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Clinical and Histopathological Findings in Mice Heavily Infected with *Fibricola seoulensis*

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Abstract: To observe the clinical course and intestinal histopathology in heavy infection of *Fibricola seoulensis*, an experimental study was performed in mice. Clinical, hematological and histopathological observation was done on 1, 3, 7 and 12 days after experimental infection with 1,000 metacercariae. On the 11th day after infection, the mice began to die and all of the infected mice were dead by the 16th day. The infected mice showed gradual weight loss. Occult blood was detected after the 10th day. Diarrhea occurred after the 9th day and was recognized in all of the infected mice on the 12th day. Hemoglobin and mean corpuscular hemoglobin decreased significantly after the 12th day, and mean corpuscular hemoglobin concentration decreased in all infected mice. The histopathological changes were more marked in the duodenum than in the jejunum or ileum. Major changes were villous atrophy and crypt hyperplasia, with decreased villus/crypt ratio, inflammatory cell infiltration and stromal edema. The present results suggest that the cause of death of mice heavily infected with *F. seoulensis* should be malnutrition and severe fluid loss due to malabsorption, together with intestinal bleeding.

Key words: *Fibricola seoulensis*, heavy infection of mouse, intestinal bleeding, malabsorption, intestinal histopathology

INTRODUCTION

After the discovery of acute human infection of *Fibricola seoulensis* in 1982, total 27 cases have been reported in Korea. They all had the history of having ingested raw snakes or frogs (Seo *et al.* 1982; Hong *et al.*, 1984 & 1986; Hong *et al.*, 1985). Among the human cases, 26 complained of no specific gastrointestinal symptoms, whereas the first case had an episode of acute abdominal pain, diarrhea and fever. Asymptomatic cases are considered to be chronic case or repeated infections with small number of metacercariae (Hong *et al.*, 1984). However, severe clinical symptoms are suggested to occur in acute stage of heavy infections.

For the purpose of understanding the pathogenicity of *F. seoulensis*, Lee *et al.* (1985) performed a histopathological study on experimental fibricoliasis in rats and mice. Villous atrophy and crypt hyperplasia were major histopathological findings in their duodenum. In general, the mucosal derangement was limited to the duodenum or upper jejunum. However, in heavy infections, the mucosal change was extended to the entire small intestine in which case severe clinical manifestations would occur. They also suggested occurrence of luminal bleeding due to severe destruction of intestinal mucosa by the flukes and a possibility of ectopic fibricoliasis.

Other intestinal trematodes, such as *Metagonimus yokogawai* (Chai, 1979), *Pygidiopsis*

summa (Seo *et al.*, 1986) and *Echinostoma revolutum* (Bindsell and Christensen, 1984; Huffman *et al.* 1986), were also reported to cause similar mucosal changes to those by *F. seoulensis*.

In parasite-host relationships, good adaptation of parasites to a host means to maintain their life cycle successfully, doing least harm to their host. In fibricoliasis, chronic, repeated infection with small number of *F. seoulensis* is thought to cause no serious damage to the host. However, if many worms, about 1,000 metacercariae per mouse for example, were infected at once, the mice might die in early stage of infection.

In order to obtain more detailed information on fibricoliasis, the authors attempted to observe some clinical and histopathological features in mice heavily infected with *F. seoulensis*. The survival rate and weight change of mice, occurrence of diarrhea, occult blood in stool, and hematological and histopathological changes in small intestine were observed chronologically.

MATERIALS AND METHODS

Over 4 week-old, male or female ICR mice, weighing 20~30g were obtained from Animal Husbandary of Seoul National University. Active *F. seoulensis* metacercariae were obtained by peptic digestion of viscera and muscle of the snake, *Natrix tigrina lateralis*, purchased at Hongchon, Kangwon-do.

To study some clinical features, ten mice were fed 1,000 metacercariae each in 0.2~0.4 ml of 0.85% saline using a syringe with ball-tip needle. Ten control mice were kept uninfected. All mice were fed *ad libitum*. To know the clinical course, each mouse was observed every day for their survival, fecal consistency, occult blood in stool, and was measured their weight until 28 days after infection. When the stool lost its normal consistency and contained watery content, it was determined as diarrhea. Occult blood was tested using commercially available tablets (Ames Division Miles Lab.). If the mouse died during the experimental period, its whole small intestine was resected, washed thoroughly with 0.85% saline, and *F. seoulensis* worms were collected and counted under stereoscopy.

