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Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea V. Intestinal Pathology in Experimentally Infected Albino Rats

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INTRODUCTION

Metagonimus yokogawai is the most frequent and prevalent species of the heterophyid trematodes in most areas of South-east Asia. Also in Korea, endemic foci of human infection with this fluke have been reported in various parts of South Kyongsang Do (Yeo et Seo, 1971; Loh et Kang, 1971), South Cholla Do(Soh et al., 1976; Chai et al., 1977 and Soh et Ahn, 1978) and Cheju Do (kang et al., 1964). The prevalence rates in the inhabitants were reported to be 30~60 per cent by egg detection method. Because of the high infection rates of the inhabitants in endemic area of Korea, attentions tend to recently be paid to this minute intestinal fluke.

The normal habitat of *M. yokogawai* is intestinal tract, especially the small intestine of human or animals. Diarrhea and/or abdominal pain has been known as the most frequent clinical complaint both in natural (Yokogawa, 1913; Koga, 1938 and Seo et al., 1971) and experimental(Koga, 1938 and Komiya et al., 1958) human cases. However, few papers have contributed to the satisfactory making out of the genesis of such symptoms and few have been available in the description of pathologic changes of the intestine, except for those of Taki(1936), Koga(1938) and Cho et al. (1978).

Koga(1938) reported transient catarrh in light infection and severe catarrh sometimes with bloody mucous diarrhea in heavy infection cases of cats or dogs. But he could not observe any invasion of worms to submucosa or blood vessels. Cho et al. (1978) also observed intestinal changes in cats by experimental infection each with 80,000 metacercariae of *M. yokogawai* and found mucosal edema with changed rugae pattern, lining epithelial cell necrosis, small round cell infiltration (especially the eosinophils, lymphocytes and plasma cells) etc. According to them, many worms were found from the slits of intestinal crypts with oral sucker facing the base of the crypts, but there was no evidence of worm invasion to deeper level.

Invasiveness of heterophyid flukes to deeper level than the base of crypts is thought to be very important in the pathogenesis of extraintestinal or visceral heterophyidiasis stressed by Africa et al. (1935 and 1948), the eggs of the flukes being transported via blood vessels to visceral organs such as heart, brain and spinal cord. They reported that *Haplorchis* sp., *Heterophyes* sp., *Stellantchasmus* sp., etc. caused visceral spread of their eggs, all of which normal habitat is intestinal tract.

From this respect, intestinal pathology should be thoroughly checked in metagonimiasis with a variety of animals to rule out the possibility

of visceral involvement and to check any difference of pathologic changes by host difference. In this study, gross and microscopic changes of small intestine of albino rats were observed by experimental infection with metacercariae of *M. yokogawai*.

MATERIALS AND METHODS

1. Collection of metacercariae and infection to rats:

Metacercariae of *M. yokogawai* were isolated from muscles of sweetfishes, *Plecoglossus altivelis*, which had been caught from the main stream of Tamjin River, South Cholla Do, Korea, by digestion technique with artificial gastric juice and counted under dissecting microscope to prepare the doses to be challenged.

A total of 24 adult albino rats, male or female, were examined for parasitic infections and subjected in this study. Rats were divided into 4 groups, one being control and others infection groups (500, 2,500 and 12,500 metacercariae in each group). Infection of the rats were performed by inserting the polyethylene capillary tube to stomach under ether anesthesia. Number of rats in each group was 3, 8, 7 and 6 respectively (Tabe 1). The rats had been kept in this laboratory for 1, 2, 3 and 4 weeks according to the schedule until sacrificed.

Table 1. Worm recovery rates in experimental groups No. control rates: 3

Week	Dose of *Mc	No. rats	No. recov. worms(%)	Dose of Mc	No. rats	No. recov. worms(%)	Dose of Mc	No. rats	No. recov. worms(%)
1	500	2	$\frac{1}{275}$(27.6)	2,500	2	$\frac{6}{1,600}$(32.1)	12,500	2	$\frac{1}{9,000}$(36.0)
2	500	2	$\frac{12}{19}$(3.1)	2,500	2	582(24.0)	12,500	0	—(—)
3	500	2	$\frac{31}{33}$(6.4)	2,500	1	$\frac{620}{416}$(16.6)	12,500	2	$\frac{286}{8,500}$(35.1)
4	500	2	$\frac{1}{14}$(1.5)	2,500	2	$\frac{102}{950}$(20.1)	12,500	2	$\frac{27}{50}$(0.3)
Total	500	8	384 (9.6)	2,500	7	4,276(24.4)	12,500	6	17,864(23.0)

