기 생 충 학 잡 지 제25권 제2호
The Korean Journal of Parasitology
Vol. 25, No. 2, 110-122, December 1987

Histopathological Changes of the Liver after Praziquantel Treatment in *Clonorchis sinensis* Infected Rabbits

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INTRODUCTION

Clonorchis sinensis is one of the important helminths of human in Korea because of its wide distribution and high prevalence rate among the inhabitants (Seo *et al.*, 1981). Adult flukes live in the bile duct of human or other mammals, and they make characteristic histopathological changes of the duct. The changes observed in various experimental animals are dilatation and thickening of bile duct, hyperplasia of bile duct epithelium, goblet cell metaplasia, inflammation and fibrosis around the duct, *etc.* (Kang, 1963; Hou, 1965; Lee, 1978a & 1978b; Min and Han, 1985).

Praziquantel is recommended as the drug of choice for the treatment of clonorchiasis (Rim *et al.*, 1981; Lee, 1984). Cure rate (or egg negative conversion rate) of clonorchiasis was reported as high as 85~90% when the drug was used in dose of 75mg/kg body weight, and the rate increased to almost 100% when the same dose was repeated to the failed cases by the first treatment (Rim *et al.*, 1981; Seo *et al.*, 1983).

However, there is no concrete information on whether the characteristic histopathological changes of the bile duct in clonorchiasis are recovered or not after praziquantel treatment. Some of the changes may be recovered but others may not. For this, Yang(1986) observed that the liver pathology in clonorchiasis of guinea pigs was almost unchanged even after a course of praziquantel treatment. But there were some limitations in the interpretation of the results because of incomplete deworming and relatively short period of follow-up.

In this context, it is necessary to observe the evolution of liver pathology in clonorchiasis after a complete treatment using other animal model. The present study intended to observe the sequential histopathological changes of the liver after chemotherapy in rabbit clonorchiasis.

MATERIALS AND METHODS

1. Collection of metacercariae of *C. sinensis*

The most well-known fish as the second intermediate host of *C. sinensis*, *Pseudorasbora parva*, were collected at Nakdong river, Pusan. The metacercariae of *C. sinensis* were isolated under the stereomicroscope from artificially digested material of the fish. The metacercariae were washed in normal saline and stored in a refrigerator.

2. Experimental infection to rabbits

Rabbits, *Oryctolagus cuniculus*, were infected with the metacercariae, 500 to each animal, into the stomach through a rubber tube.

3. Praziquantel treatment

The rabbits infected with *C. sinensis* were kept in laboratory and treated with praziquantel 50 mg/kg×2×2 days dose after 1, 2, 4 and 8

Table 1. Number of rabbits infected by *C. sinensis** and treated with praziquantel**

Duration of infection up to treatment	Not treated	Weeks after treatment		
		1	4	12
1 week	2	2	3	—
2 weeks	2	2	2	1
4 weeks	2	2	3	3
8 weeks	2	2	3	2
7 months	1	—	1	—

* No. of metacercariae of *C. sinensis*/rabbit : 500
** Dose of praziquantel was 50 mg/kg×2×2 days.

weeks and 7 months from infection as shown in Table 1.

4. Histopathological observation

The rabbits were killed after 1, 4 and 12 weeks from chemotherapy (Table 1). Their livers were removed and five segments were taken from each liver for tissue preparation. The segments were sectioned in 5~6 μm thickness and stained with hematoxylin and eosin solution.

Bile ducts, divided into two groups larger and smaller than 0.4 mm diameter, were graded for each histopathological change; 0 for normal, 1 for slight and focal changes, 2 for moderate changes, 3 for severe changes and 4 for very severe changes. The grades of 10~15 sectioned ducts were summed by group, and mean numerical grades of each change were obtained by group.

RESULTS

1. One week of infection

1) Infected control: Infected but not treated group showed slight inflammation and edema in portal spaces (Fig. 1). Slight proliferation and stratification of biliary epithelium were found. Small immature worms were found in bile ducts.

2) One week after treatment: There found no worm in bile ducts. Periductal edema, stratification and proliferation of bile duct epithelium were observed slightly in a few sections.

3) Four weeks after treatment: There was no worm in bile ducts. Little change was found (Fig. 2).

