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EFFECT OF PARASITISM ON CARBOHYDRATE RESERVES OF FRESHWATER SNAILS (Lymnaea and Bellamya) IN AND AROUND MEERUT REGION

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ABSTRACT

Snails act as an intermediate host for several species of larval trematodes. Snails belong to a large group of invertebrates such known as the phylum – Mollusca, class – Gastropoda. Snails were collected during the monsoon period from various water bodies like ponds, pools, ditches, lake reservoirs, rivers (Garh Ganga) and crop fields in and around the Meerut region which were Ram Taal Vatika, Chittora Power House, Diggi Shastri Nagar, Village Shobhapur, Village Kunda, Kankerkhera, Bhola Power House, Pond near railway Cantt. Station, Nanu ki Neher near Sardhana, Pond in Ganga Nagar near Hastinapur. Snails were collected and identified by the method suggested by reference book of Subha Rao (1989) Hand Book of freshwater Molluscs of India. Identified snails were found to be Lymnaea and Bellamya. Collected snails were thoroughly washed in running tap water, arranged species wise, counted and then kept in laboratory containers and beakers and maintained in aquariums and fed with natural food like lettuce leaf. 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 changes in the carbohydrate reserves of these hosts during parasitism have been studied with the help of histochemical and biochemical techniques.

Keywords- Trematode, cercaria, *Bellamya*, *Lymnaea*, carbohydrate reserves

Introduction:

The effects of trematode parasites on the definitive hosts have been studied extensively in the past, but relatively little is known about the relationship between the larval stages of helminths and their intermediate hosts. **Karyakarte, Yadav. (1978); Choubisa (1990); Das Gupta, B. (1973); Christie et al (1974).** Carbohydrate analysis by high performance thin layer chromatography of Cerithidea californica (Gastropoda: Prosobranchia) reported by **Marsit, C. J. et al. (2000 b).**

In general, the major pathological and physiological effects of larval trematodes on the hepatopancreas of the molluscan hosts include accumulation of fat bodies in the cells; vacuolization of cell cytoplasm, karyolysis, mechanical damage of tubules and intertubular tissues and histolysis of tissues by the excretory products of the larvae, secondary effects such as the release of pigment resulting from the destruction of the gland and the rupture of tunica propria are also quite common.

A number of histochemical changes have also been found to occur with the infection in molluscs, notably a decrease in the level of host carbohydrate reserves. Notable contributors in this field are- **Lal & Premvati, (1955); Cheng & Snyder, (1962); Cheng & Burton, (1966); Porter et al., (1967); Negus, (1968); Patnaik, (1968); Moore & Halton, (1973); Livingstone and Zwaan (1983); Horak, (1995).**

The present investigation is concerned with the histopathological effects of parasitism by Amphistome cercaría Echinostome cercaria, Xiphidio cercaria and Furcocercus cercarlae on Lymnaca luteola and Bellamya bengalensis. The changes in the carbohydrate reserves of these hosts during parasitism have been studied in detail with the help of histochemical and chemical techniques.

Material and Method :

The Digestive gland from the infected snail were taken out of the shells and drop immediately into the Bouins fluid.

After fixation material was thoroughly washed and dehydrated in a graded series of alcohols and then we used xylene for clearing the material. After clearing, the material was embedded in paraffin wax (M.P.58 °C). Paraffin embedded section were stained with PAS (Periodic Acid Schiff's) technique.

Discussion & Result:

The histological studies revealed that the hepatopancreas of the infected snails, L.luteola and B. bengalensis was completely occupied by the larval trematodes and thereby causing destruction to the hepatopancreas.

	TOTAL CARBOHYDRATES	**REDUCING SUGARS**
Normal *(L. luteola)*	3.665 ± 0.852	0.351 +0.062
B. *bengalensis*	1.104 ± 0.341	0.347 +0.059 $^{N.S}$

Table-1: Total carbohydrates and reducing sugar levels of Lymnaea luteola infected with E. cercaria and B. bengalensis infected with F. cercaria. The weights are expressed as gm% of wet weight of the hepatopancreas. Values are Mean ± S.D. of 20 estimations.

