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Comparative study on the eggshell of American *Paragonimus* through the scanning electron microscope*

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Introduction

On the American continent the following four species of *Paragonimus* are considered to be valid: i. e. *P. kellicotti* Ward, 1908, *P. caliensis* Little, 1968, *P. mexicanus* Miyazaki et Ishii, 1968 and *P. peruvianus* Miyazaki, Ibáñez et Miranda, 1969. *P. rudis* (Diesing, 1850), the oldest lung fluke, is regarded as a *nomen nudum* at present. However, the causative agent of human paragonimiasis in Central and South America remains unknown. The authors are rather skeptical about previous reports that it was caused by *P. westermani* or *P. kellicotti*. Miyazaki & Ishii (1968) compared eggs deposited in the lung tissue from a human case in Mexico reported by Martinez Báez & Jiménez Galán (1961) with those of *P. westermani*, *P. kellicotti* and *P. mexicanus*, and reported that they were very similar to those of *P. mexicanus*, stating the possibility of human infection by this lung fluke. Little (1968) entertained a suspicion that eggs found in sputum from a local Ecuadorian patient were not *P. westermani* but *P. caliensis* and that they were identical with those from a patient in Peru. Thereafter, Miyazaki *et al.* (1969) reported that eggs seen in the sputum of a Peruvian patient were similar to both *P. caliensis* and *P. peruvianus*. On the basis that *P. peruvianus* was detected from a cat in the same district as the Peruvian patient, they considered that the patient's infection was also caused by *P. peruvianus*. Thus, interesting information has accumulated concerning human paragonimiasis in Central and South America.

For diagnosis of paragonimiasis, detection of eggs is most reliable. Generally speaking, however, species identification by eggs only is not always easy. Criteria for differentiating *Paragonimus* eggs are described by previous investigators such as Chen (1940), Miyazaki(1954), Isshiki(1954, 1962), Yokogawa (1955), Kamo *et al.* (1961), Terauchi *et al.* (1961), Hatsushika (1967), Yoshida & Nishimura (1968), Miyazaki & Ishii (1968), Miyazaki *et al.* (1969), etc. Differences in eggs have been found in size, shape, ratio of the longitudinal and horizontal diameter, position of the maximum width, thickness and contour of the eggshell, opercular form and the contour of connection between the operculum and the egg body. However, these have been done with a light microscope except for an electron microscopic observation of *P. westermani* egg by Inatomi (1962) with the result that the surface structure still remains unclarified in many aspects. If differences were to be found in surface structure, it would contribute to the taxonomy and diagnosis of the species. Therefore, eggs of three kinds of American *Paragonimus* were studied with the scanning electron microscope.

Materials and Methods

Eggs observed were of *P. kellicotti*, *P. mexicanus* and *P. peruvianus*. *P. kellicotti* metacercariae were obtained from crayfish

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collected near Ann Arbor, Michigan, U. S. A. and were fed to cats and dogs. Uterine eggs were obtained from the cats ten months later and from the dogs 59 months after infection. For *P. mexicanus*, they were obtained from adult worms naturally infected in a Mexican opossum, and for *P. peruvianus*, they were from a naturally infected Peruvian cat. These samples were fixed in 10 % neutral formalin or 3 % glutaraldehyde (Millonig's buffer) and 1 % osmium tetroxide (Millonig's buffer to pH 7.4). After fixation, they were dehydrated with increasing concentrations of acetone, dried on a glass slide and coated with carbon and gold in a vacuum. A scanning electron microscope (JSM-2) was used for observation at 800–30,000 magnifications with a beam accelerating voltage of 25 kV.

