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Changes in Histopathological and Serological Findings of the Liver after Treatment in Rabbit Clonorchiasis*

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= Abstract =In order to elucidate the recovery course and the residual change of clonorchiasis after praziquantel treatment, the changing pattern of histopathological findings of the liver, along with the serological, biochemical and hematological parameters, were evaluated in experimental rabbit clonorchiasis. Twenty rabbits were infected each with 300 metacercariae of *Clonorchis sinensis* and treated with praziquantel 200 mg/kg 14 weeks after infection. Until one year after infection, a widening of the bile ducts, proliferation of biliary epithelium, and periductal fibrosis were observed in the liver, although the lesions became much milder than those of the untreated rabbits. The levels of anti-*C. sinensis* IgG antibody in the sera by ELISA decreased continuously after treatment. Biochemical items and hematological parameters showed no consistent changing pattern after infection or after treatment. It can be suggested that the histopathological lesions of rabbit clonorchiais, *i.e.*, duct dilatation, hyperplasia of biliary epithelium and periductal fibrosis, may be hardly reversible. However, the level of circulating specific IgG antibody decreased significantly according to the healing process of inflammation.

Key Words: ***Clonorchis sinensis*, Rabbit, Praziquantel, Histopathology, ELISA, Biochemistry, Hematology**

INTRODUCTION

Up until nine weeks after chemotherapy with praziquantel, the histopathological changes of the liver with clonorchiasis, such as glandular hyperplasia of biliary epithelium, cystic dilatation of the bile ducts and periductal fibrosis, were known to persist in guinea pigs, although the gross findings revealed a more or less recovering feature (Lee *et al.*, 1988). In rabbit clonorchiasis, the pathologic changes in the bile ducts became milder to some degree, but persisted up to 12 weeks post-treatment. The older lesion showed more residual changes after treatment. When treated earlier than four weeks after infection, pathological changes of the bile duct were not found (Lee *et al.*, 1987).

It is uncertain whether the persisting histopathological lesions after treatment will recover at last. It is also not certain whether the pathologic changes are correlated with any derangement of the liver function. Checked by ELISA, the circulating anti-*Clonorchis sinensis* IgG antibodies in the sera were lowered in 6 to 18 months post-treatment with praziquantel 40 mg/kg in human clonorchiasis (Lee, 1986; Kim *et al.*,

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1987), and attained a pre-infection level four months after treatment in experimental rabbits (Yang *et al.*, 1984). When infected patients were treated with praziquantel (Distocide^{R}, Shinpoong Pharm. Co., Korea) 75 mg/kg, the level of serum IgG antibody against crude antigen became negative between 9 weeks and 7 months (Hong, 1988).

Do the lesions of clonorchiasis recover after treatment? When do they recover after treatment? Does the serological conversion have any relationship with the histopathological changes, or with any biochemical and hematological data? To answer these questions, the changes in the histopathology, serology, blood chemistry and hematology of clonorchiasis, pre- or post-treatment with praziquantel, were investigated in experimentally infected rabbits.

MATERIALS AND METHODS

The metacercariae of *Clonorchis sinensis* were collected from the pepsin digested debris of *Pseudorasbora parva*. Twenty New Zealand white rabbits, 4-5 weeks old, and sexually randomized, were fed with 300 metacercariae through Levine tubes. Fourteen weeks post-infection (PI), all rabbits were treated with praziquantel 50 mg/kg *bid p. o.* for two days, total 200 mg/kg. The animals were reared *ad libitum*.

The livers were resected from the rabbits, which were sacrificed seven months and one year post-treatment respectively. One animal, which was infected but untreated, was used as the control. After gross observations, three paraffin blocks from one liver were prepared at different loci. The blocks were cut in five μm thicknesses. Haematoxylin and eosin (H & E), periodic acid Schiff (PAS), Alcian blue (pH 2.5) and Gomori's rapid one step trichrome stains were adapted to each section.

