기 생 충 학 잡 지 제23권 제 2 호
The Korean Journal of Parasitology
Vol. 23, No. 2, 203-213, December 1985

In situ posture of anterior body of *Metagonimus yokogawai* in experimentally infected dog

Young Kee Jang, Shin-Yong Kang, Suk-Il Kim and Seung-Yull Cho
Department of Parasitology, College of Medicine,
Chung-Ang University, Seoul 151, Korea

INTRODUCTION

The movement of digenetic trematodes has been described as "measuring movement" because the worms rhythmically protrude and retract their anterior body by placing acetabulum near to the oral sucker (Bennett, 1975; Beaver *et al.*, 1984). Unlike the anterior body, the portion behind the acetabulum moves much less; waves of peristalsis are extended to. Therefore, the posterior body is carried like a sac by the anterior during movement. Because most of sexual organs are situated in posterior body in most species of digeneans, Skrjabin (1947) teleologically thought that the benefit of such movement is one of the essential features in trematode physiology. It may protect the sexual organs free from violent movement to facilitate the egg production.

Another view for such movement of anterior body is its relation with trematode feeding (Smyth, 1966). In many of digenetic trematodes, anterior body is more densely armed with single-pointed or serrated spines at the tegument than the posterior. The rhythmic movement of the anterior body, therefore, results in the abrasion of host tissues at the contact areas of micro-niche. As Dawes (1962) observed in the tissue sections of *Fasciola hepatica* in experimental mice, the mechanically destroyed tissues of the host were ingested through oral cavity.

Because the movement of digenetic trematodes has been described in the freed adult/larval worms in fluid media, it has not been clarified yet whether such pattern of movement represents the sole way even at host habitat. The actual movement pattern at the niche has been rarely described.

The purpose of this paper is to describe the various postures of *Metagonimus yokogawai* which were fixed *in situ* in the mucosa of the small intestine of experimentally infected dog. It is hoped that, by this way of observation, the actual movement patterns or postures could be indirectly projected.

MATERIALS AND METHODS

1. Infection of dogs with *Metagonimus yokogawai*:

A week before the experimental infection, all dogs were treated with 10mg/kg of pyrantel pamoate and 40 mg/kg of bithionol to remove the possible helminthic infections. A total of 8 dogs of 4~6 kg was infected with 10,000 metacercariae of *M. yokogawai* respectively. The metacercariae were collected from naturally infected sweetfish (*Plecoglossus altivelis*) by peptic digestion. The fish were purchased in Jangheung Gun, Chollanam Do, Korea in October 1984.

2. Collection of *M. yokogawai* from experimentally infected dog:

Two dogs were killed by cardiac bleeding under penthotal anesthesia on 3 days, 9 days, 4 weeks and 10 weeks after the experimental infection. Peritoneal cavity was immediately opened. Small intestine from pylorus to ileocaecal valve was removed, and was cut into pieces of every 30 cm long; and the pieces were opened along the mesenteric border. The opened intestinal pieces were fixed on a flat wood board by placing serial pins along the margin. The intestines were fixed in 10% formalin.

The process from removal to fixation of intestine was completed within 20 minutes. After 2 days of formalin fixation, a 3 cm long longitudinal sections of 3 mm thickness were taken from each intestinal piece for histological observation.

The fixed mucosal tissues were removed from muscle layer with a spoon. The mucosal fragments were examined under dissecting microscope to isolate juvenile or adult *M. yokogawai*. Every spaces between villi were examined; crypt of Lieberkuehn were teased with dissecting needles, and impacted worms were released from glandular lumens of crypt.

Two dogs, killed on the 9th day after infection, were processed differently; at first, 3 cm long intestinal pieces were taken at every 30 cm for histology, and were fixed in 10% formalin; living worms were collected from remaining intestine, and were fixed in 10% formalin. After trimming the fixed intestinal pieces for histology, some of fixed worms were additionally collected.

3. Observation of the collected worms:

The collected *M. yokogawai* from the fixed intestinal pieces showed various postures. The postures were generally three dimensional. At first, some of unstained worms were flattened under cover glass pressure and were photographed.

For observation with scanning electron microscope (SEM), some of worms were fixed again in 2.5% cold phosphate buffered glutaraldehyde (pH 7.4) solution overnight, and post-fixed in 1% osmium tetroxide before freeze-drying. Then, the worms were coated with carbon and platinum for 3 minutes. The photographs were made with scanning electron microscope (Dual-130, ISI-Korea).

RESULTS

1. Recovery rate of *M. yokogawai* by infection period:

The recovery rates of *M. yokogawai* by infection period were shown in Table 1. The rates were 42.6% on the 3rd day, 55.0% on the 9th day, 33.2% on the 4th week and 8.9% on the 10th week respectively. Decreasing recovery rates were shown by length of infection period.

