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Granuloma Formation Around Inoculated *Schistosoma japonicum* Eggs in the Liver of ddY Mouse

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Abstract

In order to analyze the process of ganuloma formation around *S. japonicum* eggs, we devised a new method to inoculate schistosome eggs via the cecal vein directly into the liver of mouse. About 5,000 *S. japonicum* eggs in 0.5 ml of Hank's solution were injected via the caecal vein into the livers of ddY mice. From 1 to 50 days P.I. the livers were removed and examined histologically. From 5 days P.I., granuloma formation around the eggs was recognized and the mean granuloma volume reached its peak at 21 days P.I. The cellular compositions of granuloma was predominant with mononuclear cells and eosinophiles. In later stages, fibroblasts and multinucleated giant cells of the foreign body type were seen. The granulomas were almost the same as those in the mice with a natural *S. japonicum* infection, except for size.

Key words: *Schistosoma japonicum*, egg granuloma, mouse

Introduction

A female worm of *Schistosoma japonicum*, lying within the gynecophoral canal of the male worm, produces 3,500 eggs per day in the portal and mesenteric vein of the host and may do for years (Warren, 1982). The host inflammatory granulomatous reactions to embolised eggs and subsequent fibrosis are major pathological factors of schistosomiasis. However, it is very difficult to analyze the process of granuloma formation in experimental animals infected with *S. japonicum* or *S. mansoni*, because the worms discharge eggs daily and old and new eggs mixed within the granulomas are only recognizable by the staining pattern of the miracidium within the egg.

von Lichtenberg (1962) succeeded in developing the isolated lung granuloma model around *S. mansoni* eggs which were intravenously inoculated and by this method, the process of granuloma formation due to *S. mansoni* eggs was thoroughly analyzed (Davis *et al.*, 1974; Moore *et al.*, 1977). However, in the case of *S. japonicum* eggs, it has been extremely difficult to develop the granuloma in the mouse lung (Warren and Domingo, 1970; Warren *et al.*, 1975; Old and Mahmoud, 1981).

We devised a technique of injecting *S. japonicum* eggs into a branch of the mesenteric vein of mouse, succeeded in developing the granulomas around eggs, and were able to analyze the process of granuloma formation shown as follows.

Materials and Methods

Mice and *S. japonicum* eggs

ddY mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu).

A Yamanashi (Japanese) strain of *S. japonicum* was maintained in mice and *Oncomelania hupensis nosophora*. Four week old female ddY mice were exposed to 30–40 cercariae per mouse percutaneously on their shaved abdomens. At 8 weeks post-infection, those in-

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fected mice were sacrificed by diethyl ether anesthesia and perfused with 0.2 M NaCl solution to exclude blood from the tissues. The livers and intestinal walls were cut up, stocked in 0.2 M NaCl solution overnight at 28°C, and homogenized 2 times in a Warning blender (Nihon Seiki Co., Tokyo) at a low speed for 15 seconds. The homoginate was successively sieved through stainless wire meshes of 500, 297, 177, 125, 108 μms and finally *S. japonicum* eggs were collected on a 44 μm mesh filter. The collected eggs were washed repeatedly with 0.2 M NaCl solution and stocked in phosphate buffered solution of 0.2 mg/ml streptomycin sulfate (Meiji Seika Kaisha, Ltd., Tokyo) and 2×10^2 U/ml penicillin G potassium (Meiji Seika Kaisha, Ltd., Tokyo) for one hr. After centrifuged at 1,000 r.p.m. for one minute, schistosome eggs were diluted in 10,000/ml of Hank's solution (Nissui Pharmaceutical Co., Ltd., Tokyo).

Injection of schistosome eggs

Six week old male ddY mice were anesthetized by an intraperitoneal injection of 0.3 ml of 0.5% sodium pentobarbital (Dainippon Pharmaceutical Co., Ltd., Osaka). After the abdominal skin was shaved, a median longitudinal incision was made and the caecum was drawn out to expose the caecal vein, a branch of the mesenteric vein. About 5,000 eggs in 0.5 ml of Hank's solution were injected slowly into the caecal vein using a 26 gage needle connected to a 1 ml sterile plastic tuberculin syringe (Fig. 1). As soon as the needle was withdrawn, the wound was pressed with a piece of Gelathrombin (Green Cross Company, Osaka) for several minutes to prevent bleeding from the injection focus. After no hemorrhage was confirmed, the intestine was reinserted into the abdominal cavity and 1.0 ml of phosphate buffered solution of 1×10^2 U/ml penicillin G potassium were injected intraperitoneally. The peritoneum and skin were sutured separately. The mice were then maintained in a sterile environment.