* Mc: Metacercariae of *Metagonimus yokogawai*

** Total worm recovery rate from 21 rats $= \frac{\text{Total No. recov. worms}(22,524)}{\text{Total No. introd. worms}(96,500)} \times 100 = 23.0\%$

2. Observation of gross and microscopic findings of the intestine:

Rats were sacrificed individually and intestinal loop configuration was observed. Then, the small intestine was resected and the diameter of the loop was measured in the largest portion to check the degree of intestinal loop dilatation. The intestinal lumen was opened to observe the intraluminal contents and mucosal appearance.

For microscopic examination, 0.5 cm segments of duodenum, jejunum and ileum were resected and fixed in 10% formalin. The fixed tissues were dehydrated by successive changes of alcohols, cleared in two changes of xylene, embedded in paraffin, and sectioned by 7 microns in thickness. Routine Hematoxylin and Eosin stain was done.

3. Observation of worm development and recovery rate:

The remainder of the small intestine was put in 0.85% saline solution and the escaped worms from mucosa were counted under dissecting microscope. The worms were preserved

in refrigerator at 5°C for 2~3 days for better fixation. Well fixed worms in each experimental group were stained with Semichon's acetocarmine and the developmental status of the worms was observed.

RESULTS

1. Clinical finding and egg detection:

Almost all of the infected rats started defecating loose stool from 6~12 days after infection and it lasted, in most cases, continuously or intermittently until sacrificed. None of the 6 rats (1 week group) revealed eggs in their stool before sacrifice but 11 out of 15 rats (2, 3 and 4 week group) revealed eggs on the 14th day after infection.

2. Recovered worms and worm recovery rate:

Some of the recovered worms from 1 week group showed evidence of maturation; testes, ovary and eggs in uterus, but others were under developing. The size of the mature worms from 2, 3 or 4 week groups revealed variation (0.3 ~0.7mm in length) but nearly all of the worms from heavily infected rats were rather small (0.4~0.5mm in length) compared with the worms from human cases (0.88~0.98 in length by Seo et al., 1971). Recovered worms were shown in Plate I according to ages of infection. Worms did not become larger in size after 1 or 2 weeks from infection.

Worm recovery rates from individual rats varied greatly as shown in Table 1. The introduced dose and the number of successfully parasitized worms were not so correlated with each other. However, the recovery rate was higher in the heavily challanged rats and became lower as infection progressed. The overall worm recovery rate was 23.0% as calculated in Table 1.

3. Gross findings:

Most of the infected rats revealed dilated and translucent loops (Plate II) and the dilation was more pronounced in the distal half of the small intestine but sometimes observed in cecum or the proximal part of large intestine. Degree of the dilation was measured and presented in Table 2. The diameter of the bowel appeared to increase progressively with the duration of infection, but the increase did not necessarily correlated with the number of parasitized worms.

Table 2. Degree of intestinal dilation by diameter of lumen (mm)

Duration of infection (Week)	No. of recovered worms								Total	
	1~100		101~500		501~2,000		Over 2,000			
	No. exam.	Mean diam.	No. exam.	Mean diam.	No. exam.	Mean diam.	No. exam.	Mean diam.	No. exam.	Mean diam.
1	3	4.5	1	4.8	1	4.4	1	4.4	6	4.5
2	2	4.4	0	—	2	5.5	0	—	4	4.9
3	2	5.0	2	5.4	0	—	1	4.5	5	5.0
4	4	5.0	1	5.9	1	6.3	0	—	6	5.4
Total	11	4.7	4	5.4	4	5.4	2	4.5	21	5.0

* Control group: No. exam. ; 3 rats, Mean diameter ; 4.0mm

Luminal content was characterized by loose content consisted of mucous stool and numerous air bubbles. In a few cases of later week group, watery content rather than mucous filled the entire intestinal lumen. These foamy mucous stool was found most frequently in jejunum

Table 3. Presence of diarrheal stool according to experimental groups

Duration of infection (Week)	No. of recovered worms								Total	
	1~100		101~500		501~2,000		Over 2,000			
	No. exam.	No. posit.	No. exam.	No. posit.	No. exam.	No. posit.	No. exam.	No. posit.	No. exam.	No. posit(%)
1	3	1	1	1	1	1	1	1	6	4 (66.7)
2	2	1	0	0	2	2	0	0	4	3 (75.0)
3	2	2	2	2	0	0	1	1	5	5(100.0)
4	4	2	1	1	1	1	0	0	6	4 (66.7)
Total	11	6	4	4	4	4	2	2	21	16 (76.2)

* Rats whose any small portion of small intestine contained diarrheal stool were considered positive.

