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PATHOLOGICAL CHANGES OF BIOMPHALARIA  
ALEXANDRINA DURING ASEXUAL CYCLE OF SCHISTOSOMA  
MANSONI INFECTION

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A B S T R A C T

Several days after exposure to S.mansoni miracidia, many snails appeared inactive and partly withdrawn. By 15 days post infection, the daughter sporocysts concentrated in the region of the digestive gland and to some extent in the ovotestis. There was also infection in the various anteriorly situated organs like tentacles, head-foot and oesophagus. By 30 days post infection, daughter sporocysts increased in size and

number which occupied almost all of the interlobular connective tissue of the digestive gland and ovotestis. Necrosis of glandular tissues was noted when glandular lobules were compressed and invaded by granulomatus tissue formed around daughter sporocysts and cercariae. Atrophy, degeneration and ulceration of glandular epithelium then resulted. By 45 days post infection displacement of the digestive gland tubules by the sporocysts was observed. Ovotestis were most severely damaged, the acini lost their regular shape and were occupied by the parasite.

## I N T R O D U C T I O N

Schistosomiasis is one of the most important public health problems in many countries in the tropics and subtropics. It is a water - borne disease which is contracted by the cercarial stages of the parasites penetrating the skin of individuals who comes in contact with infested water.

Schistosomiasis is still considered as one of the most important endemic diseases in Egypt, its impact on health and economy is tremendous ( Farooq and Samaan, 1967) . During the last few decades, there has been a steady and significant change in the epidemiology and pattern of distribution of both species of schistosomes and their respective intermediate host with S.mansoni and its snail host B.alexandrina spreading into new areas which have hitherts free of the parasite and its snail intermediate host. In view of this change and due to the higher morbidity of Shistosoma mansoni , wide interest and attention are now directed to the study of S.mansoni and its relationships with the snail intermediate host B.alexandrina.

The histopathological effects of the parasites on the snail tissues have been studied by many authors, Pelseneer (1906) , Labour (1911) , Faust (1920), Rees (1936), James (1965), Malek and Cheng (1974), Jordan and Webbe (1982) , Kamel et al. (1986) . The most commonly encountered histopathological changes in molluscs, especially gastropods , are there associated with the presence of larval trematodes (sporocysts and / or rediae). This range from extremely severe to minor alterations. This topic has been comprehensively reviewed by Cheng and Snyder, (1962), malek (1980) and only a few additional studies have been reported since these reviews.

The present work has been planned to investigate the pathological changes of the snail Biomphalaria alexandrina during asexual cycle of Schistosoma mansoni infection.

## MATERIAL AND METHODS

Biomphalaria alexandrina snails were obtained from laboratory inbred stock of approximately same size (6-8 mm in shell diameter) . Snails were classified into two groups. The first was infected with S.mansoni miracidia while the second was left as a control group. Snails were infected individually, the eggs of S mansoni were isolated from the intestine of mice with mature infection of S.mansoni according to the method used by Mohamed (1987).

Each snail was placed with a little distilled water in 2.5 cm cup about 10 mm in diameter and 15 mm in depth. Snail then was exposed to 6-8 newly hatched miracidia of S.mansoni.

The containers were left 3-4 hours to ensure the penetration of miracidia.

For histopathological examinations, infected snails were randomly selected after 15,30 and 45 days post infection. The soft parts of infected and control specimens were removed from their shells and immediately fixed in alcoholic Bouin's fluid. Tissues were then dehydrated, cleared, paraffin embedded and serial sections were cut at 6-8 microns in thickness. Sections were stained with Haematoxylin and eosin.

## R E S U L T S

For the several days after exposure to miracidia, many snails appeared inactive and partly withdrawn. There after , most infected snails behaved normally until about 5-6 weeks when cercariae began to emerge. Then snails again became relatively inactive, withdrawn and appeared weak .

Transverse sections through the soft parts of uninfected B.alexandrina showing the digestive gland tubules ( Figs.1 and 2 ), ovotestis ( Figs.3 and 4 ), the head-foot ( Fig.5 ) are given to illustrate the architecture of uninfected specimens.

By 15 days post infection, the daughter sporocysts of S.mansoni concentrated in the region of the digestive gland (Fig. 6 ) and to some extent the region of the ovotestis (Fig.7 ) and also present in the various anteriorly situated organs as tentacles, head-foot and oesophagus ( Figs. 8 , 9 and 10 ). The digestive gland in which individual lobules are separated by a small amount of loose connective tissue ( Figs. 1 and 2 ).

