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CAPILLARIA HEPATICA: A CAUSE OF SEPTAL FIBROSIS OF THE LIVER

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Fine, long, fibrous septa were observed as a late change developing in the acinar zone III of the liver of rats experimentally infected with the helminth Capillaria hepatica. *Hepatic septal fibrosis begun 30 days after inoculation of embryonated eggs into the stomach of rats and became clearly evident from the 40th day onwards. Experimental observation was undertaken for 170 days. Septal fibrosis increased progressively with time and was most marked when the parasitic nodules formed around larvae, disintegrating worms and eggs were involuting.*

Septal fibrosis of the liver has not been previously recognized as a manifestation of hepatic capillariasis.

The presence of sequestered parasite antigens, probably being slowly released within the liver, appears to be a major factor in the pathogenesis of hepatic septal fibrosis observed in rats with C. hepatica *infection.*

Key words: *Capillaria hepatica* – hepatic fibrosis – septal fibrosis

Capillaria hepatica is a frequent and cosmopolitan nematode that parasitizes mammals, mainly rodents and especially rats. Rarely human infections have been reported, the cases being usually fatal (Cochrane et al., 1957; Ward & Dent, 1959; Piazza et al., 1963). Parasitological and pathological aspects of capillariasis have been extensively studied in natural and experimental infections. Good sequential morphological descriptions of the hepatic changes observed in several animal hosts have emphasized the focal nature of the lesions that become encapsulated, fibrotic and calcified with time. In such lesions the migrating larvae, adult worms and their eggs die and progressively disintegrate causing chronic inflammation and fibrosis (Solomon & Soulsby, 1973; Slais, 1973; Raybourne et al., 1974; Solomon & Grigoms, 1976). End stage cirrhosis can be the outcome of severe infection (Luttermoser, 1938), but as Lammler et al. (1974) pointed out, interpretation of these late lesions is difficult, since, at this time, egg production is ceasing and acute focal lesions are no longer observed.

During histological examination of the liver of rats with natural *C. hepatica* infection a peculiar change was frequently observed. It consisted in the formation of thin and long fibrous septa that dissected the liver parenchyma creating a cris-cross pattern reminiscent of the pig-liver. Apparently, this change had no direct anatomical relation to the focal parasitic lesions and sometimes occurred in areas remote from parasite nodules.

Septal fibrosis is a non-specific reaction of the liver to chronic injury (Andrade, 1991). It appears as a prominent finding in certain chronic liver diseases of man such as schistosomiasis (Cheever & Andrade, 1970), chronic septal hepatitis (Gerber & Vernace, 1974) and incomplete septal cirrhosis (Sciot et al., 1988). Its pathogenesis is poorly understood.

The present morphological investigation was planned to see whether septal fibrosis could also be observed in experimental *C. hepatica* infection and, if so, to describe its morphol-

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ogy, to follow its kinetics and to investigate into its pathogenesis.

MATERIALS AND METHODS

C. hepatica eggs were collected from the livers of naturally infected rats (*Rattus novergicus* and *Rattus rattus*) captured in the city of Salvador, Bahia-Brazil. The infected livers were homogenized in a domestic blender and the eggs isolated through repeated washing and sedimentation in saline. Clean eggs were placed on filter paper previously humidified with 0.5% formalin in a Petri dish and left at room temperature (24-26 °C) for 28 days. After this time the majority of eggs became embryonated. An inoculum of approximately 1,200 eggs per animal was suspended in 1 ml of saline and administered by gavage to normal young Wistar rats (initial weight: 180 g) of both sexes.

Animals were kept in individual cages with free access to a balanced commercial pellet diet (Nuvital, Curitiba, PR-Brazil) and water.

A few animals presented external signs of disease, such as weight loss, brittle hair and diminution of mobility, during the first two weeks after inoculation, and two of them died, otherwise infection was well tolerated throughout the time of experiment.