2. *In situ* postures of *M. yokogawai* recovered on the 3rd infection day:

Many juvenile worms were collected from intervillous spaces in duodenum and jejunum. A considerable number of worms were also released from the lumens of glandular crypt at the same portion of small intestine. The exact proportion of worms from intervillous spaces and from glandular crypt was not compared. The term "juvenile worm" in this paper meant the worms of 3 day-old infection, which developed testes, ovary and vitellaria. The juvenile worms of 3 day-old rarely have eggs in uterus.

In histological preparations, many transverse, sagittal or oblique sections of worms were recognized. In well prepared sagittal sections, anterior to posterior ends of worm were located uprightly

Table 1. Number of worms collected from intestinal mucosa in dogs infected with 10,000 metacercariae of *M. yokogawai*

Duration of infection		Number of worms recovered(%)
3 days	Dog 1	4,263(42.6%)
	Dog 2	4,266(42.7%)
9 days	Dog 3*	3,804(38.0%)
	Dog 4*	7,192(71.9%)
4 weeks	Dog 5	2,903(29.0%)
	Dog 6	3,733(37.3%)
10 weeks	Dog 7	687(6.9%)
	Dog 8	1,088(10.9%)

* Worms were collected in live state from unfixed intestine, and less than 10% were collected from trimmed tissue of fixed intestine that were prepared for histologic observation

from upper part of crypt to lower intervillous space. However, such section was only infrequently found. The majority of worm sections, either transverse or oblique, revealed ovoid or elliptical shapes. Sagittal sections of anterior body were located at crypt.

Some of worm sections comprising the anterior body showed unusual shapes, different from usual ovoid sections. A worm section in Fig. 1 showed a ventral sacculation of mid-portion of the worm at villo-crypt junction and a branched section of anterior body at dorsal aspect. A section of a worm with oral sucker was extremely tortuous along the body length (Fig. 2). One juvenile worm section (Fig. 3) showed its anterior body sectioned 3 times. Fig. 4 revealed a worm section which was bifurcated at anterior body.

These histological findings, either ovoid or branched sections, were compatible with the postures of whole worms that were isolated from the fixed intestinal mucosa. The postures of isolated whole worms, flattened by cover glass pressure, were illustrated in Figs. 7-1 to 7-36.

Worms shown in Figs. 7-1 to 7-9 showed ovoid shapes of dorsoventrally flattened; many with ventral concavity or with dorsoventral curvatures. These worms were usually collected at intervillous spaces; the ventral concavity or curvature were considered to be formed to adapt the local conformity of intervillous spaces. The anterior body of worms may be elongated while posterior body remained dorsoventrally flat (Figs. 7-10 to 7-18). Fig. 7-12 showed a worm of which anterior body was ventrally curved. This recurved anterior body actually encircled a villus *in situ*. Some of worms (Figs. 7-13 to 7-18) protruded their anterior body with or without transverse wrinkling at the base of protrusion. In some of them, either at the base of protrusion or at mid-portion of protruded anterior body, one or two additional protrusions were recognized, sometimes making itself like a key (Figs. 7-19 to 7-30). The length of protruded anterior body could be two times or longer than posterior one (Figs. 7-30 to 7-36).

The protrusions mimicked the amoeboid movement of anterior body of worms. To explore the mechanism of protrusion, we made observations with scanning electron microscope and with transverse sections of intestinal mucosa.

The SEM findings of protrusions were depicted in Figs. 8 to 16. The postures of worms appeared generally flattened or showed various shapes. The protrusion was observed as an elongated prominence and its ventral surface showed a longitudinal groove (Fig. 8), which extended from near the oral sucker to posterior end of body. As shown in Fig. 9, the protruded anterior body had many of short and shallow longitudinal grooves. Adjacent to the oral sucker a saccular process was protruded (Fig. 9). The dorsal surface showed rounded appearance with anterior protrusion, which tapered gradually (Fig. 10). Another worm (Fig. 11) with protruded anterior body had multiple saccular protrusions; one at the base of protruded anterior body and two at the terminal portion of main protrusion. In higher magnification of Fig. 11, tegumental spines in three saccular protrusions were arrayed in posterior direction (Figs. 12 & 13). Transverse foldings were frequently observed in ventral surface of the posterior body (Fig. 14). Magnification of Fig. 14 showed that the folding margins of anterior protrusion was discernible (Fig. 15). Fig. 16 illustrated a typical folding line of lateral portion of the anterior body.

In transverse sections of intestinal mucosa up to crypt level, many worm sections of anterior body revealed the folded form of V-shape (Fig. 5). The anterior body situated in a destroyed crypt lumen and the epithelial cells of glandular crypt were totally necrotized.

