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Pathology and Immunology of *Clonorchis sinensis* Infection of the Liver

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ABSTRACT

The existence of clonorchiasis cases among the Asian immigrants and the major clinical and pathologic features encountered in those patients is emphasized. Owing to the longevity of the parasite, clinical symptoms may occur long after endemic exposure. The common manifestations among the immigrants are recurrent pyogenic cholangitis and pancreatitis. The relationship between clonorchiasis and cholangiocarcinoma and the possible pathogenesis of this tumor are discussed. The specific immunologic phenomenon in clonorchiasis is described.

Epidemiology

Clonorchiasis is endemic in the Far East, mainly Hong Kong, China, Taiwan, Japan, Korea, and Vietnam.[6] It was estimated that 19 million people were infected in endemic areas.[22] However, the incidence of clonorchiasis dropped markedly owing to environmental changes in recent years. In Japan, for instance, water pollution eliminates most of the snail hosts of *Clonorchis sinensis*.[22] The composition of manure before use in China kills *Clonorchis* ova in stools, thus interrupting the life cycle.[22]

The infection is caused by consumption of raw, infected fish. The metacercariae in fish flesh are released in the duodenum whence the parasite migrates along the common bile duct and finally stays in the intrahepatic bile ducts.[23] It takes about one month for the metacercaria to mature, when *Clonorchis* eggs can be detected in stools. Eggs hatch only after ingestion by a suitable snail host. In the snail host, the hatched miracidium passes through stages of sporocyst, redia, and cercaria. The cercariae invade the flesh of a suitable fish host and become encysted metacercariae. Approximately 100 species of fresh water fish have been incriminated as the intermediate host of *C. sinensis*. On the other hand, the snail host of *C. sinenis* is more specific: only snails of the genus *Parafossarulus, Bulimus, Alocimua* and *Melanoides* can act as intermediate host. Since these snails have not been found in North America, clonorchiasis will not become endemic in the United States in spite of the influx of Asian refugees. However, Americans may obtain clon-

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orchiasis through long-term and short-term visits to endemic areas.[21]

The long life span of *C. sinensis* (20 to 30 years) creates a problem for the Asian immigrants. There are well-documented cases in which the patients developed clinical symptoms many years after leaving the endemic area.[16] Most of the patients reported are from Hong Kong. Less frequently seen are patients from China and Korea. Laotian refugees usually carry *Opisthorchis viverrini* rather than *C. sinensis*. However, the ova of these two parasites are practically indistinguishable. Accordingly, cases of clonorchiasis are most frequently seen in large cities where there are many Chinese immigrants, such as New York City,[10] Los Angeles,[28] and Montreal.[15] Even in South Carolina, seven to 43 cases of clonorchiasis were reported annually since 1978.[13]

Pathology

The major pathologic findings are seen in the liver. The mechanical injury caused by the suckers of the parasite and chemical stimulation by its metabolic products may contribute to the changes of the epithelium of the bile ducts.[27] The two basic morphologic changes are adenomatous proliferation and goblet cell metaplasia (figure 1).[6,7,8] In chronic infections, the adenomatous tissue is gradually replaced by fibrous tissue, thus causing thickening of bile ducts which can be visible grossly.

The most common complication in clonorchiasis is recurrent pyogenic cholangitis (RPC) or oriental cholangiohepatitis (figure 2).[6,22] This is also the most important clinical finding in immigrants in North America, leading to the diagnosis of clonorchiasis.[1,2,7,17,28] Owing to