Two animals of each sex were killed on the 10th, 20th, 30th, 40th, 60th, 90th, 120th and 170th days after infection. The liver was removed and small fragments were fixed in three different ways:

a) For *histological studies* the fragments were fixed in Bouin's fluid and routinely embedded in paraffin. The sections were stained with hematoxylin and eosin, picrosirius-red for collagen, PAS, Weigert-Van Gieson for collagen and elastic tissues and Gomori's silver impregnation method for reticulum.

b) For *immunochemical studies* the fragments were immediately placed in liquid nitrogen for a few minutes and them kept frozen in air-tight boxes until the moment they were sectioned in a cryotome at –20 °C. The sections were submitted to the indirect immunofluorescence technique for the demonstration of *C. hepatica* antigen(s), collagen isotypes (I, III and IV), laminin, fibronectin and elastin. Anti-*Capillaria* antibodies were obtained from the sera of rabbits which were experimentally infected with *C. hepatica* five months previously. Antibodies in sera recognized eggs and adult worms in both paraffin and cryotome sections up to a dilution of 1:64. Normal rabbit sera were used as controls. Antibodies against collagen isotypes, fibronectin, laminin and elastin were a gift from Dr J. A. Grimaud and Mme Sylviane Guerret, Institut Pasteur, Lyon, France. Details concerning their preparation and tests of specificity appear elsewhere (Andrade et al., 1992).

c) For *electron microscopic investigation,* tiny pieces of liver were fixed in 2% glutaldehyde in 0.2M cacodylate buffer and post fixed in 1% osmium tetroxide. Epon-embedded, semi-thin sections were stained with toluidine blue and studied by high resolution optical microscopy. Selected areas were sampled for ultra-thin sectioning in an Ultracut Reichert ultramicrotome. Sections were placed on uncoated copper grids, contrasted with lead nitrate and uranyl acetate and examined using a Zeiss E9 electron microscope at 50 mv.

RESULTS

Gross hepatic lesions were uniform in number and distribution for all the animals, and consisted of whitish dots and small nodules which were regularly spread throughout the external and cut surfaces of the liver. No evident gross changes were noted in the spleen, intestine or any other organ. Animals killed on the 40th day of infection showed a mild diffuse thickening with wrinkling of the liver capsule and an increased consistency of the organ thereafter.

Microscopically, the lesions were essentially focal and first occurred around disintegrating larvae. They consisted of hemorrhagic, hyaline and lytic necrosis plus infiltration by numerous eosinophils, polymorphonuclear neutrophils and a few lymphocytes and macrophages. One month after infection adult worms and eggs were present. By this time parasite-related lesions became granulomatous, with predominance of macrophages, fibroblasts, giant cells and some eosinophils. By the 40th day a thick fibrous capsule was formed encircling each focal lesion. A progressive shrinkage and fibrous replacement, with resorption of worm remnants and foci of calcification, marked the evolution of these focal lesions. Eggs persisted and became concentrated within the condensed fibrous scars, as seen from the 90th day on.