3. **Postures of adult worms:**

Most of sectioned adult worms recovered on 9 days, 4 weeks and 10 weeks after the infection were found in intervillous space than in 3 days old worms were (Fig. 6). They showed a nearly ovoid shape. However, the contour of the ventrodorsal surface of these adults was similar with those of 3 day-old juveniles. A single to three protrusions of anterior body were observed (Figs.

Table 2. Frequency of *M. yokogawai* with protrusions of anterior body by infection period

Duration of infection		No. of worms observed*	No. (%) of worms		
			without P.**	with a single P.	with multiple P.
3 days	Dog 1	1,426	519(36.4)	293(20.5)	614(43.1)
	Dog 2	1,637	352(21.5)	524(32.0)	761(46.5)
9 days	Dog 3	262	141(53.8)	80(30.5)	41(15.7)
	Dog 4	171	123(71.9)	35(20.5)	13(7.6)
4 weeks	Dog 5	1,611	1,315(81.6)	144(8.9)	152(9.4)
	Dog 6	1,200	478(39.8)	375(31.3)	347(28.9)
10 weeks	Dog 7	180	115(63.9)	28(15.6)	37(20.5)
	Dog 8	81	44(54.3)	9(11.1)	28(34.6)

*Only unbroken worms were observed **P. : protrusion(s)

17, 18 & 19).

The frequency of worms with protruded anterior body was decreased by infection period (Table 2). In 3 day-old worms, almost two thirds of recovered worms had a single or multiple protrusions of anterior body while their frequencies in later infection periods were 36.2% to 39.0%.

DISCUSSION

The various postures of juvenile and adult *Metagonimus yokogawai* identified in this study were considered to represent the natural *in situ* postures at the niche. We believe that these postures could not be produced as an artifact during the tissue fixation in 10% formalin, since the worms normally retract their body during the fixation rather than relax or protrude their bodies. Also it appeared to be highly improbable that the protrusion of anterior body occurred during the fixation because the tegumental spines directed posteriorly, and the already protruded anterior body to the crypt lumens could not be easily withdrawn to intervillous spaces. Evidences obtained from freed worms of fixed mucosa and from the histological sections supported that the postrures of *M. yokogawai* in the mucosa were one of normal *in situ* postures in experimental dog metagonimiasis. So far, few worms in experimental metagonimiasis of cats showed multiple protrusions of anterior body.

The peculiar postures of juvenile and adult worms observed in this study could not be related with any of three kinds of sensory papillae described by Lee *et al.* (1984) at the tegumentum of *M. yokogawai*, because the papillae distribution was rather confined around the oral and ventral suckers while the various protrusions were made at random areas of the anterior body.

Protrusions were found more frequently in early developmental stage than in later period of infection. Also, it has been observed that in early stages of experimental metagonimiasis, the juvenile worms destroyed mainly villo-crypt junction to cause the deficit in supply of covering enterocytes(Rho *et al.*, 1984). In these respects, the protrusion of anterior body of juvenile *M. yokogawai* to glandular lumens of crypt of Lieberkuehn appeared to be well related with the microscopic destruction of villo-crypt junction. One peculiar finding of this study was that the posture of anterior body of worms at glandular lumens of dog's intestine was not such simple as a single protrusion. One protrusion frequently accompanied by one or more branching protrusions. Even when a long single protrusion was impacted to glandular lumen, other saccular protrusions may be made at the base of the protrusion. These findings suggested that the protrusion was made whenever nearby spaces of glandular lumen of crypt were available. This meant that the destruction of immature enterocytes by the protruded anterior body occurred not in a single lumen of glandular crypt, but it might occur in multiple lumens of crypt at the same time by a single worm.

The protruded anterior body could not be regarded as a mere amoeboid projection of the anterior body which consisted of parenchymal tissue with covering tegument. Rather, as shown in Figs. 5, 8, 14, 15 and 16, the protruded portion was formed by folding their lateral portion of anterior body to make a reversible tube. Secondary saccular protrusions seemed to be made in a similar manner. Forceful impact of folded protrusion into glandular lumens might result in the formation of some of short longitudinal wrinkles as shown in Fig. 9.

As shown in Fig. 5, the impacted lumen exhibited necrotized enterocytes. Since many of glandular crypts supplied the enterocytes to cover a villus, the destruction of one or two crypt lumens by a worm would not exert any of serious effects on maintaining a villus integrity, if lightly infected.

This study made it rather clear that the *in situ* postures of *M. yokogawai* were not as simple as previously considered, and many protrusions were formed to adapt the lumens of glandular crypt in small intestine of dogs.

SUMMARY

The *in situ* posture of anterior body of *Metagonimus yokogawai* was observed in experimental metagonimiasis of dog.

The metacercariae were collected from naturally infected sweetfish by peptic digestion; a total of 8 dogs was orally infected with 10,000 metacercariae respectively. Two dogs were killed on 3 days, 9 days, 4 weeks and 10 weeks after the infection. The postures of worms in histological section of small intestine and of whole worms collected from the fixed intestinal mucosa were examined by light and scanning electron microscope.