Table 2. Histopathological scoring* of liver in rabbit clonorchiasis after treatment in 1 week duration of infection

Findings	Not treated	Weeks after tx.	
		1	4
Large ducts(over 0.4 mm diameter)			
dilatation	0.8	0.4	0.9
epithelial proliferation			
papillary	0.5	0.4	0.5
glandular	0.1	0.3	0.2
stratification	0.6	0.5	0.5
goblet cell metaplasia	0.1	0	0
periductal edema	1	0.4	0
periductal inflammation	0.8	0.5	0.5
periductal fibrosis	0.2	0	0.2
desquamation	0	0	0
Small ducts			
adenomatous hyperplasia	0	0	0.5
stratification	0.3	0.2	0.1
periductal edema	0.4	0.3	0.2
periductal inflammation	0.4	0.2	0.4
periductal fibrosis	0	0	0

* Mean scores of 10~15 sections of each group; negative : 0, slight : 1, moderate : 2, severe : 3, very severe : 4.

Table 2 summarized the findings of one week infection group.

2. Two weeks of infection

1) Infected control: The liver sections of infected but not treated group showed many sectioned worms in dilated bile ducts (Fig. 3). The epithelium began to proliferate in papillary or glandular shape. Periductal edema and inflammation were observed moderately and slight periductal fibrosis was also found.

2) One week after treatment: No worm was found, however, the ducts were dilated. Papillary or glandular proliferation of bile duct epithelium, stratification of epithelial cells and periductal edema were still found slightly.

3) Two weeks after treatment: There were inflammatory cells, lymphocytes and plasma cells, in the portal space. Bile ducts were dilated slightly without a worm.

4) Four weeks after treatment: The lobular architecture of the liver was well-preserved, and

Table 3. Histopathological scoring* of liver in rabbit clonorchiasis after treatment in 2 week duration of infection

Findings	Not treated	Weeks after treatment		
		1	4	12
Large ducts (over 0.4 mm diameter)				
dilatation	1.1	1.3	1.0	0.4
epithelial proliferation				
papillary	1.3	0.9	0.1	0.4
glandular	0.8	0.4	0	0
stratification	1.1	0.7	0.3	0.2
goblet cell metaplasia	0.1	0.1	0	0
periductal edema	2.0	0.7	0.1	0
periductal inflammation	0.5	0.2	0.2	0.2
periductal fibrosis	0.6	0.3	0.1	0
desquamation	0.8	0	0	0
Small ducts				
adenomatous hyperplasia	0.5	0.3	0	0
stratification	0.2	0	0.2	0
periductal edema	0.9	0.4	0.1	0
periductal inflammation	0.2	0.2	0.1	0.2
periductal fibrosis	0.1	0	0	0

* Mean scores of 10~15 sections of each group; negative : 0, slight : 1, moderate : 2, severe : 3, very severe : 4.

bile ducts showed no abnormality. Epithelial layer of the ducts were evenly arranged.

5) Twelve weeks after treatment: There were slight inflammatory cells in portal area but others were found normal (Fig. 4).

The histopathological findings of the liver in the group of 2 week duration of infection were summarized in Table 3.

3. Four weeks of infection

1) Infected control: The liver sections showed well demarcated lobular structure by infiltration of cells and fibrosis along the lobular margins. The sectioned worms were mature to have many eggs, and the bile ducts were dilated and thickened of their wall (Fig. 5). Epithelial proliferation was papillary or glandular in moderate degree, and adenomatous hyperplasia and goblet cell metaplasia were noted. Periductal inflammation and fibrosis were found, and compression of hepatic cells around the dilated and thickened ducts was observed also.

2) One week after treatment: Slight dilatation of bile ducts and slight papillary proliferation of biliary epithelia were found. Periductal edema and fibrosis persisted, but no worm was found (Fig. 6).

3) Four weeks after treatment: Slight dilatation of bile ducts, thickening of duct wall and epithelial proliferation were still observed slightly and focally. No worm was found.

4) Twelve weeks after treatment: There remained slight dilatation of the ducts or epithelial proliferation in a few sections (Fig. 7). There was no worm.

The findings were summarized in Table 4.