(Plate II), but also seen in ileum or duodenum although lesser in degree. Number of rats which revealed mucous stool among 21 infected rats was 16 (76.2%) as shown in Table 3. All of the rats, from which more than 100 worms were found parasitized, showed loose diarrheal stool in their intestinal lumen.

Mucosal hyperemia of moderate degree was sometimes seen in jejunum, however, there was not a single case with mucosal hemorrhage throughout the cases. Lymphoid tissues of the small intestine, i.e., Peyer's patches appeared slightly prominent in most of experimental animals.

4. Microscopic findings:

The pathologic changes were mainly limited in mucosa of jejunum, villus and/or sometimes the crypt, not extended to submucosa or deeper level.

Villous height was found generally to be low resulting in decreased villus/crypt height ratio and these low villi were often blunted at their tip portion. Some epithelial cells of villi particularly at tips of them became flat and, in a few cases, showed epithelial erosion. Number of goblet cells were 10~16 in one villus of control rats but increased to 20~40 in 2 heavily infected rats (1 week group), when counted at almostly the same portions of jejunum. However, this finding was not so consistent enough in each experimental group, although the goblet cells tended to increase in number in earlier groups (1 or 2 week group) and decreased in later groups (3 or 4 week group).

Adult worms of *M. yokogawai* were sometimes seen floating in intervillous space or top of villi without significant tissue damage. The majority of worm lining villous epithelia appeared intact, although epithelial erosion was observed in a few parasitized villi of a heavily infected rat. The causal relationship between the erosion and the worm was not clear or evident. Villi appeared to be, sometimes, compressed by worms and adhered to each other in severe cases.

Stroma, or lamina propria showed some recognizable findings. Edema or lymphatic dilation was almost always present at least in one segment of small intestine throughout the cases. Eosinophilic aggregation was also observed together with other inflammatory cells such as lymphocytes and plasma cells. A few small round cells were seen in control rats, however, the degree of infiltrate, particularly eosinophils and plasma cells was quite comparable. Fibroblast proliferation was sometimes accompanied with the above findings in center of stroma. In spite of apparent inflammatory reactions, no erosion reaching to lamina propria level was found throughout the animals.

Table 4. Histological findings of mucosa by duration of infection

Pathologic findings		Duration of infection (Week)			
		1	2	3	4
Villous					
Appearance	blunting	±	±	+	−
	reduction of height	±	±	+	−
	adhesion	±	−	+	+
Epithelium	flattening	+	±	+	−
	degeneration	+	−	±	−
	ulceration	+	−	±	−
	No. of goblet cells	increased	increased	unchanged	decreased
Stroma	edema	+	+	++	+++
	cell infiltration	+	+	+	++
	congestion	±	+	++	+++
	fibrosis	−	±	++	+
Loss of villi		±	−	±	−
Crypt					
Epithelium	mucoid change	±	±	±	−
	flattening	−	−	±	−
	Worm invasion	−	−	−	−

** '−': no change '±': minimal, not distinct
'+': minimal, distinct '++': moderate '+++': marked

Above microscopic findings were summarized in Table 4 and shown in Plate III and IV. Villous atrophy and blunting appeared more marked in the earlier stage of infection but these became less prominent in later stage, while the stromal edema, cell infiltration, congestion or fibrosis seemed more prominent in later stage. The severity of pathologic changes did not always correlated with the worm burdens.

DISCUSSION

The worm recovery rates from individual rats were much variable and the total worm recovery rate was 23.0% as presented in Table 1. This rate was somewhat higher than that reported by Yokogawa et Sano(1968), who recovered 11.2% of the introduced amount from 8 rats during 1～6 weeks after infection. But as this study revealed decreasing worm recovery rate as infection time went by and it was performed during 1～4 weeks after infection, the higher rate of this study could be interpreted as almost the same as the above workers' result. On the one hand, Takahashi(1929) and Gushima (1939)reported that the development of *Metagonimus* worms in mouse took place more slowly than in dog and Ito(1964) suggested that mice and rats were not considered to be suitable hosts. This study also agreed the poor susceptibility of the rats to *Metagonimus* infection, considering the low worm recovery rate, possibly short life span, smaller size of mature worms, etc.