Blood was sampled before infection, 2, 4, 8, and 12 weeks PI, and 2, 4, 8, 12, 16 weeks, 7 months and one year after treatment. The collected sera were preserved at −70°C. Enzyme linked immunosorbent assay (ELISA) was used as the serological test following the method of Hong (1988). The anti-*C. sinensis* IgG antibody titers were checked by an ELISA reader (Dynatek Co.) at 492 nm.

Biochemical parameters were checked in the laboratory of the Department of Clinical Pathology, Seoul National University Hospital. The items were total protein, albumin, cholesterol, total bilirubin, alkaline phosphatase and glutamine phosphotransferase. A hematological study was executed with a Coulter Counter in the hematology laboratory of the Department of Clinical Pathology for hemoglobin, hematocrit, leukocyte count, leukocyte differential count, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count.

RESULTS

The resected livers of the treated rabbits showed grossly normal appearance when compared with that of the untreated control, which had cirrhotic changes of white discoloration, scattered nodular elevation, and firmness. Microscopically, the H & E stained sections of the infected but untreated control livers revealed the typical features of chronic clonorchiasis, *i. e.*, dilatation of the bile ducts, hyperplasia of biliary epithelium, goblet cell metaplasia, periductal inflammation with granulation tissue, periductal fibrosis, and necrosis of adjacent hepatocytes to the bile ducts (Figs. 1, 2).

In the seven-months post-treatment livers, dilatation of the bile ducts, hyperplasia of biliary epithelium, and periductal fibrosis were observed, although the degree of lesions was milder than that of the control. One year after treatment, the lesions to a mild degree still remained. The dilatation of the bile ducts, and hyperplasia of biliary epithelium were much recovered compared to the infected control, but periductal fibrosis persisted almost unchanged (Figs. 3, 4). The hyperplastic epithelium of bile ducts of the control was positive to PAS staining especially in the adenomatous region (Figs. 5, 6). A few cells in the biliary epithelia remained still PAS positive after six months post-treatment (Fig. 7), but they turned negative one year post-treatment (Fig. 8). The epithelial cells at the basal region near lamina propriae of the control were positive to Alcian blue (Figs. 9, 10). A few cells of biliary epithelia of the 6-month group were still positive to Alcian blue (pH 2.5)(Fig. 11), but none of the cells was stained blue in the one-year post-treatment rabbit (Fig. 12). The Gomori's rapid one-step trichrome staining showed an increased amount of periductal fibro-

Table 1. Biochemical findings of rabbits pre- and post-treatment

Post-infection	0	2	4	8	12	14	16	18	22	26	45	64w
Post-treatment							2	4	8	12	31	52w
No. of exam	9	9	7	11	6	5	2	1	1	3	1	2
T-bil	0.5	0.7	0.5	0.6	0.8	0.6	0.4	0.7	0.2	0.3	0.7	0.4
(mg/dl)	± 0.25	± 0.22	± 0.09	± 0.19	± 0.44	± 0.13	± 0.07					± 0.00
Alb	2.3	2.3	2.4	2.2	2.3	2.4	2.3	2.5	2.0	2.6	2.5	2.8
(g/dl)	± 0.26	± 0.20	± 0.12	± 0.24	± 0.39	± 0.07	± 0.28			± 0.29		± 0.21
TP	4.7	5.0	5.1	5.0	5.6	5.3	5.2	5.2	4.6	5.4	6.1	6.1
(g/dl)	± 0.56	± 0.65	± 0.37	± 0.87	± 1.28	± 0.34	± 1.13			± 0.75		± 0.78
Chol	67.2	53.9	58.6	48.7	59.2	135.8	27.5	29.0	57.0	46.0	75.0	31.5
(mg/dl)	± 23.66	± 33.78	± 28.70	± 16.49	± 42.24	± 79.21	± 4.95			± 28.85		± 9.19
ALP	126.4	97.3	92.0	79.0	38.3	41.0	32.5	23.0	13.0	57.3	16.0	30.0
(IU/L)	± 16.89	± 40.83	± 34.69	± 19.90	± 18.14	± 11.47	± 13.44			± 27.21		± 0.00
GPT	9.3	12.9	13.7	7.3	28.4	14.0	15.0	9.0	61.7	4.0	30.5	
(IU/L)	± 5.85	± 7.65	± 4.27	± 6.28	± 9.32	± 0.00			± 32.56		± 0.71	

