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Development of the Japanese Liver Fluke and Pathological Changes in the Snail Host *Lymnaea truncatula*

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Introduction

Fascioliasis is prevalent among livestock in the northern and northeastern districts of Hokkaido, Japan, where *Lymnaea ollula,* the common snail host of the liver fluke in Japan, is not distributed (Itagaki and Imai, 1964), and the intermediate host had not been determined until experimentally confined to be *L. truncatula* by Kamiharako *et al.* (1986).

The present paper deals with the migration and development of *Fasciola* larvae and the histopathological changes in the host snail.

Materials and Methods

Fasciola eggs were obtained from the uterus of the flukes collected from cattle slaughtered at Toyotomi-machi, Teshio-gun, Hokkaido. The eggs were incubated at 20°C for 31 days in darkness, and then the temperature was raised to 30°C on the day before infection. The embryonated eggs were exposed to light for miracidia to hatch simultaneously.

The Teshio strain of *L. truncatula* has been raised in our laboratory and snails 5.5–8.0 mm long were used. Snails were exposed to 50 miracidia each at 25°C for 8 hours in a test tube, 15 cm long and 1.5 cm in diameter, with about 1 ml of dechlorinated tap water. Infected snails were kept in plastic aquaria of a size of 22 x 15 cm and 14 cm deep at 25°C. Snails were supplied with proper quantity of the modified Standen's food (Standen, 1951). Two infected snails each were fixed in Bouin's solution 8 and 24 hours, 3, 5, 7, 10, 15, 20, 25 and 30 days after exposure, and were then transfered to 70% ethanol, in which fine sand grains were removed from the gizzard by dissecting the side wall. Snail specimens serially sectioned 8–10 μm in thickness and stained with hematoxylin and eosin by the routine procedure.

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Results

Development of fluke larvae in snails

1. Sporocysts

The total number of sporocysts detected was almost constant from 8 hours to 5 days after exposure; but they decreased rapidly after day 7, when rediae were released from sporocysts, and were hardly detected on day 15 (Fig. 3).

Ten to 20% of the sporocysts recovered showed such nuclear abnormalities as karyopyknosis and karyolysis in the sporocyst-wall cells and germ cells (Fig. 5). In the digestive gland region and heart, however, all the sporocysts were normal.

The development of sporocysts is described in each part of the snail body (Fig. 1).

1) Head-foot and mantle

Many sporocysts were observed in the hemocele and the loose connective tissue of the

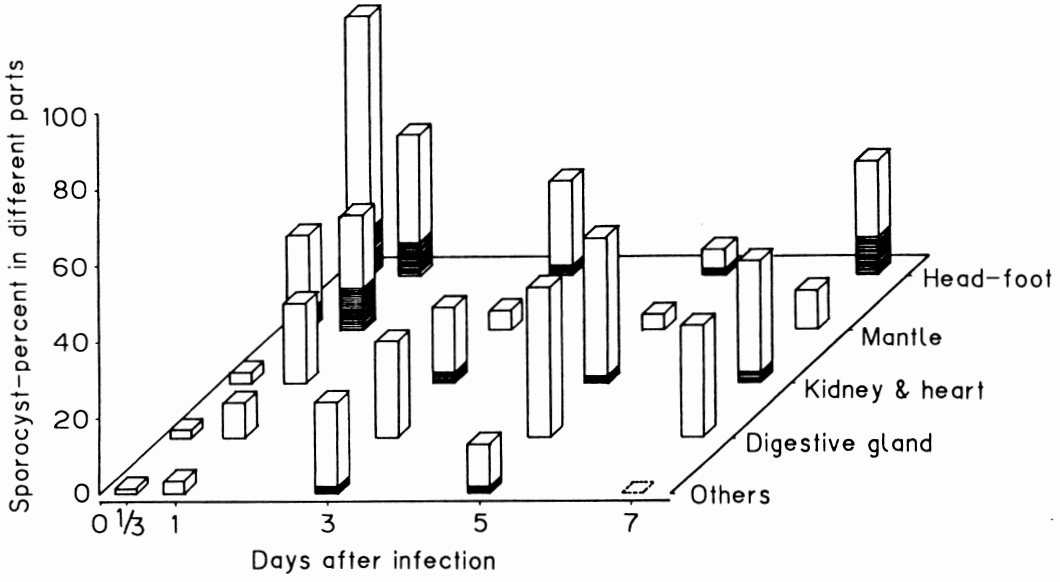

Fig. 1 Change in the number of sporocysts in different parts of snail body after exposure to miracidia. The shadowed portion shows that of abnormal sporocysts.

