HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 34, 85–90 (1980)

Histopathological effects of the adult digenetic trematode *Proctoeces ichiharai* on the kidney of top shell *Batillus cornutus*

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ABSTRACT: The histopathological effects of the adult digenetic trematode *Proctoeces ichiharai* (Fellodistomatidae) on the renal tissue of the marine gastropod *Batillus cornutus* (Turbinidae) collected from Misaki (Kanagawa Prefecture, Japan) were examined. The infected top shells showed such pathological changes as (1) discoloration of the kidney, (2) metaplasia and hyperplasia in the epithelium and subepithelial tissue of the renal coelom, (3) cyst formation by accumulation of excretory materials in the collecting ducts and renal tubules, and (4) appearance of eosinophilic granular cells.

INTRODUCTION

The majority of digenetic trematode larvae are parasites in the digestive gland of molluscs, while the adults are parasites of vertebrates. However, some species of the genus *Proctoeces* show an abbreviated life cycle by restricting themselves to and attaining maturity in the molluscan hosts (Freeman & Llewellyn, 1958; Stunkard & Uzmann, 1959; Ichihara, 1965; Loos-Frank, 1969; Lang & Dennis, 1976; Shimura & Egusa, 1979).

Previous studies on the pathology of the helminth infection in molluscs (reviewed by Wright, 1966; Cheng, 1967) have been mostly concerned with the pathological effects of the larval Digenea on the digestive gland of pelecypods and gastropods, and little attention was paid to the pathology of molluscan hosts infected by adult trematodes. To fill this gap in our knowledge, this paper describes the histopathological effects of the adult digenetic trematode *P. ichiharai* on the renal tissue of the marine gastropod *Batillus cornutus*.

MATERIALS AND METHODS

Top shells *Batillus cornutus* were collected from Misaki (Kanagawa Pref.) and Kominato (Chiba Pref.) in Japan between February 1974 and October 1975. Although the specimens obtained from Misaki showed a high incidence of infestation, those from Kominato harbored no parasite.

The soft parts of the top shells were carefully removed from their shells and preserved in either Bouin's solution or 10 % formalin. After fixation, the specimens were dehydrated in ethanol, embedded in paraffin and sectioned at 4–6 μm. The sections were

 0174-3597/80/0034/0085/$ 02.00

stained with H-E procedure. When necessary, azan, Masson's trichrome, Giemsa and periodic acid-Schiff (PAS) stainings were also employed.

RESULTS

Kidney of *Batillus cornutus*

The external appearance of the soft body and the infection site of the parasite have been described by Shimura & Egusa (1979). The parenchyma consists of the renal tubules and collecting ducts and is located between the epidermis and the renal coelom (Fig. 2A). The coelom is lined with a single layer of epithelium. The renal tubules, collecting ducts and epithelium of the renal cavity are composed of ciliated cells. The renal tubules are connected to the collecting ducts which form a large duct opening into the coelom (Fig. 2B).

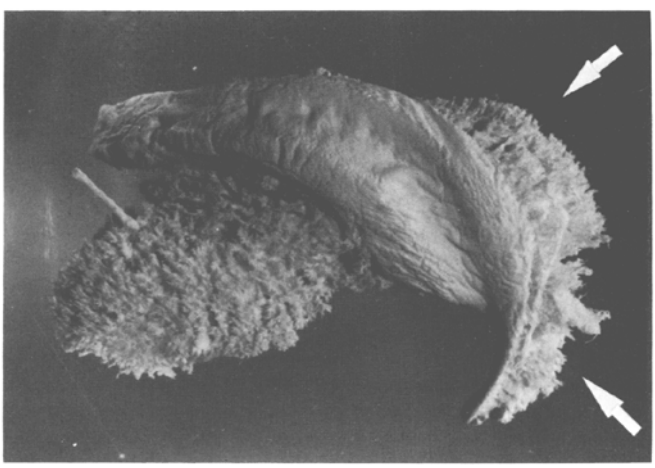

Fig. 1. A cast of the renal coelom of *Batillus cornutus* made with Neoprene Latex 601A; arrows indicate the most common site of infection (x 2.5)

A cast of the renal coelom made with Neoprene Latex 601A is shown in Figure 1. The openings of the collecting ducts to the cavity form villous protrusions. The right side of the cast is the distal end of the cavity. Arrows indicate the most common site of infection.

