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Studies on Intestinal Trematodes in Korea

XIX. Light and Scanning Electron Microscopy of *Fibricola seoulensis* collected from Albino Rats Treated with Praziquantel

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INTRODUCTION

Praziquantel, an acetylated isoquinolino-pyrazine, is a promising drug in the treatment of various kinds of trematode and cestode infections in man and animals (Andrews *et al.*, 1983). The therapeutic efficacy in terms of cure and egg reduction rates has been reported to be satisfactory, however, the detailed mechanism of drug action on the worms is not sufficiently known so far. Two striking phenomena observed from the worms exposed to praziquantel *in vitro* were, both in the trematodes and cestodes, immediate tetanic contraction of their musculature and vapid vacuolization of the syncytial tegument (Becker *et al.*, 1980 a & b; Mehlhorn *et al.*, 1981 & 1983; Kim *et al.*, 1982; Lee, 1985).

It was already reported that *Fibricola seoulensis* (Trematoda: Diplostomatidae), an intestinal trematode of the rodents and man in Korea (Seo *et al.*, 1964 & 1982), was susceptible to the treatment by praziquantel *in vitro* (Lee, 1985) and *in vivo;* man (Hong *et al.*, 1984) and albino rats (Lee *et al.*, 1985). The morphological changes on the worms exposed to the drug were also studied *in vitro* (Lee, 1985), but not *in vivo*. The present study was performed to observe the morphological changes, by light and scanning electron microscopy, on the tegument and/or in the parenchymal tissue of *F. seoulensis* collected from the experimental albino rats after treatment with praziquantel.

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MATERIALS AND METHODS

1. Preparation of the Metacercariae

The metacercariae of *F. seoulensis* were obtained from the viscera of the snakes, *Natrix tigrina lateralis*, which were purchased at Hoengseong-gun, Kangweon-do in June and July 1984. The procedure of metacercarial isolation was the same as described by Lee *et al.* (1985). The isolated matacercariae were grouped into 1,000 in numbers for preparing the challenging dose.

2. Infection and Treatment of Albino Rats

Total 15 rats (Sprague-Dawley strain) were orally given with the metacercariae under slight anesthesia with ether. Seven days later, when the worms were grown to adults, 10 mg/kg single dose of praziquantel was administered orally to each of 10 rats. After 1, 2, 6, 12 and 24 hours following the treatment 2 rats each were sacrificed by cervical dislocation and the worms were searched for from their small intestine. The remaining 5 rats were served as the source of untreated control worms.

3. Light and Scanning Electron Microscopic Observations

Both the control and drug-exposed worms were subjected to light microscopy with whole mounts and sectioned preparations, and to scanning electron microscopy on the tegument of worms.

For whole mounts, 10 specimens each of the control and drug-exposed worms (1, 6 and 24-hour groups) were fixed with 10% formalin under slight pressure. They were stained with Semichon's acetocarmine and mounted in canada balsam. The size, shape of the worms and their internal organ structures were observed. For sectioned preparations, the same numbers each of the control and drug-exposed worms were fixed with formalin without pressure, cut into 5~7 μm sections, stained with hematoxylin and eosin (H-E) and observed.

For observation of the ultrastructural changes on the worm tegument, total 120 specimens (30 each for control and 1, 6 and 24-hour groups) were washed three times with phosphate-buffered saline and fixed in 2.5% glutaraldehyde (pH 7.4) at 5°C. They were dehydrated in graded ethanols, freeze-dried, gold-coated and observed with SS-60 scanning electron microscope (ISI Korea) at an accelerating voltage of 10 KV.

RESULTS

1. Light Microscopic Findings

The major changes in the light microscopic morphology of *F. seoulensis* treated with 10 mg/kg praziquantel *in vivo* were early contraction followed by relaxation of their body, vacuolization of the tegumental and subtegumental layers, and damage in the worm intestine such as narrowing of the lumen and thickening of the wall both in whole mounts and sections, up to 24 hours of observation.