Measurement of granuloma

The livers were examined at time intervals of from 1 to 50 days post-injection (P.I.). Each

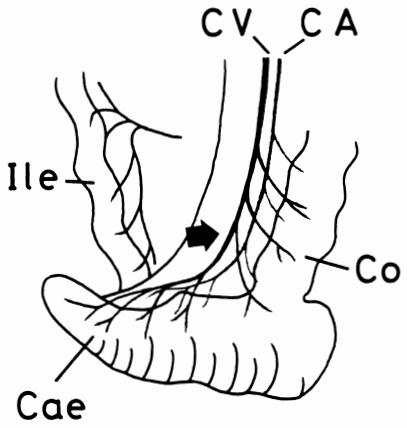

Fig. 1 A schema showing the point of injection place on the caecal vein, a branch of the mesenteric vein: The needle is inserted from the arrow point.
Ile: Ileum, Cae: Caecum, Co: Colon, CV: Caecal Vein, CA: Caecal Artery.

time 6 or 7 ddY mice were sacrificed by ether anesthesia and the removed livers were fixed in Bouin solution. The specimens were embedded into paraffin, routinely sectioned at 4 μm in thickness and stained with hematoxylin and eosin. The granuloma size around a single egg was measured by its largest diameter and its perpendicular diameter with an ocular micrometer. The granuloma volume was roughly calculated from an average of the two measurements based on its spherical nature (Warren, 1966). Per mouse more than 40 granulomas were measured and the mean volume of these granulomas was then calculated. Means and standard errors of the granuloma volume for each group were then calculated from these values.

Results

Without Gelathrombin treatment, more than half of the mice operated on died. Treatment of the injection wound with Gelathrombin succeeded in preventing the mice from bleeding to death.

A chronological change of the mean granuloma volume in the liver after inoculation of the eggs into the cecal vein was depicted in Fig.

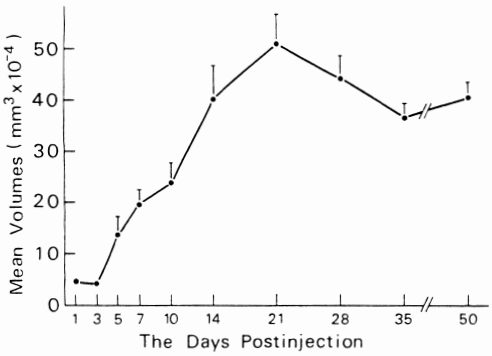

Fig. 2 The mean volume of granuloma around *S. japonicum* eggs post-injection. About 5,000 schistosome eggs in 0.5 ml of Hank's solution are injected into the caecal vein. The volume of granuloma is determined from measurements taken from 6 or 7 mice in each group at time intervals of from 1 to 50 days P.I.
Each point represents the arithmetic mean at the indicated time and each bar represents the limit of standard error.

2. The granuloma around the injected eggs appeared after 5 days P.I. The data at 1 and 3 days P.I. shows the mean volume of schistosome eggs alone. At 24 hrs P.I., no granulomatous reaction around the eggs was noticed (Photo. 1), but in some cases, hepatocellular necrosis was found around aggregated eggs. At 3 days P.I. a single layer of lymphocytes was observed around the eggs, but the granuloma was not yet formed (Photo. 2). From 5 to 21 days P.I., the granuloma began to develop around the eggs within mature miracidium, showing the maximum volume of 50.8×10^{-4} mm^3 at 21 days P.I. (Photos. 3, 4, 5, 6 and 7). After then the granuloma gradually shrinked, however, at 50 days P.I. the granuloma around degenerated eggs was still recognizable (Photo. 8).

The cellular compositions of the granuloma varied in the course of P.I. At 3 days P.I., a layer of small lymphocytes surrounded the entrapped egg in the terminal portal vein. At 5 days P.I., lymphocytes and neutrophiles were main granuloma cells (Photo. 9). From 7 days P.I., eosinophiles were mobilized around the eggs. At 10 and 14 days P.I., plasma cells and macrophages were seen near around the eggs and periphery of granuloma (Photo. 10). At this time, some granulomas were full of eosinophiles. Later, plasma cells and eosinophiles became predominant and simultaneously abundant fibroblasts were also recognized around eggs and multinucleated giant cells of the foreign body type were occasionally seen in the granuloma (Photos. 6, 11 and 12).