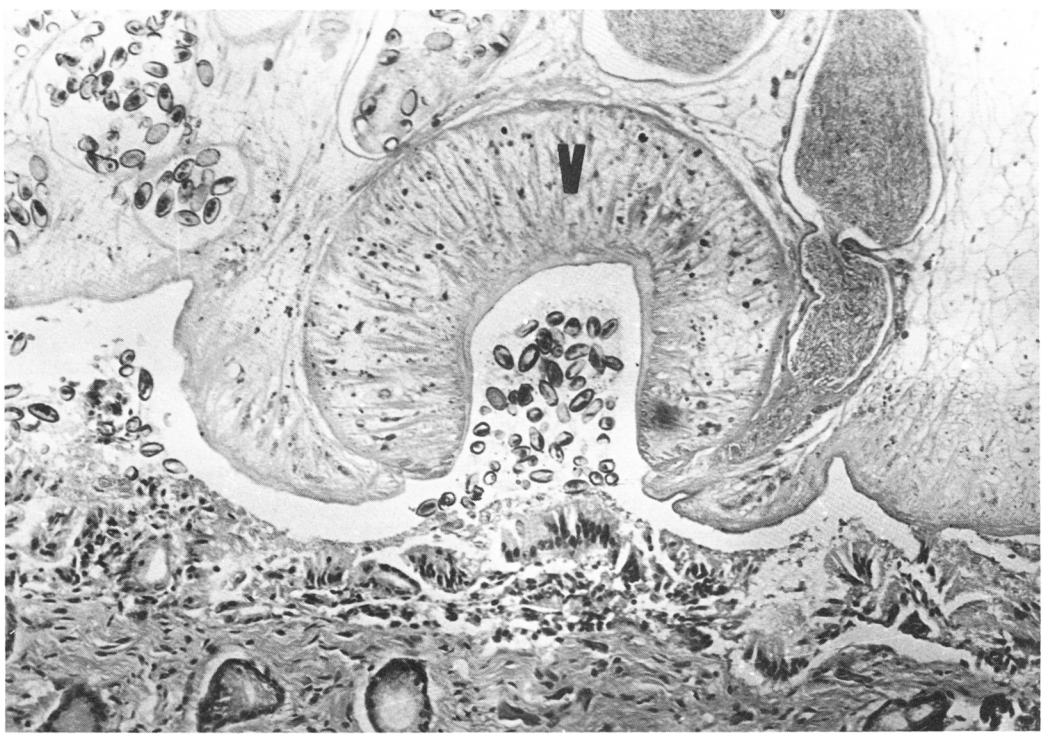


FIGURE 1. An adult *C. sinensis* in an intrahepatic bile duct. The sucking force of the ventral sucker (V) obviously causes mechanical injury to the ductal epithelium with resultant desquamation and superficial ulceration. Adenomatous proliferation of epithelium, as shown here, is the consequence of such damage. Hematoxylin and eosin. (×200). From Sun[22] with permission.