Dilation of loops might have been caused by increased intraluminal pressure probably due to foamy content and/or mucous or watery secretion. However, the exact nature of the foamy content was uncertain. They might have been

originated from the metabolic process of the worms themselves or of, if any, the associated gas-forming bacterial flora, etc. Koga(1938) mentioned his finding of bloody mucous content from severely infected cats and dogs and explained they might be due to exudative hemorrhage from inflammatory process, as there was no evidence of blood vessel damage. In spite of strong evidence of inflammatory reactions in stroma of infected rat intestine in this study, no bloody content was seen from the luminal surface and no blood vessel involvement was recognizable in tissue sections.

Reduction of villous height was a prominent microscopic finding. This finding could be thought correlated with inflammatory reactions and fibrosis of stroma which led to villous atrophy and blunting, and furthermore, impairment of absorptive function. Villous height comparison was made in the same segments of control and infected rats, for Altman et Enesco (1967) reported that reduction of ileal villous height to half of that of duodenal villi could be interpreted as normal. The cause of inflammatory reactions with edema and fibrosis in stroma of villi could not be clearly explained. A suggestion could be that the increased intraluminal pressure due to large amount of foamy and mucous contents might have collaborated with the worms in compressing the villi.

In spite of the various changes in stroma of villi, the epithelia appeared relatively intact. Some ulceration at tip portion of the epithelia was seen in 1 or 2 week groups but rarely seen in 3 or 4 week groups. Considering the rapid turn over rate of the villi or crypt epithelia (1.1 days in each part) in adult rats (Altman et Enesco, 1967), the damaged epithelia might have been replaced rapidly with new ones from mitotic division. Anyhow, epithelial cell changes by worms might be less important in the pathogenesis of metagonimiasis from the above consideration.

The decrease in number of goblet cells in some cases of later week groups was hardly explainable except for a rough assumption that it might have been due to exhaustion of the precursor cells in crypt by long, continuous reproduction of mucous cells. The precursor cells might be the poorly differentiated mucus-free columnar cells according to Merzel et Leblond(1969) in mouse small intestine. In later week groups of this experiment, goblet cells of villi were sometimes decreased and intraluminal content was less mucoid but much watery.

Worms seemed to have migrated between villi, since intraluminal or floating worms between intervillous space were frequently seen but intruded worms into the slit of crypt were not found in sectioned preparations. It should be clearly ruled out that the worms might have migrated out from the slit of crypt within a few minutes from resection of the segment until fixation, although this possibility could be lessened considering that the oral sucker indicates the direction of worm movement and that the intruded worms faced the base of crypt according to Koga(1938) in cats and dogs and Cho et al. (1978) in cats. Koga(1938) reported that the intrusion of the worms into slits of crypt was mostly seen at the beginning of infection in the upper part of small intestine. However, it should also be considered that the narrowness of the slit of crypt in rats compared with cats and dogs might have prevented the worms from intruding into the slit and let the worms migrate about in the intervillous spaces. If this concept were accepted, less suitability of rats as final host of *M. yokogawai* could be more probable from the consideration of poor ecological niche of the parasite in rat.

胞浸潤(特히, 好酸球, 淋巴球 및 形質細胞), 浮腫形成, 血管充血 또는 纖維化가 觀察되었다. 基質의 變化는 蟲體感染負荷에 依한 差異는 없었으나 感染期間이 經過될수록 더욱 뚜렷하였다. 全 實驗白鼠를 通하여

Katsurada, 1912 infected Plecoglossus altivelis Collected in Chezu Province(Quelpart Island). J. Korean Med. Ass., 7(5):470, *1964 (in Korean).*

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Although incomplete, an explanation of the genesis of diarrhea as a symptom could be made from the results of this experiment. The worms small (0.4～0.5mm).