head-foot and mantle 8 hours after exposure (Fig. 4); but they began to decrease in number from 24 hours and a small number of them retained on day 5.

2) Kidney

Most of the sporocysts were observed in the hemocele around the kidney from 24 hours to 3 days after exposure.

3) Heart

Sporocysts were occasionally detected from the lumen of the heart, especially of the atrium, from 3 to 10 days after exposure.

4) Digestive gland region

Sporocysts were located in the hemocele among the tubules of the digestive gland and around the ovotestis, but not in the parenchyma of these organs. More sporocysts were located in the digestive gland region than in the other regions from 3 to 7 days after exposure.

5) Other organs

Sporocysts were observed in the hemocele around the stomach, intestine and reproductive organs.

2. Rediae

On day 5 after exposure, the germ balls in sporocysts began to develop to rediae with the pharynx. Rediae released from sporocysts were found on day 7 (Fig. 3). On day 15 most mother rediae had daughter rediae but no cercariae. Mother rediae producing a small number of daughter rediae were detected even after day 20.

In some of the rediae recovered the cells of the body wall and the germ cells were shrunken and their nuclei showed karyopyknosis or karyolysis (Fig. 7). The rate of abnormal rediae was closely related to their location: in the head-foot and reproductive organs, most rediae were abnormal in morphology, whereas abnormal rediae were hardly seen in the digestive gland region (Fig. 2).

The development of rediae is described in each part of the snail body (Fig. 2).

1) Digestive gland region

Rediae were located in the hemocele and connective tissue among the tubules of the digestive gland and around the ovotestis, but not in the parenchyma of the digestive gland (Fig. 6). The number of rediae began to increase from day 7 after infection, especially from day 15 to 20.

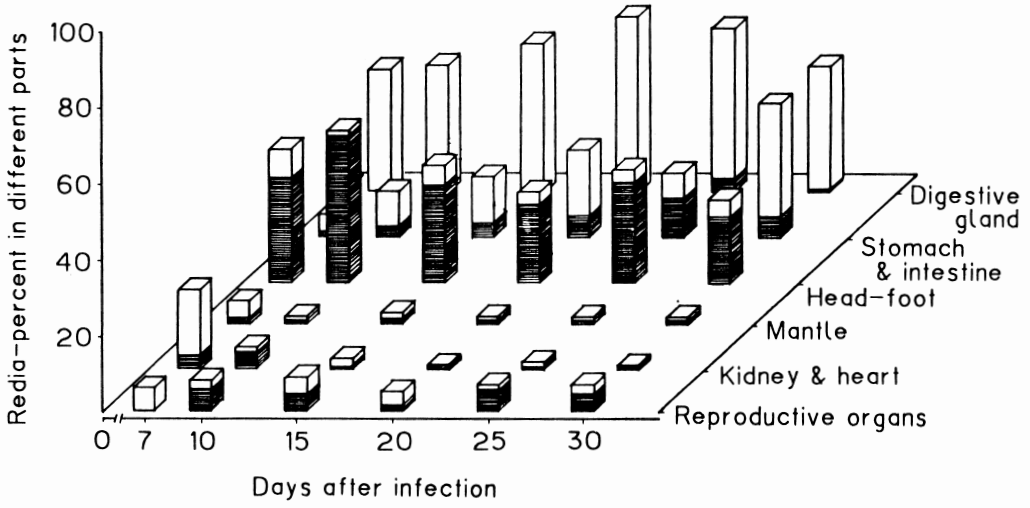

Fig. 2 Change in the number of rediae in different parts of snail body after exposure to miracidia. The shadowed portion shows that of abnormal rediae.