Abbreviations: T-bil, total bilirubin; Alb, albumin; TP, total protein; Chol, cholesterol; ALP, alkaline phosphatase; GPT, glutamine phosphotransferase

sis as green fibers (Figs. 13, 14). The amount of periductal fibrosis appeared to decrease but still persisted after treatment by one year (Figs. 15, 16). Those special-stained sections revealed the goblet cell metaplasia in the biliary epithelium in clonorchiasis. However, the population of goblet cells decreased at 6 months and disappeared at one year post-treatment. Also, the resorption of fibrous tissue to some degree could be recognized.

The serum levels of anti-*C. sinensis* IgG antibodies measured by ELISA were 0.165 ± 0.016 before infection; 0.198 ± 0.034 two weeks PI, 0.336 ± 0.067 four weeks PI, 0.453 ± 0.034 two weeks PI, 0.336 ± 0.067 four weeks PI, 0.453 ± 0.084 eight weeks PI, 0.523 ± 0.102 12 weeks PI, and after treatment; 0.435 ± 0.160 two weeks, 0.460 ± 0.093 four weeks, 0.342 ± 0.074 eight weeks, 0.360 ± 0.025 12 weeks, 0.428 ± 0.064 16 weeks, 0.308 seven months and 0.161 ± 0.302 one year post-treatment (Fig. 17). The biochemistry data are summarized in Table 1. Any meaningful changing pattern was not found. The hematological values did not changed significantly according to infection or treatment (Table 2, $P > 0.05$).

DISCUSSION

The early histopathological changes of the liver in rabbit clonorchiasis are reversible if treated in two to four weeks post-infection (Lee *et al.*, 1987). However, it is practically impossible to diagnose clonorchiasis in such an early stage of infection, because the eggs are discharged from about one month after infection (Rim, 1986). Lee *et al.* (1987) also described the residual lesions of epithelial hyperplasia, duct dilatation and periductal fibrosis at 12 weeks after treatment. The present finding reveals that those histopathological lesions caused by the infection of *C. sinensis* persist one year post-treatment in experimental rabbits. The lesions remaining one year after treatment seem to be almost irreversible. Especially, the periductal fibrosis and epithelial hyperplasia of the bile ducts may be the features which are difficult to recover completely, as they were in guinea pigs (Lee *et al.*, 1988). We can extrapolate that the human liver with chronic clonorchiasis may only very slowly recover even after complete elimination of the worms. However, the number of goblet cells in the biliary mucosa decreased distinctively at seven months after treatment, and the cells disappeared completely one year after treatment. The goblet cell metaplasia is a reversible change of very slow recovery over six months after treatment.

Why do periductal fibrosis and epithelial hyperplasia of the bile ducts persist so long and to a mild degree? The fibrosis is a scar tissue, the

