

EFFECT OF TREMATODE PARASITE ON EXCRETORY METABOLISM OF FRESHWATER SNAILS (*Lymnaea luteola* AND *Bellamya bengalensis*) IN MEERUT REGION

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ABSTRACT

Snails are known to serve as an intermediate host for several species of larval trematodes. Snails belong to a large group of invertebrates such as the phylum – Mollusca, class – Gastropoda. Snails were collected from July 2018 to June 2019 from various water bodies like ponds, pools, ditches, lake reservoirs, rivers (Garh Ganga) and crop fields of the Meerut region. They were collected in laboratory containers and beakers and maintained in aquariums and fed with natural food like lettuce leaf and ad libitum with Hydrilla. A Total 200 snails were collected with different species like *Bellamya bengalensis*, *Lymnaea luteola* and *Lymnaea acuminata* in the Meerut region. The occurrence of the larval trematode parasite was found. The effect of parasitism by excretory metabolism of the host revealed that it excreted more urea when it is parasitized by E. cercaria.

Keywords - Excretory Metabolism, *Bellamya bengalensis*, *Lymnaea luteola*, cercaria

Introduction:

Delaunay, (1931) has reported that Ammonia and Urea are the excretory products in gastropod molluscs. The characterization of the excretory patterns of molluscs by ammonotelic, urcotelic or uricotelic has oversimplified the study, in many cases. In gastropod molluscs, a number of metabolic end products have been categorized, and a summary of work on this group was reported by **Campbell and Bishop, (1970)**.

The nitrogenous degradation products, ammonia, urea and uric acid, are generally transported with the body fluids to places where they are excreted or further processed. The occurrence of these substances in the haemolymph of molluscs has been studied by **Delaunay, (1931)**; **Friedl, (1961a)**; **Potts, (1965)**; **Becker and Schmale, (1975)**; **Geraerts, (1992)**; **Curtis et al., (2000)**; **Arokelova, (2004)**.

The amount of Nitrogenous degradation products produced are indicative of the activity of the protein, nucleic acid metabolism of an animal.

Stress imposed on the latter must make itself apparent, in a change in the amount of Ammonia, Urea and Uric Acid produced. Such stress can be brought about by phases of strong reproductive activity, starvation or by parasitic invasion. The changes in the Ammonia and Urea amounts of *Biomphalaria glabrata* under different physiological conditions, such as starvation and infection with *Schistosoma mansoni* were observed recently by **Becker, (1968), (1970); Becker and Schmale, (1975), (1976), (1978); Schmale and Becker, (1975), (1977); Stanislawski, (1976).**

In the present investigation the author has observed the changes in the Nitrogenous excretory products of the two fresh water snails viz., *Lymnaea luteola* and *Bellamya bengalensis* during different larval trematode infections.

Material and Method:

The snails, *L.luteola* and *B.bengalensis* were collected from the nearby places of Meerut region. They were acclimatized to laboratory conditions for a week before experimentation. They were fed ad libitum with Hydrilla.

At a time a batch of 100 snails weighing 20+ 3.1gm. was exposed to 4l of water containing malathion for 48 hours. Equal no. of animals kept in tap water for the same interval served as controls. After exposure 3 tissues, viz foot, mental and hepatopancreas were isolated and homogenates were prepared in ice cold distilled water for the estimation of ammonia, uric acid, proteins and in 50 % Perchloric acid for urea.

Ammonia content was estimated by the method of Bergmeyer² and urea by the method of Natelson³. The uric acid was estimated by the method of Lowry et al. The statistical analysis was done according to standard statistical procedures.

Results: -

The ammonia (NH₃) and Urea excretion of *L.luteola* and *Bellamya bengalensis* different infections are presented in (Table 1 & 2). The analysis of haemolymph for the Nitrogenous products have also been included in (**Table 1 & 2**). The results reveal that the Urea concentration increased significantly (P<0.001) in all the infections in the haemolymph of *Lymnaea*.

