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DEVELOPMENT AND MIGRATION ROUTE OF *ANGIOSTRONGYLUS SIAMENSIS* IN MICE¹

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Third stage larvae of *Angiostrongylus siamensis* were inoculated orally into ddY mice. After entering the wall of the colon and cecum, the larvae migrated into the mesenteric lymph nodes and lymph vessels, where the third and fourth molts occurred. The third molt took place between 30 and 72 hours post-infection. The fourth molt began on day 4 and was completed by day 7 post-infection. Between days 6 and 10 post-infection, the young adults moved from the lymphatic vessels to the arterioles in the colon and mesentery. From there they migrated up to the mesenteric arteries, their definitive habitat. Oviposition began on day 22 post-infection. The eggs were released into the blood stream and lodged in the capillary of the lower portion of the small intestine, the cecum and the upper colon. The first stage larvae appeared in the feces of mice 31 days post-infection.

Key words : abdominal angiostrongylosis, *Angiostrongylus siamensis*, development, migration route, mouse

INTRODUCTION

Angiostrongylus siamensis OHBAYASHI, KAMIYA et BHAIBULAYA, 1979, was found in the mesenteric arteries of rodents belonging to the genus *Rattus* in Thailand.¹¹⁾ The aquatic snail, *Biomphalaria glabrata*, can serve as an experimental intermediate host of this parasite. The development of the parasite in the above snail has been studied,⁴⁾ and several laboratory animals, including mice, have been reported as the experimental final host.³⁾ Recently we found a suspected case of *A. siamensis* in a crab-eating monkey, *Macaca fascicularis*, imported from Malaysia to Japan.¹³⁾ This paper presents our observations of the development and migration route of *A. siamensis* in mice.

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MATERIALS AND METHODS

Parasite

The *A. siamensis* used in all of the experiments was originally obtained from *Rattus sabanus*, which was caught in Thailand, and has been maintained in our laboratory by using *B. glabrata* as an intermediate host and the following laboratory animals as definitive hosts: mice, rats, cotton rats (*Sigmodon hispidus*) and Mongolian gerbils (*Meriones unguiculatus*).

Experiment I Migration route of A. siamensis in mice

Twelve male, 4–6 week-old ddY strain mice were each orally infected with 500–1000 third stage larvae obtained from the snail, *B. glabrata*, by digestion with artificial gastric juice containing 1% pepsin (1 : 10000 Nakarai Chemicals, Ltd.) and 1% HCl at 37°C for 2 hours. The mice were killed under ether anaesthesia by bleeding the femoral artery at 21, 30, 48 and 72 hours, and at 4, 5, 6, 7, 8, 9, 12 and 15 days post-infection (PI). Blood was collected in a petri dish containing saline solution or distilled water and examined for worms under the dissection microscope. The peritoneal and thoracic cavities were washed with saline solution and washing fluid were also examined for worms. The stomach wall, small intestinal wall (divided into three equal portions), wall of the cecum and colon, mesentery, mesenteric lymph nodes, pancreas, liver, lungs, heart, kidneys, spleen, abdominal wall muscle, femoral muscle, diaphragm, thymus and brain were separately macerated and fragmented with pointed forceps and digested with artificial gastric juice at 37°C for 1–1.5 hours. Worms were collected from the centrifuged sediments of the digested materials under the dissection microscope. The remaining sediments were pressed under glass plates and examined for worms.

Experiment II Morphological observations

Thirty-four mice were infected with 20 larvae each, and 1–7 mice were killed at 20, 22, 24, 26, 28, 30, 32, 36 and 43 days PI. Worms were collected from the mesentery, aorta and intestinal wall. After fixation in 10% formalin, the worms were treated with lacto-phenol solution for microscopic observation. Morphological features of the worm recovered in experiment I and the third stage larvae from snails were also studied. Samples of the intestinal wall and feces of the mice were microscopically examined for the presence of eggs and first stage larvae.

Experiment III Histological examinations

Fifteen mice, each infected with 500–2500 larvae, were killed at 2, 7, 12, 24, 48, 72 and 84 hours, and at 4, 5, 6, 7, 8, 10, 13 and 15 days PI. Seven mice, each infected with 20 larvae, were killed at 17, 20, 24, 28, 32, 36 and 43 days PI. After

gross inspection, the tissues of each mouse were separated as in experiment I, except for the mesenteric lymph nodes. The small intestines and colon were slit open and made into a so-called swiss roll.⁸⁾ All the tissues were then fixed in 10% formalin, dehydrated in alcohol series, cleared in xylene, embedded in paraffin, sectioned and stained with hematoxylin-eosin.

