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Histopathology of Small Intestines of Rats and Mice Experimentally Infected with *Pygidiopsis summa*

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= Abstract =A histopathological study was performed on the intestine of rats and mice experimentally infected with *Pygidiopsis summa*. Total 16 rats and 12 mice were infected orally each with 1,000 metacercariae collected from the mullets. After 3 days to 5 weeks their small intestines were studied.

While controls showed only a slight degree of non-specific inflammation in the villous stroma, those infected with *P. summa* revealed diffuse and remarkable mucosal changes during the early stage up to 2-3 weeks of infection. The mucosal pathology was chiefly villous atrophy (thickening, blunting of tips and fusion) with severe stromal inflammation, accompanied by crypt hyperplasia. No invasion of worms into the submucosa was recognized.

Later than 3 weeks, however, the diseased mucosa began to restore their normal features. At 4-5 weeks mucosal layer became normal, except for slight stromal inflammation and/or crypt hyperplasia.

Key words: ***Pygidiopsis summa, Intestinal fluke, Heterophyid fluke, Intestinal histopathology***

INTRODUCTION

Pygidiopsis summa is one of the heterophyid flukes infecting the small intestine of birds and mammals including man. In such heterophyid infections, host-parasite relationships especially the pathogenicity of worms and response of the host are important, since erratic parasitisms were reported in heart, brain and spinal cord of man, by embolized eggs of several kinds of heterophyids; *Haplorchis, Stellantchasmus* and *Procerovum* (Africa *et al.* 1940). Details of the pathogenesis of these erratic parasitisms are still not certain, although it was once suggested by Yokogawa (1940) that in immunity-impaired patients the flukes should have invaded easily into the submucosa where they laid eggs and allowed egg transfer to other organs through the blood stream.

In *P. summa* infection, there has been no study in relation to the pathological features (intestinal or erratic) as well as its clinical significance. In human infection cases reported so far (Yokogawa *et al.* 1965; Seo *et al.* 1981a), no significant symptoms and signs were observed except for slight eosinophilia. But it seems to have been largely due to relatively low worm burdens in the cases encountered and it is highly possible that severe symptoms may occur in heavy infection cases.

As to the intestinal pathology of hosts infected by heterophyid flukes in Korea, studies have been mainly focused on *Metagonimus yokogawai* (Chai 1979; Lee *et al.* 1981; Kang *et al.* 1983; Rho *et al.* 1984), for which no erratic parasitism has been reported yet. According to them the most prominent pathological feature in the small intestine of host animals was mucosal atrophy accompanied by crypt hyperplasia, and many worms were found intruded into the intervillous space, facing the outlet of the Lieberkühn's crypt. No further penetration of worms into deeper level was observed. It is a question whether *P. summa* could penetrate into the submucosa or underneath, in the small intestine of final hosts. Therefore, in this study, attempts were made to observe the intestinal histopathology of

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rats and mice infected experimentally with *P. summa*.

MATERIALS AND METHODS

The metacercariae of *P. summa* were obtained from young mullets (*Mugil cephalus*), 15-20 cm in length, caught in the waterways within a large salt farm located at a western coastal area of Okku-gun, Jeonrabuk-do. The area is known to be an endemic focus of human pygidiopsiasis (Seo *et al.*, 1981a). The mullets were digested artificially with gastric juice for 1-2 hours, and the metacercariae of *P. summa* were collected under dissecting microscopy.

Each of total 16 rats (Wistar) and 12 mice (ICR) of either sex, was infected equally with 1,000 metacercariae through a gavage needle inserted into the stomach. Two rats and 2 mice were used for uninfected controls. To observe the intestinal pathology chronologically, the infected animals were divided into 7 groups of 2-3 animals, according to the age of infection; 3 days, 5 days, 1 week, 2 weeks, 3 weeks (rats and mice), 4 weeks and 5 weeks (rats only). After each scheduled period the rats and mice were sacrificed by cervical dislocation, their abdomen opened, and whole small intestine was resected.

The resected small intestine was divided into three parts; the duodenum, jejunum and ileum. Each segment was fixed in 10% neutral formalin, dehydrated and embedded in paraffin, cut into sections of 5-7 μm thickness, and processed for routine hematoxylin-eosin (H-E) stain.

RESULTS

1. General Changes in Mucosa

In uninfected control rats and mice, their intestinal sections revealed tall and slender villi, short and well-arranged Lieberkühn's crypts, and normal features of submucosa and other layers (Figs. 1 & 2). However, especially in villi, there was slight to moderate degree of stromal inflammation. Main cellular components were small round cells such as lymphocytes and plasma cells, with few eosinophils.

