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Histochemical findings of the tribocytic organ and tegument of *Fibricola seoulensis*†

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Abstract: The tribocytic organ and tegument of *Fibricola seoulensis* were examined histochemically for the detection of carbohydrates, mucosubstances, amyloid, collagen and alkaline phosphatase. The surface, secretes, gland cells of the tribocytic organ, and the tegument of the worms were positive to periodic acid Schiff (PAS) and PAS with diastase stain but negative to other stains. It was inferred that the tribocytic organ and tegument of *F. seoulensis* comprise neutral mucopolysaccharides, which may take a protective role against host enzymes. The surface and secretes of the tribocytic organ, and the tegument of the worms were also positive to double bridge PAP for alkaline phosphatase. This fact suggests that they may play a role as both self protective and host tissue lytic functions.

Key words: *Fibricola seoulensis*, tribocytic organ, tegument, histochemistry, neutral mucopolysaccharide, alkaline phosphatase

INTRODUCTION

The major histopathological findings of fibricoliasis were villous flattening and crypt hyperplasia (Lee *et al.*, 1985a), which is one of the common features in intestinal trematodiases such as metagonimiasis (Chai, 1979) and echinostomiasis (Lee *et al.*, 1990). The mice heavily infected with *Fibricola seoulensis* suffered from weight loss, diarrhea, intestinal bleeding, malnutrition and even death(Huh *et al.*, 1988). There are two organs or structures in *F. seoulensis* which are thought important to evoke such symptoms and pathological lesions. One is the tegument and the other is the tribocytic organ. Scanning electron microscopical studies showed that *F. seoulensis* is covered with the tegument and tegumental spines like cobbles or hands. They are explained to be present for an expansion of their metabolic surface (Lee *et al.*, 1985b). The tribocytic organ is a characteristic structure in strigeid trematodes and is known to have adsorptive and secretory functions (Smyth, 1976). It can protrude as well as retract. It is armed with sharp spines which are believed to take the role of an anchor to the host intestinal mucosa, as shown by electron microscope studies(Lee *et al.*, 1985b; Seo *et al.*, 1984).

Are there any metabolic or chemical functions in these two organs of *F. seoulensis*? So as to provide an answer to this question, we observed some histochemical natures of these two organs, especially for chemical substances such as carbohydrates, mucosubstances, amyloids and collagens, and enzymes such as alkaline phos-

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phatase.

MATERIALS AND METHODS

The metacercariae of *F. seoulensis* were collected from pepsin-digested debris of visceral membranes of the American grass snake, *Rhabdophis tigrina*, purchased in Hongchon-up, Kangwon-do. Seven mice, weighing over 20 g and sexually randomized, were fed 300 metacercariae each through a ball-tip needle. Eight days post-infection all mice were sacrificed by cervical dislocation. Immediately after that, we opened the peritoneum and resected the duodenum. The duodenum was fixed with 10% neutral formalin (pH 7.2). Adult worms were collected from the remaining intestinal segments and fixed in 10% neutral formalin. Fixed samples were kept at 4°C for 24 hours, and dehydrated. They were embedded in 56°C paraffin, hardened at room temperature, cut into 5 μm thickness, and attached to slide glasses for stain.

In order to find out carbohydrates and mucosubstances, special histological stains such as periodic acid Schiff (PAS), PAS with diastase control, alcian blue (pH 2.5), toluidine blue, aldehyde-fuchsin and Sudan black B were executed (Drury and Wallington, 1980). For the detection of extracellular substances and collagen, Congo red and Gomori's rapid one step trichrome stains were performed (Drury and Wallington, 1980).

The double bridge peroxidase anti-peroxidase (PAP) technique was followed to figure out the distribution of alkaline phosphatase. The procedure was as follows. Treat the slides with 0.01 M phosphate buffered saline (PBS, pH 7.2) including 0.05% triton-X 100. Wash three times each for ten minutes. Treat with PBS including 0.3% peroxidase and wash for thirty minutes. React with normal goat serum(1 : 1,000) for one hour and remove the serum. React with monoclonal anti-alkaline phosphatase (1 : 1,000) overnight and wash for thirty minutes. React with goat anti-rabbit IgG(1 : 40) for one hour at room temperature and wash with PBS for 30 minutes. React with mouse PAP for thirty minutes and wash for 30 minutes. Repeat the reaction with goat anti-rabbit IgG (1 : 40) and mouse PAP (1 : 100). React with peroxidase substrates (0.2% peroxide and 0.1% DAB in PBS) for 2~15 minutes. Stop the reaction by washing with PBS for five minutes. Counterstain with hematoxylin.