RESULTS

Migration route of A. siamensis in mice

Distribution of *A. siamensis* in the mice is shown in figure 1, which includes the percentages of the number of worms recovered from the mesentery, mesenteric lymph nodes, colon, cecum, peritoneal cavity and other tissues combined in the course of observation between 21 hours and 15 days PI.

The total recovery rate was about 20–40% (31.5% on the average) during the period from 21 hours to 15 days PI. Between 21 and 72 hours PI, half of the total recovered larvae were from the mesenteric lymph nodes. The number of larvae found in the colon gradually increased and reached a peak (58.3%) on day 7 PI, whereas that in the mesenteric lymph nodes correspondingly decreased. Finally, between days 8 and 15 PI, the worms were recovered mainly from the mesentery at the rate of 61.1% on day 15 PI.

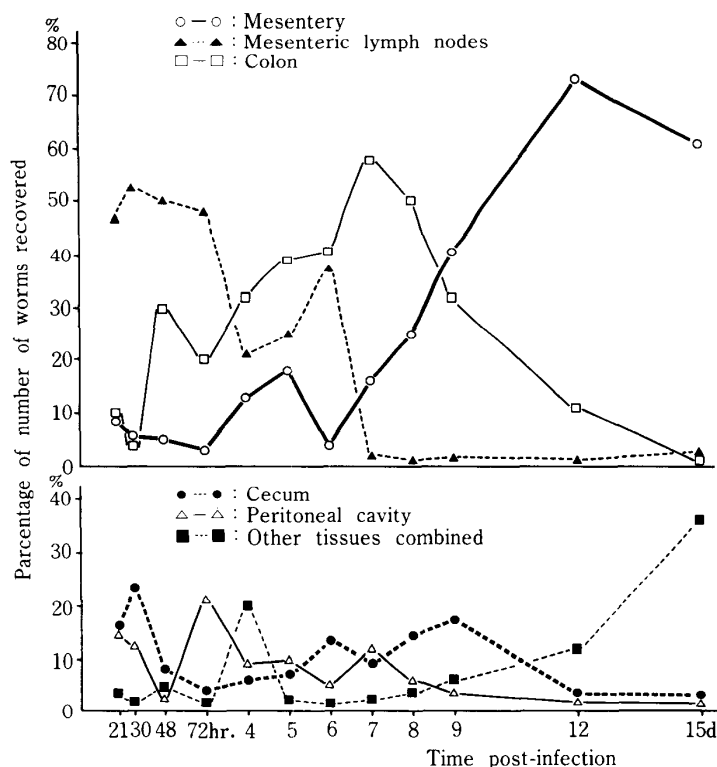


FIGURE 1 *Distribution and recovery rate of Angiostrongylus siamensis in mice*

Morphological observations

Measurements of the third and fourth stage larvae are presented in table 1. The third stage larva, which is 0.464–0.571 mm long, possesses two well-developed chitinous rods at the anterior end (fig. 2). The third molt took place between 30 and 72 hours PI.

The chitinous rods are absent in the fourth stage larva. The male fourth stage larva, which is 0.675–0.795 mm long, can be distinguished from the female, which is 0.781–0.886 mm long, by the shape of the tail. A swelling mass, which contains the developing accessory organs, can be seen in the tail of the male (figs. 3 & 5). In the female the tail is conical without any swelling mass (figs. 4 & 6). The female showed a developing genital primordium which extends to the rectum. This female organ contains a vacuole in its posterior end. The fourth molt began on day 4 and was completed by day 7 PI. The male observed on day 4 PI has a tiny bursa. In the female observed at the same time, the vacuolated structure opens anteriorly to the anus, and the vulva can be identified. The young adults recovered on day 7 PI are shown in figures 7 & 8.

The description of the adult worm was based on observations of 14 males and 20 females recovered on day 43 PI (tab. 2, figs.9–11). The wall of the mouth cavity is not chitinized. The male is 9.38–11.7 mm long. The bursa is reduced, the reduction of which is conspicuous in the lateral, externo-dorsal and dorsal rays. The dorsal ray possesses a pair of short digitiform projections. The female is 12.2–14.9 mm long and has a bluntly pointed tail end without any projection.

The eggs were first found in the squash preparation of the cecum of the mice on day 22 PI, and the first stage larvae appeared in the feces on day 31 PI.