The body size of the worms was decreased until 1 hour after the contact with the drug but thereafter it enlarged up to 24 hours (Fig. 1). The initial decrease of the body length was mainly due to the contraction of the forebody. Reversely, the hindbody appeared to be continuously elongated after the exposure to the drug, when compared with the control worms (Fig. 1 & 2). The later increase of the whole body length was due to the expansion of the parenchymal portions (Fig. 3), as a result of extensive vacuolization and relaxation, especially at their forebody.

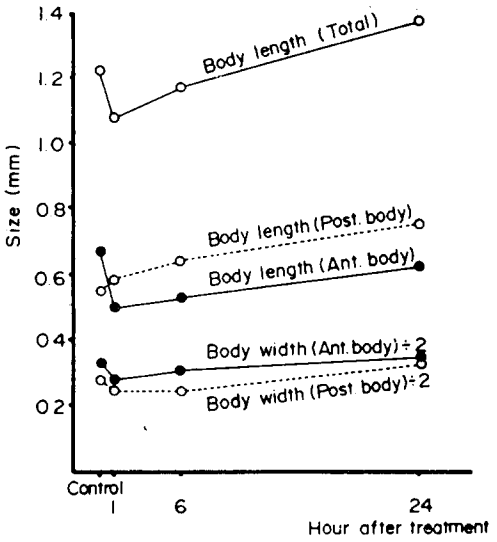

Fig. 1. Changes in size of *F. seoulensis* after exposure to praziquantel *in vivo*.

At 1 hour after the treatment, the vacuolization of the parenchymal portions, in whole-mounted specimens, was not severe in extent. However, the vacuoles increased in number and became larger in size after 6 hours and these changes became more severe after 24 hours (Fig. 4 & 5). In sectioned preparations, the vacuoles were also seen to be minimal in size and in number after 1 hour but they enlarged in size, increased in number, and extended to deeper portions after 6 (Fig. 6) and 24 hours (Fig. 7). The intestinal lumen of the control worms was wide and well lined by the villus-like structures protruding from the intestinal wall both in whole mounts and sectioned specimens (Fig. 8). But in the drug-exposed worms, the intestinal lumen became markedly narrower and nearly occluded at as earlier time as 1 hour after the treatment. The luminal occlusion was due to the protrusion of the enlarged and elongated villus-like structures towards the lumen. These intestinal

changes were almost consistently seen from 1 hour to 6~24 hours (Fig. 9) after the treatment.

2. Ultrastructural Changes on the Worm Tegument

By scanning electron microscopic observation on the tegument of the drug-exposed worms, the most prominent feature was the formation of numerous blebs of various size (Fig. 10~14), followed by rupture. The complete destruction of the tegumental surface including the protoplasmic processes, sensory papillae and even the spines ensued following the death of worms (Fig. 15~17). The tegumental changes, chiefly the bleb formation and rupture, occurred as early as one hour after the treatment (Fig. 10, 11 & 13). Thereafter up to 24 hours, such changes became more severe in extent. However, some of the worms which survived until 6 or 24 hours after the treatment revealed not marked destruction on their teguments. They are considered to have escaped from the direct contact with the drug or to have restored their teguments after receiving a slight damage on them. In comparison, 5 dead worms found from the caecum of a rat treated 6 hours before revealed complete obliteration and destruction of their teguments (Fig. 15~17), and were considered to be in autolyzing process. The sensory papillae, spines and smooth protoplasmic processes were no more recognizable on the whole surface of the worms, and the tegument no more revealed its normal velvety texture.

In the alive worms recovered after 1 hour exposure to the drug, the tegumental changes such as the bleb formation and rupture were most prominent between the portion of the oral and ventral suckers and the lateral margins of the forebody (Fig. 10). The tegument around the oral, ventral sucker themselves and the tribocytic organ was also damaged. The dorsal surface of the forebody also showed occasional blebs. However, the sensory papillae and the spines were nearly not affected through the whole forebody up to 1 hour after the exposure (Fig. 14). Not only in earlier stage but later than 6 or 24 hours after the exposure, the hindbody of the worms, in their majority, was not severely affected (Fig. 12) except for dead ones recovered from the caecum of the rat, in which extensive destruction of the whole tegument was recognizable.