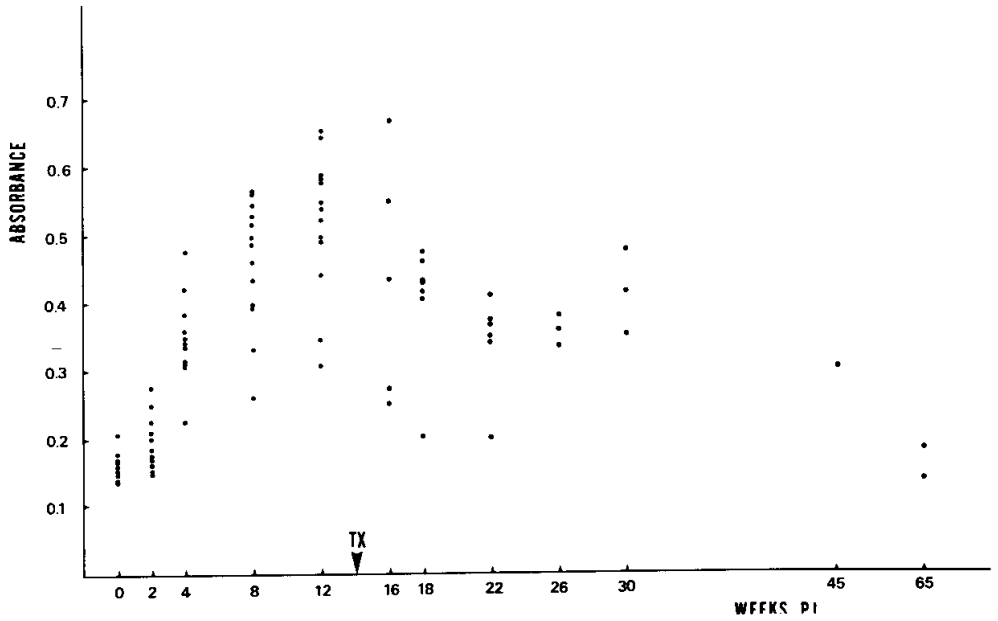

Fig. 17. Serum anti-*C. sinensis* IgG antibody of the rabbits by ELISA. Tx: Treatment with praziquantel.

collagen fiber of which would be resistant to the resorption activity of the scavenging cells. Although the reason for persistent hyperplastic epithelium after treatment is uncertain, the hyperplasia may supposedly be a hypersensitive reaction of longterm memory. We don't know the mechanism of the biliary hyperplasia in clonorchiasis, but the increase of goblet cells in the intestinal mucosa in nippostrongylosis is known to be mediated by T-lymphocytes (Roitt, 1988). This reaction may help the host expel the parasite by an increased secretion of mucus. The mechanism of host reactions, hyperplasia and metaplasia in clonorchiasis is an interesting subject for further study.

The changing pattern of anti-*C. sinensis* IgG antibodies in this rabbit clonorchiasis is similar to the results of Yang *et al.* (1983). It began to increase four weeks post-infection, reached a peak, at 12 weeks post-infection, and decreased gradually to the pre-infecton level by one year post-treatment. According to the explanation the IgG antibodies react with the metabolic antigen of *C. sinensis* during the infection but react mostly with the somatic antigen after treatment. In ELISA, the worm burden or EPG is one of the important factors that affect the antibody titers (Hong, 1988). In this experiment, the infection dose of 300 metacercariae is thought to be enough to evoke the antibody response. These results coincide well with that of previous paper which states that the antibody level could play a role as not only as an index of cure but also as a long-term follow-up tool (Hong, 1988).

In the development of clonorchiasis in the early stage of infection, the lesion becomes granulation tissue histopathologically around four weeks after infection (Lee *et al.*, 1987 & 1988). This period is also required for the significant increase of the specific IgG antibody in the serum in primary infection. Such a phenomenon is also observed in experimental anisakiasis (Hong and Lee, 1987) or in sparganosis (Hong *et al.*, 1989). In those parasitic infections, the formation of granulomas may be related with the synthesis of the circulating IgG antibody. The serological data showed a pattern of slow decrease after elimination of the worms. It turned negative after about 6-12 months. This decreasing pattern seems to be correlated with the heal-