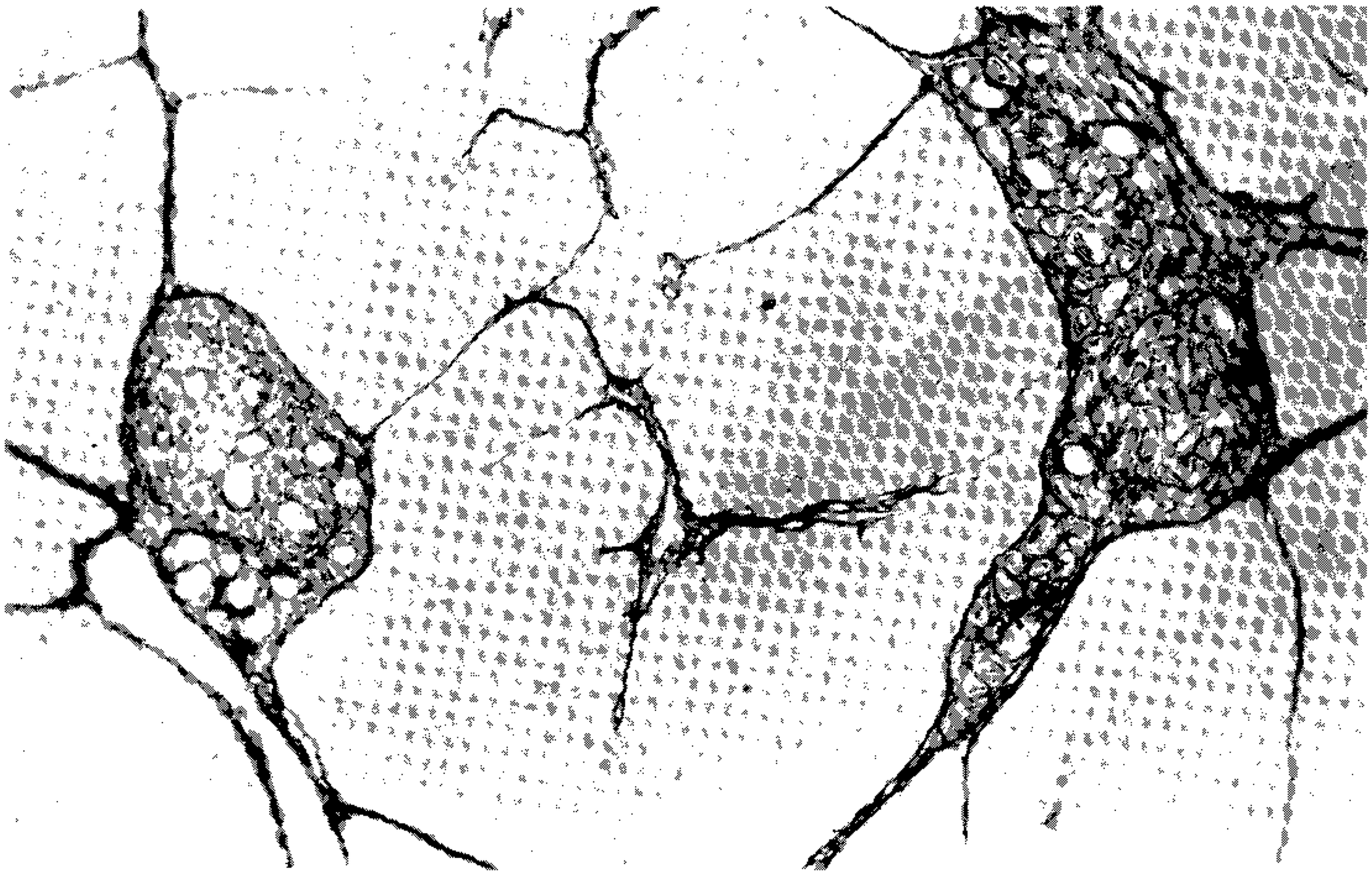

Fig. 1: two focal fibrotic nodules containing *Capillaria hepatica* eggs in the liver of a rat. Note the presence of fine, long fibrous septa dissecting the liver parenchyma. Picrosirius-red staining, 150 X.