To study the hematological and histopathological findings, 20 mice were fed 1,000 metacercariae each, and bred same as above. Five mice each were killed by decapitation on 1, 3, 7 and 12 days after infection, respectively. Another five mice were used for uninfected controls. Blood volume of 0.5ml was drawn from the decapitated site of each mouse into an EDTA tube (Becton Dickinson Vacutainer System). WBC count, RBC count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using a Coulter Counter.

Immediately after decapitation, the peritoneal cavity of mice was opened and the small intestine was resected. The small intestine was divided into 3 segments, which were referred here to as the duodenum (from the duodenal bulb to the ligament of Treitz), jejunum (proximal 1/3 of the remained segment) and ileum (another distal 2/3). The proximal 2 cm of each segment was resected for tissue preparation. The resected tissues were flushed with 0.85% saline intraluminally and then immersed in hot 10% neutral buffered formalin (60~70°C). After a fixation without opening the lumen, the tissues were dehydrated, paraffin-embedded, and sectioned at 5μm thickness and finally stained with hematoxylin and eosin.

The clinical, hematological and histopathological findings of infected mice were compared with those of controls. Mean and standard deviation of hematological values were analyzed by Wilcoxon rank sum test.

RESULTS

1. Clinical findings

Of the *F. seoulensis* infected mice, two died at day 11 of infection, and then the mice began

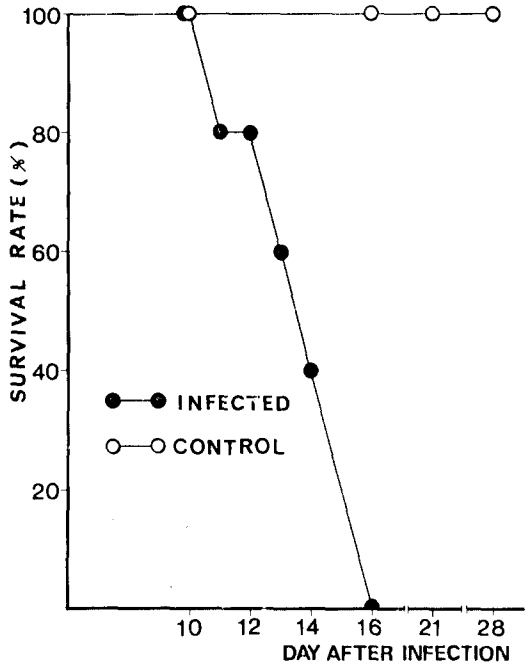

Fig. 1. Survival rate of mice fed 1,000 metacercariae of *F. seoulensis*, in comparison with control group.

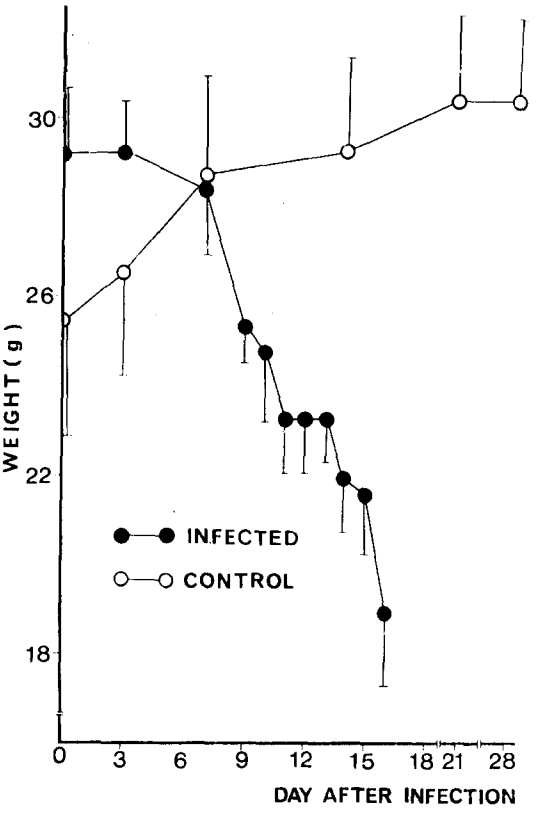

Fig. 2. Weight change of mice fed 1,000 metacercariae of *F. seoulensis*, in comparision with control group. (±; standard deviation)