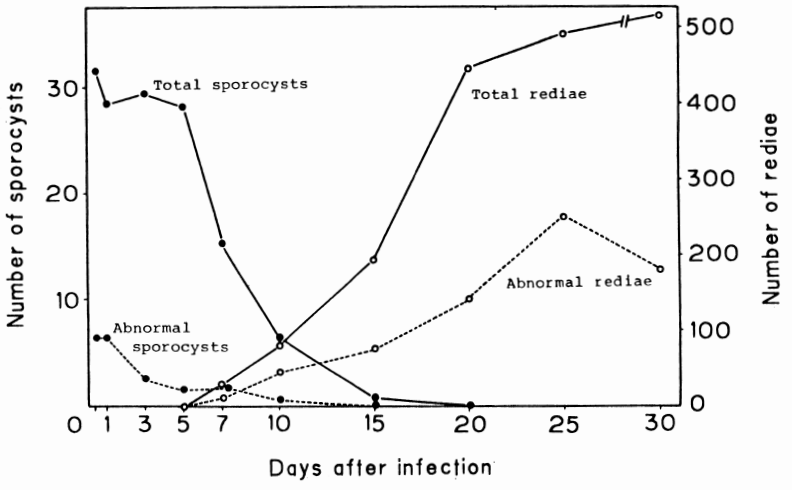

Fig. 3 Change in the average number of sporocysts and rediae in a snail host after exposure to miracidia.

2) Region of stomach and intestine

Rediae were located in the hemocele and connective tissue around the stomach and intestine, and their number increased markedly from day 25 to 30.

3) Head-foot and reproductive organ region

In the head-foot, most of the rediae were present in the cephalopedal sinus. In the region of the reproductive organs, rediae were present in the connective tissue and hemocele around the seminal vesicle, hermaphrodite duct, albumen gland and prostate gland and in the parenchyma of the ovotestis. The rediae in the ovotestis contained many yellowish granules, but no germ cells (Fig. 8).

4) Other parts of body

Only a small number of rediae were found in the mantle, kidneys and heart through the