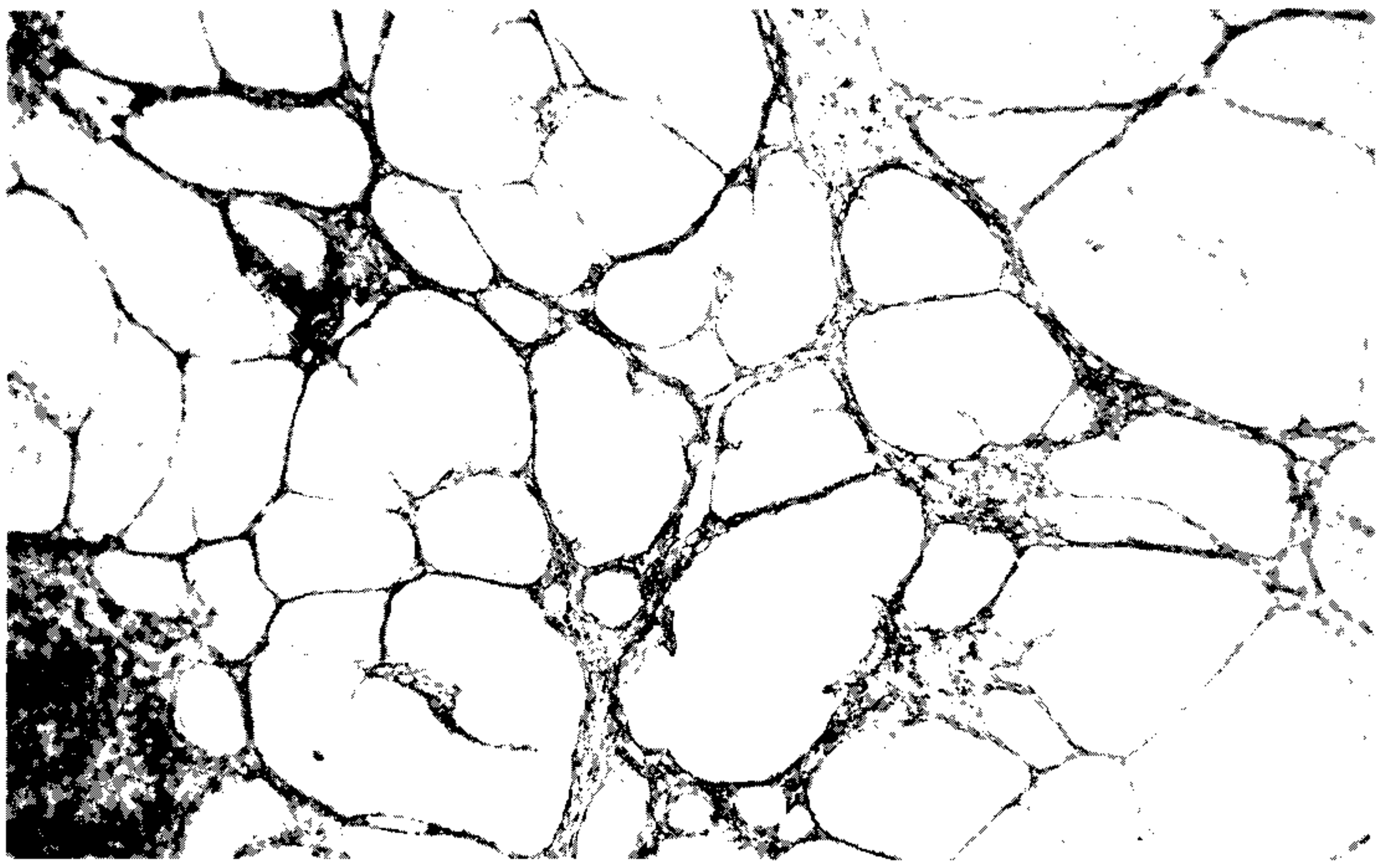

Fig. 2: advanced septum formation in the liver of a rat infected with *Capillaria hepatica*. The marked septation of the liver parenchyma has a cirrhotic appearance, however the latter maintains its acinar arrangement and is formed by one-cell thick plates. Picrosirius-red staining, 100 X.

The beginning of fibrous septation was noted on the 30th day after inoculation. Septa appeared as thin portal rays sprouting for a short distance toward the liver parenchyma and were better recognized with special stains (reticulum, picrosirius-red). However, by the 40th day they were more evident, and appeared as thin fibrous septa connecting portal spaces to portal spaces and to central canals, especially located at the zone III of the hepatic acini (Fig. 1).

The liver parenchyma maintained its normal architecture, with no signs of liver-cell degeneration or regeneration. From the 60th day on, septal fibrosis appeared as the main lesion, while the focal parasitic nodules underwent progressive fibrous replacement and shrinkage. After the 90th day following inoculation, the liver exhibited marked septation and had the appearance of a pig-liver. Sometimes, septation was so intense that a cirrhosis-like picture was formed, but the hepatic parenchyma was normal-looking and did not present regenerative or active hepatocellular nodules (Fig. 2).

Immunofluorescence staining demonstrated the predominant presence of type III collagen in the septa and of type I collagen in the capsule of the parasite nodules. The presence of collagens belonging to Types I and IV was also detected in areas of septal fibrosis, as well as elastin, fibronectin and laminin. The staining for fibronectin gave the best results. Rabbit anti-*C. hepatica* antibodies failed to demonstrate parasite antigen in fibrous septa or within sinusoidal cells following indirect immunofluorescence reaction. However, a positive reaction was constantly observed within the parasitic nodules up to the end of the experimental period. Eggs, adult worms and parasite remnants were strongly and specifically fluorescent (Fig. 3).