Table 2. Hematological parameters of rabbits pre- and post-treatment

Pre-infection	0	2	4	8	12	16	18	22	26w
Post-treatment						2	4	8	12w
No. of rabbits	14	18	13	13	13	7	2	2	2
WBC	8.0	8.4	13.0	9.4	9.9	11.5	9.7	10.0	12.0
($\times 10^6$)	± 2.18	± 3.46	± 4.62	± 2.27	± 2.70	± 2.62	± 0.49	± 0.49	± 1.27
RBC	5.9	5.3	5.8	5.6	5.7	5.1	5.3	8.1	6.3
($\times 10^6$)	± 0.46	± 0.58	± 0.51	± 0.59	± 0.55	± 5.06	± 0.69	± 2.47	± 0.07
Hb	12.8	12.2	11.9	12.5	12.6	11.3	12.0	14.0	13.4
(g/dl)	± 0.55	± 0.97	± 1.14	± 0.11	± 0.83	± 1.01	± 1.77	± 1.34	± 0.92
Hct	38.0	35.7	35.0	37.2	36.5	33.4	36.0	39.0	39.2
(%)	± 2.01	± 3.09	± 3.62	± 3.10	± 2.31	± 2.99	± 3.89	± 2.90	± 2.55
MCV	64.7	67.0	68.0	66.1	64.0	66.1	67.1	63.7	62.5
(fL)	± 2.60	± 3.86	± 0.32	± 3.85	± 3.32	± 3.57	± 1.41	± 1.06	± 3.32
MCH	21.7	22.9	24.0	22.2	22.1	22.3	23.0	23.0	20.8
(pg)	± 1.12	± 1.72	± 1.12	± 1.40	± 1.31	± 1.22	± 0.42	± 0.92	± 0.49
MCHC	33.6	34.2	35.0	33.6	34.5	30.8	34.0	35.0	34.0
(g/dl)	± 0.57	± 1.01	± 0.60	± 0.54	± 0.49	± 7.74	± 1.27	± 0.92	± 0.14
RDW	13.0	14.1	14.1	13.5	13.1	13.7	5.2	12.2	12.7
(%)	± 1.80	± 1.90	± 0.27	± 1.62	± 2.06	± 0.93	± 1.48	± 0.28	± 1.06
PLT	490.8	701.7	742.4	695.2	609.2	717.4	650.0	521.0	511.0
($\times 10^6$)	±206.60	±318.90	±287.52	±111.08	±205.44	±199.55	± 24.04	± 71.42	± 3.54
Pct	298.3	332.9	394.5	363.6	355.4	350.3	339.0	254.0	269.0
(%)	± 70.30	± 90.49	±132.09	± 74.37	± 90.38	± 45.07	± 21.92		
MPV	5.4	5.5	5.6	5.7	6.2	5.4	5.2	5.4	5.3
(fL)	± 0.62	± 0.35	± 0.43	± 0.80	± 1.26	± 2.94	± 0.14		
PDW	15.4	15.7	15.7	15.8	15.7	15.7	16.0	15.6	15.0
(%)	± 0.26	± 0.51	± 0.61	± 0.73	± 0.56	± 7.14	± 1.06		
sN	55.0	42.8	52.5	48.2	50.2	38.4	46.0	52.0	44.0
(%)	± 13.21	± 13.62	± 10.68	± 9.25	± 13.38	± 11.79	± 10.61	± 16.26	± 11.31
Ly	40.1	51.5	39.5	45.9	43.4	55.3	46.0	45.0	51.0
(%)	± 12.27	± 13.70	± 10.32	± 0.17	± 14.9	± 12.59	± 8.49	± 14.85	± 11.31
Mo	3.5	5.0	4.5	1.9	1.7	5.5	3.0	1.0	2.5
(%)	± 2.22	± 8.22	± 2.88	± 1.42	± 1.06	± 4.59	± 0.00		± 0.71
Eo	—*	1.5	2.0	1.0	1.8	1.0	—*	1.0	2.0
(%)		± 0.84	± 1.73	± 0.00	± 1.30	± 0.00			
Ba	3.4	4.2	3.3	3.0	4.5	2.1	5.0	3.0	2.0
(%)	± 3.14	± 3.59	± 1.15	± 1.60	± 3.37	± 1.07	± 2.83	± 0.00	

*Not examined

ing process of the granulation tissue into the fibrous tissue made by the worm.