Discussion

In order to study the dynamics of granuloma formation around *S. mansoni* eggs, von Lichtenberg (1962) developed the isolated lung granuloma model. Using this model, several investigators have examined the cellular constituents of the host granulomas and elucidated their delayed hypersensitivity nature (Warren *et al.*, 1967; Boros and Warren, 1970). It has also been shown that Sepharose beads conjugated with the soluble egg antigens of *S. mansoni* eggs could induce granuloma (Boros and Warren, 1970; van Mark *et al.*, 1980; Weiss *et al.*, 1986). In contrast, the granulomas did not appear around the similarly injected *S. japonicum* eggs in the lung. Even if mice were sensitized by the intraperitoneal injection of eggs, or pre-infection with *S. japonicum*, granulomas did not appear around the eggs in the lung (Warren *et al.*, 1975).

Recently host responses to *S. japonicum* eggs and antigens were revealed to be quite different in several respects from those to *S. mansoni* eggs (Warren and Domingo, 1970; Warren *et al.*, 1975; Warren *et al.*, 1978). Example differences are that an adult female worm of *S. japonicum* deposits 10 times more eggs than *S. mansoni* and *S. japonicum* eggs are usually deposited in the tissues in aggregates. Immunologically, animals infected with *S. japonicum* display an immediate hypersensitivity reaction to the soluble egg antigens injected into their footpads while *S. mansoni*-infected animals display a delayed hypersensitivity reaction to the corresponding antigens.

Warren *et al.* (1975) reported successful lung

granuloma formation around *S. japonicum* eggs subsequently when mice were pretreated by a subcutaneous injection of eggs or soluble egg antigen. Old and Mahmoud (1981) showed that the pulmonary granulomas are dependent on the integrity of cell-mediated immune responses in the prior sensitized mice. From their results, it is suspected that in the lung model granuloma formation around *S. japonicum* eggs needs prior sensitization. However, they did not suggest whether or not the sensitization is necessary to develop granuloma formation around *S. japonicum* eggs in the mouse liver. Moloney *et al.* (1982) and Cheever *et al.* (1985) reported that antibody and immune complexes were not necessary for granuloma formation in the mice infected with *S. japonicum*. According to their data, the circumoval granuloma in the livers of B-cell depleted mice on the seventh week of infection were of normal size and composition, except for the absence of plasma cells. Further study is needed to determine whether or not granuloma formation around the eggs in schistosomiasis japonica needs prior sensitization.

Ushiyama (1953) investigated the infiltration of cells around the deposited eggs in the liver by injection of living *S. japonicum* eggs into the portal vein of albino rats. However, rats appear to be inferior experimental animals when examining the pathophysiology of schistosomiasis. In the present study, we tried to examine granuloma formation around inoculated schistosome eggs in the mouse liver without pretreatment. We tried to inoculate schistosome eggs into the caecal vein. Joky *et al.* (1978) suggested that the caecal vein was most useful vein to investigate the reactions of inoculated materials in the liver. We succeeded in producing distinct granuloma around schistosome eggs in the liver, but cannot explain why Old and Mahmoud (1981) failed to develop the liver granuloma model, injecting *S. japonicum* eggs into the portal vein. Edungbola and Schiller (1979) reported the striking difference of the nature of granuloma between those in the liver developed by injecting *S. mansoni* eggs via the mesenteric-portal system and those in the lungs developed by injecting eggs via the tail vein. They differed in the chronology of granuloma development, mean size of the granulomas, and cellular composition. In the case of *S. mansoni* the maximum mean sizes of hepatic granuloma occurred at 32 days P.I. on their data, while in the case of *S. japonicum* on the present study, the mean size of those reached its peak at 21 days P.I.

The cellular compositions of the granuloma changed gradually after injection. At an early stage, lymphocytes and neutrophiles were dominant, after which eosinophiles and monocytes took their place in the granuloma. In the later stage, plasma cells, fibroblasts and eosinophiles were the dominant cells. We also investigated multinucleated giant cells of the foreign body type in some granulomas.

Present study showed no need of prior sensitization to form the granulomas around *S. japonicum* eggs. The mean volume of the granuloma around single egg reached its peak at 21 days P.I.

Our method will provide in the future further insights into the pathology of schistosomiasis japonica, especially in the relationship between the granuloma modulation and cellular or humoral immunity.