In the rats and mice infected with *P. summa*, there were marked changes in the wall of their small intestines. But the pathological changes were confined, in most cases, to the mucosal layer, and only minimal change was observed rarely in the submucosa. The mucosa revealed consistent feature of atrophy of villi accompanied by crypt hyperplasia. Generally the intestinal pathology was more severe in rats than in mice (Table 1). Though worms were chiefly found in the duodenum or jejunum, the mucosal change extended to whole small intestine, including the ileum.

In the early stage of infection up to 2 or 3 weeks, the villi showed thickening and blunting of their tips (Figs 3-11), erosion or pressure atrophy of epithelial layers, and frequent fusion (Figs. 10, 11 & 13). They frequently showed lymphatic dilatation and vascular ectasia near the tip portions (Figs. 9, 10 & 14) and revealed occasional exfoliation of epithelial layers (Fig. 8). In the lamina propria, there was marked severe inflammatory reaction especially with infiltration of eosinophils and a large number of plasma cells and lymphocytes (Figs. 4, 7 & 9). Goblet cells were markedly depleted at 3 days of infection but gradually became normal. Later than 3 weeks of infection the majority of such villous and/or stromal changes were restored to normal (Table 1). The villous thickening, fusion and tip blunting/erosion became less severe at 3 weeks, although the stromal edema and/or inflammation were persistent. At 4 weeks of infection, both the villous and stromal changes became milder (Fig. 16).

The crypt portion consistently showed hyperplastic patterns (Figs. 11 & 13), even up to 5 weeks of infection (Fig. 16). But the crypt hyperplasia was more severe before 2 weeks and gradually restored later than 3 weeks of infection.

2. Sectioned Worms and Adjacent Mucosa

In sections of three parts of the small intestine, many or a few number of worms were found intruded into the intervillous space, or facing the basal portion of villi, during the early stage of infection up to 2 weeks (Figs. 3-9 & 11-13). After 3 weeks, however, no sectioned worm was found in the specimens studied (Table 1).

In the small intestine, worms were more frequently found in the duodenum or jejunum than in the ileum. Most of them were facing or sucking the proximal part of the Lieberkühn's crypt (Figs. 4-7), and several of them were embracing the tip of villi with their lateral borders of anterior body (Figs. 5 & 6). Young worms aged only 3 days already contained several eggs in their uteri (Fig. 4).

The villi adjacent to worms revealed various features of deteriorations. The worm-lining epithelial layers were markedly compressed and rough (Figs. 3-8), to show a feature of pressure atrophy. In some sections of the early stage of infection, parts of neighbouring villi were seen to be severely

Table 1. Summary of pathological findings in the small intestines of rats and mice infected by *P. summa*

Duration of infection	Parts of intestine	Villous change			Stromal change			No. of sectioned worms**
		Thick-ening	Fusion	Blun-ting	Vasc. ectasia	Ede-ma	Cell infil.	
3 days	D	*+++/++	++/++	++/++	+/+	+/+	++/++	++/+++
	J	+++/++	++/++	++/++	++/+	+/+	++/++	++/++
	I	+++/++	++/++	++/++	++/+	+/+	++/++	−/−
5 days	D	+++/++	+++/++	++/++	++/+	+/+	++/++	++/++
	J	+++/++	+++/++	++/++	++/+	+/+	++/++	++/++
	I	+++/++	+++/++	++/++	++/+	+/+	++/++	+/+
1 week	D	+++/++	+++/++	++/++	++/+	+/+	++/++	−/+
	J	+++/++	+++/++	++/++	++/+	+/+	++/++	+/+
	I	+++/++	+++/++	++/++	++/++	+/+	++/++	−/−
2 weeks	D	++/++	+++/++	++/++	++/++	+/+	++/++	−/−
	J	++/++	+++/++	++/++	++/++	++/+	++/++	+/+
	I	++/++	+++/++	++/++	++/++	++/+	++/++	−/−
3 weeks	D	+/+	++/+	+/+	+/+	+/+	++/++	−/−
	J	+/+	++/+	+/+	+/+	+/+	++/++	−/−
	I	+/+	++/++	+/+	+/+	+/+	++/++	−/−
4 weeks	D	+/	+/	+/	−/	−/	+/	−/
	J	+/	+/	+/	−/	−/	+/	−/
	I	+/	+/	+/	−/	−/	+/	−/
5 weeks	D	+/	−/	−/	−/	−/	+/	−/
	J	+/	−/	−/	−/	−/	+/	−/
	I	−/	−/	−/	−/	−/	−/	−/

*Results from rats/mice

*−: normal, +:mild change, ++: moderate change, +++: severe change

**−: no worm, +: a few worms, ++: many worms, +++: numerous worms

compressed by the worms so as to become nearly cleaved (Fig. 7). Some worms were found to have a direct contact with or penetrate into the lymphiod tissue of the small intestine (Fig. 12).