As for the biochemical and hematological changes in clonorchiasis, Joo & Rim (1982) showed an increase of eosinophil counts in the fifth week of infection in experimental rabbits, and a few reports mentioned abnormal liver functions in heavily infected rabbits (Rim, 1986). However, no consistent pattern of the present hematological and biochemical results was found by the infection or treatment. This may be due to the compensatory reserve. If there is a severe obstruction of the bile ducts due to heavy infection, the liver function test may be abnorma

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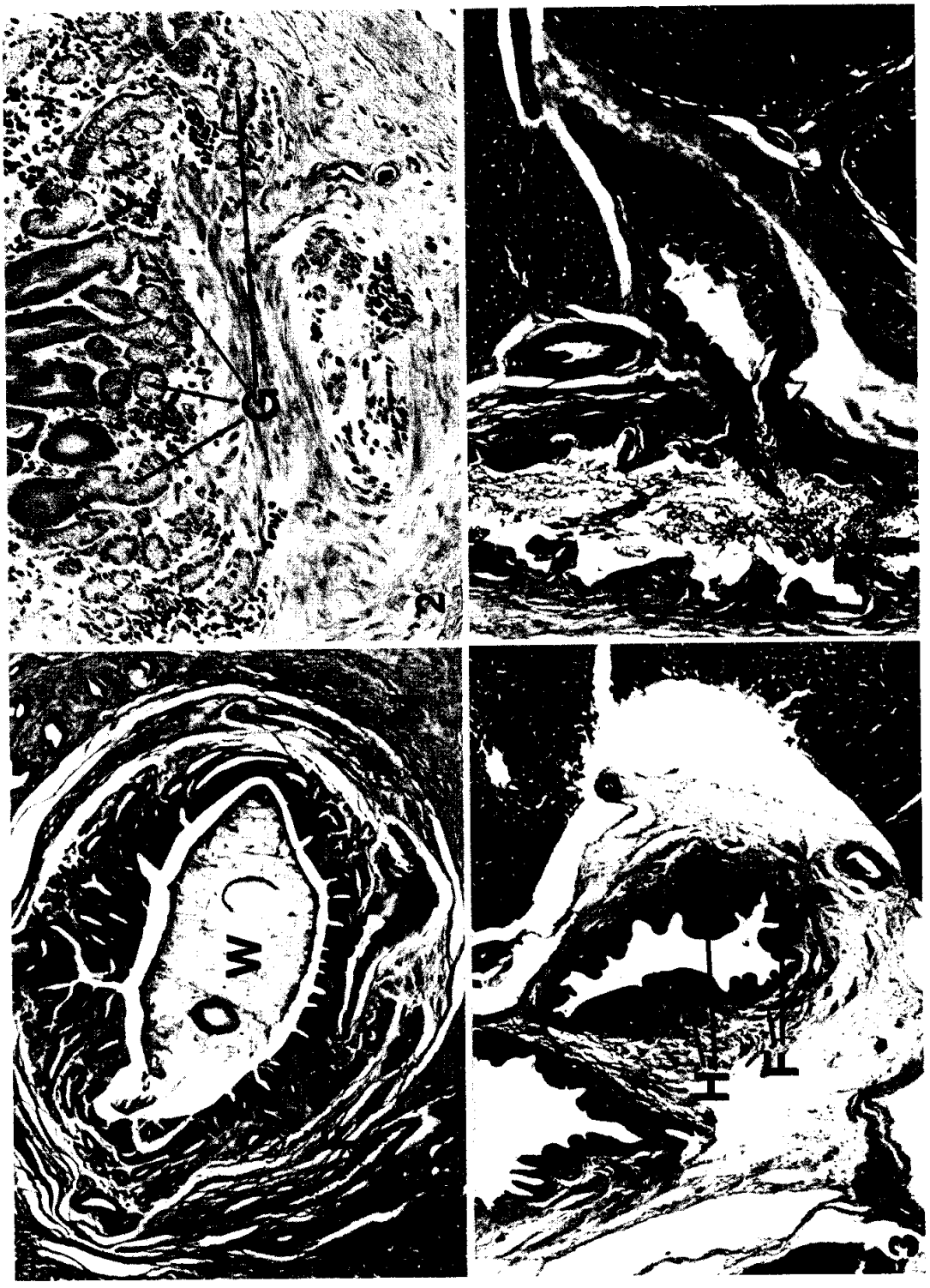
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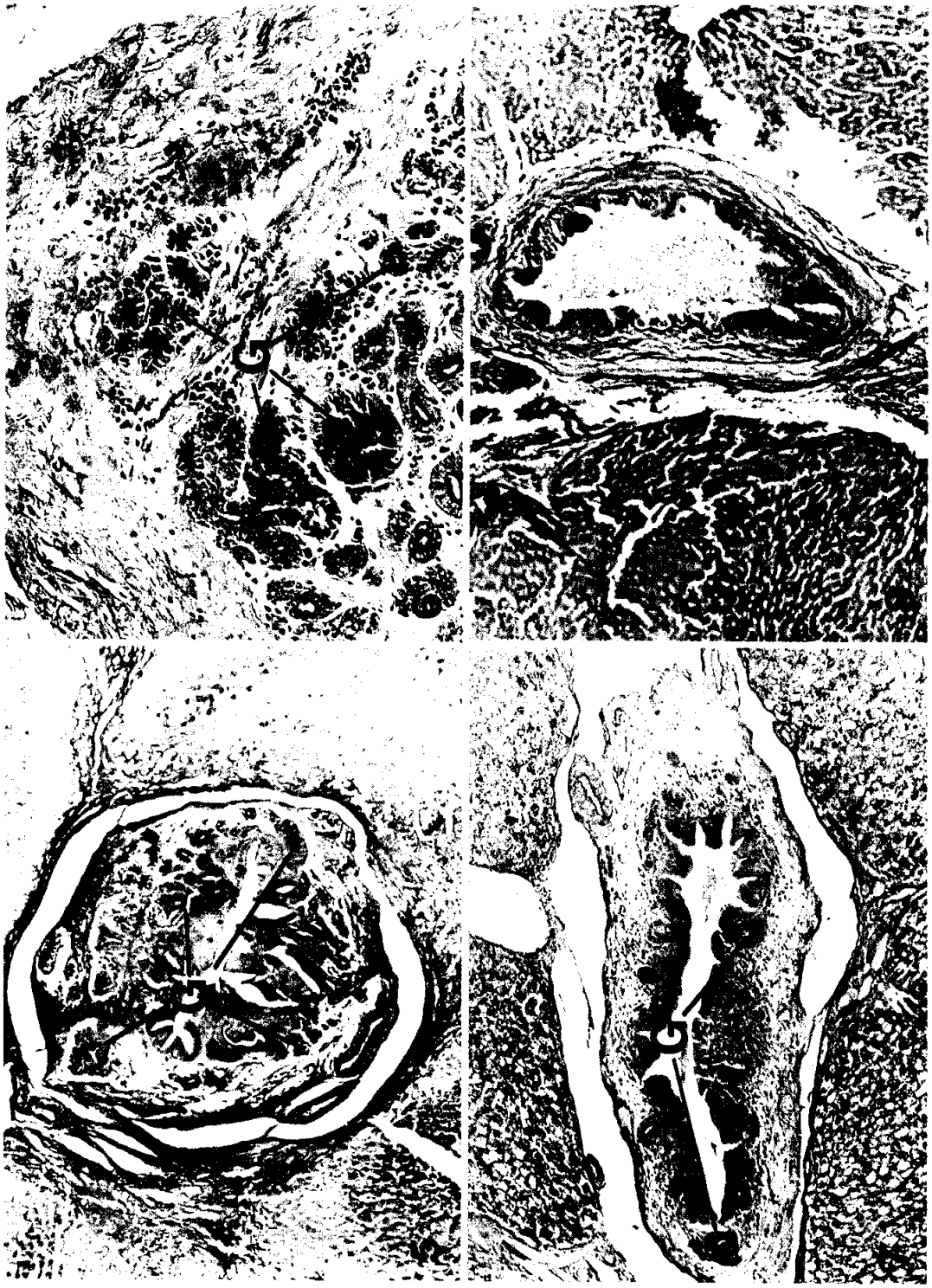