4. Eight weeks of infection

1) Infected control: Many mature worms were found in the widened and thickened bile ducts. Papillary and adenomatous hyperplasia of the duct epithelia was severe and irregular, and goblet cells were found. Periductal inflammation and fibrosis were severe to distend to neighboring portal areas and to make islets of hepatic cells. The hepatic cells around the dilated and thickened ducts were compressed (Figs. 8 & 9).

2) One week after treatment: Bile ducts were

Table 4. Histopathological scoring* of liver in rabbit clonorchiasis after treatment in 4 week duration of infection

Findings	Not treated	Weeks after treatment		
		1	4	12
Large ducts(over 0.4 mm diameter)				
dilatation	2.2	0.8	0.2	0.8
epithelial proliferation				
papillary	2.7	1.2	0.8	1
glandular	1.7	1	0.4	0.2
stratification	1.2	0.2	0.6	0.6
goblet cell metaplasia	0.2	0.3	0.7	0
periductal edema	1.3	0.8	0.6	0.3
periductal inflammation	1	0.7	0.6	0.4
periductal fibrosis	1.8	1.3	0.7	1.2
desquamation	1	0.3	0	0
Small ducts				
adenomatous hyperplasia	0.9	0.5	0.5	0.5
stratification	0.1	0.4	0.4	0.2
periductal edema	0.3	0.1	0	0
periductal inflammation	0.7	0.5	0.3	0.3
periductal fibrosis	0.1	0.3	0.3	0

* Mean scores of 10~15 sections of each group; negative : 0, slight : 1, moderate : 2, severe : 3, very severe : 4.

Table 5. Histopathological scoring* of liver in rabbit clonorchiasis after treatment in 8 week duration of infection

Findings	Not treated	Weeks after treatment		
		1	4	12
Large ducts (over 0.4 mm diameter)				
dilatation	2.8	1.0	1.1	1.4
epithelial proliferation				
papillary	3.7	2.1	1.8	1.3
glandular	2.3	1.1	1.3	1.3
stratification	1.6	1.4	1.1	0.6
goblet cell metaplasia	1.7	1.0	0.4	0
periductal edema	0.7	0	0	0.3
periductal inflammation	1.2	0.5	0.5	0.2
periductal fibrosis	2.9	2.1	2.0	2.0
desquamation	0.8	0.8	0.4	0
Small ducts				
adenomatous hyperplasia	1.6	0.3	0.6	0.7
stratification	0.8	0.9	0.5	0.2
periductal edema	0.2	0	0.1	0.3
periductal inflammation	1.0	0.5	0.5	0.4
periductal fibrosis	2.5	1.3	1.1	1.0

* Mean scores of 10~15 sections of each group; negative : 0, slight : 1, moderate : 2, severe : 3, very severe : 4.

dilated slightly and the epithelial layer was found in papillary or glandular proliferation. A few goblet cells were observed. Periductal fibrosis was found moderately (Fig. 10).

3) Four weeks after treatment: The ducts were mildly dilated, and mild proliferation of the duct epithelium was also found. Periductal fibrosis persisted (Fig. 11). Only a worm was detected in a duct, and the epithelium of the duct proliferated moderately.

4) Twelve weeks after treatment: There were slight proliferations of biliary epithelium in large ducts. Periductal fibrosis was still found (Fig. 12).

Table 5 summarized the findings of the group of 8-week infection.

5. Seven months of infection

1) Infected control: The liver consisted of islet-like hepatic cells which were surrounded by the bridges of inflammatory cells and fibrosis connecting portal spaces. Bile ducts were severely dilated with many worms in the ducts. The wall of bile ducts was severely thickened, and their papillary or adenomatous hyperplasia of biliary epithelium was very severe. Goblet cells were found many in biliary epithelia (Figs. 13, 14 & 15).

2) Four weeks after treatment: Bile ducts were dilated moderately, and epithelial proliferation was found also moderately. Portal inflammation and fibrosis were still found. No worm was detected (Fig. 16).

The results of 7-month infection group were presented in Table 6.