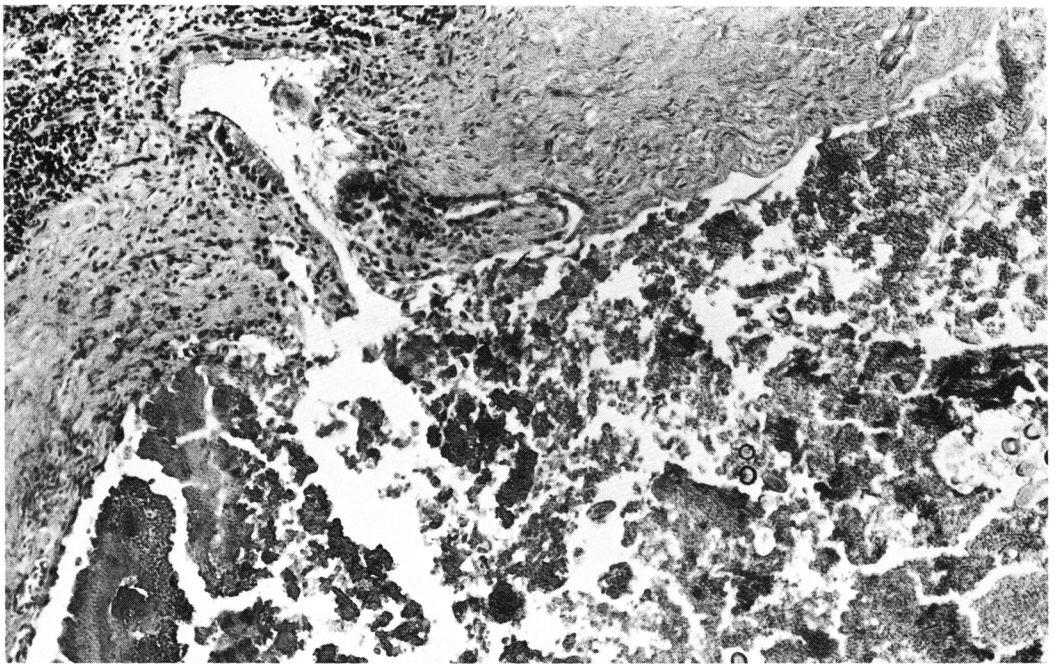


FIGURE 2. A case of recurrent pyogenic cholangitis showing heavy cellular infiltration of a denuded bile duct. The lumen contains inspissated bile mixed with *Clonorchis* ova. Hematoxylin and eosin. (×160). From Sun[22] with permission.

goblet cell metaplasia of the bile duct epithelium, the bile contains high contents of mucous secretion. This mucinous bile and the existence of the parasite and its ova in the bile duct cause cholestasis and furnish a favorable environment for secondary bacterial infection. The causative agent is usually *Escherichia coli* ascending from the intestine. The ova and bacterial colonies may thus form the nidus of an intrahepatic gallstone which is usually muddy. The lack of primary stone in the gallbladder is characteristic of this situation. Owing to cholangitis and partial biliary obstruction, the patients usually show repeated episodes of abdominal pain, fever, jaundice, and hepatomegaly, which are typical for RPC. This clinical entity can be diagnosed by a cholangiogram demonstrating the markedly dilated intrahepatic bile ducts with partial filling defects representing stones or a mass of flukes.[7]

The consequence of RPC is multiple. Most frequently, the infection spreads beyond the bile duct, causing pericholangitis and periductal fibrosis. Liver abscess may occur if the parenchyma is involved by the infectious process. A postnecrotic or biliary cirrhosis may develop on this basis; however, unlike schistosomiasis, "pipe stem" cirrhosis is never seen in uncomplicated cases. Another complication of RPC is stricture of the left hepatic duct which may or may not be accompanied by atrophy of the left hepatic lobe.[28] The most severe sequela is hepatic coma owing to extensive liver damage.

Pancreatitis is another complication which may present as a major clinical manifestation in Asian immigrants.[16] A Chinese immigrant in New York developed acute pancreatitis 25 years after endemic exposure.[16] The patient did not respond to symptomatic therapy and recovered only after anthelminthic treatment. In Hong Kong, over one-third of clonorchiasis cases had pancreatic involvement.[4] However, the pa-

tient may not have any clinical evidence of pancreatitis. On the other hand, clonorchiasis is the causative agent in 83 percent of patients with pancreatitis of unknown etiology in Hong Kong.[12] This *Clonorchis*-related pancreatitis usually develops one to three hours after a rich meal, which may have stimulated excess flow of pancreatic juice in a partially obstructed pancreatic duct, leading to autodigestion.[12] The pathology of pancreatic clonorchiasis is similar to the hepatic lesion, namely, adenomatous hyperplasia of ductal epithelium (figure 3).[6] Squamous metaplasia is also frequently demonstrated and is characteristic of pancreatic clonorchiasis.[22] When acute pancreatitis occurs, features of acute inflammation are present.

The most grave consequence of clonorchiasis is cholangiocarcinoma.[3,5,14] The causative role of clonorchiasis in the development of this tumor is supported by epidemiologic statistics, i.e., cholangiocarcinoma is more frequently seen in clonorchiasis endemic areas than non-endemic areas, and *Clonorchis* infection is seen in 94.7 percent of cholangiocarcinoma cases as compared to 31.7 percent in a control population in Hong Kong.[3] Cholangiocarcinoma has also been experimentally produced in animals infected with *Clonorchis*.[8,9] The fact that cholangiocarcinoma usually develops in the second order intrahepatic bile duct where *Clonorchis* is located and that adenomatous proliferation of ductal epithelim can be seen side by side with malignant changes (figure 4) is also supportive of the relationship between these two entities.