	Incubation medium ($\mu\text{g NH}_3/\text{gm}/\text{total wt}/24\text{hours}$)	Haemolymph ($\mu\text{g}/100\text{ml}$)
Normal (<i>L.luteola</i>)	3.481 ± 0.19	98.4 ± 12
Infected with <i>X. cercaria</i>	3.491 ± 0.21	103.2 ± 14
Normal (<i>L.luteola</i>)	3.489 ± 0.43	97.1 ± 14
Infected with <i>E. Cercaria</i>	3.494 ± 0.46	101.4 ± 13
<i>A. Cercaria</i>	3.496 ± 0.45	102.3 ± 14
Normal (<i>L.luteola</i>)	3.486 ± 4.8	98.2 ± 15
Infected with <i>F. Cercaria</i>	3.493 ± 0.50	101.2 ± 16
Normal(<i>B. bengalensis</i>)	3.107 ± 0.38	
Infected with <i>E. Cercaria</i>	3.115 ± 0.40	

Table-1 Changes in the ammonia concentration and haemolymph ammonia concentration of *L.luteola* and *B. bengalensis* during different larval trematode infection. Mean \pm S.D. of 10 estimations.

	Incubation medium ($\mu\text{g NH}_3/\text{gm}/\text{total wt}/24\text{hours}$)	Haemolymph ($\mu\text{g}/100\text{ml}$)
Normal (<i>L.luteola</i>)	62 ± 8	0.48 ± 0.026
Infected with <i>X. cercaria</i>	$119 \pm 13^*$	$1.14 \pm 0.034^*$
Normal (<i>L.luteola</i>)	64 ± 7	0.51 ± 0.07
Infected with <i>F. Cercaria</i>	$121 \pm 16^*$	$1.19 \pm 0.09^*$
<i>A. Cercaria</i>	$148 \pm 18^*$	$1.26 \pm 0.06^*$
Normal (<i>L.luteola</i>)	61 ± 5	0.49 ± 0.07
Infected with <i>E. Cercaria</i>	$152 \pm 21^*$	$1.24 \pm 0.14^*$
Normal(<i>B. bengalensis</i>)	52 ± 7	
Infected with <i>E.Cercaria</i>	$137 \pm 23^*$	

Table-2: Changes in the urea content of haemolymph and in the externally measurable urea content of *L.luteola* and *B. bengalensis* during different larval trematode infections. Mean \pm S.E of 10 estimations.

Naturally, the externally measurable Urea, i.e. the amount of Urea which has been excreted was also more in the infected snails. *B.bengalensis* also excreted more urea, when it is parasitized by *E.cercaria*. The uric acid could not be detected either in the haemolymph or in the external incubation medium of the infected snails, *Lymnaca luteola* and *Bellamya bengalensis*. No significant difference was observed in the Ammonia concentration of the infected snails, at statistical level.

Discussion: -

A comparison of the NH₃-N concentration in the haemolymph of various molluscs shown that the Ammonia concentration in the haemolymph of *L.luteola* is very low, measuring at approximately 0.1.mg/100ml found that an even lower Ammonia Values of 0.51-0.71 mg/100ml in the *L.luteola*. The highest Ammonia concentration have been found in *Helix pomatia*, 1.2 and in *Arion rufus* with 1.4 mg/100ml Ammonia - **Delaunay, (1931)**. The Ammonia concentration of *L.luteola* is approximately same as that of *B. glabrata* - **Becker and Schmale, (1975)**.

The values of Ammonia excretion (Table-1) found in the experiment for *L.luteola* of 3.46. µg/g total weight/24 hours and *Bellamya bengalensis* 3.107µg/g total weight/24hours. It corresponds well with results reported for other molluscs - **Delaunay, (1931), Bayne and Friedl, (1968); Duerr, (1968); Friedl, (1974); Becker and Schmale, (1978)**.

Only a few findings on the concentrations of urea in the haemolymph molluscs have been reported - **Friedl, (1961a)** determined. chromatographically, 0.46-0.61 mg/100ml of Urea in *L. Stagnalis*. **Becker and Schmale, (1975)** reported the urea concentration in the haemolymph of *B.glabrata* 0.16mg/100ml. The author's results on urea analysis are closely related to the findings of **Delaunay, (1931); Friedl, (1961a); Bayne and Friedl, (1968); Friedl, (1974)**.

The increase in the Urea concentration in *Lluteola* and *Bellamya bengalensis* may be due to an increase in the enzymatic activity of the Urea cycle enzymes. *Bellamya bengalensis* excreted less amount of ammonia and urea than *L. luteola*.

Uric Acid, being a typical storage excretory product, is often determined in molluscs, in homogenates of the whole body or in certain organs, such as the hepatopancreas, kidney or foot. The fact that no uric acid was found either in the haemolymph or in the incubation medium of *L.luteola* does not mean that no uric acid synthesis takes place. Since *L.luteola* is systematically close to the planorbis, the following explanations can be offered for the undetectable uric acid in author's experiment.