Table 6. Histopathological scoring* of liver in rabbit clonorchiasis after treatment in 7 month duration of infection

Findings	Not treated	4 weeks after tx.
Large ducts(over 0.4 mm diameter)		
dilatation	3.2	1
epithelial profiferation		
papillary	3.2	2.8
glandular	3.7	2.3
stratification	3.5	2.0
goblet cell metaplasia	3.7	1.3
periductal edema	0.8	0
periductal inflammation	1.3	0.3
periductal fibrosis	2.7	2.5
desquamation	1.8	0.2
Small ducts		
adenomatous hyperplasia	1.6	1.3
stratification	0.8	0
periductal edema	0.8	0
periductal inflammation	1.2	0.2
periductal fibrosis	1.6	2.2

* Mean scores of 10~15 sections fo each group; negative : 0, slight : 1, moderate : 2, severe : 3, very severe : 4.

DISCUSSION

Histopathological changes of the liver in clonorchiasis of experimental mammals are characterized by proliferation of bile ducts, especially hyperplasia or metaplasia in epithelial layer (Kang, 1963; Hou, 1965; Komiya, 1966).

The liver findings of the infected but not treated rabbits in the present study showed chronological progress of the changes. The finding at the first week after infection was acute inflammation around bile ducts. Biliary epithelium began to proliferate at the second week after infection and the proliferation progressed more and more during the experiment of 7 months. Periductal fibrosis was recognizable after 4 weeks of infection and the wall of bile ducts became more thickened as the infection progressed. Those changes of the rabbit liver were in the same pattern as previously described in rats (Lee *et al.*, 1978a; Min and Han, 1985) and guinea pigs (Lee *et al.*, 1978b).

Those changes were found to be recovered to a certain degree by chemotherapy in this study. Any kind of changes until 2 weeks after infection was found to be recovered by praziquantel treatment. The lesions were mild, 1.1~2.0 in degree after 2 weeks from infection as shown in Table 3 and they were found negligible one week after treatment.

It was found that production of collagen in the liver began to increase 2 weeks after infection in schistosomiasis (Hutadilok *et al.*, 1983).

If this finding in schistosomiasis was considered, two weeks of infection duration would also be an important period for fibrosis in the liver in clonorchiasis. Not only collagen deposition, however, but also early dilatation of bile duct and proliferation of biliary epithelium which were found during the first 2 weeks disappeared after treatment. Therefore it can be concluded that the early changes within 2 weeks of clonorchiasis are reversible.

Dilatation of bile ducts, hyperplasia of biliary epithelium and periductal fibrosis became moderate or severe, 1.7~3.7 in degree, after 4 to 8 weeks from infection (Table 4 and 5). The changes became lessened in their degree of 1~2.1 after a week from chemotherapy, and remained in mild degree from 0.2 to 2.0 until 12 weeks after treatment. The remaining changes might be healed a little more when observed longer than 12 weeks after treatment. However, it was not expected that the lesion would be healed completely because they were changed rapidly during the first week after treatment and very slowly later as presented in Tables and Figures, and the changes of bile ducts were still found after 12 weeks from treatment.

Using guinea pigs as an experimental model, Yang(1986) observed the findings of the liver in clonorchiasis after treatment. He concluded that praziquantel treatment should not be directly related with complete deworming nor histopathological healing in clonorchiasis of guinea pigs. His findings suggested that the species of experimental animal should be considered in understanding the healing process after treatment. The histopathological change of clonorchiasis in guinea pigs is characterized by saccular dilation of bile ducts, and this change seems difficult to be recovered.

Among the groups of praziquantel treatment in the present study, there was only a worm in a sectioned bile duct of 8-week infection group at 4 weeks after treatment. The worm was found living as that of control group. Also the bile duct around the worm was thickened and proliferated moderately. In the rabbits of other groups, however, the worm was not detected at all. Therefore it was proved that praziquantel 50 mg/kg×2×2 days dose was excellent for treatment of clonorchiasis in the rabbit.

The liver lesions in schistosomiasis were found recovered almost completely 4 weeks after praziquantel treatment (Mehlhorn *et al.*, 1982; El-Haway *et al.*, 1986). Contrary to healing of the liver lesions of schistosomiasis, those of clonorchiasis were not healed completely. The difference was regarded as due to different nature of histopathology. In clonorchiasis, bile duct dilatation, hyperplasia of biliary epithelium and periductal fibrosis are main changes compared with egg granuloma and fibrosis in the liver in schistosomiasis. Among the lesions in clonorchiasis over 4 weeks of infection duration, hyperplasia of biliary epithelium was healed to mild degree after deworming but not completely. Periductal fibrosis was recovered only a little, but goblet cell metaplasia was not found at all after 12 weeks from the treatment.

