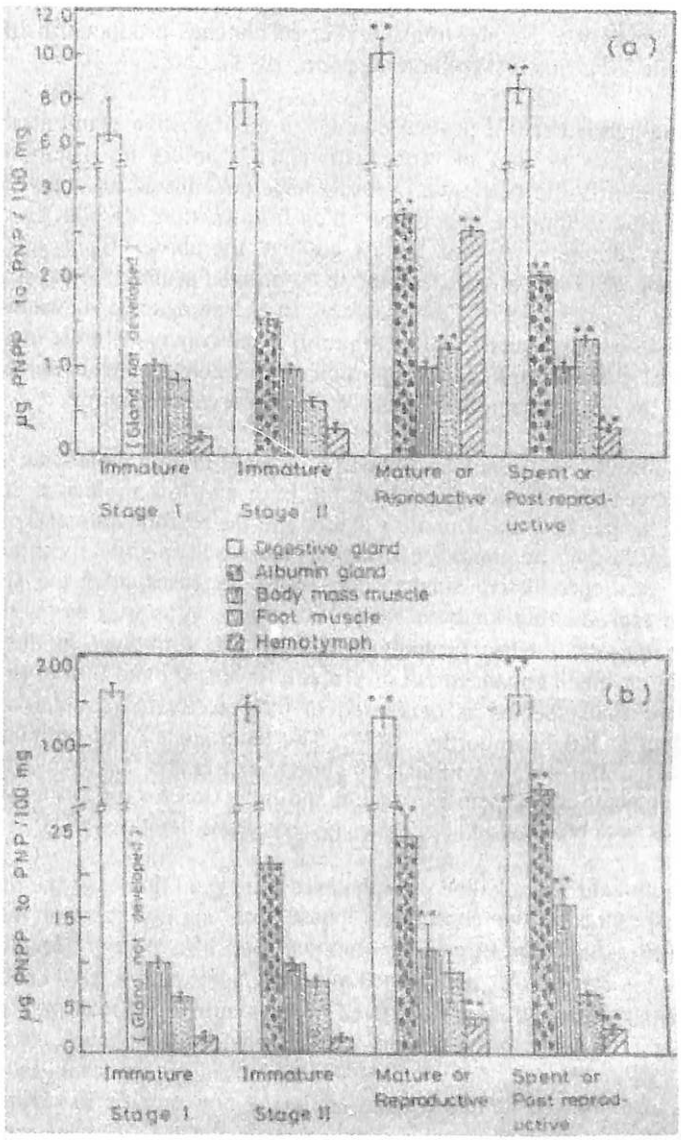

Fig. 3 : Acid and alkaline phosphatases activity in different developmental stages in *A. fulica.*

levels in the immature phase and their protein content slightly decreased in the later stages. However, their levels in both reproductive and postreproductive phases remained almost the same. In body mass muscle, it showed higher levels in the immature phases while significantly decreased after the differentiation of gonads in the reproductive phase. Its level remained the same in the postreproductive phase also. In the foot muscles, the protein remained at lower levels in the immature phases while it insignificantly increased after the maturation. The results reveal that the hemolymph protein remained uniform at all the stages of development and suggests the role of proteins in the milieu interior regulation during development. The above protein profile also reveals its heterogeneous distribution in the different tissues. The persistence of higher levels of

protein content in the albumin gland even after egg laying, reveals its importance for the future clutches, as it has been reported that the total number of clutches produced in life time of land snails, are highly variable in *A. fulica* (Wolda & Kreulen, 1973).

On the contrary the persistence of protein content in the digestive gland almost at the same level in the postreproductive as that of reproductive stage, infers its metabolic role for the inanimation period. Similarly the body mass muscle and foot muscle may have energy sparing functions for animals suspended phase of activity which follows after reproductive and egg laying in the post monsoon or summer's months. In this context, the above tissues are equipped with endogenous energy resources like phosphoarginine to be utilized at times of stress is of interest to mention (De Zwaan & Wijsman, 1976). The decrease in the percentage of water content in the reproductive and postreproductive phases in the albumin gland concur with the increased levels of protein in it. The above change implies the importance of protein synthesis during development and maturation. It also reveals concentration effect at cellular level in albumin.

