[Jpn. J. Parasitol., Vol. 40, No. 6, 607–610, December, 1991]

Research Note

Scanning Electron Microscopical Diagnosis of Paraffin-sectioned *Schistosoma japonicum* Eggs from a Patient Stomach

MASATAKA KOGA[1], KAZUYOSHI NISHIHARA[2], YOICHI ISHII[1] AND MASAZUMI TSUNEYOSHI[2]

(Accepted for publication; November 13, 1991)

Key words: schistosomiasis japonica, paraffin sections, eggs, inner surface pits, scanning electron microscopy

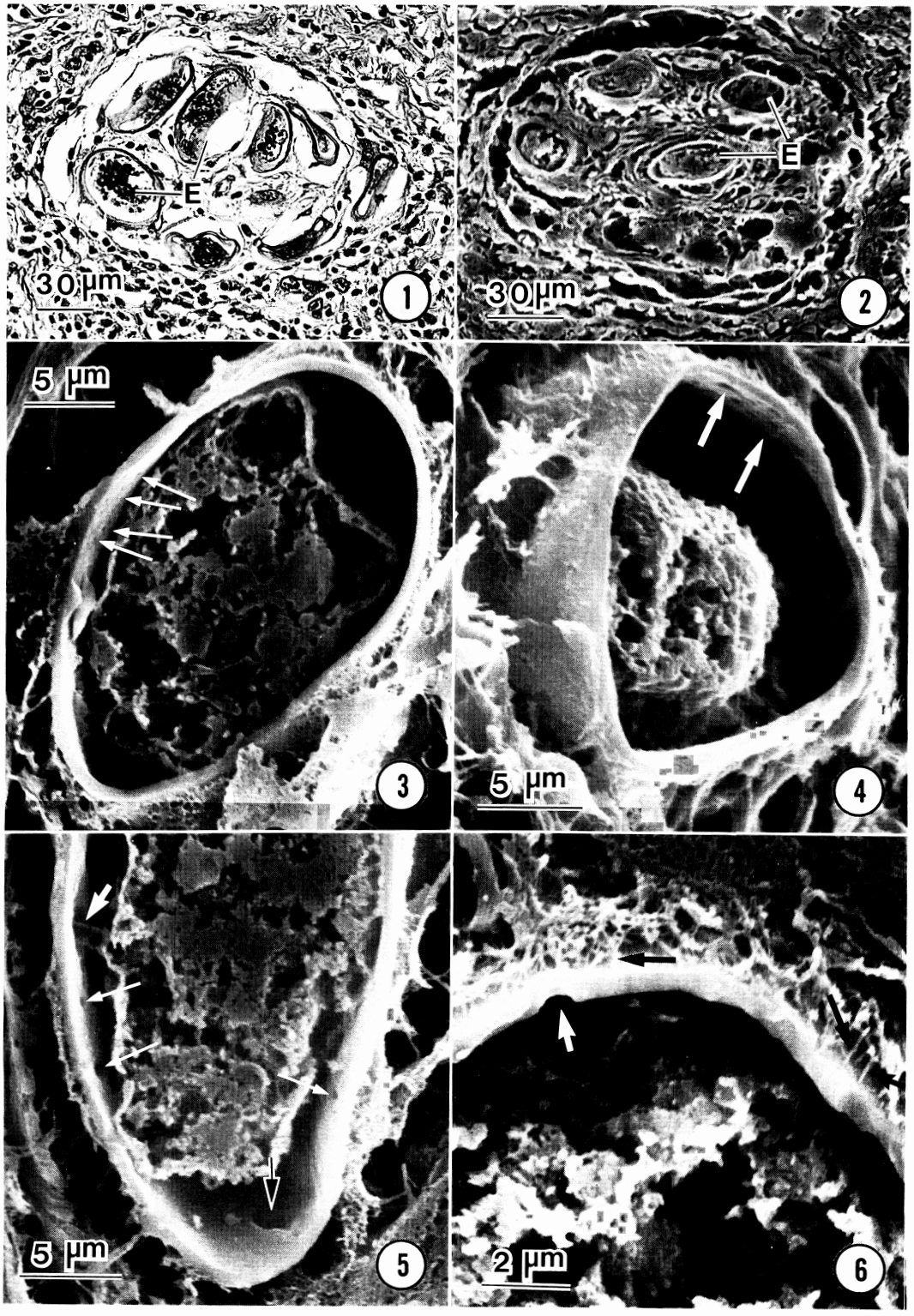
Schistosoma japonicum Katsurada, 1904 was a common parasite only three decades ago in Kyushu, Japan, but occurs quite rarely now. We encountered a patient who had eggs of *S. japonicum* in his stomach wall and regional lymph nodes. Paraffin-embedded and sectioned tissues were prepared for both light microscopy (LM) and scanning electron microscopy (SEM) to determine the value of SEM for diagnosing parasitic infections with paraffin-embedded tissue. Only one previous report has used SEM of paraffin-sectioned tissues to make a histopathological diagnosis (Ishii, 1972).