RESULTS

The surface, secretes and gland cells of the tribocytic organ were positive to PAS (Fig. 1)

Table 1. Results of stains

Organ	PAS	PASD	AB	TB	AF	CR	TR	PAP
Tribocytic organ								
surface	+	+	−	−	−	−	−	+
secretion	+	+	−	−	−	−	−	+
gland cells	+	+	−	−	−	−	+	−
Tegument of the worms	+	+	−	−	−	−	−	+

Abbreviations; PASD: PAS with diastase control, AB: alcian blue (pH 2.5), TB: toluidine blue, AF: aldehyde fuchsin, CR: Congo red, TR: Gomori's trichrome, PAP: double bridge PAP.

(→)

Fig. 5. Aldehyde fuchsin stain. Sulfated MPS-deep purple violet, T: tribocytic organ, ×140.
Fig. 6. Sudan black B stain. Glycolipid-bluish black, T: tribocytic organ, ×140.
Fig. 7. Congo red stain. Amyloid-red, T: tribocytic organ, ×140.
Fig. 8. Gomori's rapid one step trichrome stain. Collagen-greenish blue, G: gland cells, ×140.
Fig. 9. Double bridge PAP for alkaline phosphatase. Positive-golden yellow, G: gland cells, TE: tegument, ×40.