The results were summarized as follows:

1. The recovery rates of worms were 42.6% on 3 days, 55.0% on 9 days, 33.2% on 4 weeks and 9.8% on 10 weeks after the infection respectively.

2. In histological sections of small intestine, most of worms were found at intervillous spaces as ovoid sections of posterior body. However, many worms, especially in 3 day-old worms, revealed protruded anterior body in glandular lumens of crypt. Some sections of anterior body were bifurcated or sacculated.

3. The worms collected from fixed intestinal mucosa under dissecting microscope exhibited a variety of postures. Many worms showed flat shapes with a concavity or curvatures. However, in many worms, the anterior body made a single or multiple protrusions.

4. By SEM observation of protruded anterior body, a longitudinal groove was found.

5. The frequency of worms with protruded anterior body decreased in 9 days, 4 weeks and 10 weeks than in 3 day-old worms.

The above findings indicated that the anterior body of juvenile and adult *M. yokogawai* protruded to lumens of glandular crypt by folding their lateral portions to make a reversible tube-like structure. Frequent multiple protrusions were considered to be made to adapt the microniche of glandular crypts of dog intestine.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Professor W.H. Lee, Department of Physics, College of Liberal Arts and Sciences, Chung-Ang University, for advices in preparation of scanning electron micrographs. Professor Y.T. Yang, Department of Microbiology, and Dr. B.C. Yoo, Department of Int. Medicine, College of Medicine, C.A.U. kindly read and corrected our manuscript for us.

REFERENCES

Beaver, P.C., Jung, R.C. and Cupp, E.W. (1984) Clinical Parasitology, 9th ed., p. 406, Lea & Febiger, Philadelphia.

Bennett, C.E. (1975) Surface features, sensory structures and movement of newly excysted juvenile *Fasciola hepatica* L. *J. Parasit.*, **61**(5):886-891.

Dawes, B. (1962) A histological study of the caecal epithelium of *Fasciola hepatica* L. *Parasitology*, **52**: 483-493.

Lee, S.H., Seo, B.S., Chai, J.Y. and Hong, S.J. (1984) Study on *Metagonimus yokogawai* (Katsurada 1912) in Korea VII. Electron microscopic observation on the tegumental structure. *Korean J. Parasit.*, **22**(1):1-10.

Rho, I.H., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1984) Observation on the pathogenesis of villous changes in early phase of experimental metagonimiasis. *Chung-Ang J. Medicine*, **9**(1):67-76.

Smyth, J.D. (1966) The physiology of trematodes. pp. 21-28. W.H. Freeman and Co., San Francisco.

Skrjabin, K.I. (1947) Trematodes of animals and man (Fundamentals of Trematodology). Vol. 1, USSR Academy of Science, Moscow.

=국문초록=

개의 실험적 요꼬가와흡충증에서 충체의 자세

중앙대학교 의과대학 기생충학교실

장영기 · 강신영 · 김석일 · 조승열

요꼬가와흡충을 개에 감염시키고 장점막에서 분리한 초기 발육단계의 충체가 특이한 자세를 취하고 있음을 관찰하여 이를 보고하고자 한다.

은어에서 분리한 요꼬가와흡충 피낭유충을 개 8마리에 각각 10,000개씩 경구감염시키고, 감염 후 3일, 9일, 4주 및 10주에 2마리씩 도살하였다. 10% 포르말린으로 고정한 장점막에서 분리 수집한 요꼬가와흡충의 자세를 광학 및 주사현미경으로 관찰하였다. 그 결과를 요약하면 다음과 같다.

1. 충체회수율은 감염 3일에 42.6%, 9일에 55.0%, 4주에 33.2%, 및 10주에 8.9%로 감염이 경과할수록 감소하였다.