Histological examinations

Depending on the time PI, worms were found in the following organs: the mucosa, submucosa and muscle layer of the upper colon at 2 hours PI (fig. 12); the marginal and intermedial sinuses of the mesenteric lymph nodes, the lymphatic vessels peripheral to the nodes and the lymph vessels of the cecum and colon from 7 hours to 5 days PI (figs. 13 & 14); the arterioles, small arteries and lymph vessels of the mesentery, marginal sinus of the mesenteric lymph nodes, and arteries and lymphatic vessels of the colon, cecum and lower portion of the small intestine from 6 to 8 days PI (figs. 15 & 16); and the mesenteric arteries and arteries of the cecum and lower portion of the small intestine at 10 days PI and thereafter (fig. 17). In the course of the observations made between 7 hours and 5 days PI, the larvae were prominently found in the lymphatic system of the mesentery. Especially on day 5 PI, many larvae were found in the lymph vessels of the colon. Between 6 and 8 days PI, multiple small hemorrhages were seen in the colon, cecum, mesentery and mesenteric lymph nodes. The eggs and first stage larvae were first observed on days 24 and 28 PI,

TABLE 1 *Measurements of third and fourth stage larvae of Angiostrongylus siamensis (in mm)*

	THIRD STAGE	FOURTH STAGE (72 hours after infection)	
		Male	Female
Body length	0.464–0.571 (0.514)	0.675–0.795 (0.746)	0.781–0.886 (0.817)
Length of esophagus	0.170–0.205 (0.189)	0.179–0.215 (0.197)	0.179–0.218 (0.199)
Nerve ring*	0.068–0.082 (0.076)	0.075–0.097 (0.086)	0.079–0.102 (0.090)
Excretory pore*	0.070–0.086 (0.078)	0.090–0.111 (0.101)	0.093–0.109 (0.103)
Length of tail	0.025–0.036 (0.031)	0.029–0.038 (0.033)	0.038–0.045 (0.040)
Genital primordium [†]	0.168–0.225 (0.187)	—	—

All measurements are based on 20 specimens, and the means are given in parentheses

* Distance from anterior end

[†] Distance from posterior end

TABLE 2 *Measurements of adults of Angiostrongylus siamensis (in mm)*

	MALE	FEMALE
Body length	9.38–11.7 (10.7)	12.2–14.9 (13.3)
Length of esophagus	0.223–0.275 (0.251)	0.229–0.275 (0.253)
Nerve ring*	0.179–0.218 (0.200)	0.179–0.232 (0.204)
Excretory pore*	0.300–0.364 (0.343)	0.321–0.357 (0.339)
Length of spicule	0.321–0.364 (0.340)	—
Length of gubernaculum	0.037–0.050 (0.044)	—
Vulva [†]	—	0.214–0.277 (0.239)
Length of tail	—	0.071–0.089 (0.078)

Measurements are based on 14 males and 20 females recovered at 43 days after infection, and the means are given in parentheses

* Distance from anterior end

[†] Distance from posterior end

respectively. The eggs and larvae were distributed in the lamina propria, submucosa and muscle layer of the lower portion of the small intestine, the cecum and upper colon, and in the mesentery and mesenteric lymph nodes (fig. 18). Granulomatous lesions surrounding the eggs and larvae were observed in the organs mentioned above (fig. 19).

DISCUSSION

From the results, the development and migration route of *A. siamensis* in mice were deduced to be as follows. After oral infection, the third stage larvae invade the wall of the cecum and colon. Thereafter, they migrate to the marginal and intermedial sinuses of the mesenteric lymph nodes and lymphatic vessels peripheral to the nodes, where the third and fourth molts occur. The third molt takes place between 30 and 72 hours PI and the fourth molt between days 4 and 7 PI. The worms move to the colon via the lymph vessels on day 5 PI. On day 6 PI, the young adults penetrate the wall of the lymph vessels and pass into the arterioles of the colon and mesentery. Their migration terminates at the arterioles on day 10 PI. Multiple small hemorrhages occurring in the colon, cecum, mesentery and mesenteric lymph nodes are due to the penetration of the worm. Finally, the young adults reach the mesenteric arteries and the branches supplying the lower portion of the small intestine and cecum. Oviposition begins on day 22 PI. The eggs are released into the blood stream and lodge mainly in the capillary of lower portion of the small intestine, cecum and upper colon. After developing into first stage larvae here, the larvae penetrate into the lumen of the intestine, and then appear in the feces on day 31 PI.