Discoloration in kidney

The uninfected kidneys are brown, the infected ones dark green or whitish brown. The site of discoloration is typically restricted to the heavily infected area posterior to the pericardium. In the case of heavy infection, the discoloration extends through the entire kidney. The site of discoloration does not differ histologically from areas of normal color.

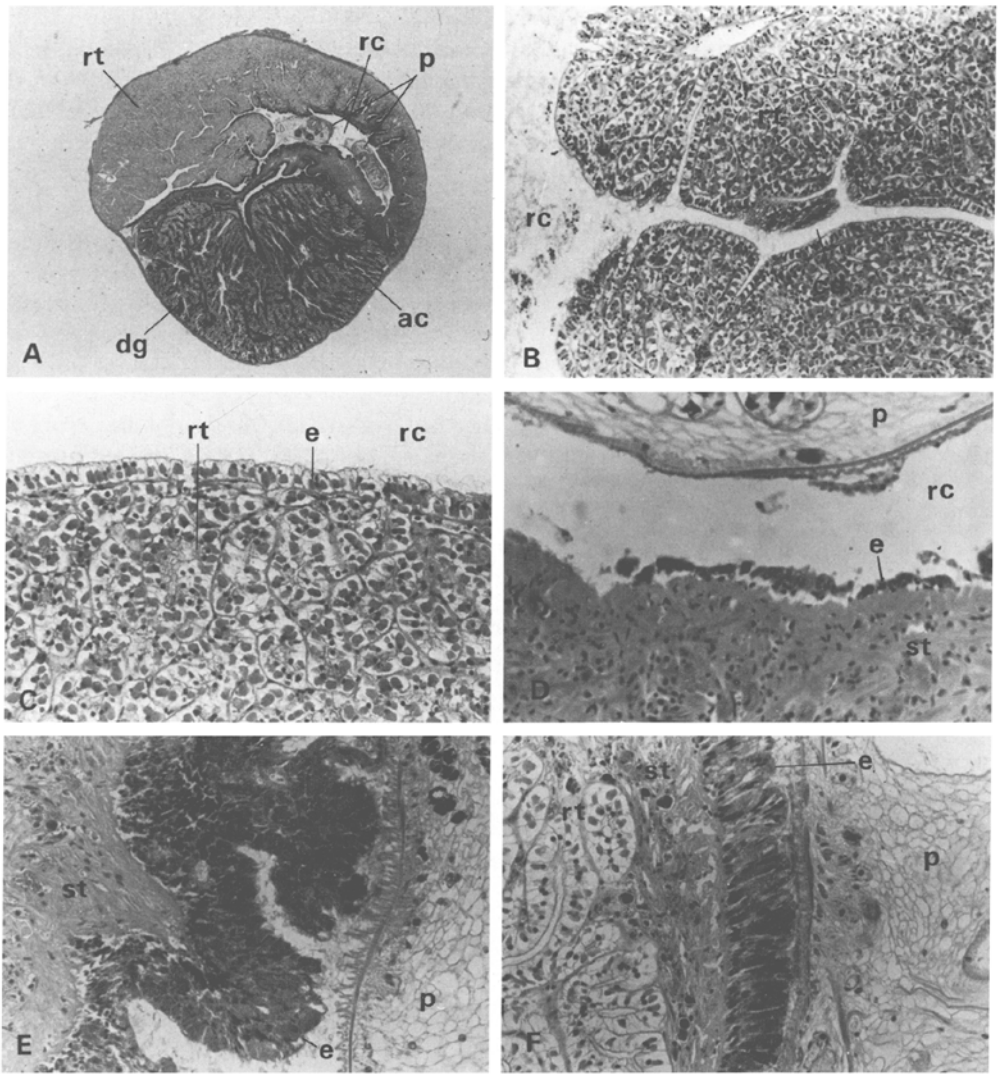

Fig. 2. Histopathological changes in the kidney of *Batillus cornutus* caused by the infection of *Proctoeces ichiharai*. (A) Transverse section posterior to pericardium through renal coelom and digestive gland of infected top shell; two parasites are visible in the coelom (x 3.7). (B) Uninfected kidney showing collecting ducts opening into the coelom (x 74). (C) Uninfected kidney showing the lining of renal coelom by a single layer of epithelium and the underlying renal tubules (x 148). (D) Damaged epithelium probably caused by mechanical stimuli of *P. ichiharai* (x 148). (E) Hyperplasia of epithelial tissues of infected kidney showing papillate and stratified epithelium with an increase of eosinophilic granular cells (x 148). (F) Infected kidney showing fibrosis between the epithelium and the renal tubules; notice the increase of eosinophilic granular cells in the epithelium (x 148). Abbreviations: ac: alimentary canal, c: cyst, cd: collecting duct, dg: digestive gland, e: epithelium, p: parasite, rc: renal coelom, rt: renal tubule, st: subepithelial tissue