The patient was a 63-year-old male living in Saga Prefecture, Kyushu, Japan. He was admitted to the hospital for epigastralgia. He was diagnosed to have stomach cancer after fibroscopic examination of the organ, and the patient underwent a complete gastrectomy and removal of the perigastric lymph nodes. The cancer tissues were fixed conventionally with formalin, dehydrated in a graded series of ethanol, cleared with xylene, and embedded in paraffin. The paraffin-embedded tissues were sectioned at 4–5 μm and stained with hematoxylin and eosin for LM. Paraffin-embedded specimens were also sectioned at 15 μm for SEM. These sections were mounted on small glass plates (10×10 mm) and deparaffinized in xylene for six hours. The sections were then transferred to 100% ethanol followed by isoamyl acetate and dried in a critical point dryer. The specimens were coated with gold in an ion sputter coater (Hitachi HCP-1100) and examined with a JSM-U3 scanning electron microscope at 15 kV.

Light microscopic histopathology revealed adenocarcinoma and several foci of parasite eggs in the submucosa of the stomach (Fig. 1, E: eggs). Foci of parasite eggs were also present in the regional lymph nodes. The foci were surrounded by infilliates of fibroblast, fibrocytes, lymphocytes and macrophages. By SEM, each parasite egg was distinctly surrounded by host fibrous tissue (Fig. 2). This was true even in foci where eggs were adjacent to each other. Eggs were 58–65×35–38 μm in size and had no operculum. The eggshell measured 0.6–0.7 μm in thickness (Fig. 3). The eggshell surface had the microvilli-like chitinous projections that are characteristic of *S. japonicum* eggs (Fig. 6, black arrows) (Inatomi, 1962; Schnitzer *et al.*, 1971; Sakamoto and Ishii, 1976; He *et al.*, 1980; Zhang *et al.*, 1986; Koga *et al.*, 1988). However, a subterminal small spine on the shell surface occurred only in one egg.

Other noteworthy observations from this study were of previously undescribed pits on the inner surface of the eggshells. The pits were of variable sizes but could be divided into three types based on size. The first type was very small

[1] *Department of Parasitology,*
[2] *The second department of Pathology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan.*
古賀正崇　石井洋一（九州大学医学部寄生虫学教室）
西原一善　恒吉正澄（同・病理学第2講座）


E
30 μm
1
E
30 μm
2
5 μm
3
5 μm
4
5 μm
5
2 μm
6

(0.2–0.3 μm in diameter) and numerous, and was difficult to recognize at low magnifications (Fig. 3, arrows). The second type was 0.6–0.8 μm in size (Fig. 4, arrows; Fig. 5, white slim arrows) and occasionally a cross sectioned pit was found on the cut surface of eggshell, showing the depth of it (Fig. 6, white arrow). The third type was 1.6–2.2 μm in diameter (Fig. 5, white stumpy and black arrows) and was completely perforated. Fibrous host tissue was within the perforations, suggesting that the host cells were attacking the eggs (Fig. 5, white stumpy arrow). The circum-oval immune precipitin (COP) reaction may occur at the sites of the large pits. The small and intermediate pits do not perforate the eggshell and might play a role in release of the miracidium during hatching of the eggs because they had no opercula. While Cao *et al.* (1982) demonstrated some micropores and microcanals in the shell layer of *S. japonicum* eggs in transmission electron micrographs. Similar shell pores are described by Race *et al.*. (1971) using TEM in *Schistosoma mansoni.* The pits displayed in the present study might communicate with these micropores or microcanals.

