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Histopathological observations on the digestive gland of *Lymnaea auricularia* infected with larval trematodes

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Abstract. Histological observations were made on the digestive gland of infected and uninfected freshwater snails, *Lymnaea auricularia*, and the varying degree of histopathogenesis of echinostome cercaria, *Cercaria itoi*, as well as strigeoid metacercaria, *Tetracotyle lymnaei*, along with *Cercaria itoi* (double infection) in the digestive gland have been observed. Basically 3 types of lesions, L_1, L_2 and L_3 were found and were associated with the types and size of trematode larvae as well as with the degree of parasitemia. In the early stage of larval development or in mild infection, type I lesions (L_1) were commonly observed in which degenerative changes were confined to individual digestive gland tubules, and neighbouring tubules mostly remained healthy or unaffected. The type II lesions (L_2) were associated with severe infection and were less common than L_1 lesions and large areas of parasitised digestive gland tubules were revealed to be necrotic. Type III lesions (L_3) were also associated with severe infection in which digestive gland tubules appeared as rudimentary tubules located between trematode rediae. In the present study, in addition to a description of the different types of lesions, the various alterations of cellular structures of the digestive gland of *Lymnaea auricularia* due to single or double infections (*Cercaria itoi* + *Tetracotyle lymnaei*) are also discussed.

Keywords. Histopathology; digestive gland; *Lymnaea auricularia*; *Cercaria itoi*; *Tetracotyle lymnaei* (metacercaria); lesions.

1. Introduction

Infection by most trematodes, adults as well as their larval stages cause considerable damage to the host tissues. Histopathological changes in molluscs caused by larval trematode infections have been described by many (James 1965; Patnaik and Ray 1966; Moore and Halton 1973; Mohandas 1974, 1977; Yoshino 1976; Sommerville 1978; Bertman 1980; Choubisa 1988), and reviewed by Cheng and Snyder (1962), Wright (1966) and Erasmus (1972). However, none of these workers has attempted to give a comparative histopathological account of the digestive gland associated with single and double infection. Although, many have reported instances of double and multiple infections (Mohandas 1971; Choubisa and Sharma 1983a, 1986) in snail host; cases of two different developmental stages (cercaria + metacercaria) of different species of trematodes occurring in the same snail species are rarely reported. In the present study this point has been highlighted along with histopathogenesis of the echinostome cercaria, *Cercaria itoi* in the digestive gland of *Lymnaea auricularia*.

2. Materials and methods

During a survey of freshwater larval trematodes and their snail hosts in southern Rajasthan (Choubisa and Sharma 1983a, 1986) specimens of the snail species,

L. auricularia, were collected and examined for larval trematode infection. The methods of rearing the snails, and collection and identification of the larval trematodes were the same as described earlier (Choubisa and Sharma 1986). The infected digestive glands either with echinostome cercaria, *C. itoi*, and its redia or strigeoid metacercaria, *Tetracotyle lymnaei* along with *C. itoi* and its redia (double infection) were fixed in 10% buffered formal saline or Bouin's fixative. Tissue blocks were prepared and sections cut at 7 μm on a rotary microtome. Sections were stained with haematoxylin and eosin.

3. Results

Of the 250 *L. auricularia* examined during winter (December to January) 32 (12·8%) were infected with echinostome cercaria, *C. itoi*, and 5(2%) with this cercarial species along with the metacercaria, *T. lymnaei*.

3.1 *Morphology and histology of uninfected digestive gland*

The healthy digestive gland of *L. auricularia* was found to be grey in colour and occupied the maximum part of the whole of the visceral mass which was enclosed by a thin membrane, the tunica propria.