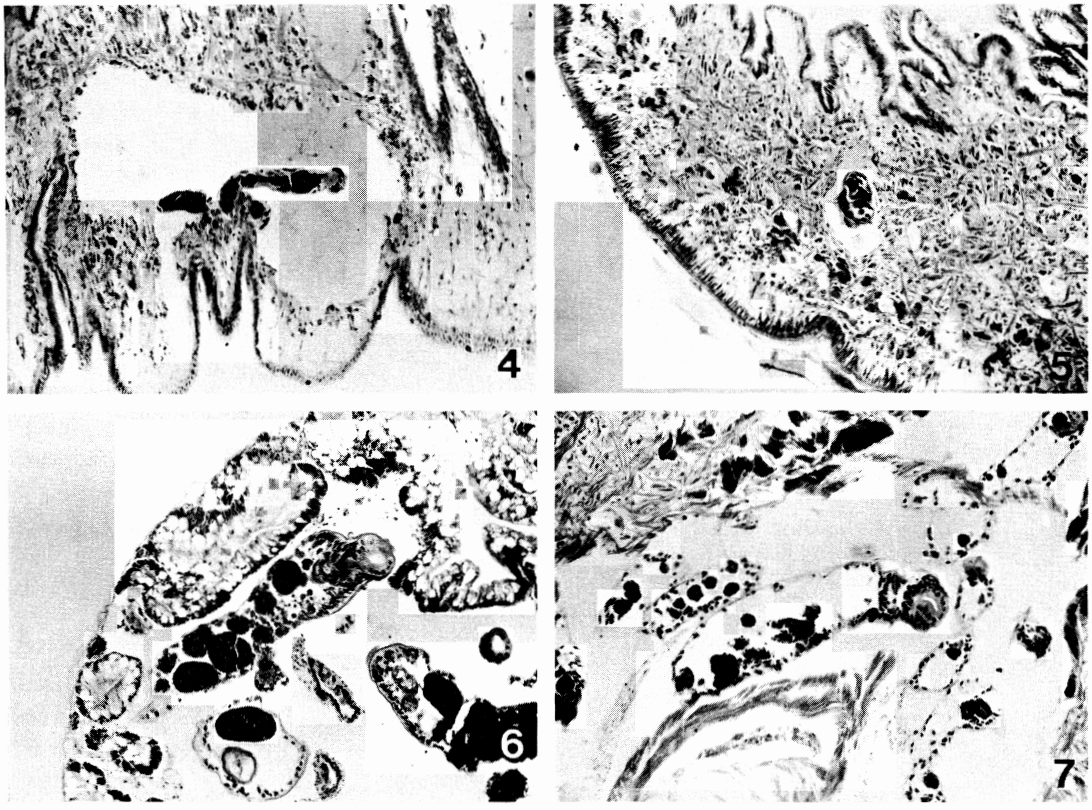

Fig. 4 Normal sporocysts in head-foot, 8 hours after exposure. × 35.
Fig. 5 An abnormal sporocysts in head-foot, 8 hours after exposure. × 35.
Fig. 6 Normal rediae in digestive gland region, 15 days after exposure. × 17.5.
Fig. 7 Abnormal rediae in cephalopedal sinus, 15 days after exposure. × 17.5.

course of development.

3. Cercariae

Cercarial development was different in different parts of the body. On day 20 after exposure, the rediae among the tubules of the digestive gland and around the stomach and intestine included germ balls producing cercariae, whereas none of those in the cephalopedal sinus and around the reproductive organs contained such germ balls. On day 30, mature cercariae were released from rediae. Cercarial shedding started on day 33 after exposure.

Pathological changes in host snails

Normal sporocysts, rediae and cercariae provoked no tissue response of host snails, but abnormal rediae were occasionally surrounded and phagocytized by amebocytes (Fig. 9).

The epithelium of the head-foot and mantle was transformed or displaced at the sites invaded by miracidia. The shrinkage and necrosis of the connective tissue were seen around sporocysts. The most marked morphological changes were provoked by rediae in the digestive gland region and ovotestis. In the digestive gland region, the tubules were reduced in number and the intertubular connective tissue often disappeared. The ovotestis became markedly atrophic and degenerate in all the snails examined from day 15 to 30. The infected ovotestis contained yellowish granules of different sizes and a small number of germ cells (Fig. 10). The changes in the digestive gland region and ovotestis became marked