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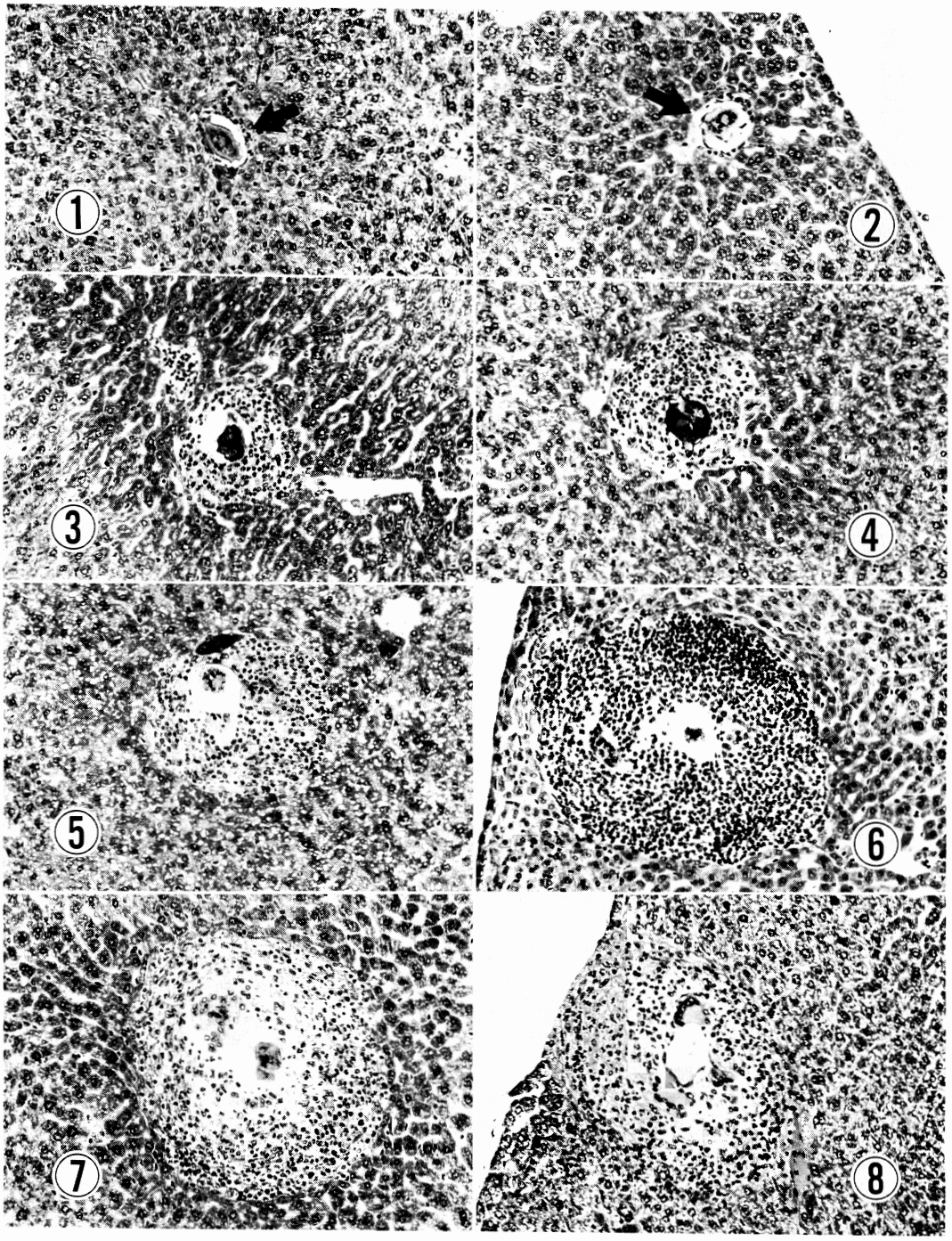
Photo. 1 At one day P.I., schistosome eggs impacts in the terminal portal vein and granuloma formation is not found (An arrow shows the egg). (×180)
Photo. 2 At 3 days P.I., single layer of lymphocytes is formed around egg (An arrow shows the egg). (×180)
Photo. 3 At 5 days P.I., granuloma is found around the eggs with mature miracidium. (×180)
Photo. 4 At 7 days P.I., the size of granuloma is increasing.
Photo. 5 At 14 days P.I., the size of the granuloma is bigger than that of 7 days P.I. Eosinophiles are significant around the eggs. (×180)
Photo. 6 At 21 days P.I., the mean volume of granuloma reached its peak. Eosinophiles and plasma cells are predominant. (×180)