DISCUSSION

From the light and scanning electron microscopic findings in *F. seoulensis* treated with praziquantel *in vivo*, it is evident the drug has its action on the tegument, underlying parenchymal tissue and intestine of the worms. In whole mounts and sections, the vacuolization of not only the tegument but also the subtegumental layers was conspicuous and the narrowing of the intestinal lumen bue to the enlargement and thickening of the intestinal wall was marked.

The change in the worm intestines after the exposure to praziquantel suggests that the drug is uptaken by the worms not only through the tegument but through the alimentary canal. At present, however, it is not clear through which route the drug uptake is more rapid and more abundant. According to the *in vitro* study with *F. seoulensis* (Lee, 1985), the tegumental changes of worms occurred as early as 5 minutes after the exposure. However, the intestinal change after 5 minutes was not simultaneously studied, therefore, comparison was not made.

The formation of the vacuoles or blebs on the tegument and/or the muscular contraction of the trematodes and cestodes treated with praziquantel has been reported by many workers (Becker *et al.*, 1980 a & b: Mehlhorn *et al.*, 1981 & 1983; Kim *et al.*, 1982; Chiu *et al.*, 1982; Lee, 1985). In the present study, the changes on the tegument of *F. seoulensis* were also characterized by the bleb formation followed by rupture, and the worms showed marked contraction especially of their forebody. However, the origin and/or the mechanism of the bleb formation and the muscular contraction has not been clearly understood so far.

In *Clonorchis sinensis* and *Paragonimus wester-*

mani, the origin of the blebs was suggested to be the nerve bulbs under the sensory papillae near the oral, ventral suckers and the excretory pore (Kim *et al.*, 1982; Chiu *et al.*, 1982). By scanning and transmission electron microscopic observations, they further suggested that the protoplasms of the tegumental nerve bulb cells, presumably the origin of the blebs, should protrude out of the tegument after rapid degeneration and passing through the space nearby the sensory cilium and through the protoplasmic membrane. However, in the present study, there was entirely no evidence supporting the above suggestion. The vacuoles or blebs were abundant on the tegumental surface between the oral and ventral suckers of *F. seoulensis*, where normally are distributed many sensory papillae (Seo *et al.*, 1984). However, around there, despite the formation of numerous blebs, the majority of the sensory papillae were found completely intact. Therefore, it is strongly suggested that the origin of the blebs may not be the sensory papillae.

The bleb formation on the tegument and the muscular contraction of the drug-exposed worms was reported to be calcium dependent. It was demonstrated that praziquantel increased the permeability of *Opisthorchis viverrini* tegument to calcium binding or transport across the membrane to result in the bleb formation (Ruenwongsa *et al.*, 1983). Similarly, in *Schistosoma mansoni*, the depletion of calcium from the culture media inhibited the bleb formation on the tegument (Xiao *et al.*, 1984) and blocked the muscular contraction of the worms (Pax *et al.*, 1978). However, it has not been elucidated whether the increased uptake of calcium by the parasites is only and directly related to the tegumental vacuolization. Sirisinha *et al.* (1984) suggested that the bleb formation of *O. viverrini* after the treatment with praziquantel may be a non-specific secondary change, since they observed the similar vacuolization in the worms exposed to fresh normal human sera.