DISCUSSION

The intestinal pathology of rats and mice infected with *P. summa* was largely confined to the mucosal layer of the small intestine, and characterized by villous atrophy and crypt hyperplasia. Similar pathological features have been reported in other heterophyid or intestinal fluke infections, such as *Metagonimus yokogawai* (Chai 1979; Lee *et al.* 1981; Kang *et al.* 1983), *Heterophyes heterophyes* (Hamdy *et* Nicola 1981) and *Fibricola seoulensis* (Lee *et al.* 1985).

As to the pathogenesis of mucosal pathology by intestinal flukes, mechanical and/or chemical (or immunological) injuries have been proposed by many workers. However, in metagonimiasis of cats (Rho *et al.* 1984), a shortage of enterocytes to cover the villous stroma mechanically invaded and destroyed by worms was suggested to be a main pathogenetic factor. A supporting paper was published by Kim *et al.* (1985) who observed the effects of prednisolone, an immunosuppresant, on the intestinal pathology of metagonimiasis of cats. According to them the pathology was not significantly different between prednisolone-injected and control groups, so that the villous atrophy seemed less closely related to immunological responses. They suggested that the pathological changes in mucosa may be chiefly caused by mechanical injury by the worms, and thus related to the number of parasites infected. At present, however, there is

no direct evidence to verify this suggestion and further studies are necessary.

Later than 3 or 4 weeks of infection by *P. summa* in rats and mice, spontaneous resolution of mucosal pathology was observed. It was the same as reported in experimental metagonimiasis of rats (Chai 1979). Such a spontaneous resolution may be related to the decreased number of parasites as infection period increased. In metagonimiasis or pygidiopsiasis of rats (Chai 1979; Seo *et al.* 1981b), the parasite number was reported to have much decreased after 1 or 2 weeks of infection. In the present study, no worms were found in sections of the small intestine of 9 mice sacrificed later than 10 days. In rats only a few were found later than 3 weeks. In comparison, a different result was reported in metagonimiasis of larger animals such as dogs (Kang *et al.* 1983). According to them, in spite of improvement of mucosal pathology after 4-6 weeks, no significant decrease in parasite number was recognized. In this respect, the restoration of damaged mucosa may not necessarily mean the removal of a majority of worms from the host intestine.

At any rate, it still remains a question whether the intestinal pathology due to *P. summa* or other heterophyids is confined to the mucosal layer, even when normal immune response of the host is severely impaired. The pathogenesis of extraintestinal egg embolism by heterophyids in human host (Africa *et al.* 1940) is hardly explained if the flukes had failed to penentrate into the submucosa or underneath of the small intestine. As the host response to the parasites is different by host species, it would be interesting to study the pathogenecity of *P. summa* in larger hosts such as dogs, cats or hamsters. Studies on simultaneous infection of *P. summa* with other gut-infecting organisms are also necessitated so as to understand the sophisticated details of host-parasite relationships in human heterophyidiasis.

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=국문초록=

*Pygidiopsis summa*에 실험감염된 흰쥐와 마우스 小腸의 組織病理學的 硏究

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인체 腸吸蟲의 하나인 *Pygidiopsis summa*를 흰쥐와 마우스에 實驗感染시키고 腸病變을 관찰하였다. 被囊幼蟲은 崇魚(mullet)의 아가미·食道 등으로부터 획득하였고 흰쥐 16마리와 마우스 12마리에 각각 1,000개씩 감염시켰다. 감염시키지 않은 흰쥐와 마우스 각각 2마리를 대조군으로 하였다. 감염후 3일부터 5주까지 이들을 희생하고 小腸의 절편을 채취하여 組織病理學的 관찰을 시행하였다.