In conclusion, the histopathological changes of the liver in clonorchiasis are reversible after treatment only for the first 2 weeks of infection. However, the changes found 4 weeks or more after infection are not healed completely. Furthermore, more severe changes remain after treatment as the duration of infection becomes longer. The conclusion suggests the importance of early treatment as possible in clonorchiasis.

SUMMARY

The rabbits were infected with *Clonorchis sinensis* and were treated with praziquantel at the dose of 50 mg/kg×2×2 days after 1, 2, 4, 8 weeks and 7 months from the infection. Their livers were observed histopathologically 1, 4 and 12 weeks after treatment. The findings are summarized as below.

1. The changes of the liver in control rabbits were relatively mild until 2 weeks after infection. However, widening and thickening of bile ducts, proliferation of biliary epithelium and periductal

fibrosis were moderate after 4 weeks from infection and those changes were severe after 8 weeks and 7 months. Goblet cell metaplasia was found after 8 weeks from infection.

2. The mild changes of 2-week infection group were completely recovered by 4 weeks after the treatment. In the groups of 4 or more weeks after infection, the changes of bile ducts became milder in the degree after the treatment, but were still found 12 weeks after the treatment. As the infection duration was passed, more severe changes were observed after the treatment.

In this context, it is concluded that the liver changes of acute clonorchiasis in the early two weeks are reversible by treatment while chronic biliary epithelial changes are irreversible. Therefore, early treatment should be recommended as possible to minimize the remaining histopathological changes of liver in clonorchiasis.

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=국문초록=

토끼의 간흡충성 간병변의 프라지콴텔 치료 후 변화 양상

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간흡충(*Clonorchis sinensis*)에 감염된 토끼에 프라지콴텔을 50 mg/kg×2회×2일의 용법으로 투여하여 12주 후까지 간의 조직병리학적인 소견을 관찰한 바 아래의 결론을 얻었다.

감염 2주까지 관찰된 간내 담관 상피층의 미약한 증식, 담도주위 염증과 부종 등은 치료 4주 후에 모두 소실되어 이 때 까지의 변화는 가역적인 것으로 판단된다. 그러나 감염 4주 이후에는 치료 후 1주에 이미 상당히 정도가 약해졌으나 12주까지도 담관과 담관상피층의 증식이 많이 완화된 수준으로 계속 관찰되었고 담관주위 섬유화도 지속되었다. 이러한 잔존 병변은 감염 후 경과한 기간이 길수록 정도가 심하였다.

간흡충증이 감염 초기가 아니면 치료 후에도 간에 형성된 병변이 잔존하는 것으로 미루어, 감염자의 감염 후 2~4주 이내의 조기치료가 중요하다고 판단된다.

EXPLANATION FOR FIGURES

Fig. 1. Infected control of 1 week infection showing a juvenile *C. sinensis* in a small bile duct, and periductal edema, hematoxylin-eosin (HE) stained, ×140.

Fig. 2. Two large ducts after 4 weeks from treatment of 1 week infection with slight periductal inflammation, HE stained, ×100.

Fig. 3. Infected control of 2 week infection showing a worm in a dilated bile duct. Papillary proliferation of the duct epithelia and periductal edema and inflammation were observed, HE stained, ×40.

Fig. 4. High power view of a bile duct 12 weeks after treatment of 2 week infection with slight periductal inflammation, HE stained, ×200.

Fig. 5. Infected control of 4 week infection, 2 sections of mature worms were found in a dilated bile duct. Biliary epithelial layer was compressed, flattened or desquamated near the worms, HE stained, ×48.

Fig. 6. Small bile ducts 1 week after treatment of 4 week infection with slight epithelial proliferation and periductal fibrosis, HE stained, ×72.

Fig. 7. A large bile duct 12 weeks after treatment of 4 week infection showing slight epithelial proliferation and periductal inflammation, HE stained, ×48.

Fig. 8. Three sections of *C. sinensis* in a severely dilated bile duct after 8 week from infection. Epithelial proliferation and compression, desquamation and periductal inflammation were observed, HE stained, ×40.