There seems to be many direct and indirect causes of worm death after they are exposed to praziquantel. Since the immediate tetanic contraction of the worms is a unique feature in many kinds of trematodes and cestodes (Andrews *et al.*, 1983), muscular paralysis is considered to be one of the direct cause of the worm death. In *F. seoulensis*, the immediate worm death was observed after the contact with higher praziquantel concentrations than 1 μg/ml *in vitro* (Lee, 1985), and the worm death is assumed to be due to the muscular and/or nerve paralysis. The tegumental, parenchymal and intestinal damages may also participate in the worm death in case that they are exposed to an insufficient drug concentration for the immediate death. The dislodgement from their normal habitats following the contact with praziquantel seems to be an indirect factor for a delayed death of several hinds of trematodes. The shift of *S. mansoni* from the mesenteric venule to the hepatic portal vein after the treatment (Andrews *et al.*, 1983) or that of the intestinal fluke such as *F. seoulensis* from the duodenum to the ileo-caecal portion (Lee *et al.*, 1985) seems to be detrimental for the worms to survive. Fortunately in this study, 5 dead worms recovered from the caecum 6 hours after the treatment were subjected to scanning electron microscopy. They were found to be already in autolyzing process and their teguments were so severely destroyed that it was unable to discriminate any of the normal structures.

SUMMARY

An experimental study was performed to observe the *in vivo* effects of praziquantel on the light and scanning electron microscopic morphology of *Fibricola seoulensis*. The metacercariae were obtained from the snakes and 1,000 in each number was orally given to total 15 albino rats; 5 controls and 10 treatment group. Seven days later the 10 rats were treated with 10 mg/kg praziquantel and sacrificed 1~24 hours later to search for the worms from their small intestines.

The major light microscopic changes in the drug-exposed worms were early contraction

followed by relaxation of especially their forebody, vacuolization of the tegument and subtegumental parenchymal layers, and narrowing of the intestinal lumens. The scanning electron microscopic findings were characterized by formation of numerous blebs followed by rupture and subsequent destruction of their whole teguments. These results show that the change in worm body is not confined to the tegument but extends to deeper parenchymal portions and also occurs in their intestines. It is suggested that the drug uptake by the worms should be either through their tegument or through the digestive tract.