대조군의 경우에는 小腸 융모 基質層(stroma)의 가벼운 非特異性 炎症反應을 제외하고 정상적인 組織所見을 보였으나, *P. summa*를 감염시킨 흰쥐와 마우스에서는 감염 2~3주까지 매우 심하고 광범위한 粘膜(mucosa)의 病變을 보였다. 이러한 粘膜의 病變은 융모위축(thickening, blunting of tips, fusion) 및 基質의 심한 炎症反應, 이에 동반된 腸腺增殖(crypt hyperplasia)으로 특징지울 수 있었다. 그러나 蟲體가 粘膜下 (submucosa)로 침입한 증거는 찾을 수 없었다.

이러한 심한 病變에도 불구하고 감염 3주 이후부터는 粘膜 病變이 다시 正常化되는 경향을 보였다. 감염 4~5주째에는 거의 正常에 가까운 粘膜 形態를 나타내었다. 다만 몇 개의 腸組織에서 감염 5주까지도 基質의 炎症과 腸腺增殖이 남아있는 경우가 관찰되었다.

이상의 결과는 *P. summa*가 흰쥐나 마우스에서 腸管의 粘膜 이하부위로 깊이 침입하지 않음을 나타내고 있었고 감염초기의 粘膜病變이 매우 심하지만 3주 이후부터 자연적으로 正常化됨을 보였다.

LEGENDS FOR FIGURES

Figs. 1-2. Intestinal sections of control animals (H-E stain).

Fig. 1. Duodenum of a control mouse. Villi are tall and slender, which is their normal contour, but in the stroma non-specific inflammatory reactions are seen. x100.

Fig. 2. Jejunum of a control rat. Nearly normal villi except for some infiltration of inflammatory cells. x200.

Figs. 3-16. Intestinal sections of rats and mice infected with *P. summa*(H-E stain).

Fig. 3. Duodenum of a mouse, 3-day group. One worm is seen sectioned between two villi, of which lining borders are markedly compressed and show atrophy. x100.

Fig. 4. *Ibid,* another portion. A villi is seen to be thickened, shortened and its stroma shows severe inflammatory reaction. A worm is vertically sectioned to show its internal structures and a few eggs. x200.

Fig. 5. Jejunum of a mouse, 3-day group, showing two sectioned worms. The left worm is destroying the base of a villus with its oral sucker. x100.

Fig. 6. *Ibid,* with a sectioned worm. The worm is embracing a part of villus with the ventral cavity of its anterior body. x100.

Fig. 7. *Ibid,* a magnification near the worm. The wormlining villus (arrows) is much compressed at its basal portion, to be nearly cleaved. The sectioned worm shows its various internal organs. x200.

Fig. 8. Jejunum of a rat, 3-day group. A worm is sectioned between two villi, which are severely deformed and inflamed. x100.

Fig. 9. Jejunum of a mouse, 5-day group. Villi show tip and/or stromal edema and a part of epithelial layer (tip portion) adjacent to a worm is compressed and destroyed. x100.

Fig. 10. Ileum of a rat, 5-day group. A villus is much flattened, thickened and inflamed. Crypts show hyperplasia. x100.

Fig. 11. Jejunum of a mouse, 1-week group, showing a sectioned worm, with villous and crypt changes. x100.

Fig. 12. *Ibid,* another portion. A worm is intruding into the base of an intervillous space in direct contact with the lymphoid tissue. x100.

Fig. 13. Jejunum of a mouse, 2-week group. A worm is sectioned on the top of an intervillous space. Villi are severely deformed and crypts show marked hyperplasia. x100.

Fig. 14. *Ibid,* another portion. Villi show edema on their tips and inflammations in the stroma. x100.

Fig. 15. Ileum of a rat, 4-week group. Villi are tall and slender, and revealing their normal contour. x100.

Fig. 16. Ileum of a rat, 5-week group. The mucosa show its nearly normal morphology except for stromal inflammation and crypt hyperplasia. x100.