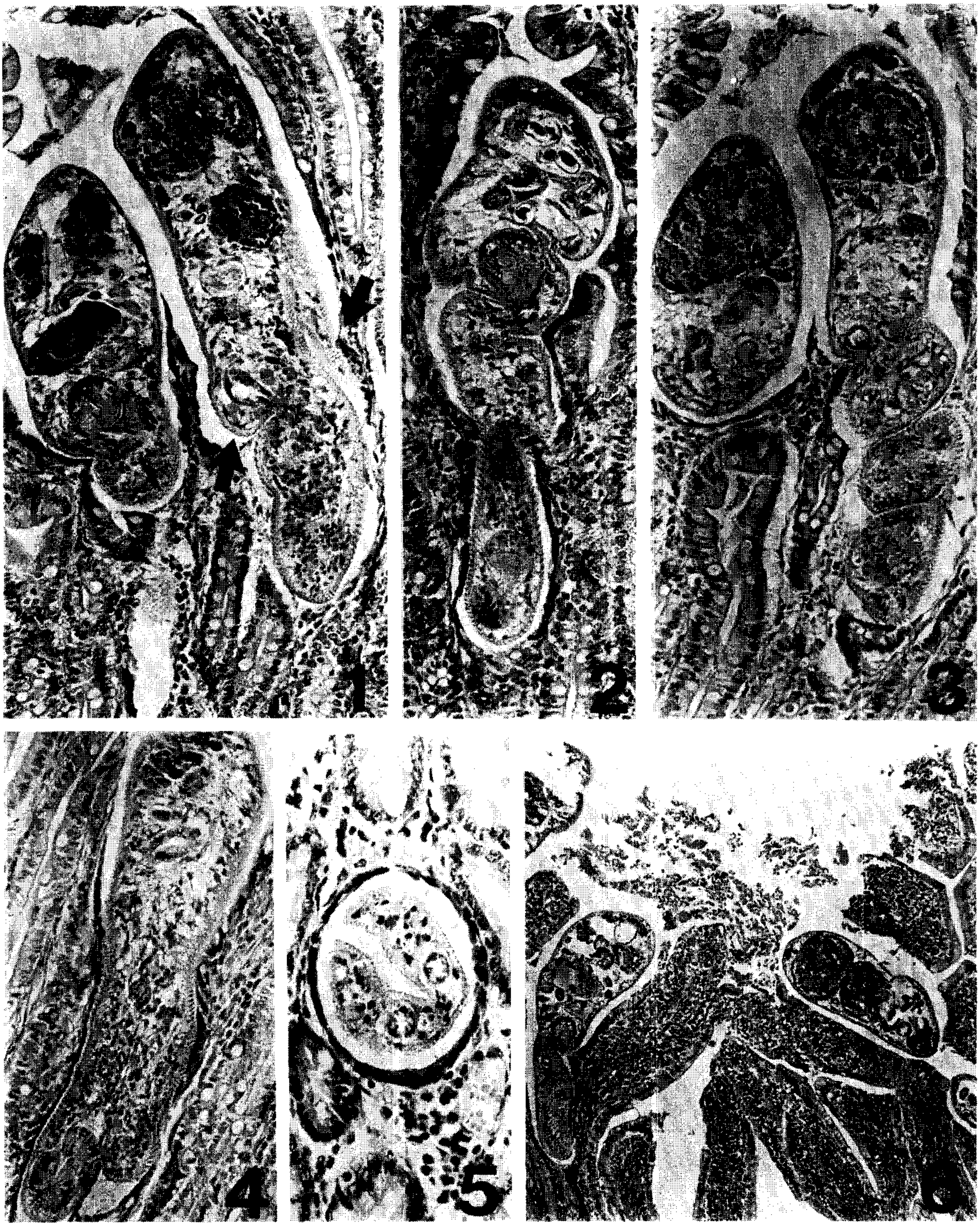
2. 감염후 3일된 개의 소장 점막의 조직절편에서 미성숙 충체가 융모사이에 위치하거나 장은와에 침입함을 관찰할 수 있었다. 이때 장은와 상부에 침입한 충체절편중에는 하부로 향한 충체 전반부가 2분지된 모양으로 절편이 발견되는 경우가 있었다.