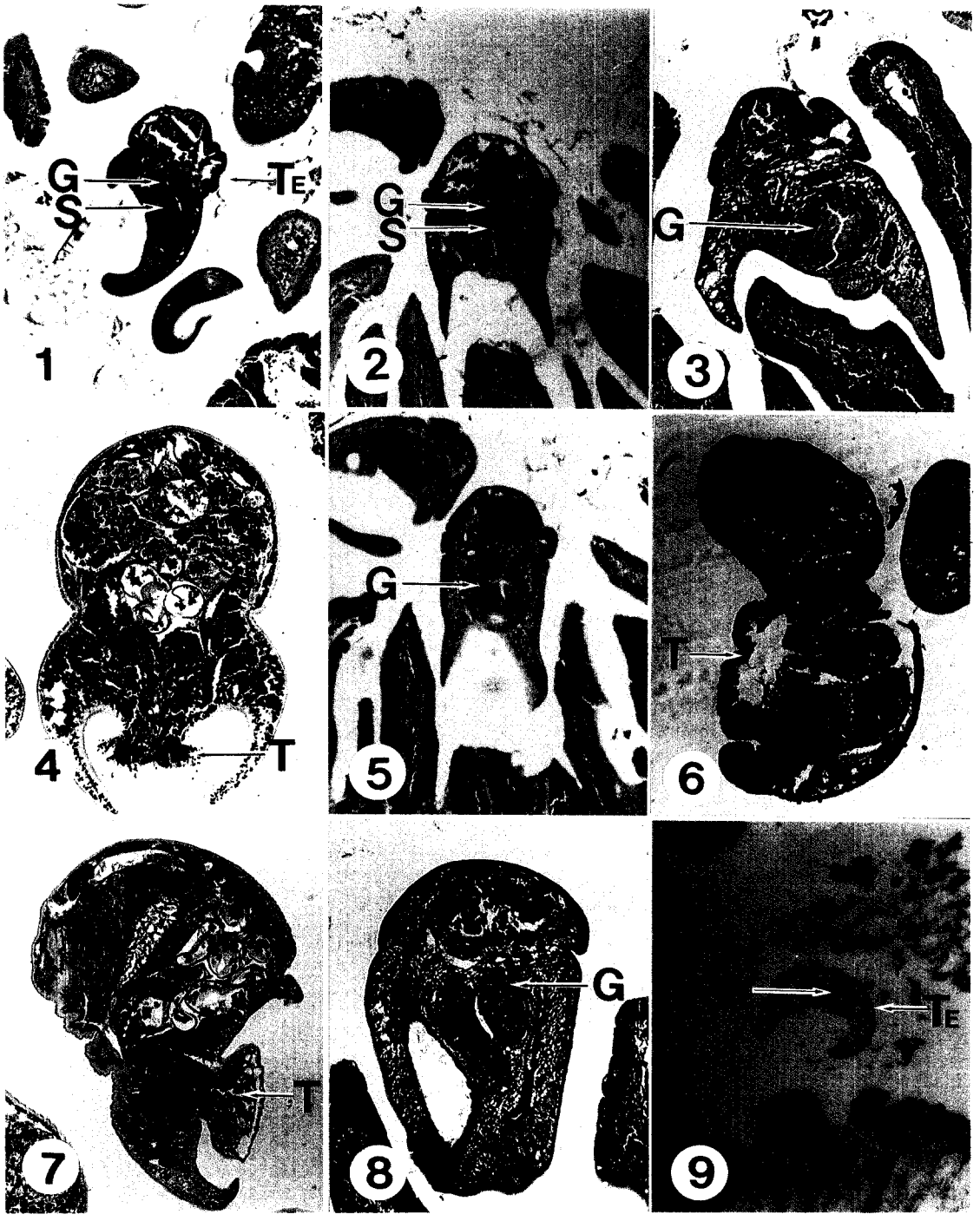

Fig. 1. PAS reaction. Positive-red, G: gland cells of the tribocytic organ, S: secretes of the gland, TE: tegument of the worm, ×140.

Fig. 2. PAS reaction with diastase control. Positive-red, G: gland cells, S: secretes of the gland, ×140.

Fig. 3. Alcian blue(pH 2.5) stain. Positive-blue, G: gland cells, ×100.

Fig. 4. Toluidine blue stain. Metachromatic substance-red, T: tribocytic organ, ×140.

and PAS with diastase stain (Fig. 2) The tegument of the worms was also positive to these stains. There was no difference between these two stains. Both of the two structures were negative to alcian blue (pH 2.5) (Fig. 3), toluidine blue (Fig. 4), aldehyde fuchsin (Fig. 5), Sudan black B (Fig. 6) and Congo red (Fig. 7). The interstitial tissue of gland cells of the tribocytic organ was positive to Gomori's trichrome stain (Fig. 8). The surface, secretes of the tribocytic organ, and tegument of the worms were all positive to double bridge PAP for alkaline phosphatase (Fig. 9). The results were as summarized in Table 1.

DISCUSSION

According to the above findings and staining properties of carbohydrates and mucopolysaccharides(MPS), it is suggested to be neutral MPS which was positive to PAS with diastase stain but negative to other stains(Tables 2 & 3) (Drury and Wallington, 1980). In other words, the surface secretes and gland cells of the tribocytic organ are considered to comprise neutral MPS. Neutral MPS is thought to be secreted from the gland cells, since the cytoplasm of gland cells and secretes were strong positive to PAS. MPS is now renamed as proteoglycan, which is a complex of glycosaminoglycan and protein (Murray *et al.*, 1988). Above structures were also positive for the activity of alkaline phosphatase. The interstitial tissue of gland cells of the tribocytic organ appeared to contain collagen.

What will be the function of neutral MPS for *F. seoulensis*? Probably it will take a protective role against host enzymes. It was argued that acid MPS present in the tegument of *Diplostomum spathaceum*, a fish trematode, protect the digestion of their tegument from proteolytic enzymes of the host (Öhman, 1965). In case of *F. seoulensis*, neutral MPS also seems to be involved in the degradation of host enzymes or metabolites in the intestine of mammalian hosts.