2. Gross findings were characterized by dilation of intestinal loop, foamy mucous content

—蔡鍾一：요꼬가와 吸蟲에 關한 硏究 V. 白鼠에서의 腸病變—

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Plate I

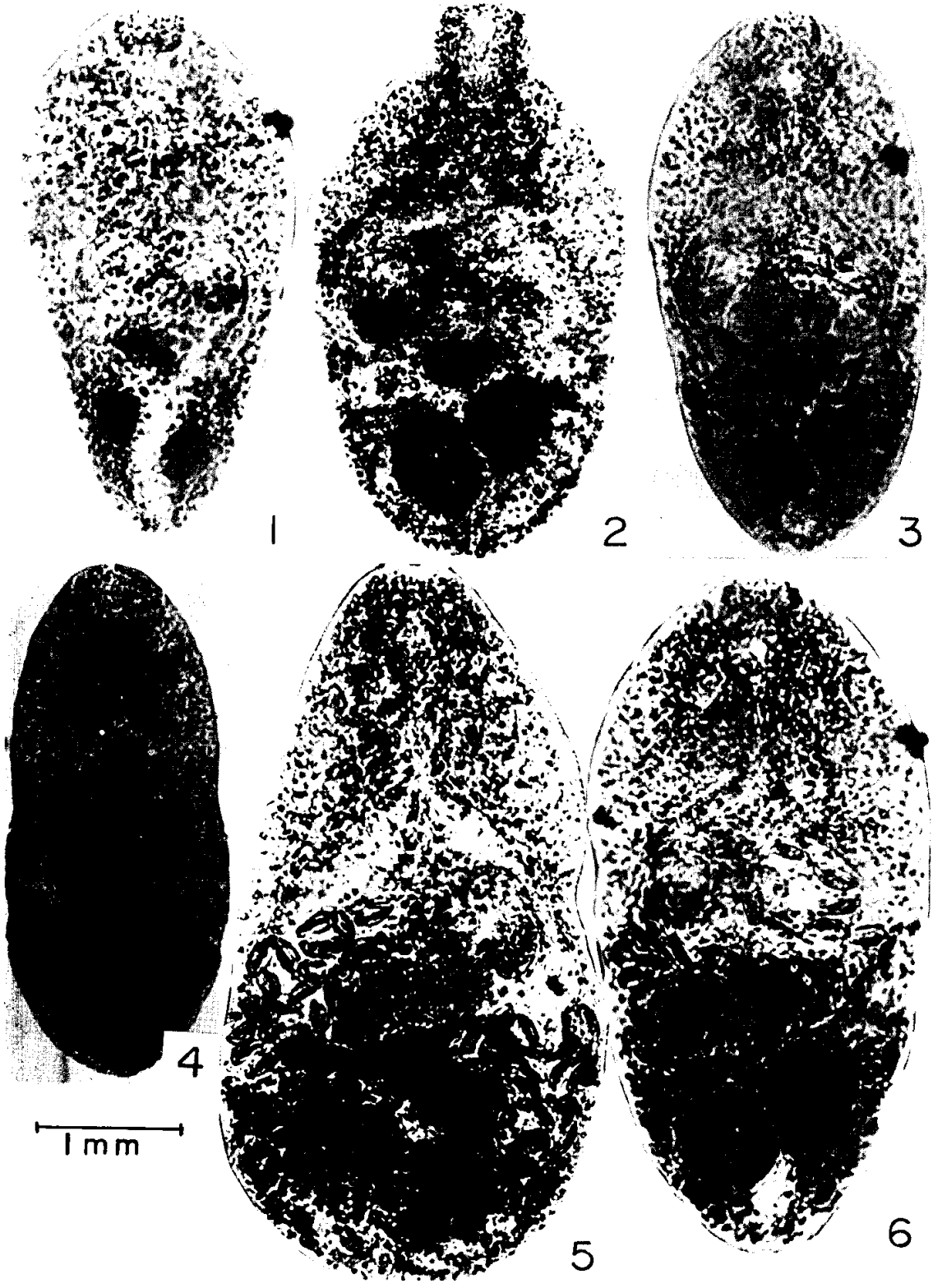


Plate II