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Daughter sporocysts appeared in the interlobular connective tissue. When B.alexandrina digestive gland becomes filled with sporocysts , the enveloping tunica propria usually become ruptured, and the larval trematodes invade the adjacent ovotestis. It seems that this is usually the mechanism for invasion of the reproductive system ( Fig. 11 ). S. mansoni sporocysts invaded the connective tissue around and between the acini of the ovotestis but none of them were found inside any acini .

As infection progressed, daughter sporocysts increased in size and number by about 30 days post infection. Numerous daughter sporocysts occupied almost all of the interlobular connective tissue of the digestive gland and ovotestis (Fig. 11 ). Development of large numbers of daughter sporocysts in the digestive gland altered the hepatic architecture. As much as two thirds of the parenchymal tissue disappeared and the normal complex lobular branching structure was lost (Fig.12 ). The architecture of the ovotestis also a compound lobular gland was less altered than that of the digestive gland because few daughter sporocysts developed there. The reproductive cells within the ovotestis become very scarce.

By 45 days post infection, the uptake of nutrients by sporocysts takes place through the tegument , the effect causes toxic changes in the digestive gland cells, the epithelium distal wall of the cell often breaks , down (Fig.13), displacement of the tubules and loss of their branched

structure were observed ( Fig.14 ). Ovotestis were most severely damaged ( Fig. 15 ). The acini lost their regular shape and occupied mostly by the sporocysts. In some cases the reduction and often the complete obliteration of the acini were observed. As a result of infection heavy production and accumulation of excretory granules were observed accompanied by concurrent proliferation of interlobular connective tissue. It was observed that there are usually several cercariae in the region of ovotestis (Fig.16) digestive gland and in blood sinuses. Complete replacement of the digestive gland cells and the ovotestis acini were detected.

## D I S C U S S I O N

By far the majority of digenetic trematodes larval stages utilize their molluscan intermediate host's digestive gland as a primary site of infection. Although if this organ becomes densely packed with sporocysts, some of these larvae may secondarily invade the adjacent ovotestis and other organs of the snail, Malek ( 1954, 1955, 1958 ) ; Cheng and Cooperman (1964) ; Pan ( 1965 ) ; Kamel ( 1979 ) and Kamel et.al.(1986).

In the present investigation, results indicated a great reduction in digestive gland and ovotestis, also some other organs as tentacles, head-foot and oesophagus as well as the connective tissues and muscular tissues. This destruction is of four types.

- 1- Mechanical destruction due to pressure exerted by the Parasite.

- 2- Extracorporeal digestion by sporocysts.
- 3- Lysis due to the parasite excreta.
- 4- Autolysis due to starvation .

Histologically, the present investigation indicated that the acinar cells affected by mechanical pressure appear extremely compressed, and if ruptured the fragments reveal jagged edges. Furthermore, their nuclei are commonly not pyknotic, unless the nucleated fragments have persisted for some time . Cells were destroyed as the result of enzymes secreted from the tegument of spore - cysts . The cells not in immediate contact with the parasite often show symptoms of destruction and/ or necrosis while in the case of mechanical damage, the compressed or cleanly ruptured cells usually occurred in the immediate proximity of the parasite.

In addition to the types of cell destruction, another histopathology feature of parasitized digestive gland is the occurrence in large numbers of two types of globules of intracytoplasmic origin, especially if host parasite association is of relatively long duration. The first type of globules, known as excretory or ferment globule are yellowish brown even in stained sections. They are un-nucleated and represent accumulated metabolic wastes in hyper action excretory or ferment cells of the digestive gland . As the cells become ruptured, digested or lysed , the comparatively large excretory globules become dispersed -

throughout the tissue. Another striking cytological feature commonly encountered in digestive gland cells of molluscs infected with larval trematodes is the reduction of the normal columnar epithelial cells of each acinus to cuboidal or even squamous epithelium . This phenomenon has been reported by Cheng and Snyder ( 1962) as has been confirmed by James ( 1965). This morphological change can be attributed to the starvation of cells as the result of the blockage haemolymph channels by the parasites ( Rees, 1936; James 1965 ).