Fig. 9. A large bile duct a without worm of a 8 week infected control rabbit, with papillary or glandular proliferation of epithelium, HE stained, ×40.

Fig. 10. A large duct one week after treatment of 8 week infection with irregular epithelial proliferation and periductal fibrosis, HE stained, ×40.

Fig. 11. A large duct 4 weeks after treatment of 8 week infection with irregular epithelial proliferation and periductal fibrosis, HE stained, ×56.

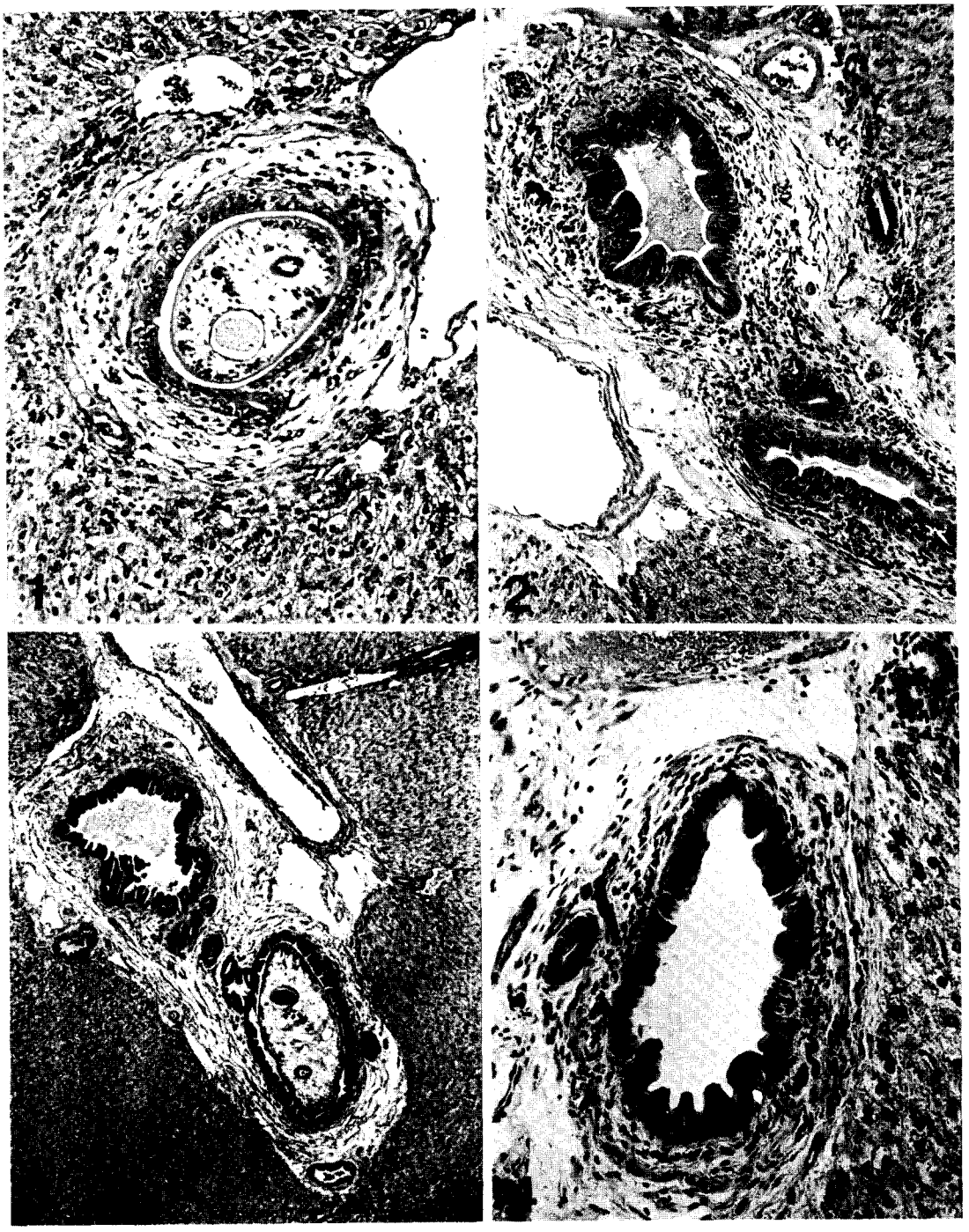
Fig. 12. A large duct 12 weeks after treatment of 8 week infection with slight epithelial proliferation and periductal fibrosis, HE stained, ×56.