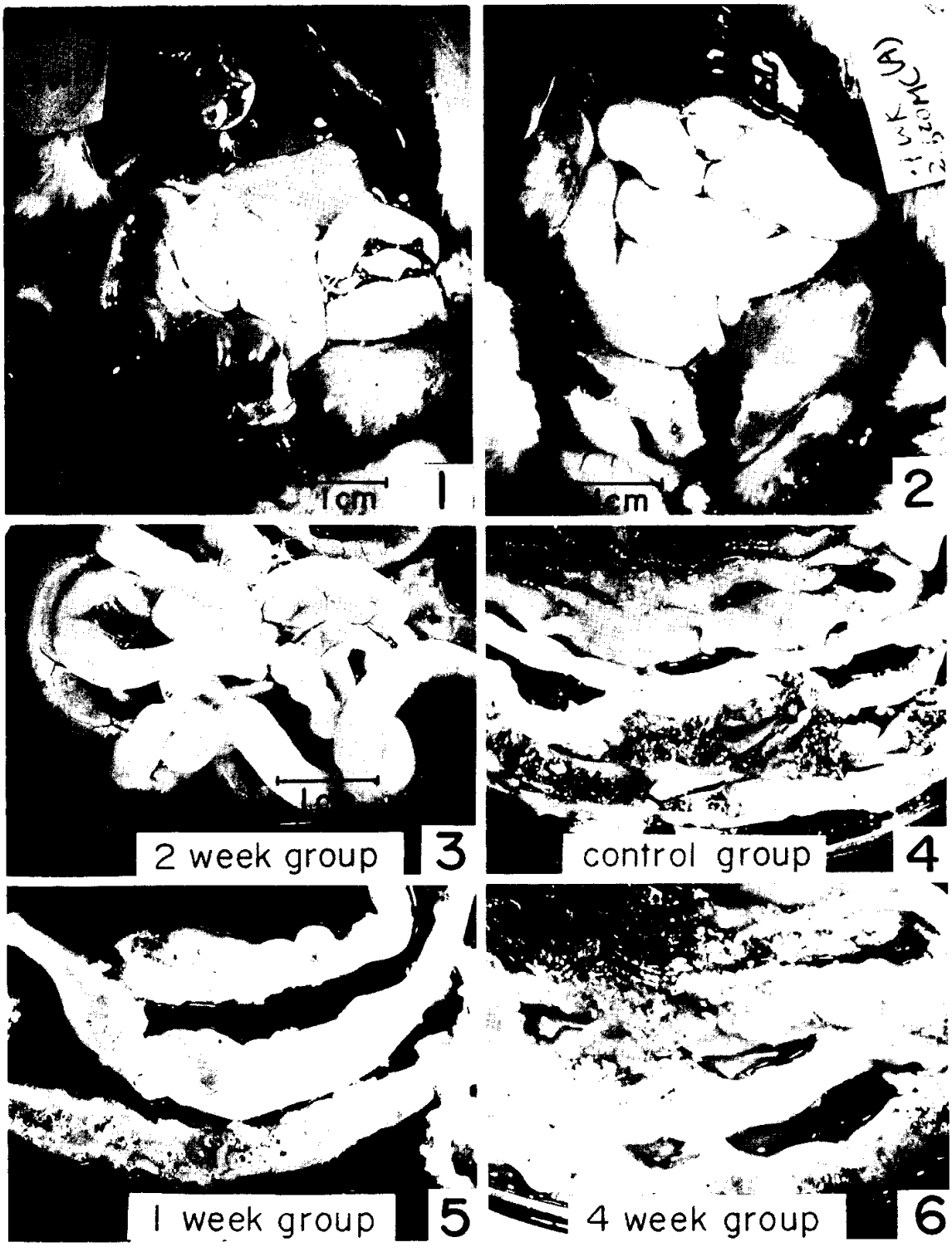
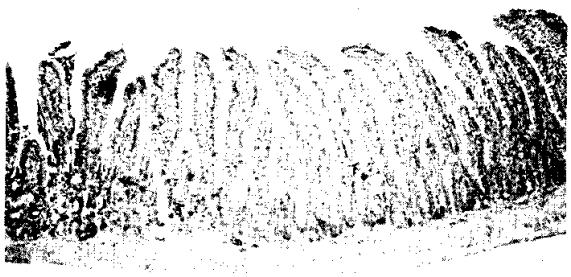

1cm
1
2
2 week group
3
control group
4
1 week group
5
4 week group
6

Plate III



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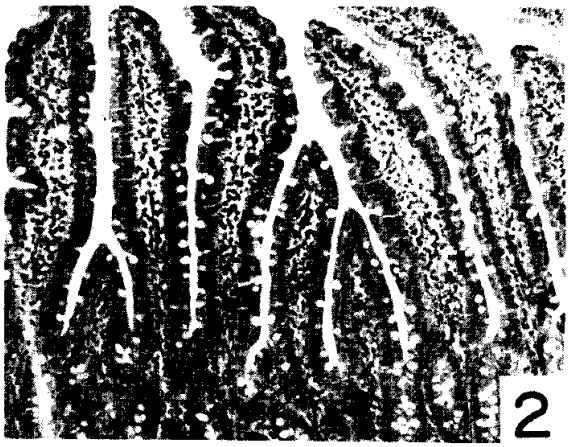