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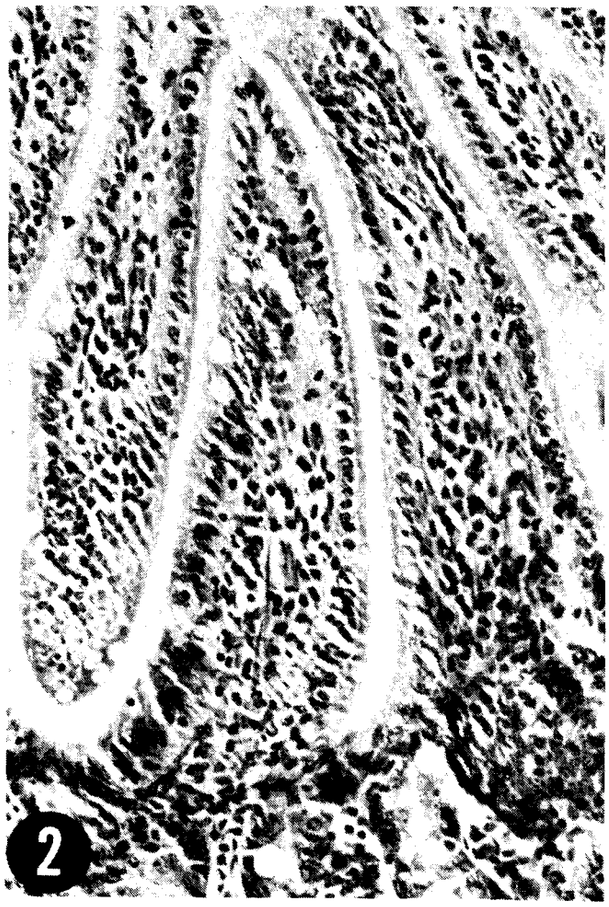

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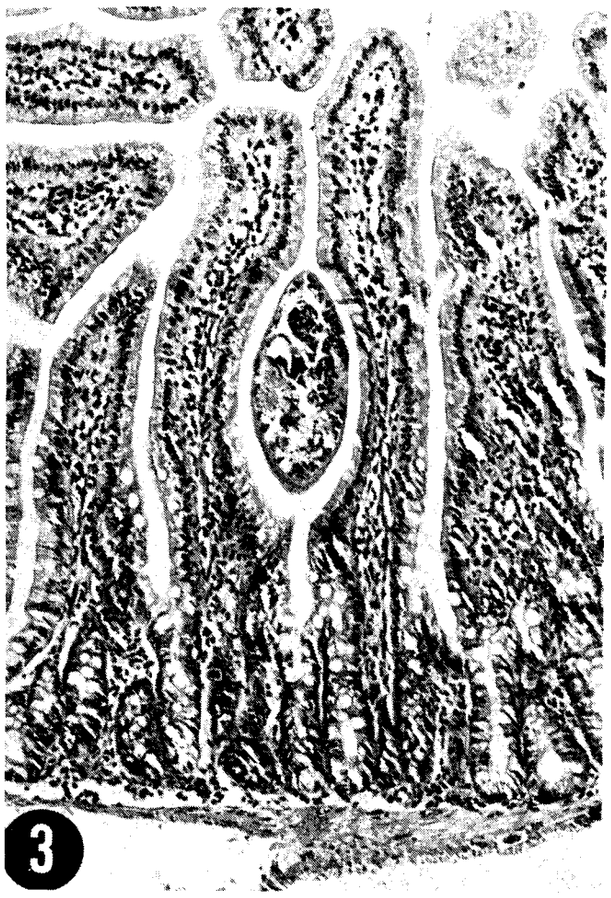

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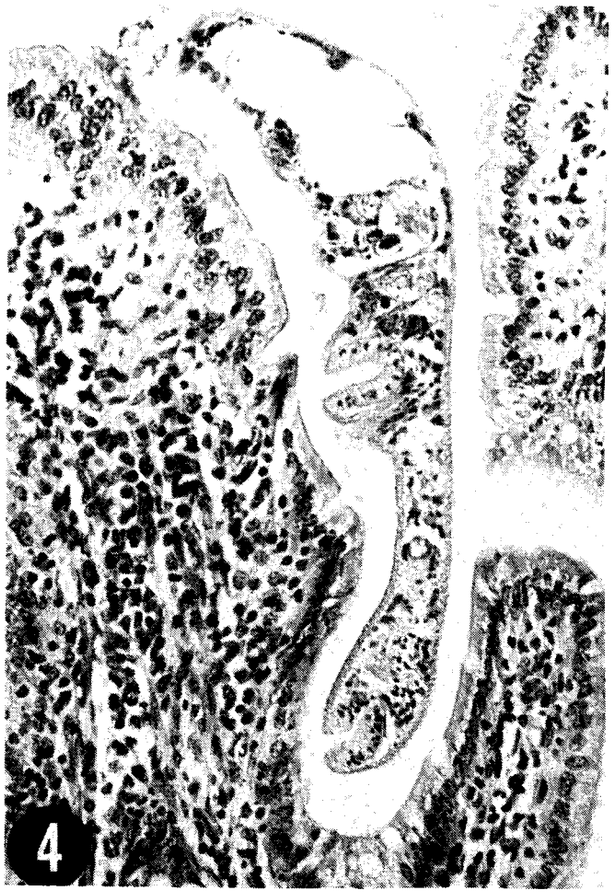