Results and Discussion

The surface of eggshell of *P. kellicotti* including the operculum is generally smooth (Fig. 1). The operculum is relatively flat and central part is slightly raised in most eggs. The shell at the connection between the operculum and egg body is thick and raised (Fig. 2). At the abopercular end a starfish-like prominence of various size and form is seen on the shell (Figs. 1 and 3). This corresponds to the description by Ameel (1934) who said "this end is more or less thickened and is occasionally provided with a nodule of varied form". It also corresponds to "watchglass-like projection" or "shadow-like black thickening" described by Isshiki (1962) and Yoshida & Nishimura (1968) (Fig. 8). The shell surface of *P. mexicanus* is rough as compared with *P. kellicotti* and many pits measuring 1.0–1.6 μ in diameter are recognized on the shell (Fig. 4). The shell at the connection between the operculum and egg body is thick and prominent but not so marked as *P. kellicotti*. The operculum is prominent as a whole and not so smooth as *P. kellicotti*. The abopercular end assumes an undulating form and the eggshell is inwardly concave as shown by arrows in Fig. 5. The surface of *P. peruvianus* eggshell is rougher than that of *P. mexicanus* and pits of 1.0–2.8 μ in diameter are densely scattered. The contour of connection between the operculum and egg body resembles that of *P. mexicanus* (Figs. 6 and 7). The undulating contour of eggshell was described by Little (1968), Miyazaki & Ishii (1968) and Miyazaki *et al.* (1969) in *P. caliensis*, *P. mexicanus* and *P. peruvianus* respectively by light microscopy (Fig. 8), and was more conspicuous in *P. caliensis* and *P. peruvianus*. The feature can be seen in sectioned or whole mounted specimens (Fig. 9). The confirmation of this peculiar surface structure in the present study is interesting as a criterion for differentiating the species.

Hasegawa (1935) made a detailed investigation of the eggshell of trematodes, especially of *Metorchis*, *Opisthorchis*, *Exorchis* and *Clonorchis*, and Hilliard (1960) observed the egg surface of eleven kinds of Diphyllobothriid cestodes. Both authors found differences among some species by light microscopy. Ishii & Tokunaga (1970a) demonstrated eggs of *Diplogonoporus grandis* using the scanning electron microscope for the first time. They compared them with those of *Diphyllobothrium latum* and found differences in the size of pits and their distribution density on the shell surface. Ishii & Tokunaga (1970 b-d) and Ishii *et al.* (1970) obtained further interesting image on the eggshell of *Clonorchis*, *Schistosoma*, *Paragonimus* and *Gnathostoma*. The authors think that studies on the structure of eggshell with the scanning electron microscope will supply interesting answers to the problems involved in taxonomic and diagnostic studies of parasitic helminths.

Summary

Scanning electron microscopy on the eggs of American lung flukes showed that the surface of the shell was smooth in *P. kellicotti*, slightly rough in *P. mexicanus* and remarkably rough in *P. peruvianus*. Differences were also found in the form of operculum and abopercular end between *P. kellicotti* and *P. mexicanus*. These differences can be recognized to some

extent through the light microscope, as shown in Figs. 8 and 9.

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走査電子顕微鏡によるアメリカ産肺吸虫の卵殻の比較研究〔特別掲載〕