to die one after another. After all, none of the infected mice survived later than the day 16. In comparison, all of 10 control mice were alive until the end of this experiment (Fig. 1). The mean weight of infected mice was decreased gradually from 29.5g to 19.0g, while control mice continuously gained their weight (Fig. 2). Before death, the infected mice showed brittle hair and were less active. One mouse dead at day 16 showed gross luminal bleeding of the gut (Fig. 3).

The occult blood in stools first appeared at day 10, and three of 4 mice were occult blood positive at day 14(Table 1). At day 9, two mice began to expel loose stools. All mice showed diarrheal stools at day 12. There once occurred diarrheal stools in a mouse, it continued until death of mouse (Table 2).

The worms recovered from each mouse were immature to fully mature adults. The number of worms per mouse ranged from 230 to 875. There was a very low correlation between the worm burden and the mouse survival time (Table 3, $r=0.01$).

2. Hematological findings

Compared with controls, the infected mice showed a significant decrease of hemoglobin concentration ($p<0.05$) and MCH($p<0.05$) at

Table 1. Occult blood positive rate in mice fed 1,000 metacercariae of *F. seoulensis* by infection day

Day after infection	Control mice	Infected mice
	No. posit./No. exam. (%)	No. posit./No. exam. (%)
1~9	0/10(0)	0/10(0)
10	0/10(0)	1/10(10)
11	0/10(0)	2/ 8(25)
12	0/10(0)	1/ 8(12.5)
13	0/10(0)	3/ 6(50)
14	0/10(0)	3/ 4(75)
15	0/10(0)	—

Table 2. Appearance rate of diarrhea in mice fed 1,000 metacercariae of *F. seoulensis* by infection day

Day after infection	Control mice	Infected mice
	No. appeared/No. exam. (%)	No. appeared/No. exam. (%)
1~8	0/10(0)	0/10(0)
9	0/10(0)	2/10(20)
10	0/10(0)	3/10(30)
11	0/10(0)	4/8(50)
12	0/10(0)	8/8(100)
13	0/10(0)	6/6(100)
14	0/10(0)	6/6(100)
15	0/10(0)	4/4(100)

Table 3. Number of recovered worms from each mouse according to duration of survival days

Duration of survival(day)	No. of mice	No. of recovered worms
11	2	230, 460
13	2	875, 810
15	2	781, 667
16	4	346, 464
		342, 536

$Y=1.159X+533$ ($r=0.01$)

Y; No. of recovered worms, X; duration of survival. Total worm recovery rate: 55.1%

day 12. MCHC appeared to decrease after 1 day of infection, which did not return to normal until day 12 ($p<0.05$). There were no recognizable decreases in WBC count, RBC count, hematocrit and MCV (Table 4).

3. Histopathological findings

1. Control: The intestinal sections of control mice revealed well-preserved mucosal integrity with well arranged finger-like villi and their epithelial linings. The villus/crypt(V/C) ratio was about 3~4:1. These findings were almost same in the duodenum, jejunum and ileum (Fig. 4).

2. One day after infection: In the duodenum, young worms were found to have penetrated between villi and located nearby the crypt layer (Fig. 5). The mucosal layer revealed no change except slight disfigurement of villi adjacent to worms. V/C ratio was about 3:1. In the jejunum, no specific abnormality was found. The ileum also showed its normal contour.

3. Three days after infection: In the duodenum, young worms were found mainly in the intervillous space, pinching the villous epithelium using their oral and ventral suckers. Flattening, disfigurement, thickening and fusion of villi as well as stromal lymphedema and lymphangiectasia, were consistently observed (Fig. 6). The jejunal villi also showed atrophy and stromal edema, although less severe than that of the duodenum. The ileum showed no specific change yet.