In heavy infections many empty spaces were observed in the digestive gland. The changes in the size and shape of the digestive cells are also observed in the infected snails. The histochemical studies revealed that the glycogen content decreased in the hepatopancreas of snails during all the infections under reference, concurrent with the depletion of glycogen in the host, an increase of the same has been observed in the bodies of parasites. The carbohydrate levels of Lymnaea luteola, infected with Amphistome cercaria, Echinostome cercaria, Xiphidio cercaria and Furcocercus cercaria showed a depletion of 66% and 49% ($p<0.001$) respectively. The hepatopancreas of B.bengalensis infected with Furcocercus cercaria showed 45% less carbohydrates. **(Table-1).**

The quantitative studies on reducing sugars revealed that during Echinostome cercarial infection, no change was observed in the hepatopancreas of L. luteola. But Xiphidio and Furcocercus cercarial

infections, the sugar levels are 27 % and 42% more ($p<0.001$). Only 9% ($p<0.005$) difference was observed in Amphistome cercaria infection. In B.bengalensis with E. cercaria, not much difference was observed in the reducing sugar levels **(Table-1.)**

Three distinct types of cells have been observed in the epithelium of the digestive gland of Lymnaea luteola and B.bengalensis. Each tubule is comprised of two types of columnar cells viz., digestive cells and mucus cells together with small groups of basophil cells which are strongly basophilic in nature (Fig.A). The digestive organs exhibit considerable variation in their detailed cytoplasmic structure and this can be due to the presence of number of parasites in the visicinity. The luminal border of the digestive cells give a positive reaction with PAS, and is unstained with alcian blue, thus indicating the presence of neutral mucoprotein. The middle portion of the digestive cell is generally occupied by a large vacuole containing a basophilic granular material. This appears to have a hypomuco polysaccharide content and stains with alcian blue and PAS glycogen deposits occur in the Sub apical and granular endoplasm of the cells and stain strongly with alcian blue, indicating high concentration of acid mucosubstances.

Basophil cells different in shape from the digestive and mucous cells occur mainly in groups of varying numbers at the blunt endings of the tubules. Since some acidophilic granules are found in the apical cytoplasm of the basophilic cells they are thought to be secretory in function.

The following morphological or histopathological changes have been observed in the infected digestive gland cells of L.luteola and B.bengalensis.

In the infected digestive gland the intertubular connective tissue was disrupted, and became structureless. The digestive epithelium became, very thin during heavy infections and was disrupted by the parasites particularly the growing cercariae **(Fig.B).** The digestive cells showed an increase in the vacuolation arrangement of pancreatic cells. Variation in the shape of hepatopancreatic cells from columnar to cuboidal and some squamous forms occured in the infected snails. The part of infected digestive cells also decreased. The appearance of cytoplasmic granules in the basal portion cells and shifting of the nucleus towards the basal portion appearance of lower but clumps of cytoplasmic granules and the occurrence of karyolysis in densely infected snails all strongly suggest, physiological reaction. Similar changes have also been reported by **Cheng & Snyder, (1962), Lymnaea trumcalulate, Moore & Halton, (1973), Indoplanorbis exustus, Karyakarte. & Yadav, (1976); Tripati & Singh (2002),** reported to the toxic effects of dimethoate and carbaryl pesticides

on carbohydrate metabolism of fresh water snail Lymnaca acuminata.

The histochemical studies on glycogen shows that both the digestive and mucous cells of the digestive gland of uninfected Lymnaca luteola and B.bengalensis are rich in glycogen, which appeared as fine granular material scattered throughout the cytoplasm **(Fig.C)** glycogen granules were also seen associated with the intertubular connection tissue network

In *L.luteola* infected with Amphistome cercaria, E.Cercaria, X. cercaria and F.cercaria and in B.bengalensis infected with E. cercaria there was a decrease in the glycogen content of the digestive cells.

Concurrent with this depletion of host glycogen, there was an increase of the same in the developing parasites when developing within the sporocysts or rediae. **Joosse, J., Van Elk, R.,(1986)** reported Trichobilharia ocellata : physiological characterization of giant growth, glycogen depletion and absence of reproductive activity in the intermediate snail host, Lymnaea stagnalis. Fully developed cercariae with deposits of glycogen in their suckers, in the parenchyma, glycogen granules were also associated with the body walls of sporocysts and stored glycogen in the body wall of sporocysts have been reported by **Ginetzinskaja, (1960).** Rediae showed heavy concentrations of glycogen in the gut as it contain the cellular debris of the digestive cells it has ingested. In heavy infection of Amphistome cercaria and E.cercaria the digestive gland has many empty spaces. Thus the damage done to the host by the larval trematodes is of two types viz., mechanical as well as lytic. In densely infected areas the cell membranes of the hepatopancreatic cells are no longer intact and no traces of glycogen are visible. In the infected foot

region of L. *luteola, Amphistome cercaria* were found to be encysted in the muscular tissue.