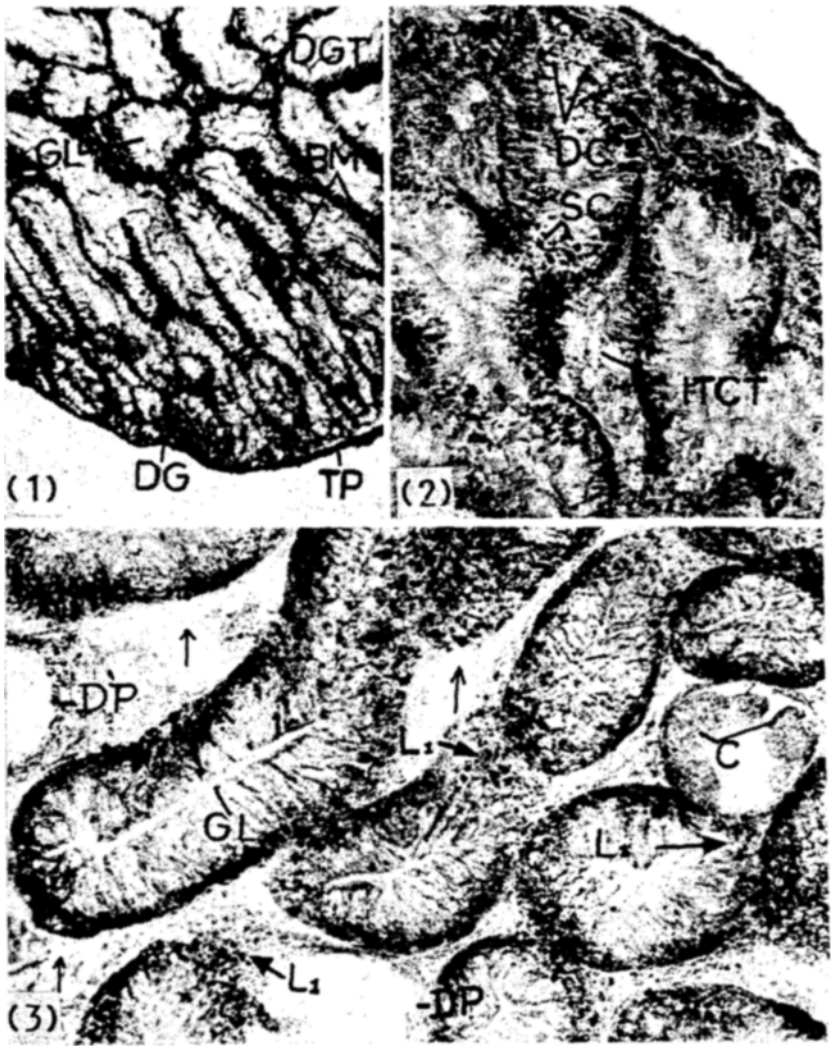
The basic histological feature was the mass of digestive gland tubules embedded in their stroma of fine connectives (figures 1, 2). The tubule consists of a single layer of epithelial cells on a prominent basement membrane. The epithelial cells are differentiated into 3 types on the basis of their morphology. The digestive cells (absorptive cells) are ciliated columnar with basal nuclei and numerous globules. These were observed most frequently. The second type of cells were the secretory cells, oval to triangular shaped with base abutting the basement membrane and the apex disposed towards the digestive lumen. Their nuclei were comparatively large and were more numerous in some digestive tubules. The third type of cells, undifferentiated cells or thin cells, were seen only in the intestinal component of the digestive epithelium.

The space between digestive gland tubules was filled with a fine and unbroken network of intertubular connective tissues (figures 1, 2). The intraglandular space or gut lumina in healthy digestive glands was found to be very reduced or compact. The unbroken intertubular connective tissues and compact gut lumina reflect healthy condition of the digestive gland.