The exact pathogenesis of cholangiocarcinoma is still unknown. It seems likely that *Clonorchis* infection is only a predisposing factor by inducing proliferation of the epithelium of bile ducts.[5]

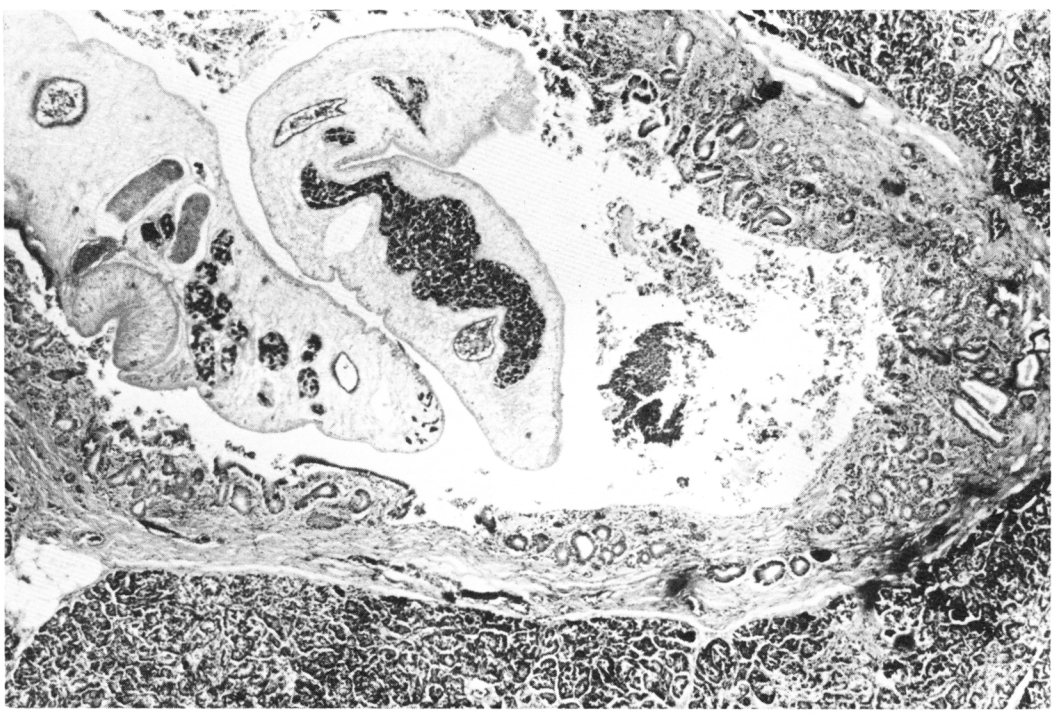


FIGURE 3. Two sections of an adult *C. sinensis* in a pancreatic duct which is partly denuded with maked adenomatous hyperplasia. Hematoxylin and eosin. (×50). From Sun[22] with permission.