Photo. 7 At 28 days P.I., a multinucleated giant cell of the foreign body type is seen and fibroblasts are increasing around the eggs. (×180)
Photo. 8 At 50 days P.I., the miracidium in the eggs is already destroyed, but granuloma around schistosome eggs is still evident. (×180)
Photo. 9 High magnification at 5 days P.I. Lymphocytes are predominant in granuloma around the eggs. (×700)
Photo. 10 High magnification at 14 days P.I. Eosinophiles are significant in granuloma around the eggs. (×700)
Photo. 11 High magnification at 21 days P.I. Eosinophiles and plasma cells are yet significant around the eggs and giant cells are seen. (×700)
Photo. 12 High magnification at 35 days P.I. Fibrous tissues are significant in granuloma around destroyed eggs. (×700)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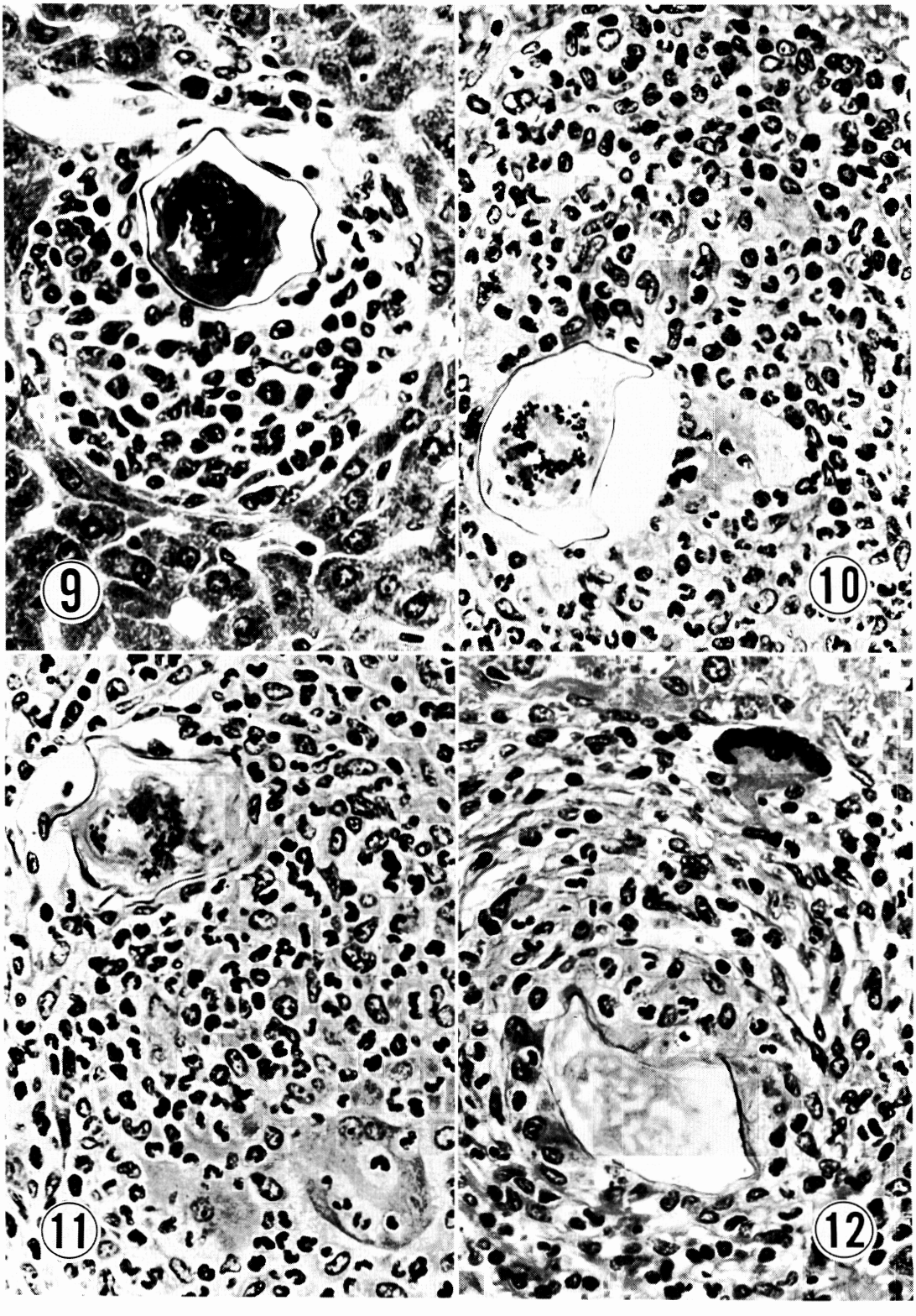
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