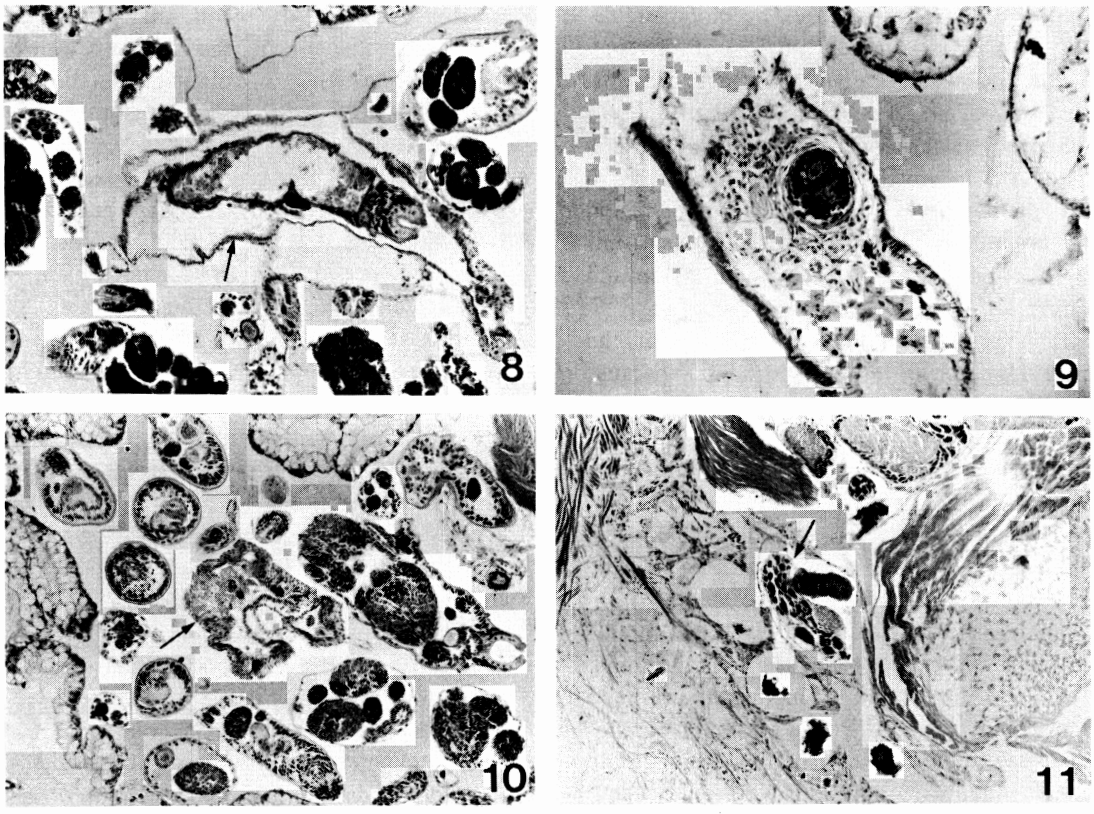

Fig. 8 A redia invading ovotestis, 15 days after exposure. x 28. An arrow shows the wall of ovotestis.
Fig. 9 A degenerated redia surrounded by amebocytes in head-foot, 20 days after exposure. x 35.
Fig. 10 Atrophy of ovotestis (arrow), 20 days after exposure. x 23.
Fig. 11 Transformation of central ganglion (arrow) by invasion of a redia, 30 days after exposure. x 23.

with the increase in the number of rediae. The central ganglia were sometimes invaded by rediae, and the ganglion cells were deformed and atrophied (Fig. 11).

Discussion

There are many informations available on the migration of larval trematodes in host snails. *Fasciola* sporocysts begin to migrate from the invading sites to the heart, kidneys and the regions around the intestine and digestive gland 24 hours after infection (Kawanaka, 1978). In *Tarebia granifera mauiensis, Philophthalmus gralli* larvae migrate by utilizing blood circulation (Alicata, 1962). In *Australorbis glabratus,* daughter sporocysts of *Schistosoma mansoni* migrate by their own vermicular locomotion (Pan, 1965). In this study *Fasciola* sporocyst hardly conducted vermicular movement. Judging from the blood circulation of *Lymnaea* snails (Bekius, 1972), it seems that sporocysts are carried principally by blood circulation to different parts of the body: sporocysts are carried through the venous system from the head-foot and mantle to the heart by way of the kidneys. They are then pumped out of the heart and carried to the anterior part of the body through the anterior aorta and to the posterior part, especially to the digestive gland region, through the posterior aorta. The sporocysts carried to the posterior part reach the hemocele where they develop rapidly, whereas most of those to the

anterior part are carried back by blood circulation to the posterior part of the body by way of the heart and kidneys. Some sporocysts, however, stay and develop in the heart.

Sporocysts developed better in the visceral sinus than in any other part of the body, so that, the hemocele in the digestive gland region and around the stomach and intestine seems to be suitable for sporocysts to develop and mature.

Rediae were not carried by way of the kidneys and heart unlike sporocysts, because the rediae in those organs did not vary in number with the time after infection. Most of the rediae released from sporocysts in the digestive gland region will develop there, but some of them will migrate to the cephalopedal sinus through the hemocele around the reproductive organs and esophagus.