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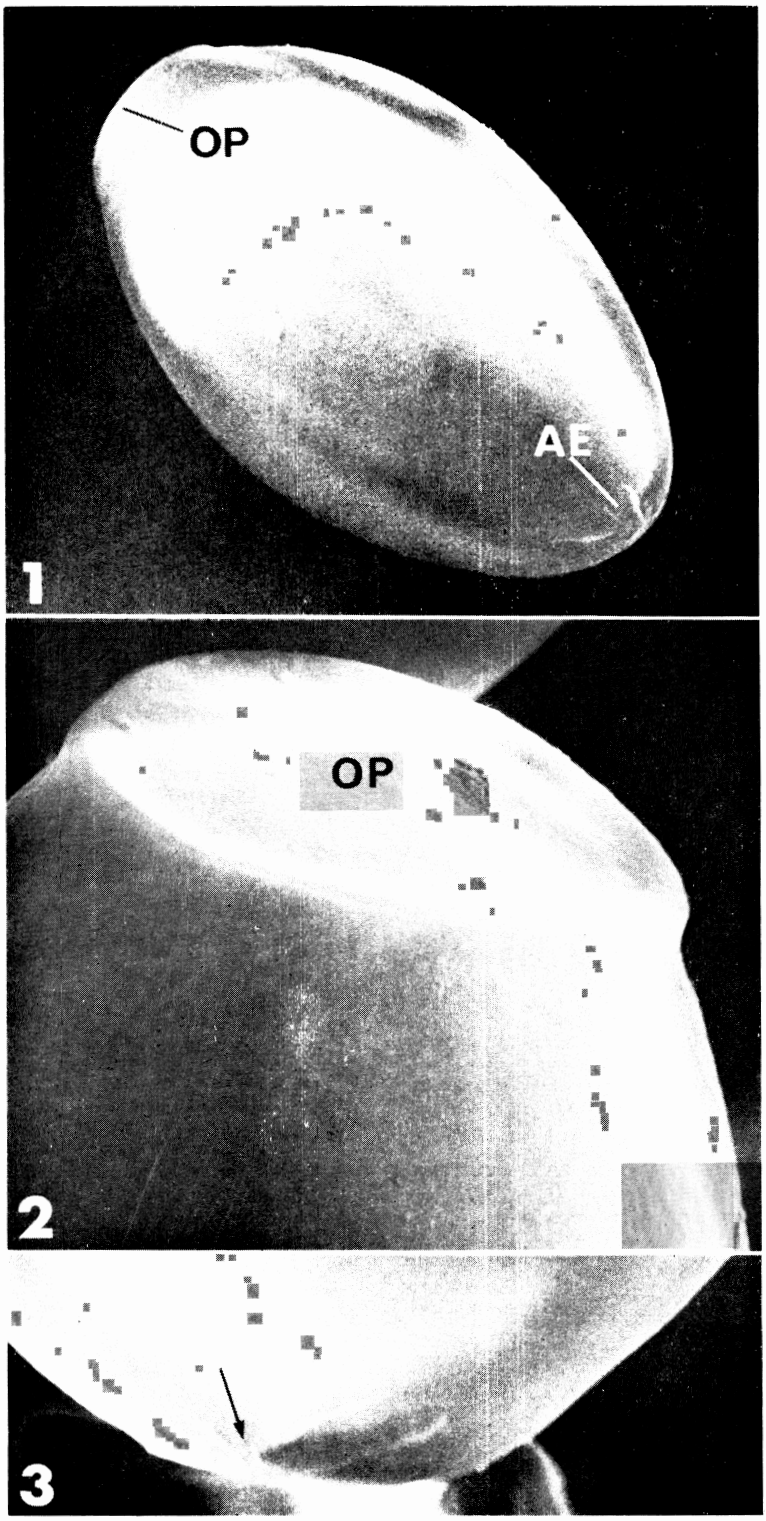
走査電子顕微鏡を利用してアメリカ産3種の肺吸虫即ちケリコット肺吸虫，メキシコ肺吸虫およびペルー肺吸虫の卵を観察した．卵殻表面はケリコット肺吸虫卵が平滑であるのに反して，メキシコ肺吸虫とペルー肺吸虫のは小さな凹みが多く見られる関係で全体に粗糙で，特にペルー肺吸虫卵はメキシコ肺吸虫卵に比較して凹みが深く凹凸の著明なものが多かつた．卵蓋はメキシコ肺吸虫，ペルー肺吸虫卵のものに比較してケリコット肺吸虫卵のものは扁平であつた．無蓋端はケリコット肺吸虫卵において大きさ，形態は多様であるが，ヒトデ様の隆起が卵殻上に認められたのに対して，メキシコ肺吸虫卵では波状構造を呈し，卵殻が内側に陥入しているものが多かつた．