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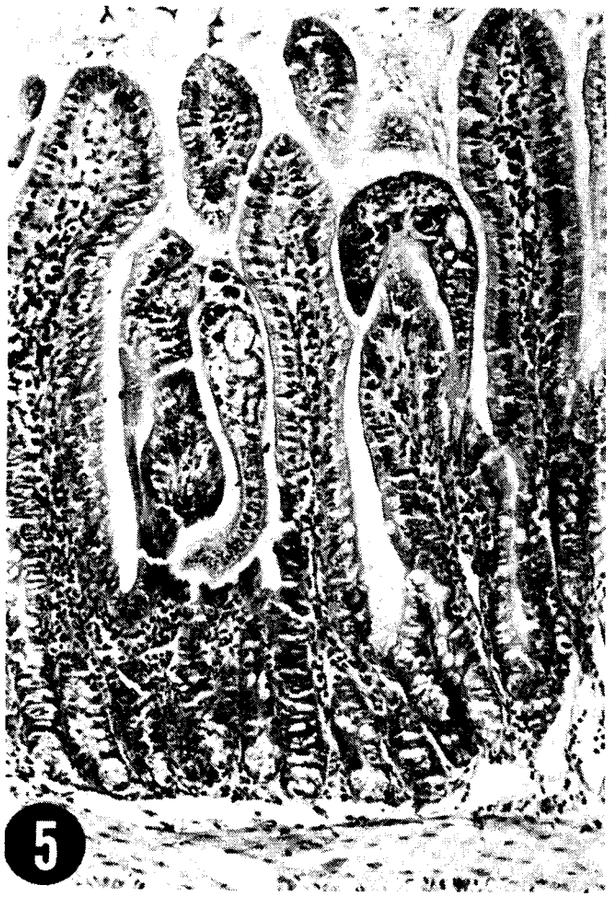

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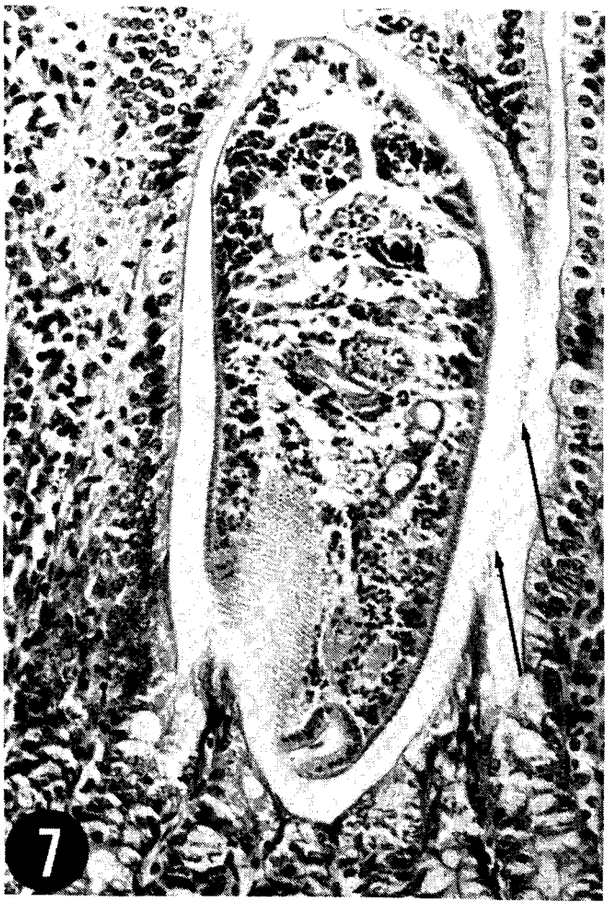