Among the *Angiostrongylus*-species, development in the final host has been reported in *A. cantonensis*, *A. costaricensis*, *A. vasorum* and *A. dujardini*.^{1,2,6,10,14)} *A. costaricensis*, which is parasitic in the mesenteric arteries of rodents in North and South Americas,^{7,15,16)} shows similarities to *A. siamensis* in its morphology, habitat, ileocecal lesion and broad range of final hosts. Recently it has been suggested that these two nematodes are closely related species, because F₁ hybrids can be produced experimentally from both of them.¹²⁾

A. siamensis is very similar to *A. costaricensis* in its development and migration route within the final host. However, there are three exceptions: the third molt and migration to the arterioles within the mice begins one day earlier than that of *A. siamensis* in cotton rats.¹⁰⁾ and the prepatent period is 7 days longer than that of *A. costaricensis* in cotton rats. In view of this fact, pathogenicity of *A. siamensis* in the monkey and its phylogenetic proximity to *A. costaricensis*, which is known to be the cause of eosinophilic abdominal granuloma in children in Costa Rica,^{5,9)} attention must also be paid to *A. siamensis* as a potential zoonotic parasite.

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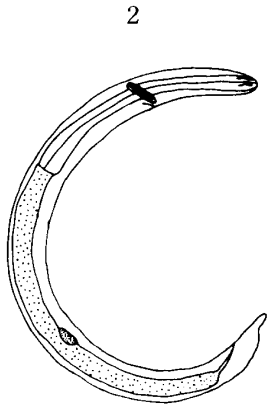
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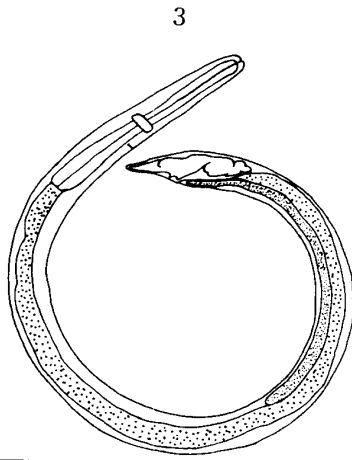
EXPLANTATION OF PLATES

PLATE I

- Fig. 2 Third stage larva
Figs. 3-6 Fourth stage larva, 72 hours post-infection (PI)
Fig. 3 Male
Fig. 4 Female
Fig. 5 Posterior end of male
Fig. 6 Posterior end of female
Fig. 7 Male young adult, 7 days PI
Fig. 8 Female young adult, 7 days PI
Figs. 9-11 Adult worm, 43 days PI
Fig. 9 Anterior end of female
Fig. 10 Posterior end of female
Fig. 11 Posterior end of male



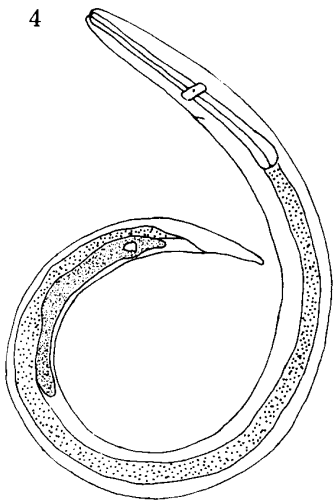
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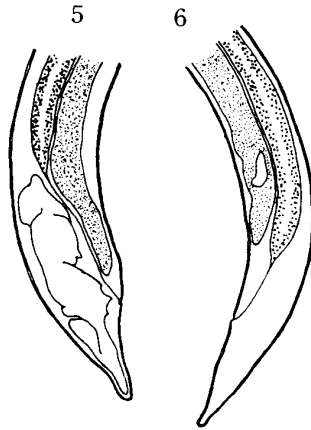
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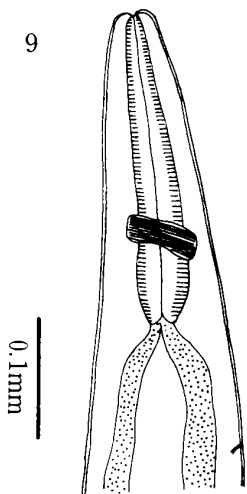
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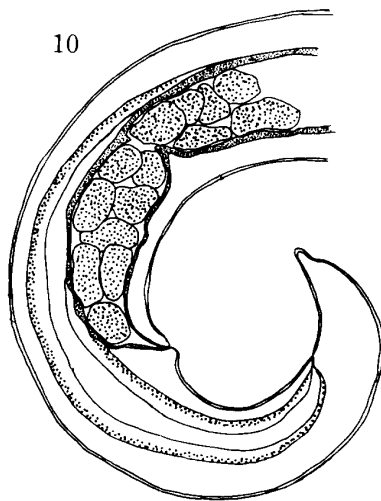
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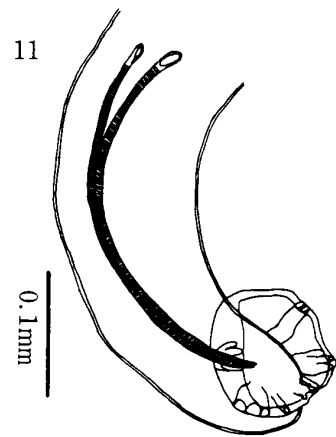
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PLATE II