Schistosomiasis was detected accidentally in this patient during histopathological examination of tissue sections. The patient has lived in an endemic area for a relatively long time, and the eggs that were examined exhibited signs of moderate degeneration. It was not known whether adult worms were still living within the mesenteric veins, but it is unlikely that viable eggs were still being produced and excreted. We suspect that the prevalence of similar occult infections may be higher than that of generally recognized in former endemic area.

Acknowledgements

We are grateful to Dr. C. T. Atkinson, U. S. Fish and Wildlife Service, Hawaii Research Group, Hawaii Volcanos National Park, for reading and correcting the manuscript.

References

1) Cao, H. M., Wang, Y. F. and Long, S. (1982): A study of ultrastructure of egg shell of *Schistosoma japonicum*. I. Transmission electron microscopic observation of *S. japonicum* egg. Ann. Parasitol. (Paris), 57, 345–352.
2) He, Y., Gong, Z. and Ma, J. (1980): Scanning and transmission electron microscopy of *Schistosoma japonicum* egg shell. Chin. Med. J., 93, 861–864.
3) Inatomi, S. (1962): Submicroscopic structure of the egg shell of helminths. Okayama Igakkai Zasshi, 74, 31–81.
4) Ishii, Y. (1972): Application of scanning electron microscope to histopathologic diagnosis in parasitic diseases. Igaku-no-Ayumi, 83, A-331–332.
5) Koga, M., Ishii, Y. and Huang, W. C. (1988): Scanning electron microscopy of egg-shell surface of *Schistosoma japonicum* (Chinese strain). Jpn. J. Parasitol., 37, 182–184.
6) Race, G. J., Martin, J. H., Moore, D. V. and Larsh, J. E. Jr. (1971): Scanning and transmission electron-microscopy of *Schistosoma mansoni* eggs, cercariae, and adults. Am. J. Trop. Med. Hyg., 20, 914–924.
7) Sakamoto, K. and Ishii, Y. (1976): Fine structure of schistosome eggs as seen through the scanning electron microscope. Am. J. Trop. Med. Hyg., 25, 841–844.

Fig. 1 Light micrograph of a cluster of *S. japonicum* eggs in the submucosa of the stomach. The focus is surrounded by infiltrated cells. E: eggs.

Figs. 2–6 Scanning electron micrographs.

Fig. 2 Same tissue as Fig. 1. Each egg is surrounded by fibrous host tissue. E: eggs.

Fig. 3 The eggshell is 0.6–0.7 μm in thickness and has no operculum. Many small pits (0.2–0.3 μm in diameter) are present inside the eggshell (arrows).

Fig. 4 A second type of internal pit measuring 0.6–0.8 μm in diameter (arrows) was present in other eggs.

Fig. 5 Two types of internal pits are present, intermediate sized ones similar to those in Fig. 4 (white slim arrows) and large pits measuring 1.6–2.2 μm in diameter (black and white stumpy arrows). Fibrous host tissue has invaded the egg through these large pit-like pores and is associated with the egg contents (white stumpy arrow).

Fig. 6 Eggs have microvilli-like chitinous projections which protrude from the outer surface of the eggshell (black arrows). Pits with intermediate dimensions (0.6–0.8 μm) penetrate to the middle of the eggshell (white arrow).

8) Schnitzer, B., Sodeman, T., Sodeman, W. A., Jr. and Durkee, T. (1971): Microspines on *Schistosoma japonicum* and *S. haematocium* egg shells. Parasitology, 62, 385–387.

9) Zhang, G. F., Cheng, X. L. and Teng, H. M. (1986): Ultrastructural observation on *Schistosoma japonicum* eggs at different development stages. J. Parasitol. Parasitic Dis., 4, 293.