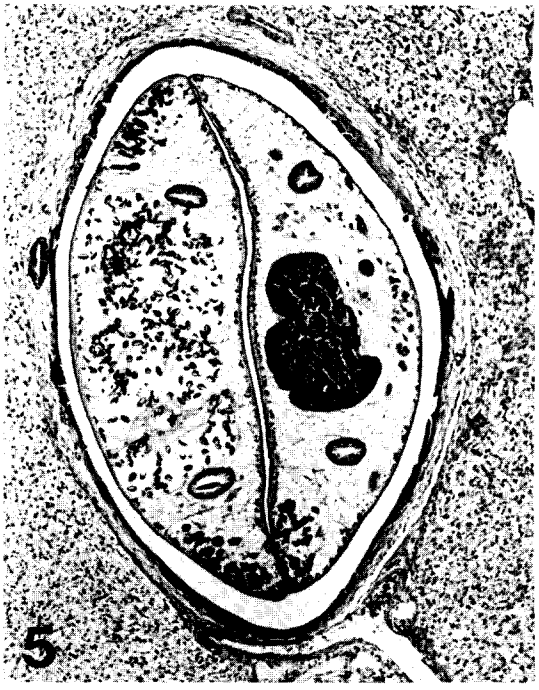
Fig. 13. A sectioned worm of mature *C. sinensis* in a dilated bile duct was found 7 months after infection. Epithelial layer of the duct was proliferated markedly and desquamated partly, HE stained, ×40.

Fig. 14. A large duct with papillary and adenomatous hyperplasia of epithelium and periductal fibrosis and inflammation, of 7 month infection, HE stained, ×40.

Fig. 15. High power of a bile duct 7 months infected control showing goblet cells in biliary epithelium, HE stained, ×200.

Fig. 16. A large bile duct 4 weeks after treatment of 7 month infection showing papillary and adenomatous hyperplasia and periductal fibrosis, HE stained, ×56.





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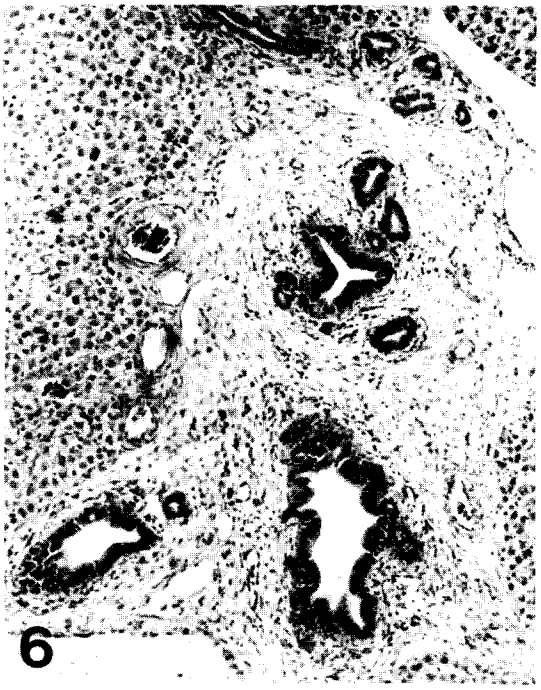

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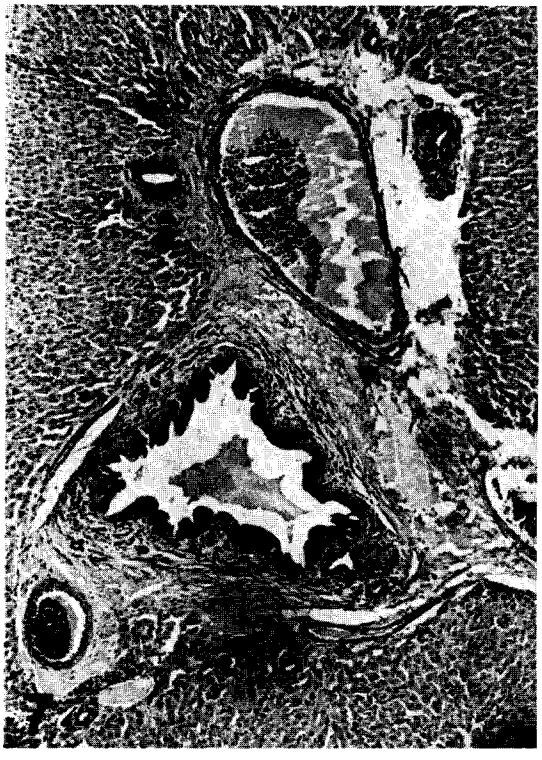