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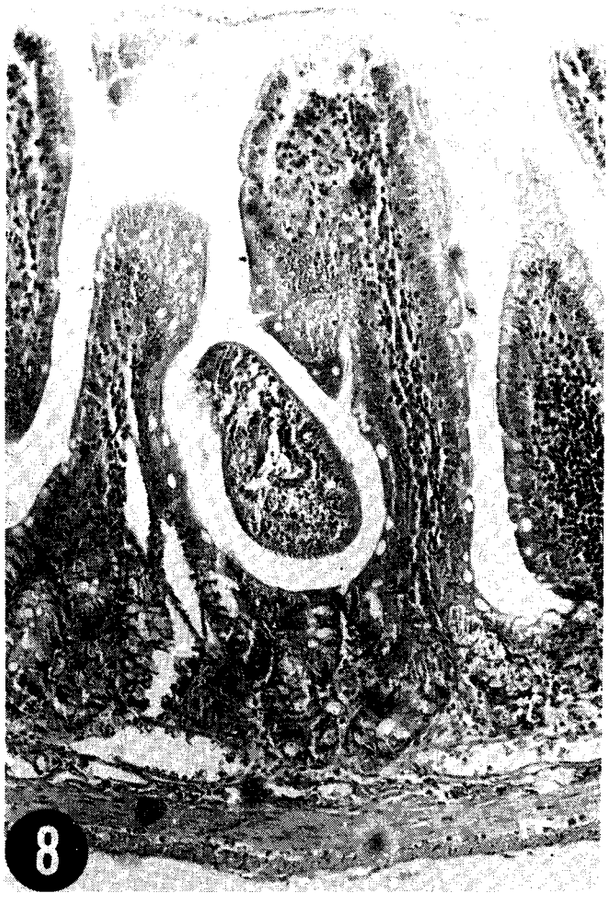

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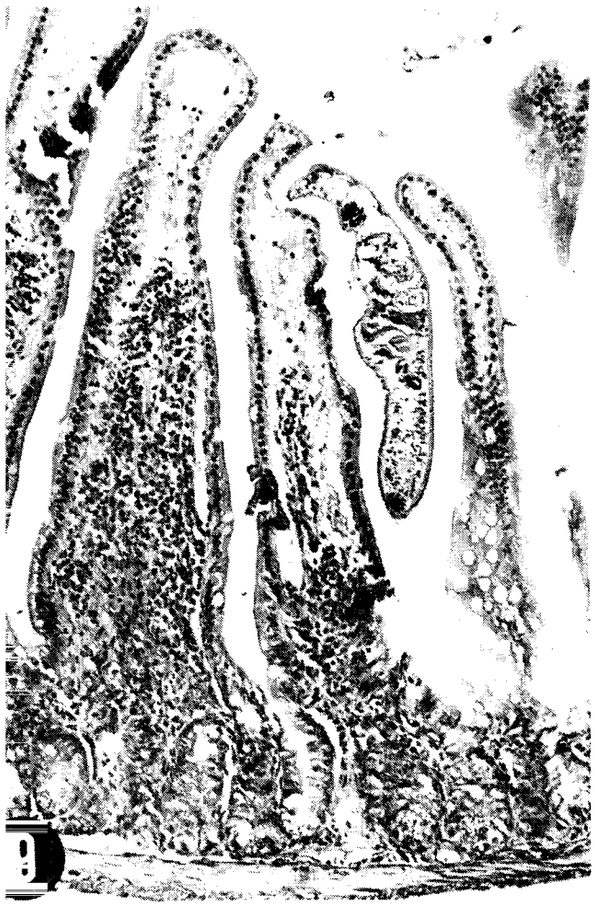

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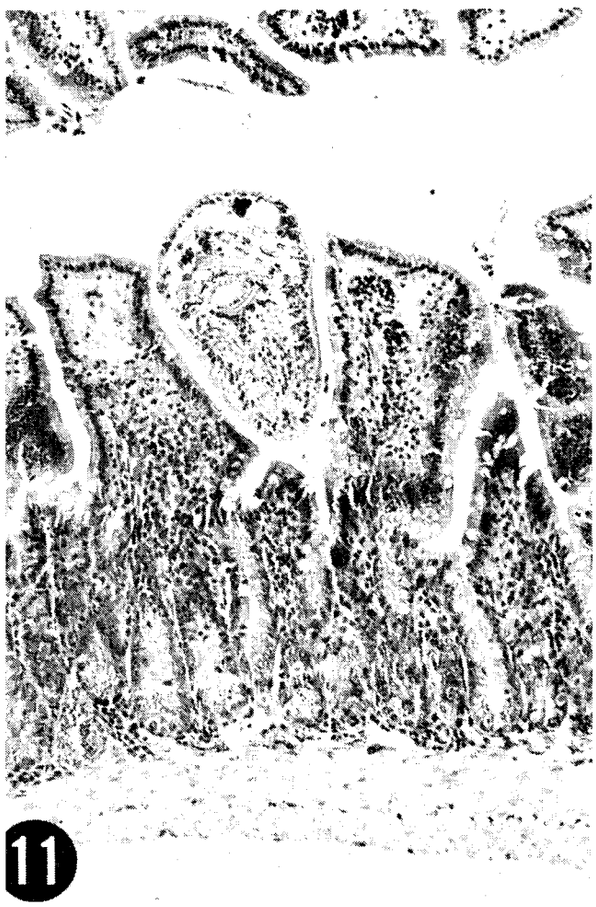