1. The transportation of uric acid may not be carried out by the haemolymph or it may be the excretory cells.
2. Uric acid synthesis may take place in the storage tissue itself.
3. Uric acid concentration may lie below the detecting sensitivity of the method used. Uric acid below 30µg/100ml cannot be detected by the method employed.

The maximum rise of urea concentration is during the infection of *E.cercarial* infection of *L. luteola*. Since this group has prominent rediae causing maximum damage to the host, the protein is catabolized to a maximum extent.

In *A. cercaria* infection which has also got prominent rediae, more protein will be catabolized than during Xiphidio and Furcocercus cercarial infections. *X. cercariae* have no rediae, therefore, little autolysis takes place resulting in little damage to the tissue comparatively. The histological examination of the host tissue during these infection also supports these conclusions.

In *Bellamyia bengalensis*, also there was not much damage in the infected tissue unlike in the case of *E.cercariae* or *A.cercaria* infection in *L. luteola* and the pathogenicity can be compared with that of Xiphidio and Furcocercus cercariae infections, thus the catabolism of protein is also limited. The results showed that urea concentration has not increased enormously during this infection.

Starvation or lack of protein diet food has resulted in the increase of urea excretion and the activity of urea cycle enzyme in *Lumbricus terrestris* consider, the raised protein catabolism to be the reason for the alternations observed in the excretory metabolism. During *S.mansoni* infection also the snail *B. glabrata* showed a decrease in its stress resulted in the increase of urea excretion- **Becker & Schmale, (1978)**. In *L. luteola* and *Bellamyia bengalensis* also during infection the protein content decreased considerably, thus the protein catabolism has resulted into an increase of urea concentration in the haemolymph. Hence, the observed changes, in the excretory metabolism of *L.luteola* and *Bellamyia* tendency as that of *bengalensis* have a similar *B. glabrata* in reaction to a varying protein supply.

Emerson, (1967), has observed the metabolism of reserve food substance during starvation. As a source of energy, Carbohydrates, proteins are first utilized and then lipids. From the beginning of a starvation period, *B. glabrata*, catabolizes proteins, besides carbohydrates and lipids- **Von Brand, et al., (1948), (1957); Christie, et al. (1974)**. Since the physiological state of parasitism is almost same as that of the physiological state of starvation, **Moore & Halton, (1973)**, in *L. hurcola* and *Bellamyia bengalensis*, also during different larval trematode infection the similar type of mobilization of reserve food substances may take place as in other molluscs. Hence, it is concluded that a stress

situation brought about by the infection in these snails, leads to an increased protein catabolism.

Finally, the following explanation can be offered to the changes in the urea concentration of the infected *L. luteola* and *Bellamya bengalensis*.

The different groups of larval trematodes obtain their carbohydrates from the host that causes a physiological state of starvation in the host. The parasites then catabolize tissue protein to meet its energy requirements.). **Andrews & Reid, (1972)**, while examining the urea cycle enzymes of molluscs, an extensive biochemical process of the formation of urea in vertebrates and molluscs was noted.

However, the urea produced in the urea cycle in the case of ureotelic vertebrates has been proved to serve for detoxification of ammonia and excretion of nitrogen. These hypotheses are not necessarily applicable to the molluscs hitherto examined viz, *L. luteola* and *Bellamya bengalensis* for two reasons.

1- Urea can be produced in other ways without playing a role in the detoxification of ammonia (enzymatic effect of exogenous arginine, uricolysis).

2- So far it has not been proved that the urea cycle found in a mollusc serves mainly for detoxification of ammonia.

Conclusion: -

The changes in the main nitrogenous excretory products of *Lymnaea luteola* and *Bellamya bengalensis* during different larval trematode infections have been studied and discussed in the light of host parasite relationship. The effect of parasitism by excretory metabolism of the host revealed that it excreted more urea when it is parasitized by *E. cercaria*. The increase in the Urea concentration in *L. luteola* and *Bellamya bengalensis* may be due to an increase in the enzymatic activity of the Urea cycle enzymes. *Bellamya bengalensis* excreted less amount of ammonia and urea than *L. luteola*. For the reasons discussed, the increase in urea concentration in the haemolymph and thereafter, excreted by *L. luteola* and *Bellamya bengalensis* during different larval trematode infections cannot be taken for granted as an indication of the mobilization of the protein of the body itself for the production of energy, as the animals were proved to be ureotelic.

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