It seems, therefore, that active digenean larvae remove glycogen from host tissues and incorporate it into their own bodies. The exact method of consumption, however, depends on the feeding habits of the parasites. Rediae of *Amphistome cercaria* & E. cercaria obtain most of their carbohydrates through direct ingestion of host cells. However rediae and sporocysts also absorb nutrients through their body walls, as revealed by the results on reducing sugar. The histochemical studies on glycogen also support this. The body wall of redia and sporocyst contain glycogen. The presence of microvilli in the ultra structure rediae of C. embricata has been reported by Reader in (1971), which enhances the absorption area of the body wall of rediae.

The increase in the reducing sugar levels of the host, L.*luteola during X. cercaria* and *F.cercarial* infections and the in-significant increase during *Amphistome cercaria* and *E.cercarial* infections and *E. cercarial* infection in B.*bengalensis*, will throw some light on the mode of food intake by these larvae. As already stated that rediae obtain most of their nourishment through direct ingestion of host cells but sporocysts cannot obtain nourishment by this method, as they lack a mouth hence the alternate pathway is, absorption. It is generally believed **Cheng, (1963)** that glycogen molecules are too large to pass directly through larval digenean body walls. It seems likely that glycogen in the host digestive gland is hydrolysed to simple sugars which are consumed by the Parasites and are used in the resynthesis of glycogen. Since the sporocysts of *X.cercaria* and *F.cercaria* obtain most of their nourishment by this process, the reducing sugar levels of the host L. *luteola* have increased ($P<0.001$), on the other hand no significant increase has been observed during *Amphistome cercaria, E.cercaria* and *F. cercarial* infections in the hosts L. *luteola* and B.*bengalensis* which shows that the rediae of these parasites obtain most of their carbohydrates by ingesting the host cells and partly by absorption.

Though, earlier workers have postulated that the parasites secrete, a glycogen digesting enzyme which hydrolyse the host glycogen to simple sugars, it is clear now that phosphatases are the enzymes secreted by the parasites and which are responsible for the conversion of glycogen into simpler sugars.

The excreta of the parasites were observed in sections of the infected snails. Since these small brownish granules were observed in the sporocysts and cercariae, they were regarded as excretory in nature. Such excretory granules were also reported by the earlier workers viz., **Cheng & Snyder, (1962).** The excreta of the parasites were found to be lytic in nature as the lysis of hepatopancreatic cells was observed in the areas where the excreta are present. The excreta of *Amphistome cercaria, E. cercaria, X.cercaria* and *E.cercaria* appeared either in rediae, sporocysts or cercariae only after these parasites have accumulated sufficient glycogen in them. Thus, it can be said that the excreta of these parasites are a result of carbohydrate metabolism.

Fig.A Periodic Acid Schiff's (PAS) Preparation of transverse section of the digestive gland of the infected snail. The secretory body exhibit a strong reaction of PAS.

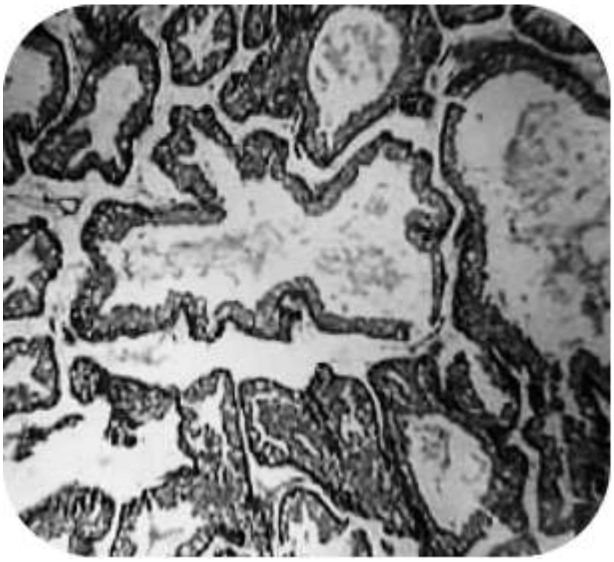

Fig.B: The digestive cells showing an increase in the vacuolation

arrangement of Pancreatic cells, appearance of cytoplasmic granules, in the basal portion of cells.