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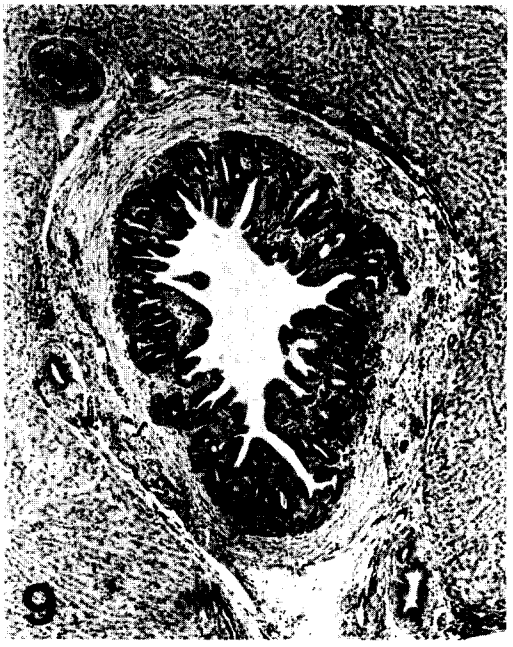
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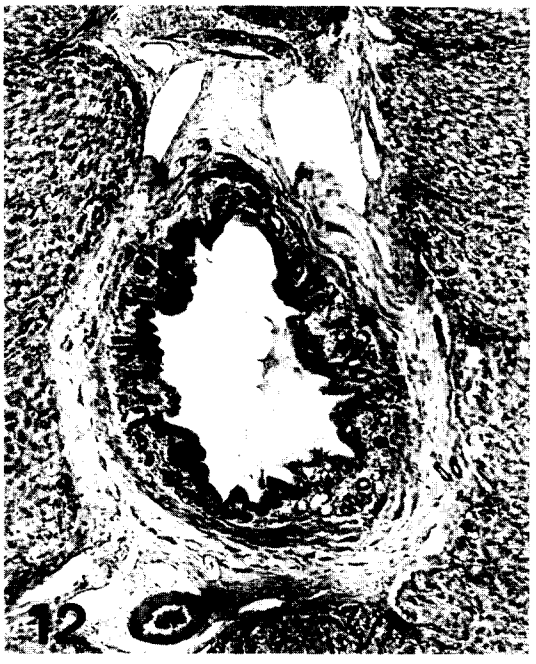
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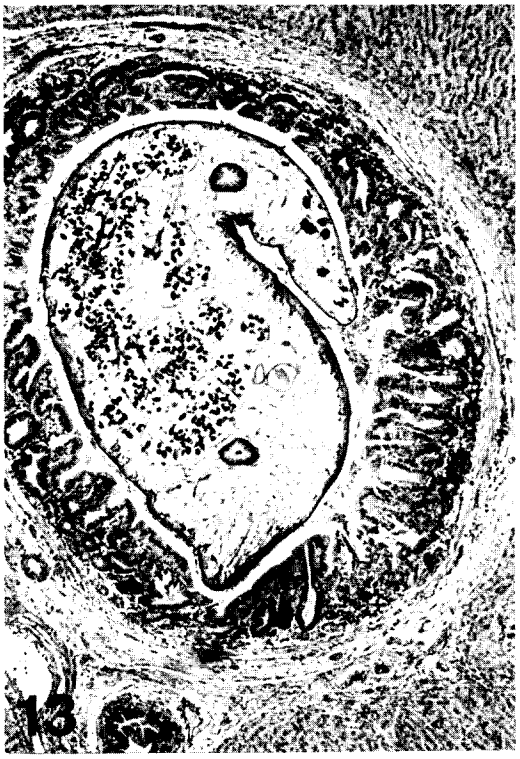

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