The results on electropherogram reveals a specific increase in high molecular weight fractions in all the tissue. However, in albumin gland both the high and low molecular weight fractions increased. The above changes between immature stages and the reproductive and postreproductive stages reveal uniformity in that the number of fractions increased specifically in all the tissues in the reproductive and postreproductive stages. The above increases infer the solubilisation of proteins at the time of reproduction for both metabolic utilization as well as for the specific egg development. It also suggests intense proteolytic activity and turn over in the tissues during growth and differentiation. Such enhancement of proteolytic activity and turn over in tissues have been reported in active snail species as compared to their aestivated counterparts in previous studies (Brahmanandam & Krishnamoorthy, 1973). The heterogeneity of protein content in the different tissues reveal that the protein synthesis by genetic expression is phasic during growth and differentiation of the snails. Such protein expression and induction for the tissue formation by the genetic mechanism has been reported in vertebrates (Zagris & Podimatas, 1994).

The increase in both acid and alkaline phosphatases activity in tissues at the reproductive and also consequently thereupon in the consequent phase may signify its role in providing the substrates or metaboilites involved in egg development and also energy for their inanimation which follows after egg laying. As phosphated or phosphorylated sugars have already been reported in gastropod tissues and they are involved in the synthesis of macromolecules essential for egg development, the increase in the activity of non-specific phosphatases may contribute to the above synthesis (Almendros & Porcel, 1993). Concurrent to the changes in protein, the phosphatases activity also showed temporal changes. Being non-specific in substrate specificity, the increased activity of both acid and alkaline phosphatase in the reproductive and postreproductive phases may imply their role in energy deviation as well as in differentiation. Such instances of enhanced phophatases activity during growth and differentiation in molluscs are not uncommon.

ACKNOWLEDGEMENTS

The first author (D. Indra) wishes to thank the Council of Scientific & Industrial Research, New Delhi for providing financial support to carry out these studies.

REFERENCES

ALMENDROS, A. & PORCEL, D. 1993. Phosphatase activity in the hepatopancrease of *Helix aspersa. Comp. Biochem. Physiol.* **118** : 455 - 460.

BERRY, A.J. & CHAN, L.C. 1968. Reproductive condition and tentacle extirpation in Malayan *Achatina fulica* (Pulmonata). *Aust. Jour. Zool.* **16** : 849 - 855.

DAVIS, B.J. 1964. Disc electrophoresis II. Method and application to human serum protein. *Ann. N.Y. Acad. Sci.* **121** : 404 - 427.

DEZWAAN, A. & WIJSMAN, T.C.M. 1976. Anaerobic metabolism in bivalvia (Molllusca) characteristics of anaerobic metabolism. *Comp. Biochem. Physiol.* **54** : 313 - 324.

LOWRY, OL.HL., ROSENBROUGH, N., FAR, A.L. & RANDALL, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193** : 265 - 275.

MANINDRA NATH, SINGH. 1986. Occurrence of the Giant African Snail (*Achatina fulica*) in Bihar State. *J. Environ. Biol.* 7(4) : 253 - 257.

PAWSON, P.A. & CHASE, R. 1984. The life cycle and reproductive activity of *Achatina fulica* (Bowdich) in laboratory culture. *Journ. Moll. Stud.* **50** : 85 - 91.

RAMALINGAM, K. 1989. Stress and carbohydrate metabolism in animals. *Indian Rev. Life Sci.* **9** : 185 - 204.

SAKAE, M. 1968. Investigations of Giant Africal snail, *Achatina fulica* in Amami-Oshima Island. Annual Report of Oshima Agricultural Centre, **1968** : 28 - 29.

TENNISWOOD, M., BIND, C.E. & CLARK, A.F. 1976. Acid phosphates antrogen dependent markers of rat prostate. *Can. J. Bio. Biochem.* **54** : 340 - 343.

TOMIYAMA, K. 1991. Reproductive behaviour of hermaphrodite land snail *Achatina fulica*. 22nd Intern. Ethol. Conf. Abs. Otani University, Kyoto, Japan. pp. 43.

VIJAYA BRAHMANANDAM & KRISHNAMOORTHY, R.V. 1973.

WOLDA, H. 1970. Variation in growth rate in the land snail *Cepaea nemoralis. Res. Popul. Ecol.* **12** : 185 - 204.

WOLDA, H. & KREULEN, D. 1973. Ecololgy of some experimental populations of the land snail *Cepaea nemoralis* II. Production and survival of eggs and juveniles. *Neth. J. Zool.* **23** : 169 - 188.

ZAGRIS, N. & PODIMATAS, T. 1994. 5-Azacytidine changes gene expression and causes developmental arrest of early chick embryo. *Int. J. Dev. Biol.* **38** : 741 - 749.

ZAR, J.H. 1974. *Biostatistical Analysis*. Prentice-Hall Inc., U.S.A.