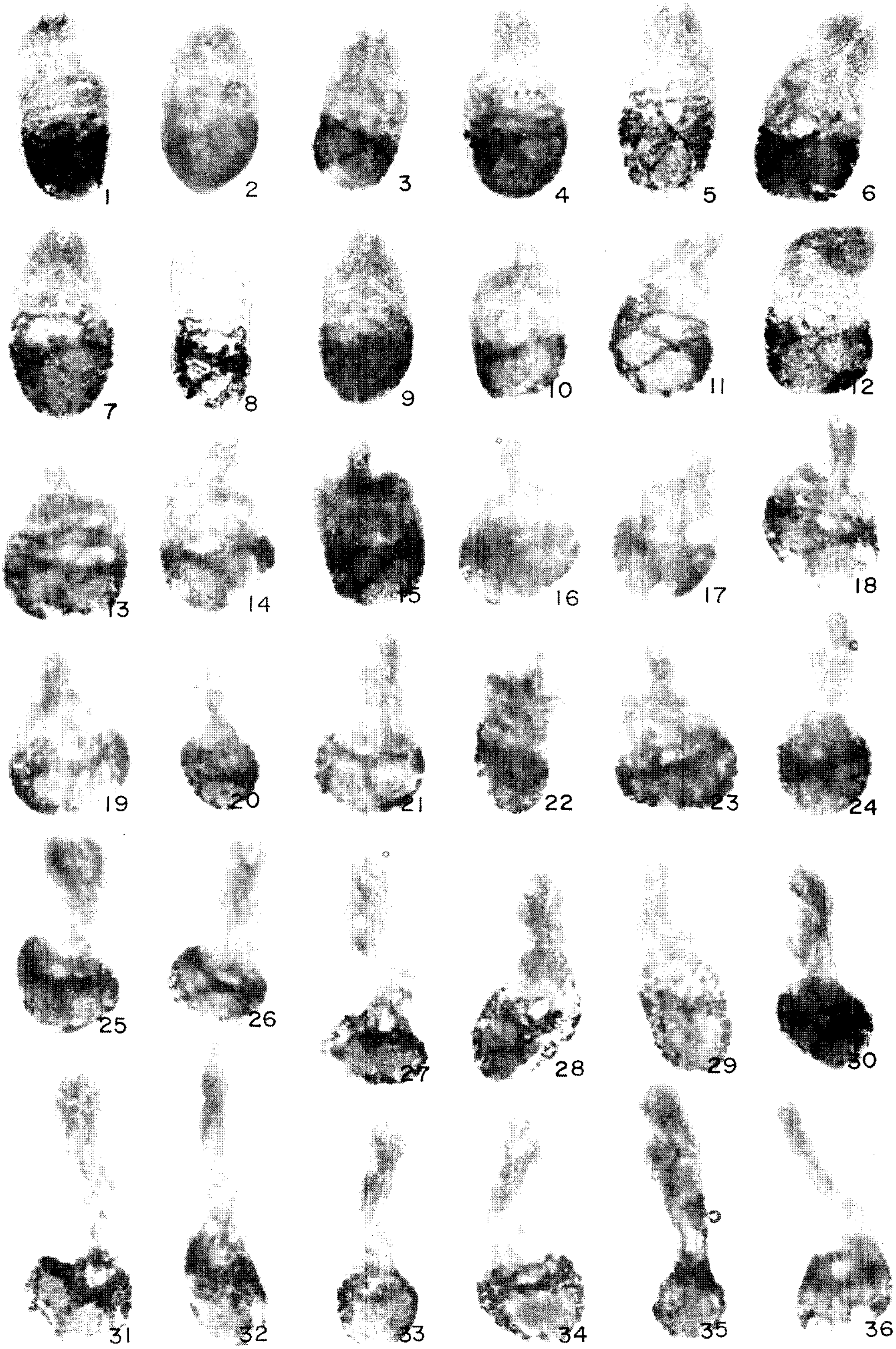
3. 고정한 소장 조직에서 장점막을 분리하고 해부침으로 점막조직을 해부하여 수집한 고정된 충체는 특히 감염후 3일째에 여러가지 특이한 자세를 보였다. 충체전반부가 앞쪽으로 매우 돌출한 충체가 많았는데 돌출한 형태는 단순히 한가닥으로 돌출한 것에서 2분지, 3분지된 것까지 있어 마치 충체 전반부가 아메바운동을 하는 것 같이 보였다.