There found recently are many kinds of enzyme activities from some trematodes or their excretory-secretory substances. Acid phosphatase activity in the ceca, alkaline phosphatase activity in the excretory bladder and Mg-ATPase activity in the tegument and parenchymal cells of *Paragonimus westermani* were recently reported (Fujino *et al.*, 1989). Acid phosphatase, alkaline phosphatase, succinate hydrogenase and non-specific esterase activities were also found in the tegument of *Orthocoelium scoliocoelium* and *Paramphistomum cervi* (Sharma and Hanna, 1988). Thiol protease was released *in vitro* by *Fasciola hepatica*

Table 2. Staining reactions of carbohydrates and mucosubstances

Substance	PAS	PASD	AB	TB	AF	SB
Polysaccharides						
Glucose(glycogen)	+	−	−	−	−	−
Acid mucopolysaccharides						
Carboxylated	−	−	+	+	−	−
Sulfated	−	−	+	+	+	−
Neutral mucopolysaccharides	+	+	−	−	−	−
Mucoprotein(glycoprotein)						
Neutral	+	+	+	−	−	−
Carboxylated	+	+	+	+	+	−
Sulfated	+	+	+	+	+	−
Glycolipid	+	+	−	−	−	+

Abbreviations; PASD: PAS with diastase control, AB: alcian blue (pH 2.5), TB: toluidine blue, AF: aldehyde fuchsin, SB: Sudan black B

Table 3. Staining reactions of amyloid and collagen

Substance	Congo red	Gomori's trichrome
Amyloid	+	−
Collagen	−	+

(Dalton and Heffernan, 1989).

The alkaline phosphatase in the mammalian small intestine is believed to participate in the degradation of organic phosphate to free phosphate. Although there is no lucid evidence, this enzyme has been known to act in the substance transportation (Kaplan, 1972). However, so far uncertain is the function of alkaline phosphatase distributed in the body of helminths. Dusanic (1959) observed alkaline phosphatase activities in *Schistosoma mansoni* and reported that alkaline phosphatase will support the absorptive and secretory functions of the parasites. In *F. seoulensis*, this enzyme will take an important role in the functions of the tribocytic organ and tegument of the worms.

Then, what functions does the tribocytic organ have? Erasmus and Öhman(1963) proposed that the functions of the tribocytic organ of *Cyathocotyle bushiensis* include secretion of tissue lytic enzymes, adsorption to the host, absorption and digestion of the nutrients. He found the activities of protease, esterase and phosphatase from the secretes of its tribocytic organ. Öhman (1966) also confirmed that alkaline phosphatase, acid phosphatase, esterase and aminopeptidase are distributed in the tribocytic organ of *Holostephanus luhei*, a bird intestinal trematode. It was also reported that the tegument of the tribocytic organ of *Diplostomum phoenix* revealed acid phosphatase activity (Erasmus and Öhman, 1963).

In *F. seoulensis*, by the facts that MPS is secreted from the tribocytic organ and alkaline phosphatase activity was observed from the secretes, the functions of the tribocytic organ could be postulated as "secretion and lysis". The protrusion of the tribocytic organ and invasion into the lamina propria of host villi in tissue sections (Lee *et al.*, 1985a) and the presence of sharp tegumental spines covering the tribocytic organ (Seo *et al.*, 1984), can support the adsorption and host tissue lytic functions of this peculiar organ. It seems necessary to find out some proteolytic enzymes from the secretes and gland cells of the tribocytic organ of *F. seoulensis*, so as to understand better the mechanisms of tissue lysis seen in histopathological sections (Lee *et al.*, 1985a).

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=국문초록=

*Fibricola seoulensis*의 tribocytic organ 및 표피의 조직화학적 관찰 소견

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우리 나라에서만 인체 감염이 확인된 장흡충 *Fibricola seoulensis*의 특징적 구조인 tribocytic organ의 기능을 파악하는 연구의 일환으로, 이 구조물과 표피의 조직화학적 성질을 밝히기 위해 특수 염색을 시행하였다. 충체의 단면을 H & E 염색, 탄수화물, mucosubstance, amyloid 및 교원질에 대한 특수 염색 및 alkaline phosphatase에 대한 면역 조직화학 염색을 시행, 관찰하였다. Tribocytic organ의 표피, 분비물 및 선세포는 PAS 및 double bridge PAP에 양성이었다. 충체의 표피 역시 같은 염색에 양성이었다. 그러므로 *F. seoulensis*에서 tribocytic organ의 표피, 분비물 및 선세포와 충체의 표피는 neutral mucopolysaccharide를 함유하고 있으며 tribocytic organ의 표피, 분비물 및 충체의 표피는 alkaline phosphatase 활성도가 있음을 알 수 있었다. 이러한 구조들은 충체의 분비 기능 및 숙주 점막에의 부착 등에 관여할 것으로 생각된다.

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