これらの差はすでに光顕的にもみとめられていたが，今回の観察によつて形像として得ることができ，一層明瞭に確認することができた．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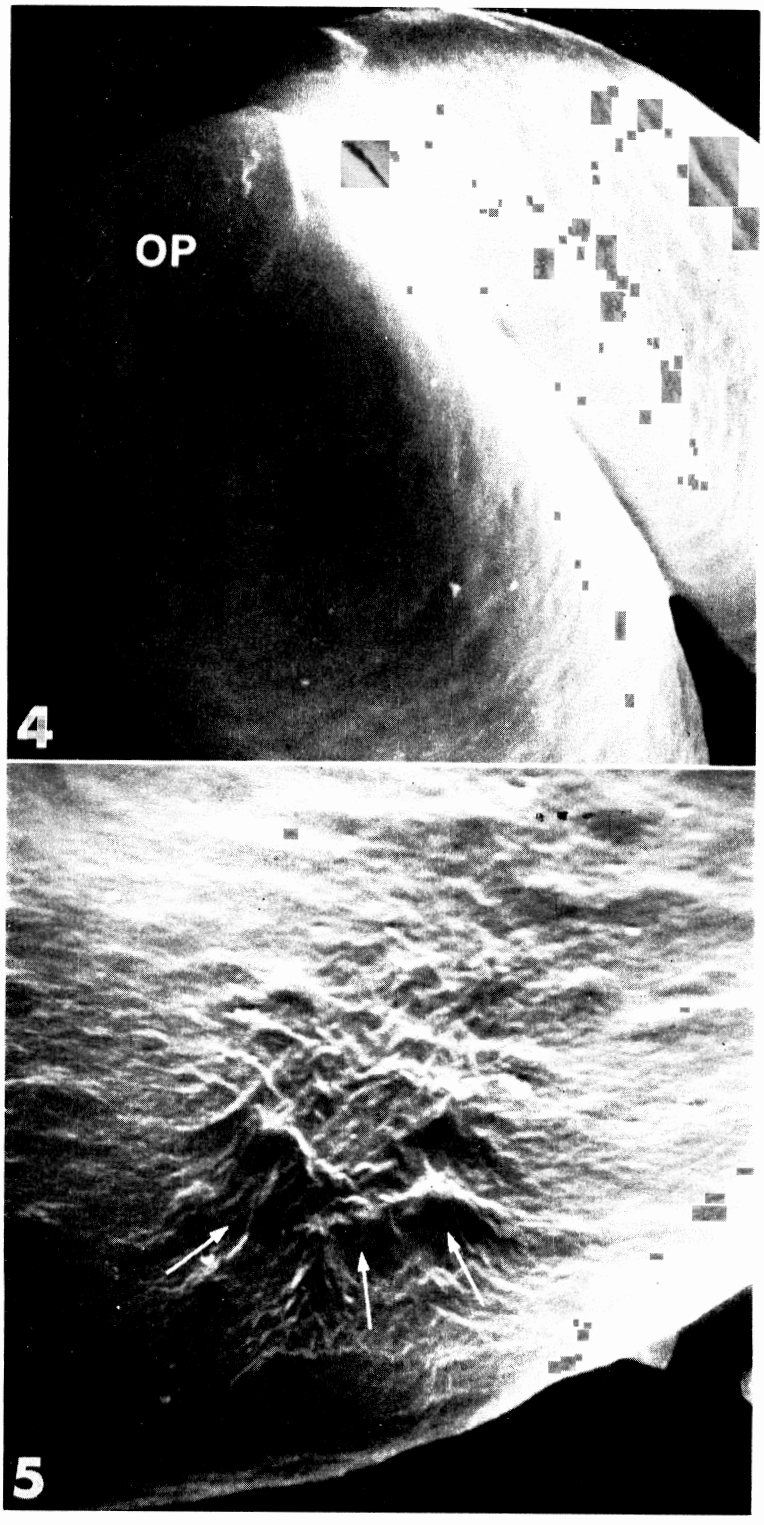
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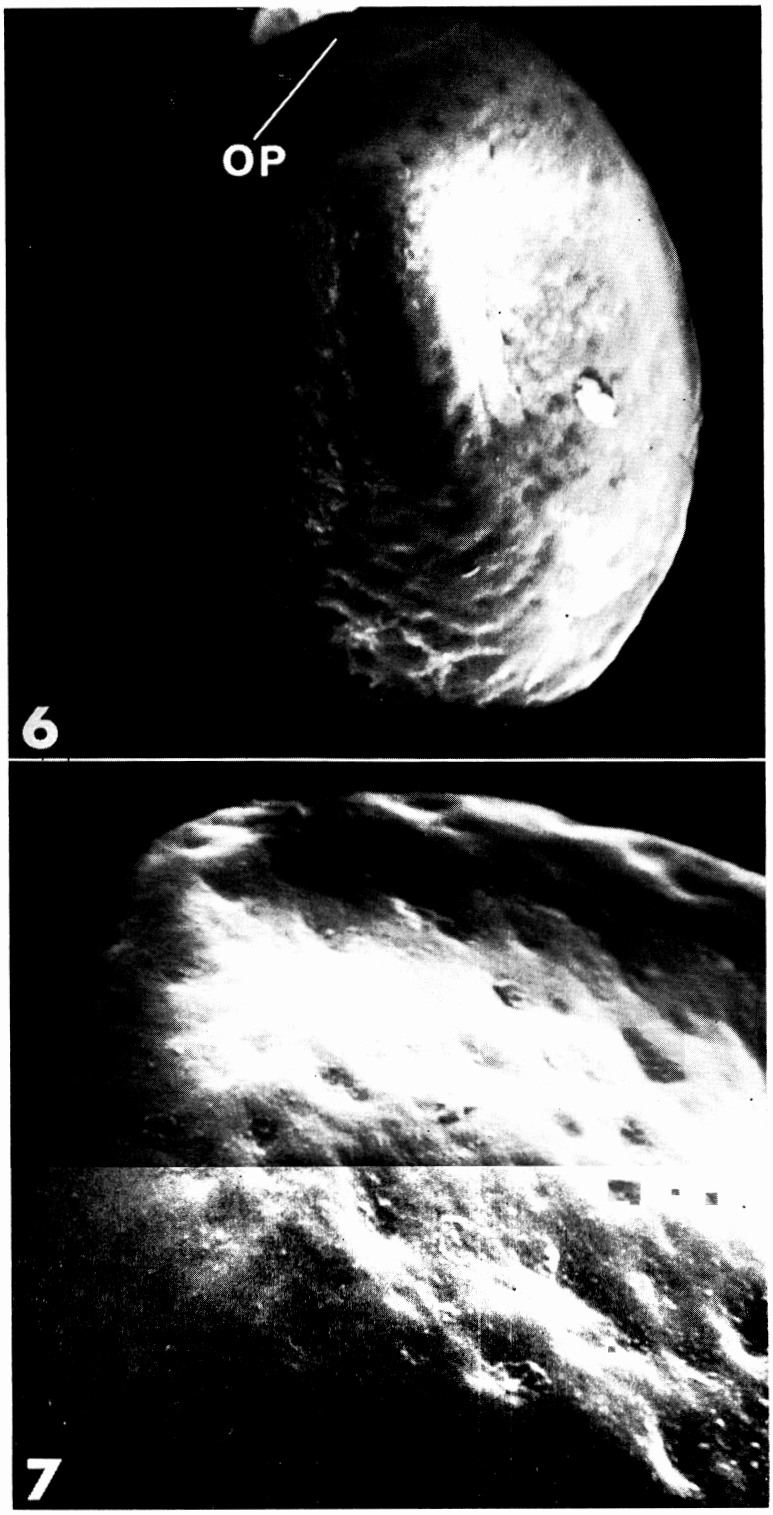
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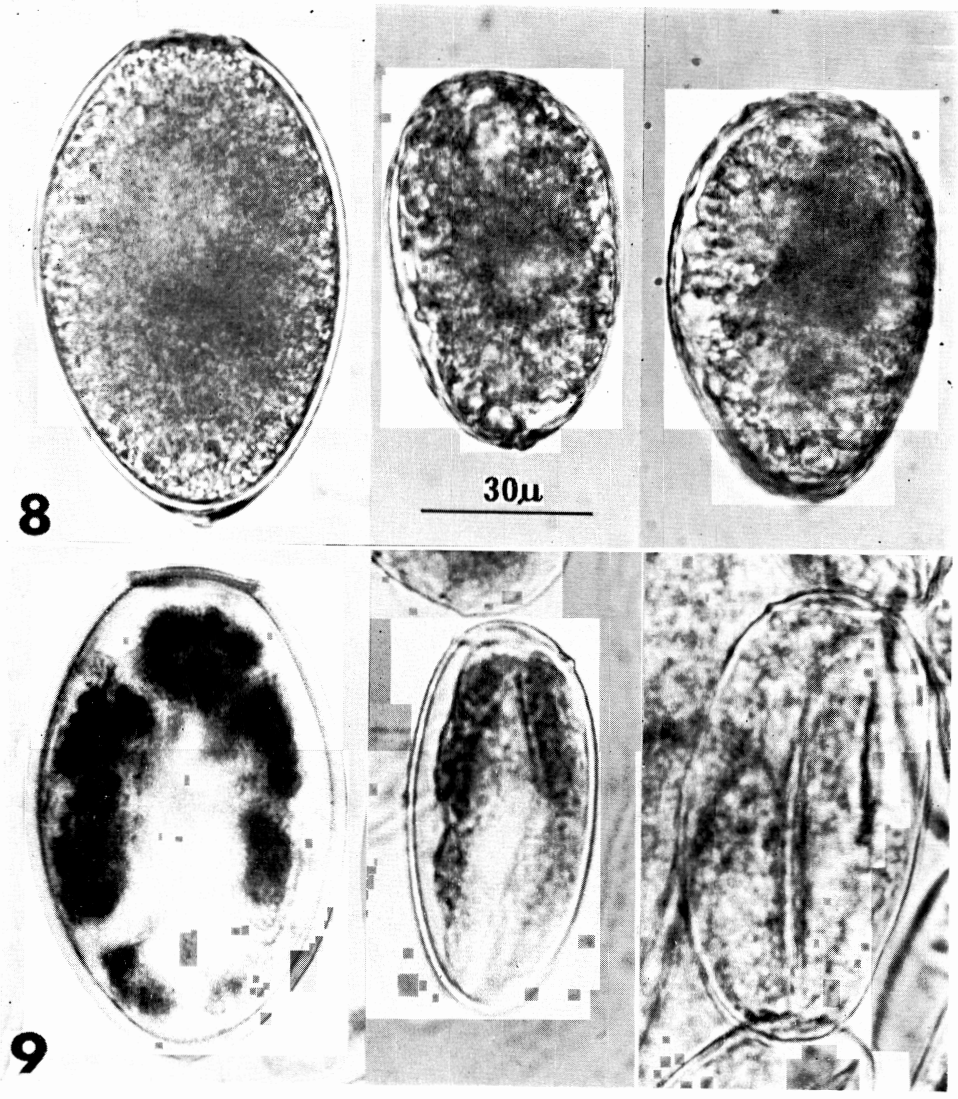

Figs. 1–7 : Scanning electron micrographs of the eggshell of American *Paragonimus*.
Figs. 8 & 9 : Light micrographs of the same materials.

Fig. 1. *P. kellicotti*, showing operculum (OP) and abopercular end (AE). × 1100
Fig. 2. *P. kellicotti*, operculum and texture of shell. × 3300
Fig. 3. *P. kellicotti*, starfish-like prominence on abopercular end (arrow). × 3300
Fig. 4. *P. mexicanus*, two, slightly undulate texture of shell. × 2200
Fig. 5. *P. mexicanus*, abopercular end with concaves (arrows). × 6600
Fig. 6. *P. peruvianus*, highly undulate texture of shell. × 1200
Fig. 7. *P. peruvianus*, details of pits. × 3300
Fig. 8. Comparison of eggs of *P. kellicotti* (left), *P. mexicanus* (middle) and *P. peruvianus* at the same magnification.
Fig. 9. Sections of the uterine eggs of *P. kellicotti* (left) and *P. mexicanus* (middle). Eggs of *P. peruvianus* (right) in the uterus of a mounted specimen. Magnification is the same as Fig. 8.