Fig.C Glycogen granules scattered throughout the cytoplasm associated with inter-tubular connection tissue network

Conclusion:

The current study is concerned with the histopathological effects of parasitism cercaria on *Lymnaea luteola* and *Bellamya bengalensis*. The changes in the carbohydrate reserves of these hosts during parasitism have been studied in detail with the help of histochemical and chemical techniques. The histochemical studies revealed that the glycogen content decreased in the hepatopancreas of snails during all the infections under reference, concurrent with the depletion of glycogen in the host, an increase of the same was observed in the bodies of parasites.

References:

[1] Choubisa, S. L. (1990). Cercaria gurayai, a new Species (Furcocercaria) from the Freshwater Snail,> Faunus ater (Linnaeus). *Records of the Zoological Survey of India*, *87*(4), 267-271.

[2] Dasgupta, A. K. (1973). Agriculture and economic development in India. *Agriculture and economic development in India.*

[3] Christie, J. D., Foster, W. B., & Stauber, L. A. (1974). The effect of parasitism and starvation on carbohydrate reserves of Biomphalaria glabrata. *Journal of Invertebrate Pathology*, *23*(1), 55-62.

[4] Marsit, C. J., Fried, B., & Sherma, J. (2000). High-performance thin-layer chromatographic analysis of lutein and β-carotene in Cerithidia californica (Gastropoda) infected with two species of larval trematodes. *Journal of Parasitology*, *86*(3), 635-636.

[5] Lal, M. B. (1955, December). Studies in histopathology—Changes induced by a larval monostome in the digestive gland of the snail, Melanoides tuberculatus (Müller). In *Proceedings of the Indian Academy of Sciences-Section B* (Vol. 42, No. 6, pp. 293-299). Springer India.

[6] Cheng, T. C., & Snyder, R. W. (1962). Studies on host-parasite relationships between larval trematodes and their hosts. III. Certain aspects of lipid metabolism in Helisoma

trivolvis (Say) infected with the larvae of Glypthelmins pennsylvaniensis Cheng and related phenomena. *Transactions of the American Microscopical Society*, *81*(4), 327-331.
[7] Cheng, T. C., & Burton, R. W. (1966). Relationships between Bucephalus sp. and Crassostrea virginica: a histochemical study of some carbohydrates and carbohydrate complexes occurring in the host and parasite. *Parasitology*, *56*(1), 111-122.
[8] Porter, C., Pratt, I., & Owczarzak, A. (1967). Histopathological and histochemical effects of the trematode Nanophyetus salmincola (Chapin) on the hepatopancreas of its snail host, Oxytrema siliqua (Gould). *Transactions of the American Microscopical Society*, 232-239.
[9] Negus, M. R. (1968). The nutrition of sporocysts of the trematode Cercaria doricha Rothschild, 1935 in the molluscan host Turritella communis Risso. *Parasitology*, *58*(2), 355-366.
[10] Patnaik, M. M. (1968). Notes on glycogen deposits in Limnaea auricularia var. rufescens and the parasitic larval stages of Fasciola gigantica and Echinostoma revolutum-A Histochemical Study. *Annales de Parasitologie Humaine et Comparée*, *43*(4), 449-456.
[11] Moore, M. N., & Halton, D. W. (1973). Histochemical changes in the digestive gland of Lymnaea truncatula infected with Fasciola hepatica. *Zeitschrift für Parasitenkunde*, *43*(1), 1-16.
[12] LIVINGSTONE, D. R., & DE ZWAAN, A. L. B. E. R. T. U. S. (1983). Carbohydrate metabolism of gastropods. In *Metabolic biochemistry and molecular biomechanics* (pp. 177-242). Academic Press.
[13] Horák, P., & Mikes, L. (1995). Cercarial surface saccharides of six trematode species from the pond snail, Lymnaea stagnalis.
[14] Joosse, J., & Van Elk, R. (1986). Trichobilharzia ocellata: physiological characterization of giant growth, glycogen depletion, and absence of reproductive activity in the intermediate snail host, Lymnaea stagnalis. *Experimental parasitology*, *62*(1), 1-13.
[15] Cheng, T. C., & Snyder, R. W. (1963). Studies on host-parasite relationships between larval trematodes and their hosts. IV. A histochemical determination of glucose and its role in the metabolism of molluscan host and parasite. *Transactions of the American Microscopical Society*, *82*(3), 343-346.