Considering the ovotestis, it has been noted earlier that a reduction in the fecundity of molluscs parasitized by larval trematodes is known . This information is the result of studies done by Hurst (1927) ; Rees ( 1936) ; Malek ( 1952) ; Coelho ( 1954 ) ; Pan ( 1965); Strock (1967); Kamel ( 1979) and Abdel Reheem ( 1985 ).

In the present study, it was observed that B.alexandrina infected with S.mansoni, sporocysts invaded the connective tissue around and between the acini of the ovotestis, but non of them found inside the gonad by this Schistosome having daughter sporocysts stage is not the result of mechanical influence of the parasite, but rather by induced physiological conditions that tend to disturb the metabolism of the snail host. The results run in full agreement with those described by Faust (1920), Agersborg ( 1924) and Malek ( 1980 ).

The present work is the first in a series dealing with the histopathological and immunological studies of Biomphalaria alexandrina infected with Schistosoma mansoni. It is hoped that the encouraging results obtained in the present study will stimulate further investigation in the field of Schistosomes control in Egypt.

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EXPLANATION OF FIGURES

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- Fig.1. Transverse section through the visceral hump of uninfected B.alexandrina showing the digestive gland tubules (X 33.3).
- Fig.2. Transverse section through the digestive gland tubules of uninfected B.alexandrina showing the digestive gland tubules cell walls and the basal nucleus ( X 66).
- Fig.3. Transverse section through the ovotestis of uninfected B.alexandrina showing the manufun - tion of both ova and sperms (X66).
- Fig.4. Magnified transverse section through the ovotestis acinus of uninfected B.alexandrina showing the germinal epithelium, oocytes , mature ovum and spermatozoa in the center of the acinus lumen ( X 132 ).
- Fig.5. Transverse section in the foot of uninfected B.alexandrina showing the foot epithelium , connective tissue and mucous cells (X 33 ).
- Fig.6. Transverse section of 15 days post infected specimen of B.alexandrina showing the location of sporocysts between the tubules ( X 66 ).
- Fig.7. Transverse section of 15 days post infected specimen showing the location of the parasite between the ovotestis acini ( X 132 ).

- Fig.8. Different stages of developing sporocysts within the head-foot of 15 days post infected specimen of B.alexandrina ( X 132 ).
- Fig.9. Section through the mantle cavity of 15 days post infected B.alexandrina showing location of the parasites ( X 33 ).
- Fig.10. Section in 15 days post infected specimen showing infected foot and connective tissue ( X 66 ).
- Fig.11. Magnified transverse section of 30 days post infected specimen showing the sporocysts within the buccal cavity ( X 66 ).
- Fig.12. Transverse section of 30 days post infected specimen showing infected digestive gland and the ovotestis ( X 66 ).
- Fig.13. Transverse section of 45 days post infected specimen showing the break down of digestive gland tubules ( X 33 ).
- Fig.14. Transverse section in the ovotestis of 45 days post infection showing the displacement of the acini by sporocysts and cercariae ( X 132 ).
- Fig.15. Transverse section of 45 days post infected specimen showing the destruction of digestive gland cells ( X 66 ).
- Fig.16. Section of 45 days post infected ovotestis of B.alexandrina showing sporocysts and cercariae between the acini ( X 66 ).



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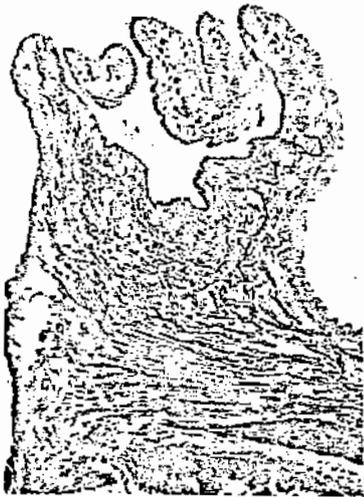
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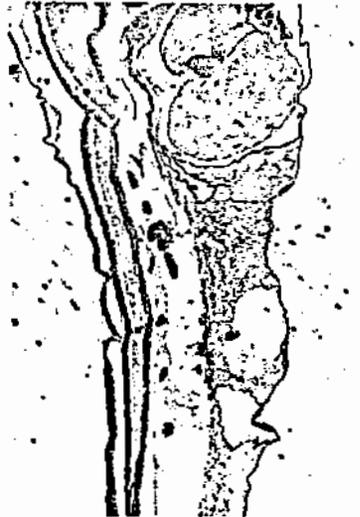
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