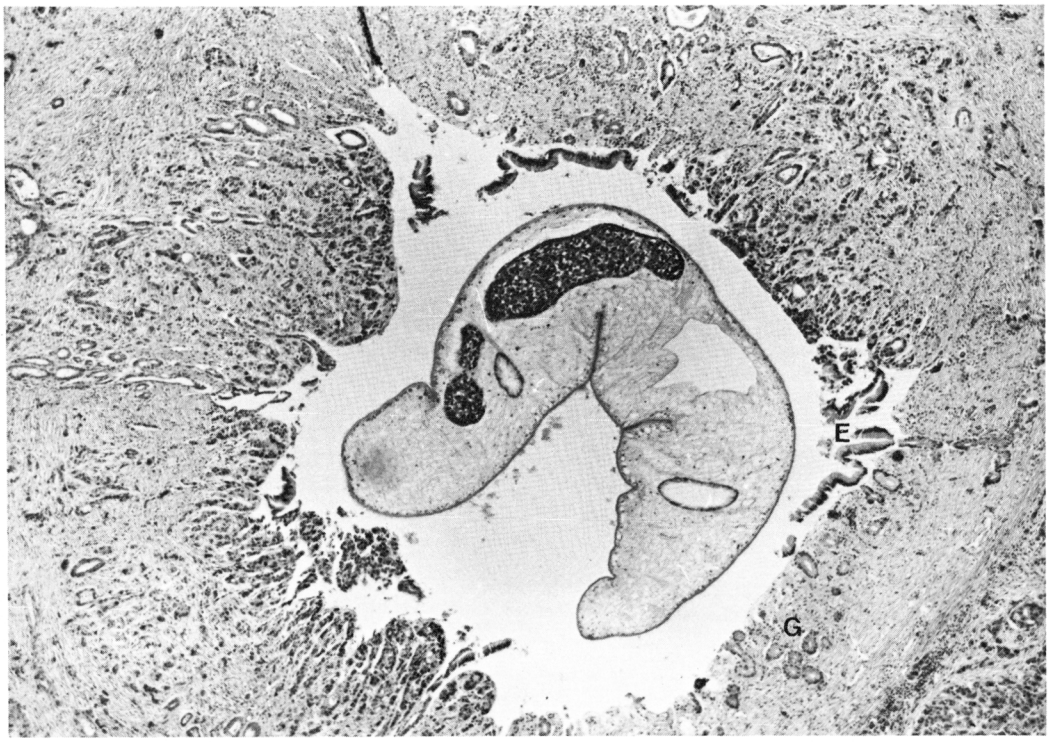


FIGURE 4. A case of cholangiocarcinoma showing a cross section of an adult *C. sinensis* in the lumen of a cancerous bile duct. Note the coexistence of normal epithelium and malignant cells. Hematoxylin and eosin. (×50). From Sun[22] with permission.

The proliferating epithelium may be susceptable to the action of carcinogen(s) present at levels insufficient to induce cholangiocarcinoma in non-infected individuals. Hou has suggested that the metabolic or degenerative products of the parasite and/or a bile component altered chemically by the parasite can be the origin of carcinogenic substances.[8,9] However, Flavell argues that if this is true, the carcinogen(s) should be accumulated in the gallbladder and induce tumor at this site.[5] Another possible carcinogen is aflatoxin, a mycotoxin commonly found in fungus infected foodstuffs.[5] Although aflatoxins can produce cholangiocarcinoma in primates experimentally, they are more frequently linked to hepatocellular carcinoma.[5] More recently, attention has been drawn to the carcinogenic effect of nitrosamines which are found commonly in preserved foodstuffs in the Far East.[5,14] In southern China and Hong Kong, it has been found in salted fish and dried shrimps. Although the pathogenesis of cholangiocarcinoma is still inconclusive, it is reasonable to assume that the etiology is multifactorial in origin.

Clonorchis-associated cholangiocarcinoma is characterized by prominent mucin secretion. It is also frequently accompanied by extensive fibrosis. Although the tumor can be multicentric, it is usually located near the hilum of the liver. The parasite is frequently found in the bile duct which is surrounded by the tumor.

Unlike schistosomiasis in which the ova are the major cause of pathologic lesions, *Clonorchis* eggs are inert as they are impermeable and the antigens se-

creted by the miracidium do not reach the host tisue. In a study by Sun of 525 *Clonorchis*-infected cases, only three cases showed egg-induced lesions: eosinophilic granuloma in the gallbladder (figure 5), giant cell reaction in the liver, and pulmonary embolism.[19] An additional case of egg-granuloma in the portal areas of the liver was reported recently by Sun.[21] In these cases, eggs were partly damaged by the acute inflammatory process; thus, the antigens released by the miracidia induced tissue reactions.