Metaplasia and hyperplasia in epithelium

A single layer of columnar and ciliated epithelium lines the renal coelom of a normal *B. cornutus* (Fig. 2C), but the epithelial tissue of an infected top shell shows partial desquamation or degeneration at the infected site in the cavity (Fig. 2D). Progressive deformation is also observed at other parts of the epithelium. This is due to epithelial hyperplasia showing stratified or papillate structure coupled with proliferation of eosinophilic granular cells (Fig. 2E).

Metaplasia and hyperplasia in subepithelial tissue

The subepithelial connective tissues of the renal coelom occur only near the alimentary canal (Fig. 3A), but there is no connective tissue between the epithelium and the glandular parts of the kidney (parenchyma) in an uninfected healthy snail (Fig. 2C). In the parasitized kidney, the collagen fibers appear between the epithelium and the parenchyma showing metaplasia of fibrosis (Fig. 2F), and the subepithelial connective tissue adjacent to the digestive tract becomes remarkably thickened (Fig. 3B). The metaplasia and hyperplasia in the epithelium and subepithelial tissue are apparently caused by the prolonged mechanical stimuli resulting from the sucking motion and locomotion of the parasite.

Cyst formation

In a heavily infected kidney, cysts are formed by an excessive accumulation of the excretory materials in the collecting ducts or renal tubules. The cyst size varied from a small one about 100 μm embedded in the parenchyma, through a medium size like a nodule beneath the epidermis (Fig. 3C), to a large one of several millimeters in diameter that pushes up the epidermis to form a swelling (Fig. 3D). The largest cyst in the samples examined is about 5 mm in diameter and appears dark red in color. A transverse section shows it is divided into compartments and consists of several smaller cysts presumably connected to one another (Fig. 3E).

Eosinophilic granular cells

Some peculiar cells filled with eosinophilic and PAS positive granules, apparently secretory in function, are observed in the infected top shells taken from the Misaki region. The cells are abundant in the epithelium (Figs 2E and 2F), but are also distributed among the cells of the collecting ducts and renal tubules (Fig. 3F). In uninfected snails obtained from the Kominato region no eosinophilic cells are detected (Figs 2B and 2C).

DISCUSSION

In freshwater mussels (*Anodonta* spp.) infected with *Aspidogaster conchicola*, host responses similar to those observed in *Batillus cornutus*, e. g. renal epithelial metaplasia and fibrosis of the underlying connective tissue, have been reported (Pauley & Becker, 1968). However, contrary to *A. conchicola*, no encapsulation of *Proctoeces ichiharai* was observed in *B. cornutus*. The proportion of the affected area to the entire kidney in *B.*

cornutus is small even in a heavily infected specimen. The remaining parts of the renal tissue, mainly the proximal and middle part of the kidney, show no significant histological difference from an uninfected kidney. Therefore, it is presumed that *P. ichiharai* causes little excretory malfunction to its host snail.