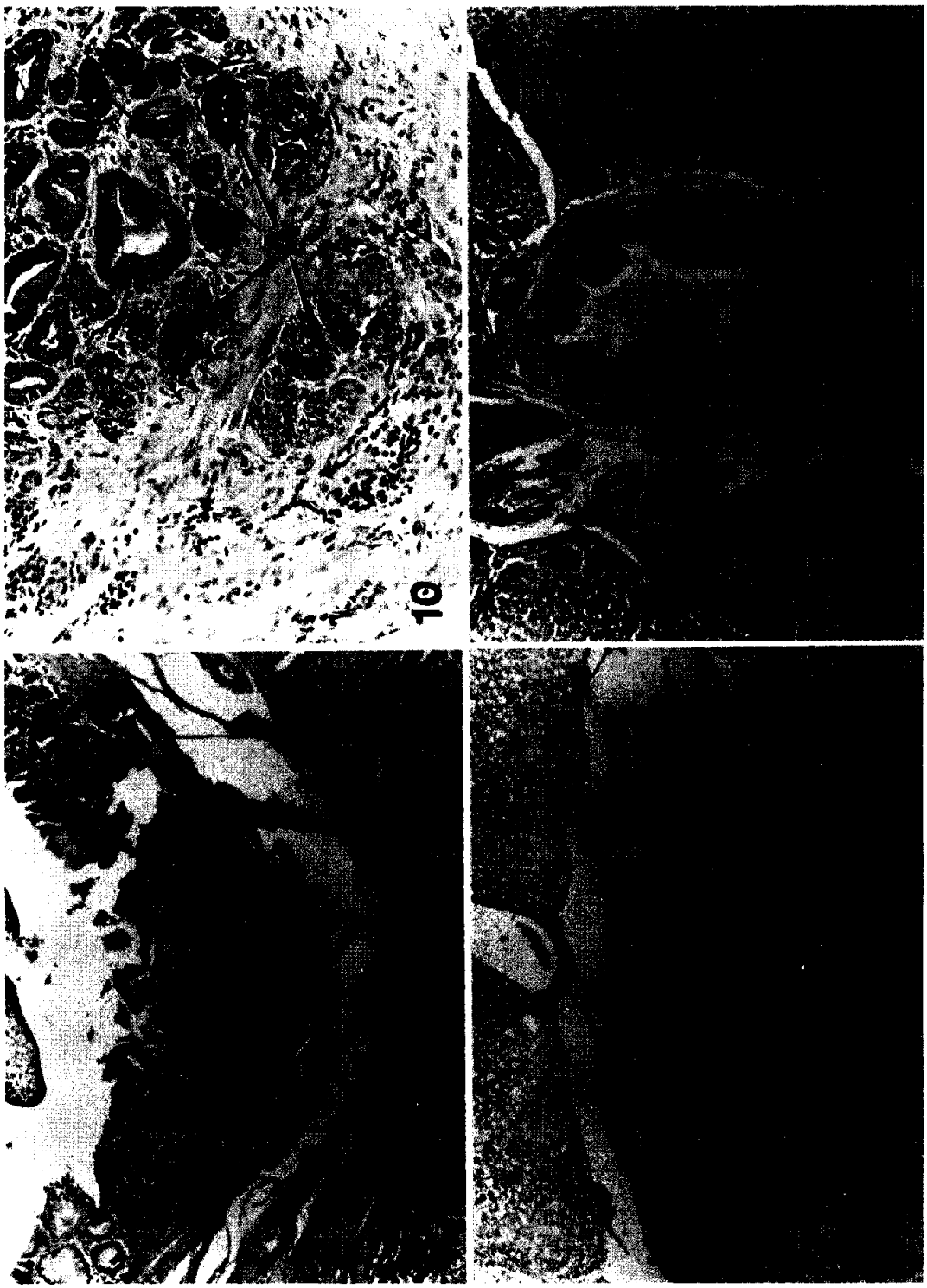
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