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韓國의 腸吸蟲에 관한 硏究

XIX. 프라지콴텔 투여 흰쥐에서 수집한 *Fibricola seoulensis*의 光學 및 走査 電子顯微鏡的 觀察

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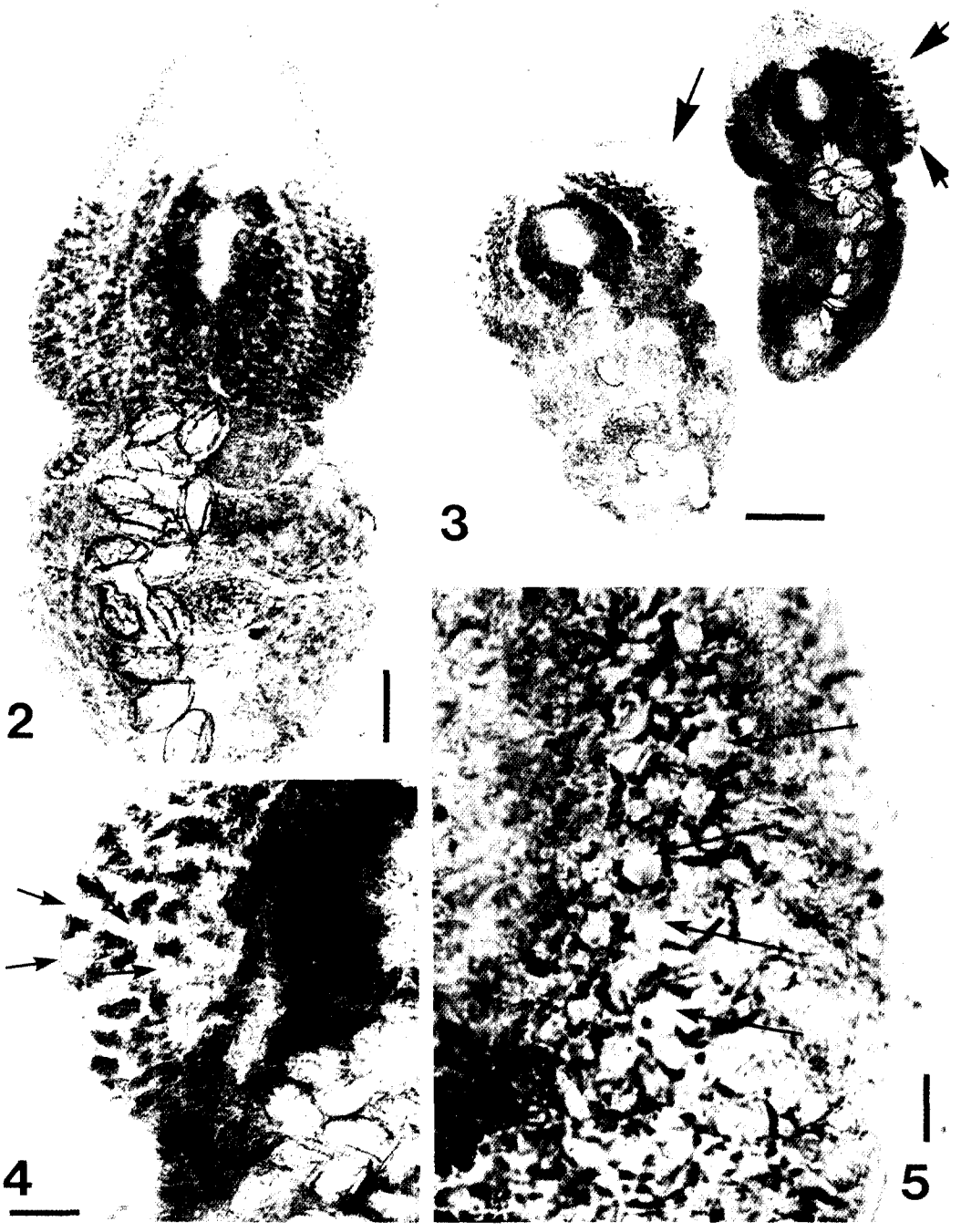
프라지콴텔 投與가 흰쥐체내에서 *Fibricola seoulensis*에 미치는 영향을 光學 및 走査電子顯微鏡을 이용하여 관찰하였다. 被囊幼蟲은 뱀의 내장에서 分離한 것을 사용하였고 5마리의 非投藥對照群 및 10마리의 投藥群 흰쥐에 각각 1,000個씩을 經口感染시켰다. 感染 7日後 投藥群 흰쥐에 10mg/kg의 프라지콴텔을 투여하고 1~24時間後 흰쥐를 희생시킨 다음 小腸으로부터 蟲體를 수집하여 對照群으로부터 얻은 蟲體와 비교관찰하였다.

投藥群에서 얻은 *F. seoulensis*蟲體는 光學顯微鏡的 관찰에서 蟲體특히 前半部의 수축 및 잇달은 이완, 表皮 및 表皮下 實質組織의 空胞化(vacuolization) 및 腸管의 內腔폐쇄 등이 특징적으로 관찰되었다. 走査電子顯微鏡的인 蟲體 表皮의 변화는 많은 水胞形成(bleb formation) 및 파열과 이에 따른 蟲體 全表皮의 파괴로 특징지울 수 있었다. 이런 결과는 프라지콴텔에 의한 蟲體의 손상이 表皮에 심하게 나타나지만 表皮에 限定되지 않고 表皮下層까지도 파급되며 또 腸管에도 뚜렷한 손상이 오는 것을 나타내고 있었다. 이런 점으로 보아 蟲體에 의한 프라지콴텔의 흡수는 表皮 뿐만 아니고 消化管을 통해서도 일어남을 의미하였다.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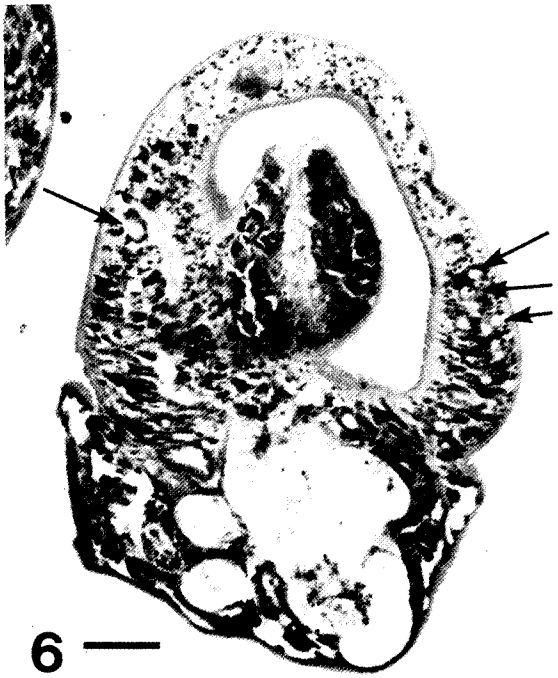