A rare complication of clonorchiasis is pulmonary hypertension.[11] This condition is usually seen in patients with RPC in whom infected necrotic tissue from the liver is carried by the blood stream to the lung and causes repeated microembolization of small pulmonary blood vessels, leading to pulmonary hypertension.

Immunology

The immunologic response in *Clonorchis* infected patients is an interesting phenomenon. Humoral antibody can be detected by different techniques such as immunofluorescent technique, gel-diffusion, indirect hemagglutination, and circum-adult precipitation.[18] These antibodies may show detrimental effects on the adult worms *in vitro*,[20] but they are not protective to the host *in vivo*.[24] Therefore, patients may contract repeated infections, and hundreds or thousands of flukes can be recovered from one patient. The non-protective nature of these antibodies is also evidenced by comparing the worm-burden in artifi-

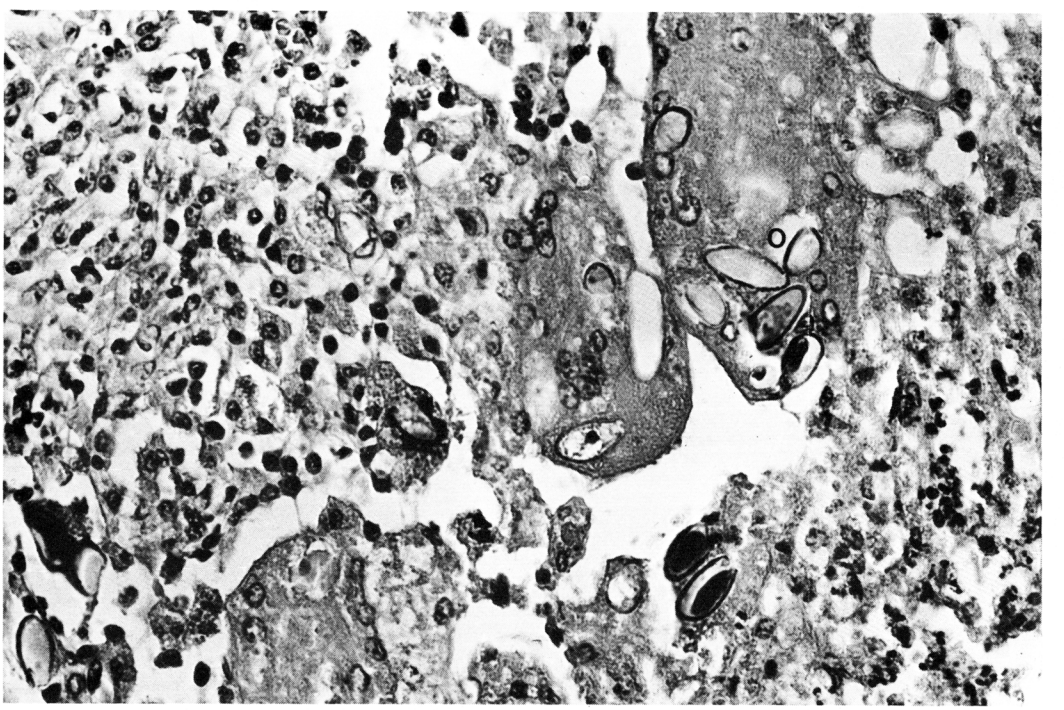
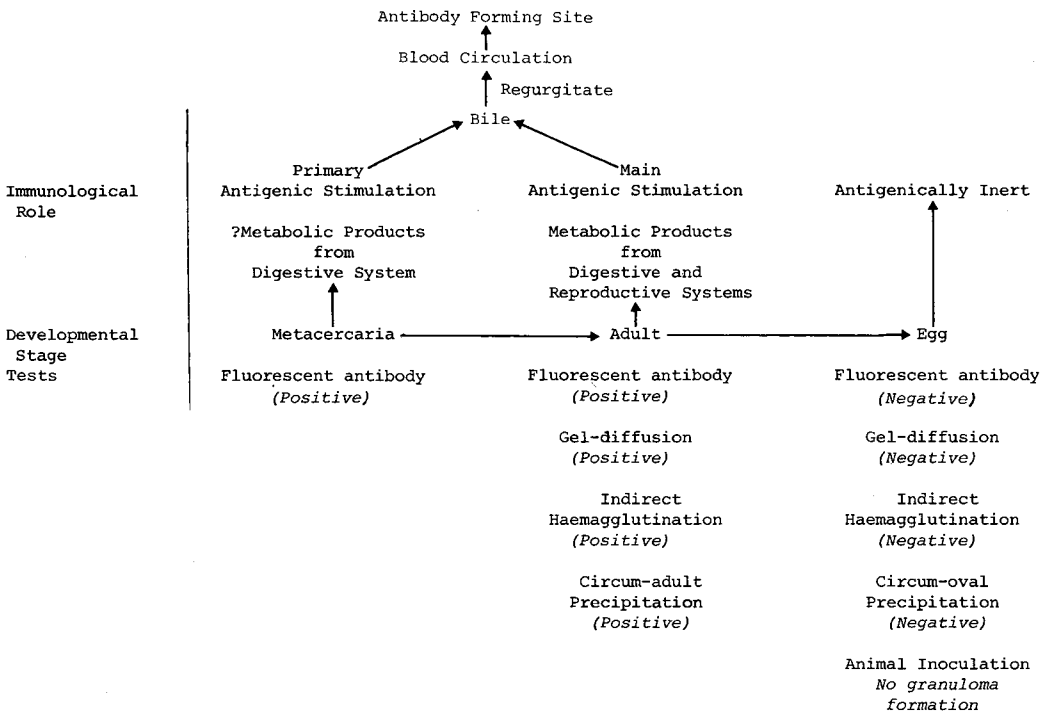