- Fig. 12 Showing the larva (↑) in the mucosa of the colon, 2 hours PI, ×190
- Fig. 13 Larvae in the marginal sinus of the mesenteric lymph node, 24 hours PI, ×240
- Fig. 14 Larvae in the lymphatic vessel peripheral to the mesenteric lymph node, 24 hours PI, ×120
- Fig. 15 Worm (↑) in the artery in the submucosa of the colon, 7 days PI, ×240

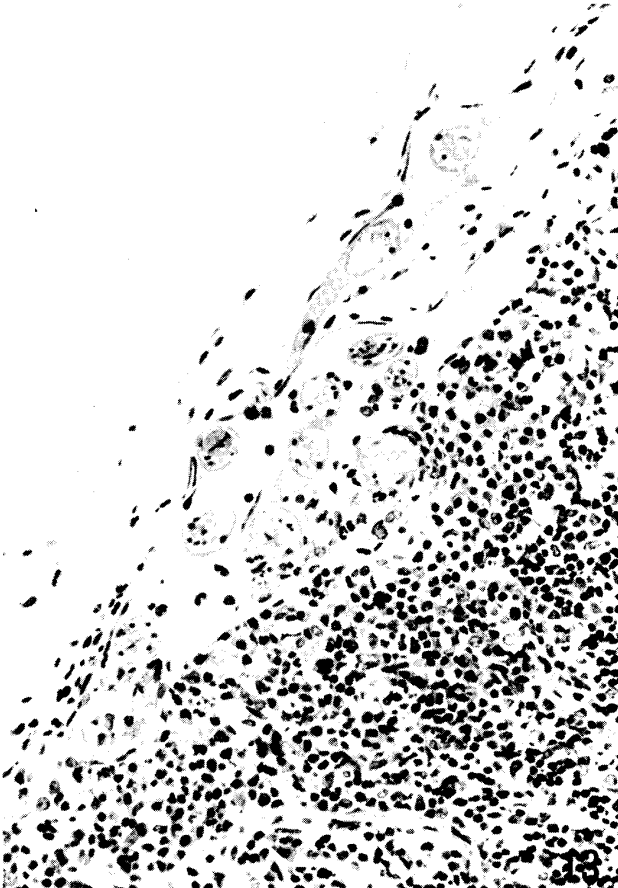
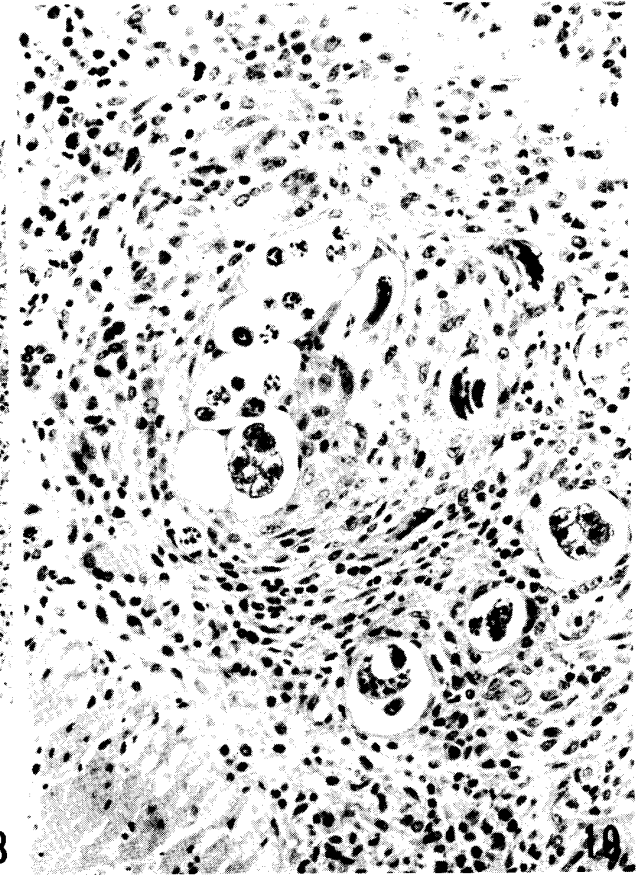