4. One week after infection: Several mature worms were found to be entrapping the villi in the duodenum sections. Disfigurement, thickening and fusion of villi and crypt hyperplasia became more conspicious. Stromal infiltration of inflammatory cells was also severe. V/C ratio remarkably decreased to about 1:1(Figs. 6, 7 & 8). V/C ratio in the jejunum was about 2:1 (Fig. 9). Villi of ileum showed their stromal edema.

Table 4. Hematological values of mice fed 1,000 metacercariae of *F. seoulensis* by day

Day	WBC($\times 10^3$)	RBC($\times 10^6$)	Hb(g/dl)	Hct(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)
Control	4.84(1.5)*	8.67(0.55)	14.8(0.65)	42.2(2.9)	48.7(1.7)	17.0(0.5)	35.1(1.4)
1	6.9 (1.2)	8.14(1.24)	14.0(1.2)	48.9(9.3)	59.7(3.1)	17.3(1.6)	29.1(3.9)
3	5.0 (1.5)	8.37(0.40)	14.2(0.8)	43.0(3.5)	51.4(2.4)	17.0(0.4)	33.1(1.0)
7	6.5 (2.2)	8.60(0.36)	14.1(0.8)	43.3(3.7)	49.4(3.2)	16.4(0.7)	33.3(1.1)
12	8.75(8.75)	7.59(1.12)	12.2(1.65)	37.1(6.5)	48.7(1.4)	16.1(0.5)	33.2(1.7)

* In parenthesis: standard deviation

5. Twelve days after infection: The worms were found embracing the villi by their ventral curvature and suckers. Above pathological conditions was more severely progressed. Sometimes complete flattening of the villi was observed (Fig. 10). The jejunum showed the same change as that of 1 week. At this time, more profound stromal edema appeared in ileum (Fig. 11).

DISCUSSION

The fact that human can be infected with *F. seulensis* and can manifest severe intestinal symptoms(Seo *et al.*, 1982) raised a question how this fluke can cause such clinical manifestations. The present study provided some answers to this question.

First, heavy infection with 1,000 metacercariae to mice clearly produced a severe clinical course and finally a fatal outcome. The infection dose of 1,000 was selected, since in a pilot study the infection of 500 metacercariae did not affect the survival of mice while that of 1,000 or 1,500 could kill the mice.

Second, heavy infection actually caused severe diarrhea in experimental mice. In the first human case who suffered from diarrhea, total 79 worms were collected(Seo *et al.*, 1982). It is speculated, however, that more than 100 worms were infected in this patient, when the heavy metacercarial burden in the snake was considered(Hong, 1982; Cho *et al.*, 1983).

Third, the marked weight loss in infeced mice is an outcome of malabsorption due to mucosal atrophy. These findings are consistent with Lee *et al.*(1985)'s suggestion that malabsorption might occur if there is heavy infection in mice enough to overload the compensation by the lower part of the intestine.

Fourth, gross bleeding was found in the lumen. Positive occult blood test and decreased levels of hemoglobin or MCV also suggest intestinal bleeding by this fluke. Bleeding seems to originate at the sites of mucosal erosion induced by attachment of the worms. Profound intestinal bleeding might be a preliminary cause of death.

Fifth, villous atrophy and crypt hyperplasia seen in this study were just the same as those of non-specific histopathological features observed in malabsorption(Shiner & Barkin, 1985). These findings were also similar to those observed in the previous study on experimental *F. seoulensis* infection(Lee *et al.*, 1985). However, in the present study, the pathological changes were not confined to the duodenum but extended to the jejunum and ileum. It can be said that such pathological changes in entire small intestine were severe enough to cause clinical manifestations such as diarrhea, weight loss and even death in experimental mouse.

Similar histopathological findings were observed in other intestinal parasitoses; hookworm infection(Seehy *et al.*, 1962), strongyloidiasis (Milner *et al.*, 1965), trichinosis in mice (Manson-Smith *et al.*, 1979), and human *Isospora belli* infection (Liebman *et al.*, 1980).