3.2 *Morphology and histology of parasitized digestive gland*

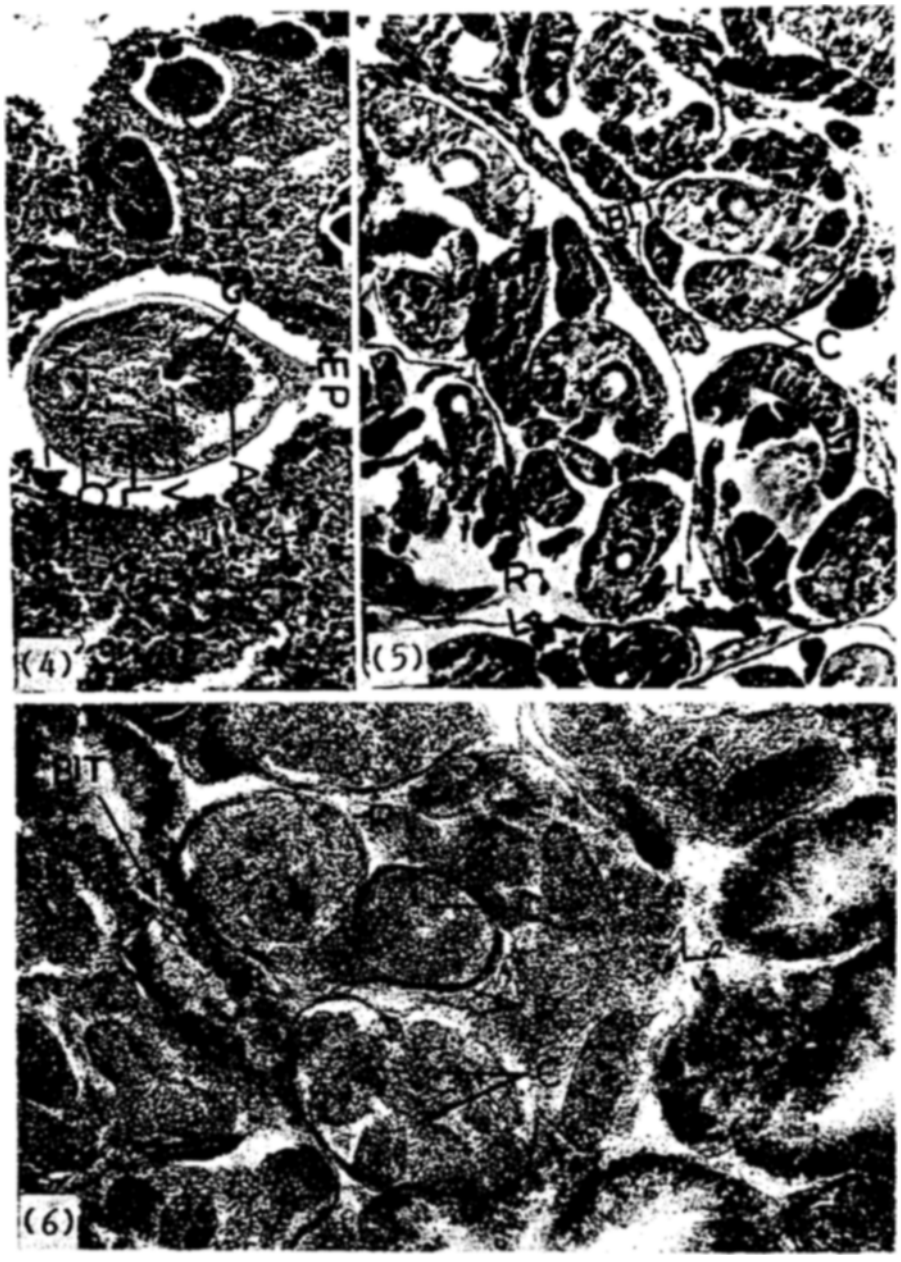
Infected digestive glands of *L. auricularia* either with single (*C. itoi*) or double (*C. itoi* + *T. lymnaei*) infections appeared to white in colour, swollen and in a loose mass. The tunica propria was also seen under pressure.

In most cases large areas of intertubular connective tissues were occupied by parthenitae. Tubules were reduced in diameter, number and irregularly shaped but their lumina were found to be enlarged (figure 3). Similarly, the intertubular spaces were also found to be enlarged. In the parasitized digestive gland, either with single or double infections, 3 types of lesions could be distinguished. In the type I lesion



Figures 1–3. Histological section of healthy digestive gland of *L. auricularia* stained with haematoxylin and eosin (× 75). **2.** Section of healthy digestive gland enlarged (× 240). **3.** Section of infected digestive gland with *C. itoi* showing lesion L_1 (× 240).

(L_1), degenerative changes were confined to individual tubules and neighbouring tubules were unaffected (figure 3 indicated by L_1). Such types of lesions occurred in mild infections with larval parasites. The number of globules in the cytoplasm of digestive cells was reduced, consequently the diameter of tubules became progressively smaller and the intertubular lumina progressively occluded (figure 6). The lateral walls of digestive cells as well as basement membrane appeared to be broken leading to complete loss of cell organization (figure 3). As a result of gradual advancement of infection, the height of columnar cells became reduced, and the lumina was found enlarged. The secretory cells were the last to lose their integrity and remained free within the lumina. Intertubular areas were found to be progressively enlarged and filled with transudate association with the destruction of



Figures 4–6. 4. Section of infected digestive gland with *C. itoi* and metacercaria, *T. lymnaei* showing lesion L_2 (×240). 5. Section of very severely infected digestive gland with *C. itoi* showing lesion L_3 (×240). 6. Section of severely infected digestive gland with *C. itoi* showing blocking of tubules along with lesion L_2 (×240).