The digestive gland region was most suitable for rediae to develop, because a large number of rediae were located in this region and more than 90% of them developed normally. Most of the rediae in the cephalopedal sinus and around the reproductive organs, on the other hand, were abnormal in morphology, so that this led us to conclude that those regions are not favorable for rediae to develop. Rondelaud and Barthe (1980) also reported that the degeneration of *F. hepatica* rediae is closely related to their locations in *L. truncatula.*

Many reports have been published on the pathological changes of snail tissues induced by larval trematodes. In *A. glabratus* infected with *S. mansoni,* the germ cells become very small in number by six weeks after infection, but neither degeneration nor atrophy is present in the ovotestis (Pan, 1965). In *L. auricularia* infected with *Echinostoma revolutum,* the ovotestis is disintegrated and markedly histolyzed by rediae and sometimes reduced in size (Patnaik and Ray, 1966). In *L. truncatula* infected with *F. hepatica,* the ovotestis is atrophied and degenerated by three weeks after infection (Hodasi, 1972). In the present study, the morphological changes of the ovotestis somewhat agreed with those reported by the previous authors. It seems that these morphological changes of the ovotestis will be produced by the ingestion or compression of the tissue by rediae or by some metabolites of rediae.

In the digestive gland of *L. auricularia,* histolysis, displacement, atrophy and reduction are present in the glandular tissue (Patnaik and Ray, 1966). In *L. catascopium* infected with *Schistosomatium douthitti,* the gland is atrophied and its tubules are reduced in number (Loker, 1979). In *L. stagnalis* infected with strigeid sporocysts, cytolysis of the gland cells is induced by sporocysts, although the tubules are not destructed even in the heavy infection (Bertman, 1980). Consequently, the changes in the digestive gland are different between the species of trematodes and snails concerned. The histological changes in the digestive gland region observed in this study will be due to the ingestion and compression by rediae.

Mother sporocysts and cercariae of *S. mansoni* frequently invade the central ganglia of *A. glabratus* to cause the compression of ganglion cells and the local constriction of the nerve trunk (Pan, 1965). In this study also, the same situation was observed and the infected snails seemed to suffer from a nervous trouble.

Summary

Sporocysts of the Japanese liver fluke were carried by blood circulation from invading sites to different regions by way of the kidneys and heart, whereas rediae were not carried. The hemocele among the tubules of the digestive gland and around the stomach and intestine (the visceral sinus) was most favorable for sporocysts and rediae to grow and mature, whereas that in the head-foot and around the reproductive organs was unsuitable for rediae to develop.

The larval stages, especially rediae, markedly injured the ovotestis and digestive gland sinus. Pathological changes in the ovotestis

were atrophy, degeneration and disappearance of germ cells, and those in the digestive gland region were the reduction and loss of the intertubular connective tissue and the decrease in the number of tubules. These pathological changes were recognized in all the snails examined from 15 to 30 days after infection.

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中間宿主における北海道天北地方産肝蛭の発育と病理学的変化

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コシダカモノアラガイ体内における北海道天北地方産肝蛭の発育と，貝体の病理学的変化を観察するため感染実験を行ない次の結果を得た．

1．肝蛭スポロシストは侵入部位から体液循環を利用して，腎臓・心臓を径て貝体各部に移行する．スポロシストの発育と成熟に最も適した部位は，中腸腺の導管周囲結合織（血体腔）である．

2．中腸腺部でスポロシストより遊離したレジアの多くは移動することはないが，一部のものは中腸腺部より生殖器官・食道周囲血体腔を通って頭足洞に移動する．レジアの発育・成熟に適した部位は中腸腺の導管周囲及び胃・腸管周囲の結合織（血体腔）である．しかし，頭足部の血体腔（特に頭足洞）及び両性腺を除く生殖器官の周囲部は不適である．

3．感染貝は肝蛭幼虫寄生により病害を受けるが，特にレジアによる両性腺，中腸腺部の病変が著しい．両性腺は感染15日後より萎縮，変性し腺内の生殖細胞はほとんど消失した．中腸腺部では導管間の結合織の減少・消失及び導管数の減少がみられた．これらの変化は感染15日以降のすべての感染貝で観察された．