The pathogenesis of above non-specific lesions was explained in relation to the T-cell immune reaction. Ferguson and Jarrette(1975) observed that V/C ratio was in the normal range in nude mice infected with *Nippostrongylus brasiliensis* and explained that this non-specific change was due to T-cell immunity. Also, Manson-Smith *et al.*(1979) observed that in thymectomized mice, worm expulsion was either delayed or absent, and villous atrophy and crypt hyperplasia were either delayed, reduced or even absent according to the extent of T-cell depletion. They inferred that the morphological change of intestinal mucosa which accompanied the intestinal phase of *Trichinella* infection in the mouse was a consequence of a local delayed type hypersensitivity reaction. However, there was another experimental result that these non-specific findings seen in trichinosis were indifferent to T-cells(Ruitenberg *et al.*, 1977). Bindsell and Christensen (1984) reported that the pathological findings of nude mouse intestine infected with *E. revolutum* was not different from that in control mice infected with the

same worms. In metagonimiasis, the lesions became less severe by administration of prednisolone (Chai *et al.*, 1984). Early lesions, however, were hard to be explained by immunity. Rho *et al.*(1984) explained the pathogenesis of intestinal metagonimiasis to be a compensation process of host to cope with the destruction of epithelial cells. Therefore, the role of T-cells in the pathogenesis of intestinal parasitoses is uncertain. The pathogenesis of fibricoliasis is thought to be similar to that of other intestinal helminthiasis such as metagonimiasis, of which the biological niche is the intervillous space.

In the future, functional approaches such as quantitative analysis of enzyme activities in the intestinal mucosa infected with *F. seoulensis* and/or histochemical studies are necessary. Also an evaluation of actual loss of nutrients using radioisotope would be necessary. To determine the amount of blood loss from the intestine or to know whether the worm ingests host blood or not, hemodynamic study is also needed.

The tribocytic organ of *Cyathocotyle bushiensis* had the activity of proteolytic esterase and phosphatase (Erasmus and Ohman, 1965). In *F. seoulensis*, the tribocytic organ may also have lytic actions on the host tissues. The interaction between tribocytic organ and host epithelial cells is also a theme for a further study.

In conclusion, the results of this study are thought to explain well the clinical findings observed in the first human case (Seo *et al.*, 1982) and also support the previous suggestions on the cause of clinical manifestations in fibricoliasis.

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=국문초록=

Fibricola seoulensis 중감염 마우스의 임상적 및 조직병리학적 소견

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인체에 *Fibricola seoulensis*가 중감염되면 심한 복통 및 설사가 초래된다. 또 과거 동물실험에서 관찰된 바로는 충체가 소장 점막에 심한 손상을 주며, 이에 따라 흡수장애가 초래되며 장출혈도 수반될 것으로 추측되었다. 이 실험에서는 이러한 사항을 보다 명백히 하는 한편 위장관 증상의 발현기전을 이해하기 위하여 마우스에 *F. seoulensis* 피낭유충 1,000개를 감염시킨 다음 나타나는 임상적 및 조직병리학적 소견을 관찰하였다.

감염 11일째부터 마우스가 사망하기 시작하여 16일째에는 모두 사망하였다. 감염군은 시간이 경과할수록 체중이 줄고, 대조군은 증가하였다. 대변내의 잠혈은 감염 10일째부터 나타나기 시작하였다. 감염 9일째부터 설사가 관찰되어 12일째에는 모두에서 나타났다. 혈액학적으로는 감염 12일군에서 혈색소 및 평균적혈구혈색소량의 감소가 보였고, 평균적혈구혈색소 농도는 감염군에서 모두 감소하였다. 병리학적 소견을 보면, 십이지장에서 가장 병변이 심하고 회장 및 공장은 덜하였다. 융모 위축, 장선와 비대, 기질내 세포침윤 및 부종이 특징적인 소견이었다. 감염기간이 경과할수록 병변이 심해지고 융모대 선와의 높이 비율도 감소하였다.

이상의 결과로 보아, *F. seoulensis*의 중감염이 마우스 전 소장에 보상하기 어려운 큰 손상을 주고 흡수장애 및 장출혈을 일으키며, 체중감소, 설사, 대변내 잠혈 출현 등의 임상증상을 일으킴으로써 결국은 사망까지 유발함을 알 수 있었다.