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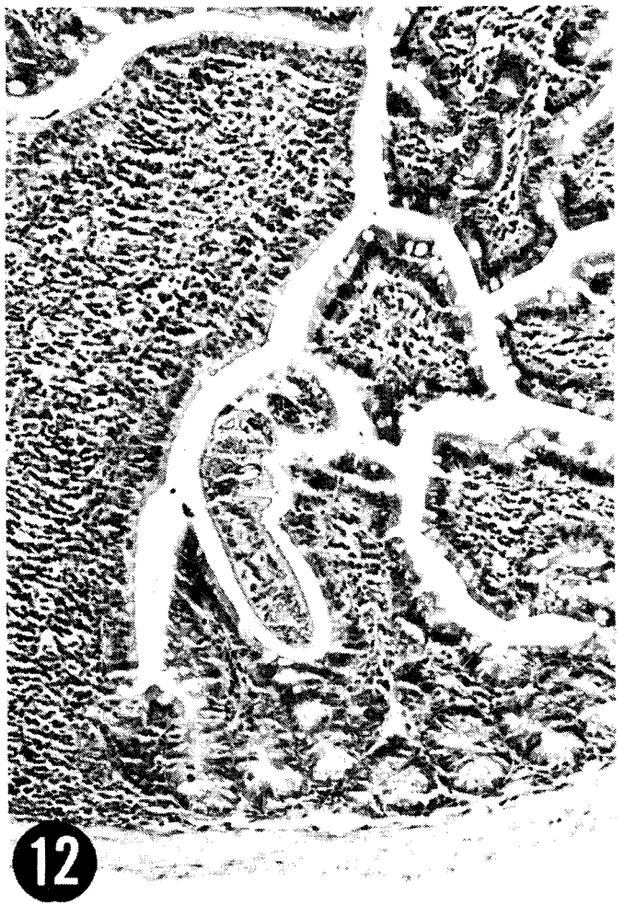

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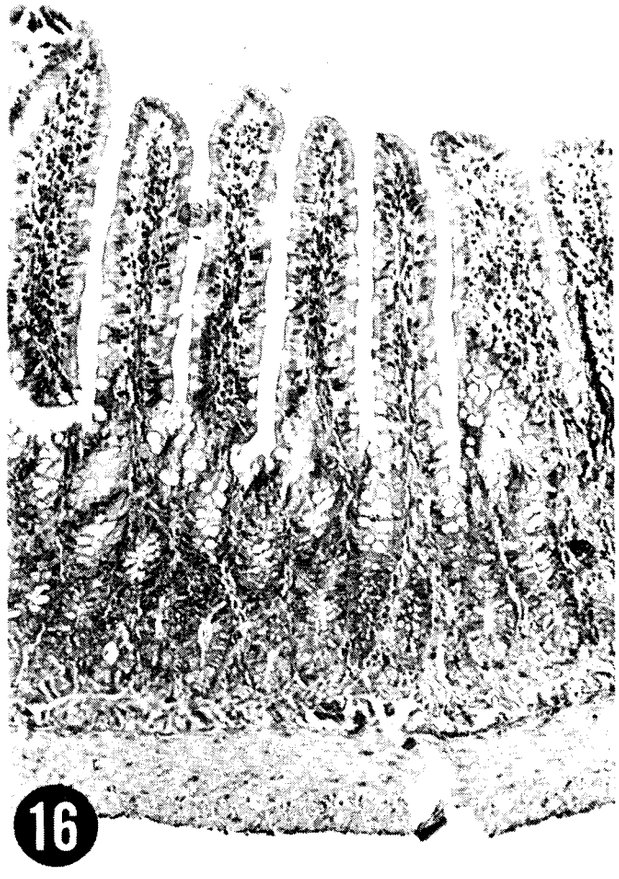

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