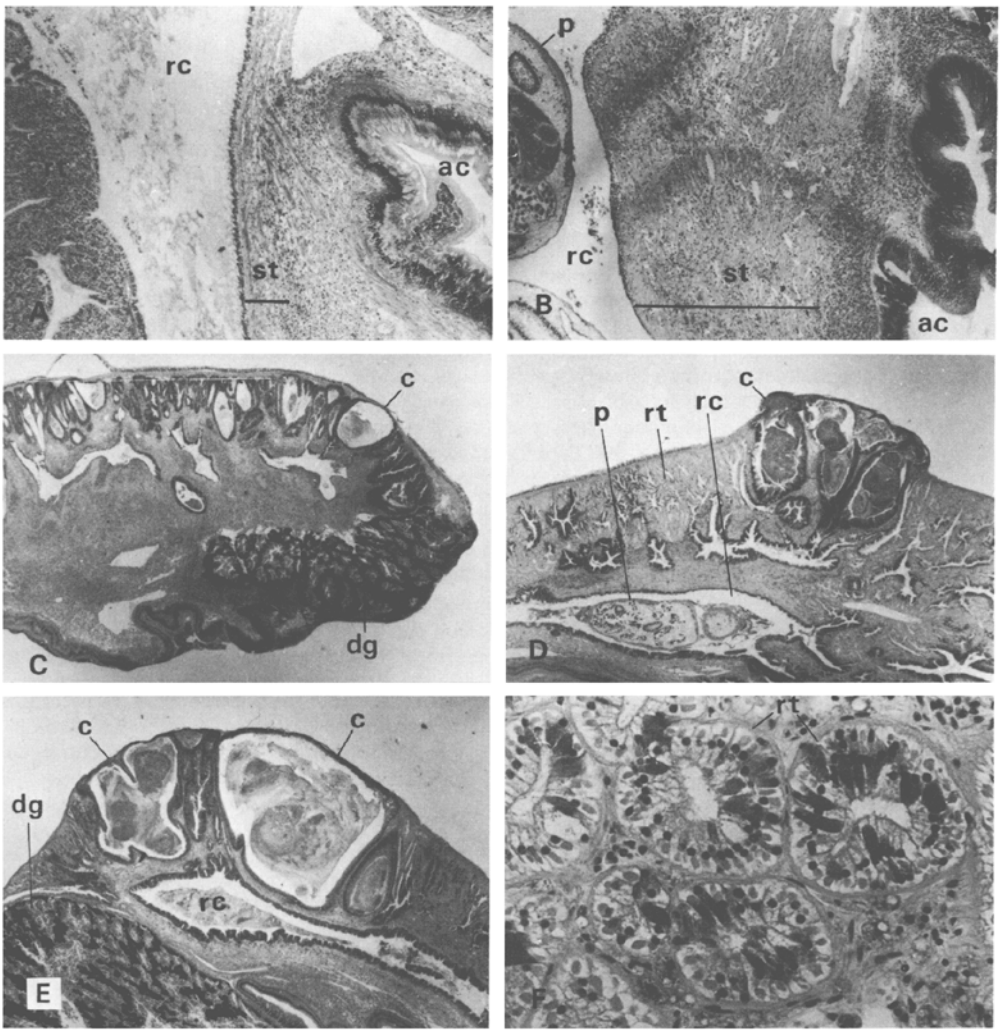

Fig. 3. Histopathological changes in the kidney of *Batillus cornutus* caused by the infection of *Proctoeces ichiharai*. (A) Uninfected kidney showing thin subepithelial tissue situated close to the alimentary canal; the line bar indicates thickness (x 30). (B) Infected kidney showing the same region in Fig. 3B; notice the remarkably thickened subepithelial tissue (x 30). (C) Numerous cysts of varying sizes beneath the epidermis (x 11). (D) Larger cysts; and increase of eosinophilic granular cells in the epithelium of the renal coelom, collecting ducts and lining of the cysts (x 11). (E) The largest cyst in the sample observed containing probably the excessive excretory materials (x 11). (F) The appearance of eosinophilic granular cells in the renal tubules of an infected kidney (x 222). For abbreviations see legend to Figure 2

Although Sakaguchi et al. (1970) reported emaciation in pearl oyster that was heavily infected with *Proctoeces* sp., an analysis of correlation between the intensity of *P. ichiharai* in a top shell and the condition factor, calculated as the ratio of soft body weight to shell height, provided no evidence of the influence of *P. ichiharai* on the growth of the host.

The process of cyst formation in the top shell kidney is thought to be as follows: initially, the metacercariae of *P. ichiharai* invade the renal coelom and settle there for a long period of growth and maturation; gradually, fibrosis occurs under the epithelial lining of the coelom due to the chronic effects of physical stimuli of the parasite; the collecting ducts then extrinsically close and the excretory materials accumulate in the ducts, which distend toward the epidermis and finally are macroscopically discernible through the epidermis.

Acknowledgements. I wish to express my deepest appreciation to Professor Dr. S. Egusa, Faculty of Agriculture, University of Tokyo, for his kind direction through the course of this study, and Professor Dr. J. S. Ho, Department of Biology, California State University, Long Beach, for his kind advice in the preparation of the manuscript. Particular thanks are due to Mr. N. Sueta for his help in preparation of specimens for histological studies.

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