EXPLANATIONS FOR FIGURES

Fig. 3. The gross feature of an intestinal segment of a mouse dead on day 16 after infection, showing gross luminal bleeding and worms (arrows).

Fig. 4. Mouse duodenum of control group, showing normal and long, finger-like villi. H-E stain, ×100.

Fig. 5. Duodenal section of a 1-day infected mouse showing a young fluke in the intervillous space. H-E stain, ×100.

Fig. 6. Duodenal section of a 3-day infected mouse showing a worm entrapping the tip of a villus. Destruction of epithelial cells adjacent to the worm, and compression of the villus is conspicious. H-E stain, ×100.

Fig. 7. Duodenal section of a 7-day infected mouse showing villous atrophy, crypt hyperplasia, cellular infiltration and decreased V/C ratio (about 1 : 1). H-E stain, ×40.

Fig. 8. Duodenal section of a 7-day infected mouse showing a mature worm entrapping the villus, and showing destroyed villi. H-E stain, ×100.

Fig. 9. Jejunal section of a 7-day infected mouse showing stromal edema and decreased V/C ratio. H-E stain, ×40.

Fig. 10. Duodenal section of a 12-day infected mouse showing marked villous atrophy, crypt hyperplasia and reversed V/C ratio (1 : 2). H-E stain, ×40.

Fig. 11. Ileal section of a 12-day infected mouse showing severe stromal edema and flattening of villi and markedly decreased V/C ratio (2 : 1 to 1 : 1). H-E stain, ×100.


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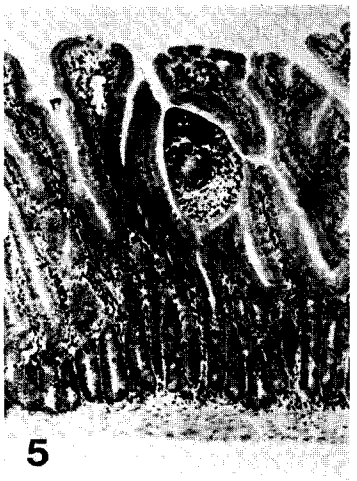
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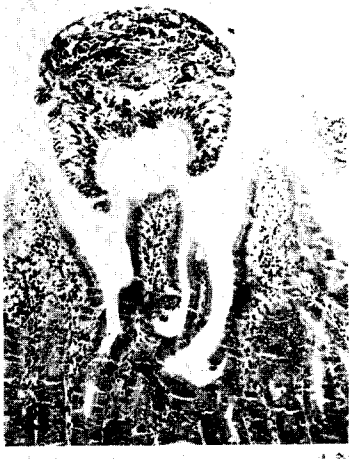


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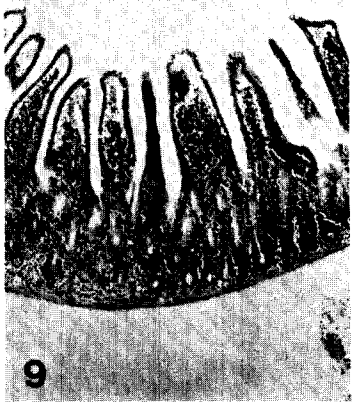
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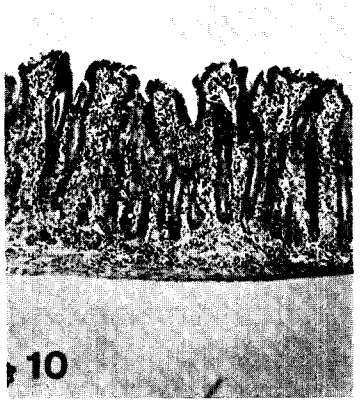
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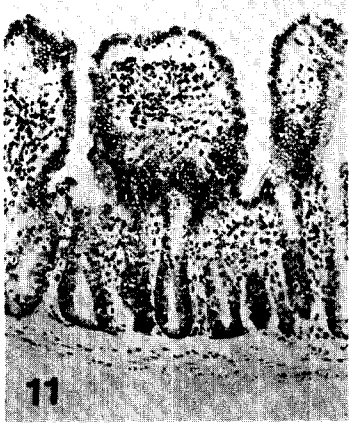
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