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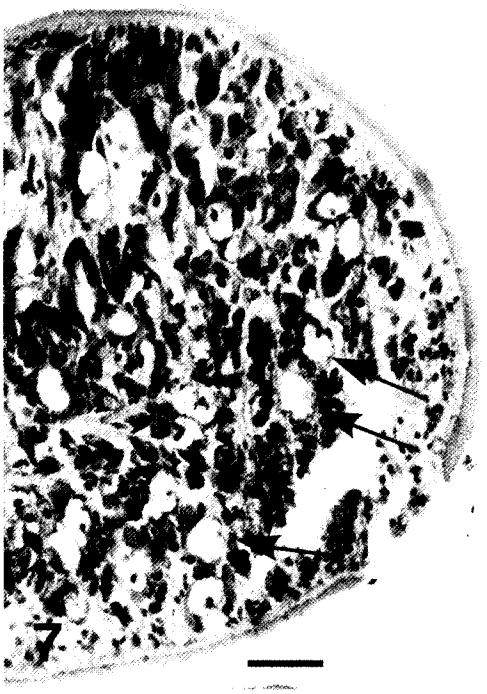

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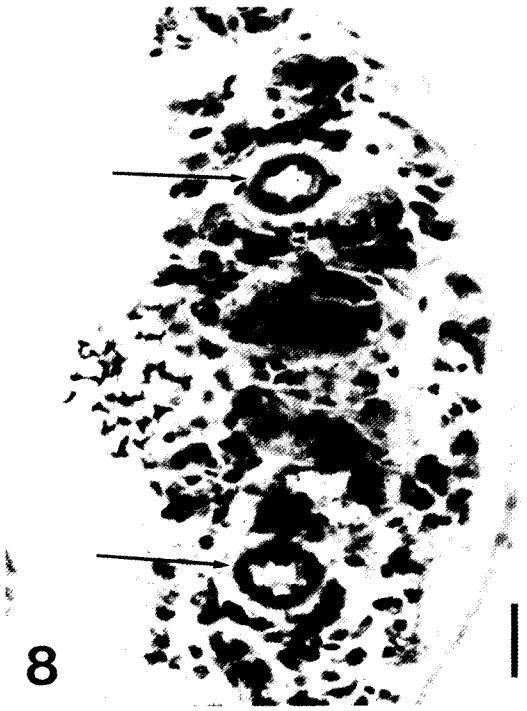

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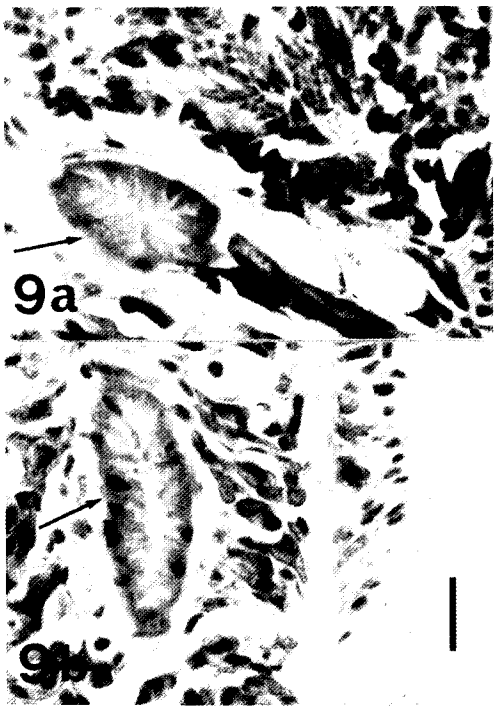