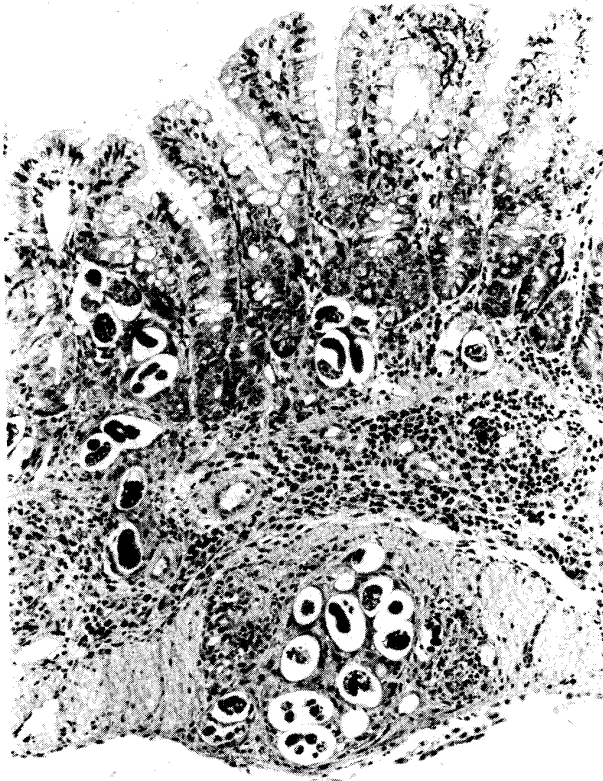
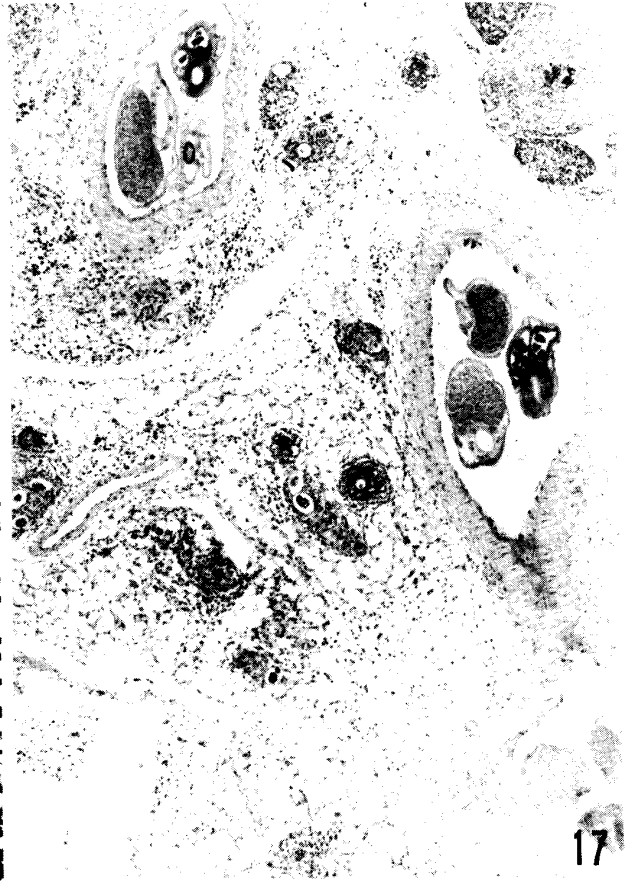


PLATE III

- Fig. 16 Worms in the artery (↑) and lymph vessel of the mesentery, 7 days PI, ×190
- Fig. 17 Worms in the mesenteric arteries, 43 days PI, ×50
- Fig. 18 Developing eggs in the cecum, 43 days PI, ×120
- Fig. 19 Granulomatous lesion including eggs inside in the cecum, 43 days PI, ×240



18

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