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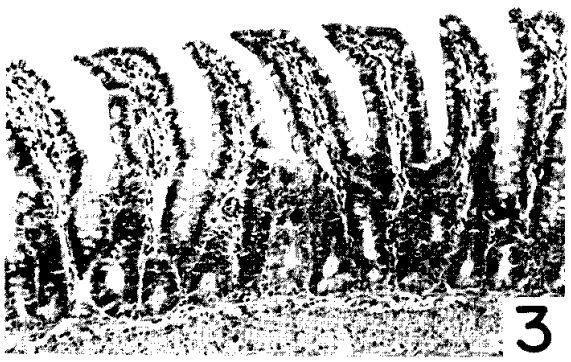

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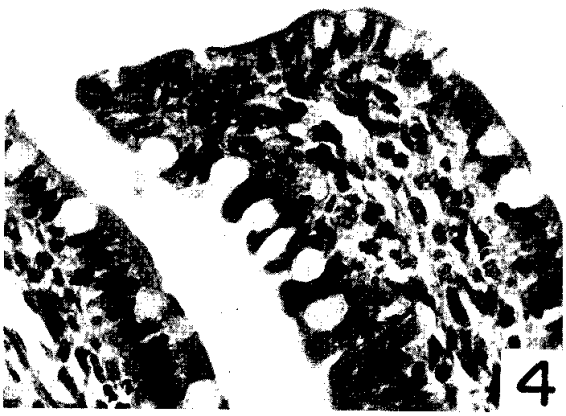

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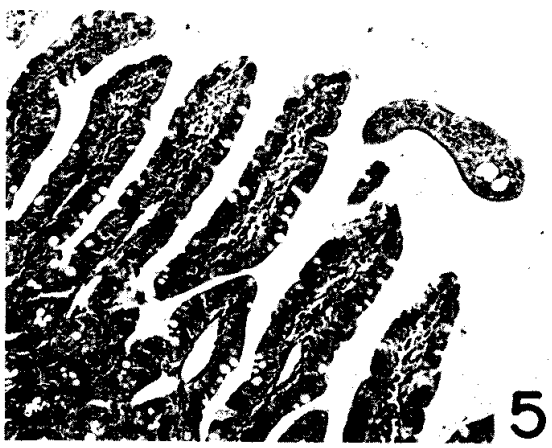

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Plate IV

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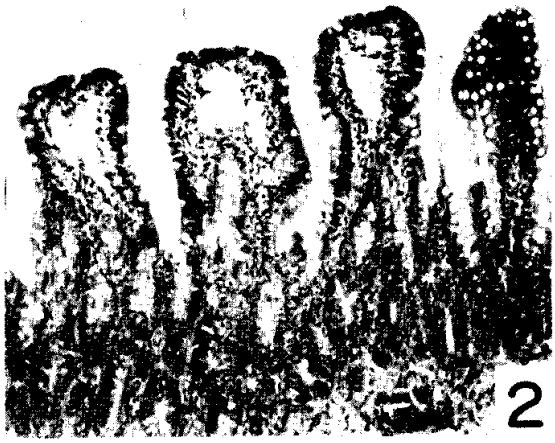

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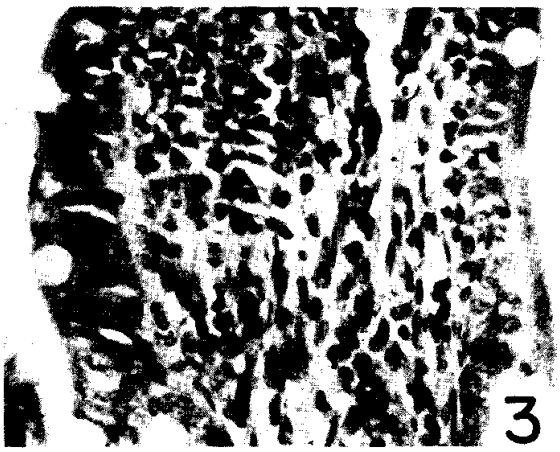