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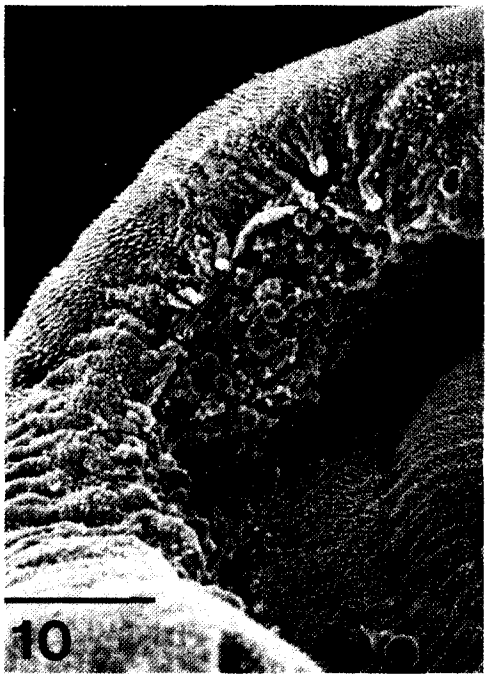
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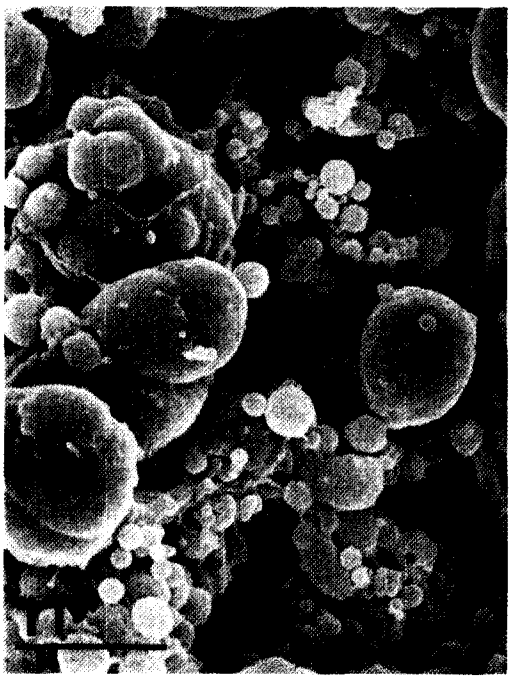