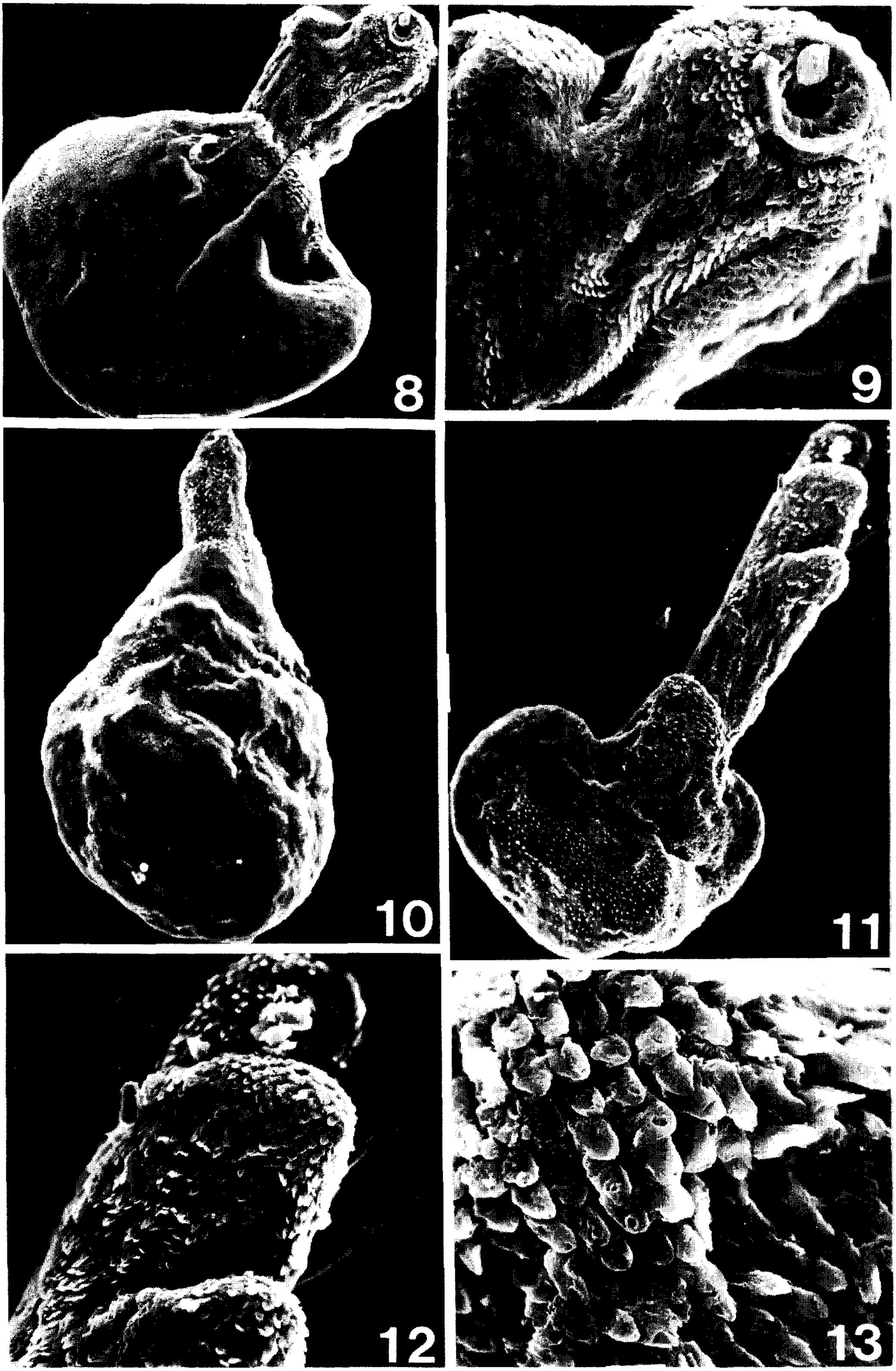
4. 이러한 형태의 충체를 주사전자현미경으로 관찰하였던 바 돌출한 충체전반부는 돌출한 전반부의 길이를 따라 길이로 홈이 파져 있음이 관찰되었다.

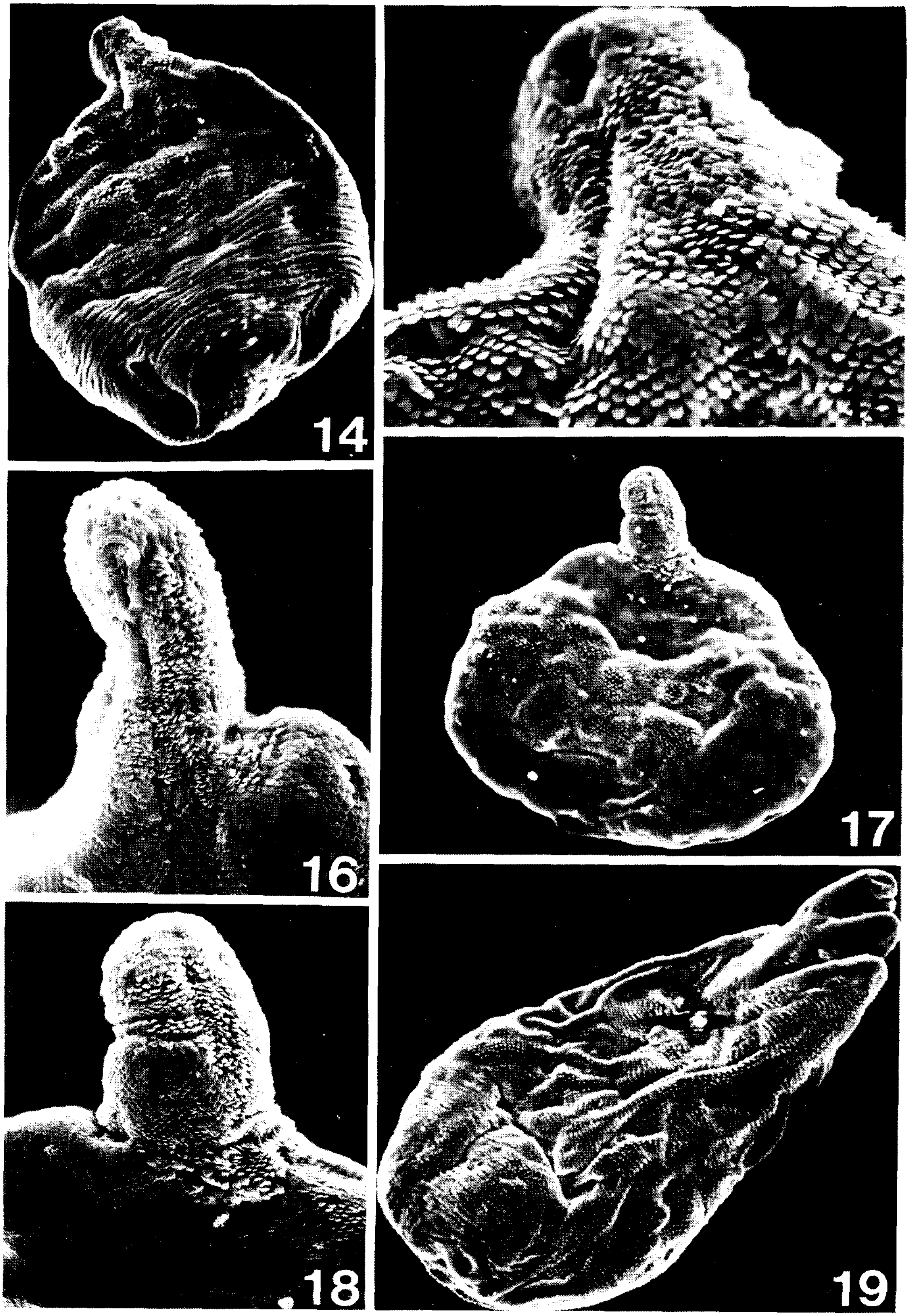
5. 감염후 9일, 4주 및 10주된 충체에서는 전반부가 돌출한 충체의 비율은 감염 3일된 충체에 비해 감소하였다.