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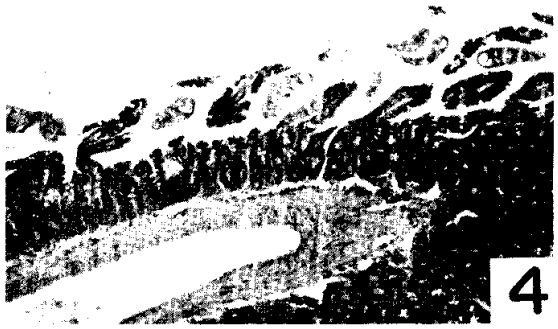

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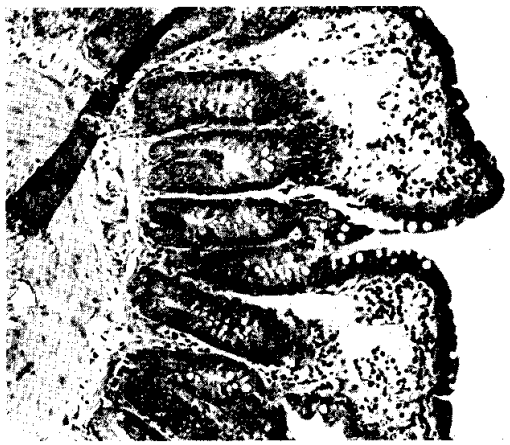

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LEGENDS FOR FIGURES

Plate I

1. Recovered *Metagonimus yokogawai* from rat, 1 week group. Ovary and testes are now developing but no shelled eggs are seen. ×200.
2. More developed one from rat of 1 week group, however, no eggs are still seen in this stage. ×200.
3. Mature worm with several eggs in uterus, ovary and testes, 1 week group. Size of the worm was not larger than worms of 1 and 2. ×200.
4. Two-week worm of *M. yokogawai*. More eggs are seen in uterus but size of the worm did not increase but rather shrinked. ×200.
5. Two-week worm of *M. yokogawai*. Abundant eggs are seen and the size of the worm increased remarkably. Other reproductive organs also fully matured. ×200.
6. Four-week worm of *M. yokogawai*. Size of the worm did not become increased compared with that of two-week group. ×200.

Plate II

1. The intestinal loop conture of control rat, 3 week group. No dilation or translucency is seen.
2. The loop conture of infected rat, 4 week grup. Marked dilation is seen through the entire intestine even the cecum. Same magnification as 1 of control rat.
3. Dilated and translucent small bowel of infected rat, 2 week group.
4. A mucosal surface view of small intestine, control rat. No foamy content is seen. In jejunum portion, a little mucous content is seen normally.
5. Foamy mucous content in jejunum portion of 1 week group rat.
6. Marked watery content is seen all over the entire lumen, with occasional foams in upper or lower portions of small intestine.

Plate III

1. Microscopic picture of small bowel mucosa in a control rat. The villi are slender and delicate with approximately 3 to 1 villus/crypt height ratio. ×50.
2. *Ibid.* ×100. The covering epithelia of the villi are tall columnar cells with basally located nuclei. Goblet cells are seen quite often.
3. One week experimental group. The villus/crypt ratio is approximately 1 to 1. The tips of villi are somewhat flattened. No worm is present. ×100.
4. Higher magnification of 3, showing an increased number of goblet cells, flattening of epithelial cells. The stroma contained increased number of eosinophils. ×400.
5. One-week group. A worm is seen characteristically in intervillous space or floating on top of villi. A focal erosion of villous epithelium is seen, however, causal relationship with the the worm could not be assured. Increased inflammtory cells are seen. ×100.
6. Two-week group. Intervillous adhesion with a remarkable stromal edema and inflammation is seen together with a *Metagonimns* worm which is impacted in intervillous space. ×100.

Plate IV

1. Three-week group showing reduced villus/crypt ratio along with blunting of the tips of villi. ×50.
2. Higher magnification of 1. The villi show blunting and stromal edema. Increased number of inflammatory cells is noted in the stroma. ×100.
3. Higher magnification (×400) of one villous stroma to show cellular components. Eosinophils, plasma cells and occasional fibroblasts are seen.
4. Three-week group. Seven worms are seen floating on tops of distorted villi. As the mucosa was tangentially cut, villi orientation is not clear. ×50.
5. Four-week group. The villi are still blunt and stroma is quite edematous. ×50.
6. Higher magnification of villi of the same rat as 5, showing lymphatic dilation and interstitial edema. ×100.