FIGURE 5. A case of eosinophilic granuloma of gallbladder caused by *Clonorchis* infection. Note *Clonorchis* ova are seen inside and outside multinucleated foreign body giant cells. The background is infiltrated by eosinophils and histiocytes. Hematoxylin and eosin. (×800).

TABLE I

Postulated Mechanism of Antibody Production in Clonorchiasis



cially immunized and non-immunized animals, which showed no statistically significant difference between these two groups.[24]

The antibodies detected in patients and experimentally infected animals are mainly induced by the metabolic products of the parasite.[24] Somatic antigens are not effective antigens, and egg antigens are not responsible for antibody production in patients and experimental animals. By immunofluorescent studies, the gastrointestinal tract and the reproductive organs of the parasite react with antibodies from patients and infected animals, thus identifying the sources of metabolic products that elicit antibody formation.[25] The exact effective fraction in metabolic products of the parasite was not identified, but they contain protease, carbohydrase, and esterase.[26] The metabolic antigens are mainly from mature adult worms, but metacercariae also show weak reaction to infected human or animal sera.[25] Since antibodies are detected four weeks after infection in rabbits, guinea pigs, and rats, i.e., soon after the metacercariae have developed into the adult form, the initial antigenic stimulation is probably coming from metacercariae. The relationship between the various developmental stages and antibody production is illustrated in table I.

Summary

Clonorchis sinensis is still an important liver fluke in Asia, affecting millions of patients. Because of its long life span, clonorchiasis can be seen in Asian im-

migrants in North America long after their endemic exposure. When patients from endemic areas show symptoms of recurrent pyogenic cholangitis, biliary obstruction, or acute pancreatitis, clonorchiasis should be included in the differential diagnosis. A diagnosis can be easily established by stool examination for eggs, but sometimes duodenal drainage is necessary for the detection of eggs. In the case of RPC, a cholangiogram showing dilated intrahepatic bile ducts with partial filling defects is characteristic and diagnostic for those from the endemic areas. In chronic cases, praziquantel can be used to eliminate the flukes.[13] In acute cases, however, surgical intervention should be considered.[28]

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