이상의 결과에서 요꼬가와흡충은 감염초기에 장은와 선강(腺腔) 상부에 충체 전반부를 밀어 넣을 때에 전반부를 튜브모양으로 만들어 침입하며 인근 장은와 선강을 동시에 침입하기 위하여 한 충체에서 여러개의 튜브모양 돌출부를 만들고 있는 것으로 사료되었다.


1
2
3
4
5
6


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36


8
9
10
11
12
13


14
16
17
18
19

EXPLANATION OF PLATES

Fig. 1. Worm sections of 3 day-old infection of *Metagonimus yokogawai.* Arrows indicated a sacculated protrusion at the base of main protrusion to glandular lumen of crypt, and a branched section of protrusion (H&E, ×250).

Fig. 2. Another view of 3 day old worm section (H&E, ×250).

Fig. 3. Another view of 3 day old worm sections showing an anterior body sectioned three times (H&E, ×250).

Fig. 4. Three day old worm section with bifurcated protrusion of anterior body (H&E, ×250).

Fig. 5. Transverse section of crypt of Lieberkuehn of small intestinal mucosa in 3 day-old infection. V-shaped, folded section of anterior body was seen. Necrotized enterocytes were also observed (H&E, ×250).

Fig. 6. Worm sections in 4 week-old infection in dog small intestine. Worms located at intervillous spaces (H&E, ×100).

Figs. 7-1 to 7-36: Unstained, three dimensional *M. yokogawai* of 3 day-old infection were flattened under cover glass pressure and were photographed (×100).

Figs. 7-1 to 7-8. Worms from intervillous spaces. Worms appeared as conventionally flattened worms.

Fig. 7-12. Ventrally recurved anterior body embraced a villus and was fixed ***in situ*** posture.

Figs. 7-9 to 7-18. Anterior body protruded a single projection. The base of the protrusion may be transversely wrinkled.

Figs. 7-19 to Fig. 7-30. Multiple protrusions of anterior body made various shapes of worms.

Figs. 7-31 to 7-36. Prolonged protrusions of anterior body. Sexual organs may be displaced to protruded anterior body in part as in Fig. 7-35.

Figs. 8 to 16. Scanning electron microscopic findings of 3 day-old juvenile worms.

Fig. 8. A main protrusion with a small saccular prominence at side was seen in this worm (×300).

Fig. 9. Magnification of Fig. 8. Longitudinal wrinklings were seen along the axis of protrusion (×1,000).

Fig. 10. Dorsal view of a worm without folding. A protrusion of anterior body was seen (×300).

Fig. 11. A long protrusion of anterior body had daughter protrusions (×300).

Fig. 12. Magnification of Fig. 11 (×800).

Fig. 13. Magnification of Fig. 12. Scale-like tegumental spines at anterior body were seen (×3,000).

Fig. 14. A worm with a small protrusion of anterior body. Multiple transverse wrinklings on posterior body were seen (×200).

Fig. 15. Magnification of Fig. 14. A groove-like folding line was seen at ventral part of the protrusion (×800).

Fig. 16. A folding line was clearly seen in this worm (×600).

Figs. 17 to 19. SEM findings of 4 week-old worms.

Fig. 17. A worm had a small protrusion of anterior body (×200).

Fig. 18. Magnification of Fig. 17, showing two transverse wrinklings (×500).

Fig. 19. A worm with short, multiple protrusions in anterior body (×200).