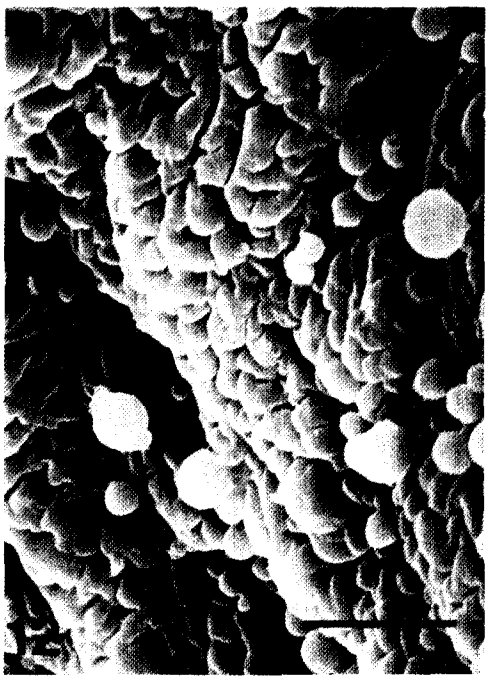

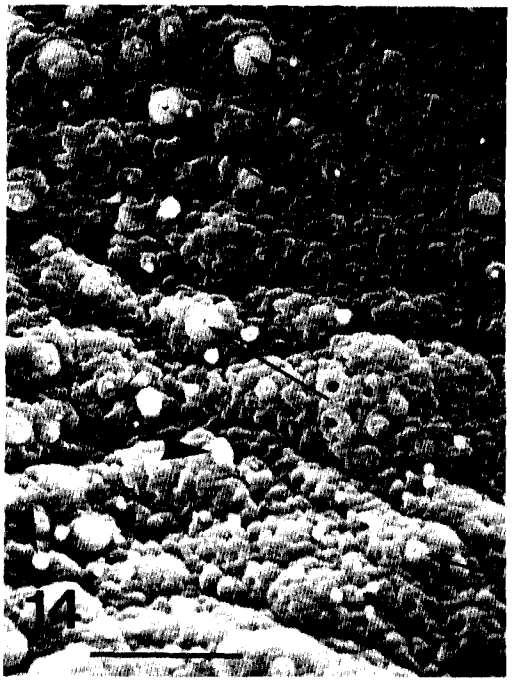
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EXPLANATIONS FOR FIGURES

Fig. 2. A normal adult worm of *F. seoulensis* showing its characteristic feature. Acetocarmine stain (scale: 0.1mm).

Fig. 3. Two drug-exposed specimens (6-hour group). The forebody is contracted while the hindbody elongated. The lateral margins show hazy appearance (arrow heads) due to severe vacuolizations. Acetocarmine stain (scale: 0.2mm).

Fig. 4. The lateral margin of a worm (24-hour group). The tegument is markedly destroyed and many vacuoles are seen in the superficial and deep layers (scale: 0.1mm).

Fig. 5. Another worm (24-hour group) showing many parenchymal vacuoles (scale: 25μm).

Fig. 6. A sectioned worm of 6-hour group. The lateral margins of the forebody show many vacuoles (arrow heads). H-E stain (scale: 0.1mm).

Fig. 7. *Ibid*, 24-hour group. The vacuolization of the parenchymal tissues is conspicuous. H-E stain (scale: 25μm).

Fig. 8. Cross section of a control worm showing two intact intestines (arrow heads) with wide lumens and thin walls. H-E stain (scale: 25μm).

Fig. 9. Section of the drug-exposed worms (a: 6-hour group, b: 24-hour group) showing their nearly occluded intestinal lumen and thickened wall. H-E stain (scale: 25μm).

Fig. 10. Scanning electron microscopic view of the lateral margin of a drug-exposed worm (1-hour group). Many tegumental blebs are seen (arrow heads) and the tegumental architecture is severely altered (scale: 35μm).

Fig. 11. Magnification of an arrow portion of Fig. 10. The tegumental blebs are much variable in size and shape (scale: 4μm).

Fig. 12. The tegumental surface of the hindbody of a not severely damaged worm (6-hour group). Many blebs are seen and the cobblestone-like tegumental integrity is a little deformed (scale: 2.5μm).

Fig. 13. The tegument around oral sucker of a drug-exposed worm (1-hour group). The ruptured blebs (arrow heads) are seen but the spines are entirely intact (scale: 4μm).

Fig. 14. Another worm in the same group as Fig. 13, showing the tegument between oral and ventral suckers. Despite the bleb formation (large arrows) on the tegument, the sensory papillae appear to be intact (small arrow heads). The blebs and the sensory papillae grossly have no relations each other (scale: 5μm).

Fig. 15. The forebody of a drug-exposed dead worm (6-hour group) recovered from the caecum of a rat. The whole tegumental surface is severely destroyed (scale: 33μm).

Fig. 16. Magnification of the worm in Fig. 15. There is no recognizable tegumental structures such as the spines, sensory papillae, *etc.* (scale: 4μm).

Fig. 17. *Ibid*, another portion. The tegumental surface shows much rough and dirty appearance (scale 4μm).