(AO, Adhesive organ; BIT, blocking of individual tubule; BM, basement membrane; C, cercaria; DC, digestive cells; DG, digestive gland; DGT, digestive gland tubules; EP, excretory pore; G, gonads; GL, gland lumen; ITCT, intratubular connective tissues; L, lappets; MC, metacercaria; OS, oral sucker; R, redia; SC, secretory cells; VS, ventral sucker).

the tubules (figure 3, indicated by everted arrows). In severe infections, cells of tubular epithelium in close apposition to redia appeared to be fragmented.

The type II lesions (L_2) associated with severe infection or greater degree of parasitemia were frequently observed in single infections (figure 6) but common in double infections (figure 4). Type L_2 in either type of infection was characterized by the development of large foci of severe acute liquetative necrosis in a gland which otherwise manifested the features of the type L_1 lesion. In the necrotic areas, unorganized columnar digestive cells along with clusters of rediae and cercariae were found, but secretory cells remained virtually intact within affected areas. In double infections, there was complete necrosis of digestive gland but in single infection some tubules still remained intact.

The type III lesions (L_3) were observed only in the case of severe single infection (figure 5). L_3 were characterized by atrophy of digestive tubules, and tubules appeared as small mass of cells but necrosis was not found.

4. Discussion

It is clear from the present investigation that the variety of histopathological changes in snail tissues induced by larval trematodes depend upon the severity or degree of infection, size and types of larvae. The mechanical damages (L_1) of the digestive tubules appear to be the cumulative effect of larval migration, feeding and asexual reproduction, whereas autolytic necrosis (L_2) is the result of the release of proteolytic enzymes from the ruptured digestive cells/or enzymatic secretion from trematode larvae (Choubisa and Sharma 1983b; Sharma and Choubisa 1985; Choubisa 1986). In heavy infection with rediae of *C. itoi* the digestive gland tubules at various places between well developed rediae became compressed or blocked. Consequently, starvation autolysis or atrophy (L_3) has taken place. However, the root of complexity lies in the degree of parasitemia, age/sizes and types of trematode larvae.

Common pathological changes, such as blocking of digestive tubules and reduction in height of columnar cells in the form of squamous or cuboidal as well as increase in inter and intratubular spaces as observed in the present study may be due to (i) intracellular digestion at the time of starvation in *L. auricularia* causes starvation autolysis and atrophy of the gland and (ii) the reduction in the amount of storage nutrients due to increasing demands of the developing host gonads as well as developing or multiplication of larvae. The former point gets support from the work of James (1965) and Mohandas (1974) and the latter from the work of Bertman (1980). The pathological alterations in the digestive gland of the snail host of the present study can be classified basically into two, mechanical and physiological. There may be several factors involved in such pathogenesis. Both types of damages may have come from the blocking of digestive tubules by growth, multiplication and movement of trematode larvae. The severity of mechanical damage also depends upon the type and size of trematode larvae as well as their developmental stages. Mohandas (1977) and Choubisa (1988) have also discussed that radial stages produce greater mechanical as well as physiological damages than sporocysts. Rediae have mouth and locomotary organs which are responsible for a great deal of mechanical damage whereas their pharyngeal glands, and digestive system are responsible for the physiological damages. Some times redia engulfs digestive cells and also uses its hydrolase enzymes for extracellular digestion

(Sharma and Choubisa 1985). Thus both types of damages take place with radial infection and this factor must also be considered. Other contributory factors are parasitic secretions and excretions which produce toxic effects. In the present study, greater pathogenesis (both types of damages) was found in the case of double infection since excysted metacercaria, *T. lymnaei* and rediae of echinostome cercaria have well developed locomotory organs and digestive system. Double infection actually contributes twice the hydrolases than single infection in the digestive gland of *L. auricularia.*

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