High resolution optical microscopy combined with electron microscopy revealed that the fibrous septa were bordered by sinusoids or directly by liver cords. They contained parallel collagen fibrils, blood vessels and several types of connective tissue cells, especially fibroblasts, mast cells and fat-storing cells (Fig. 4). Some of the latter contained few cytoplas-

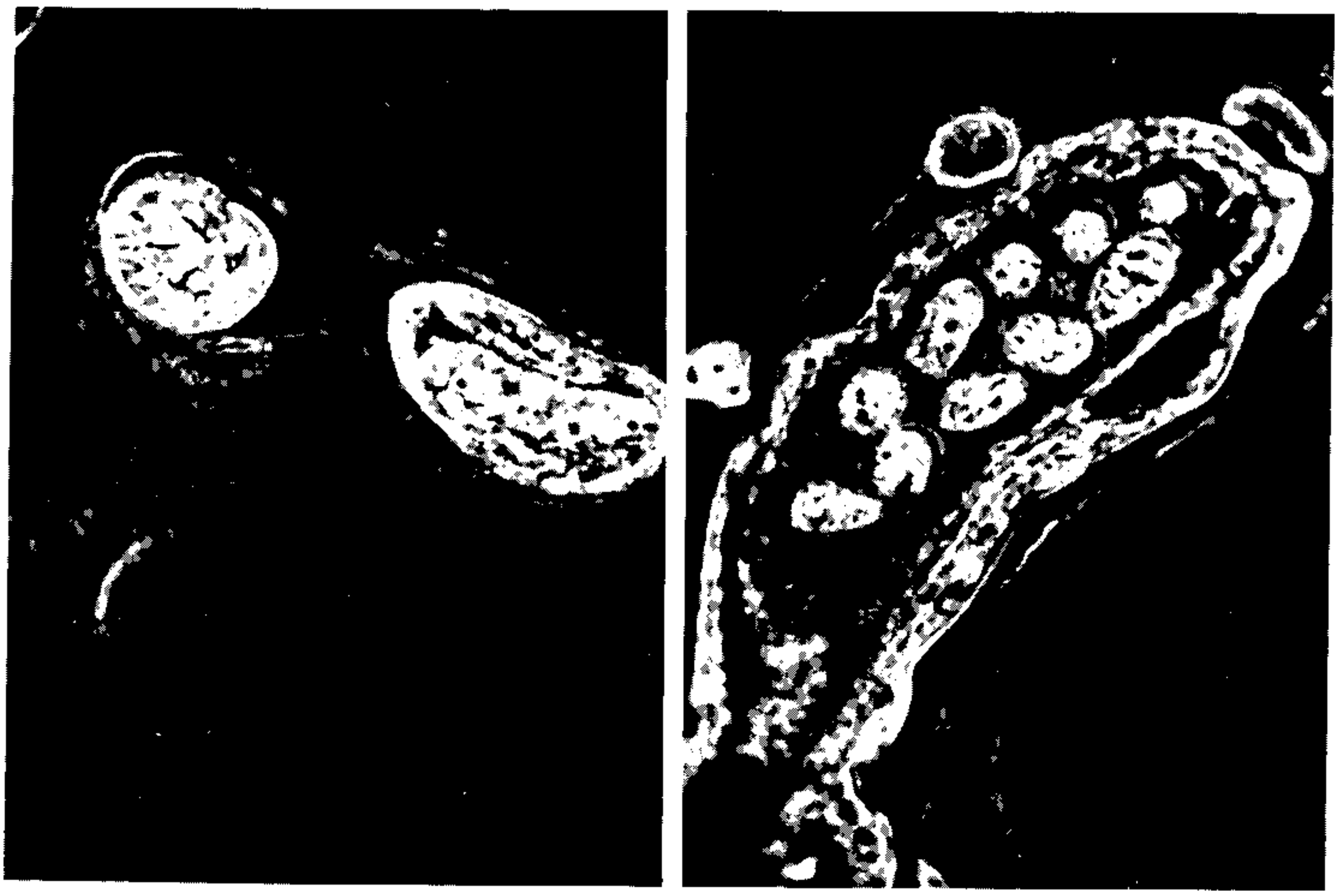

Fig. 3: developing worms (right) and eggs, within and outside a worm (left), appear fluorescent demonstrating the presence of *Capillaria hepatica* antigen. Cryostat sections of rat liver treated with rabbit anti-capillaria antibodies followed by goat-anti rabbit IgG. 400 X.

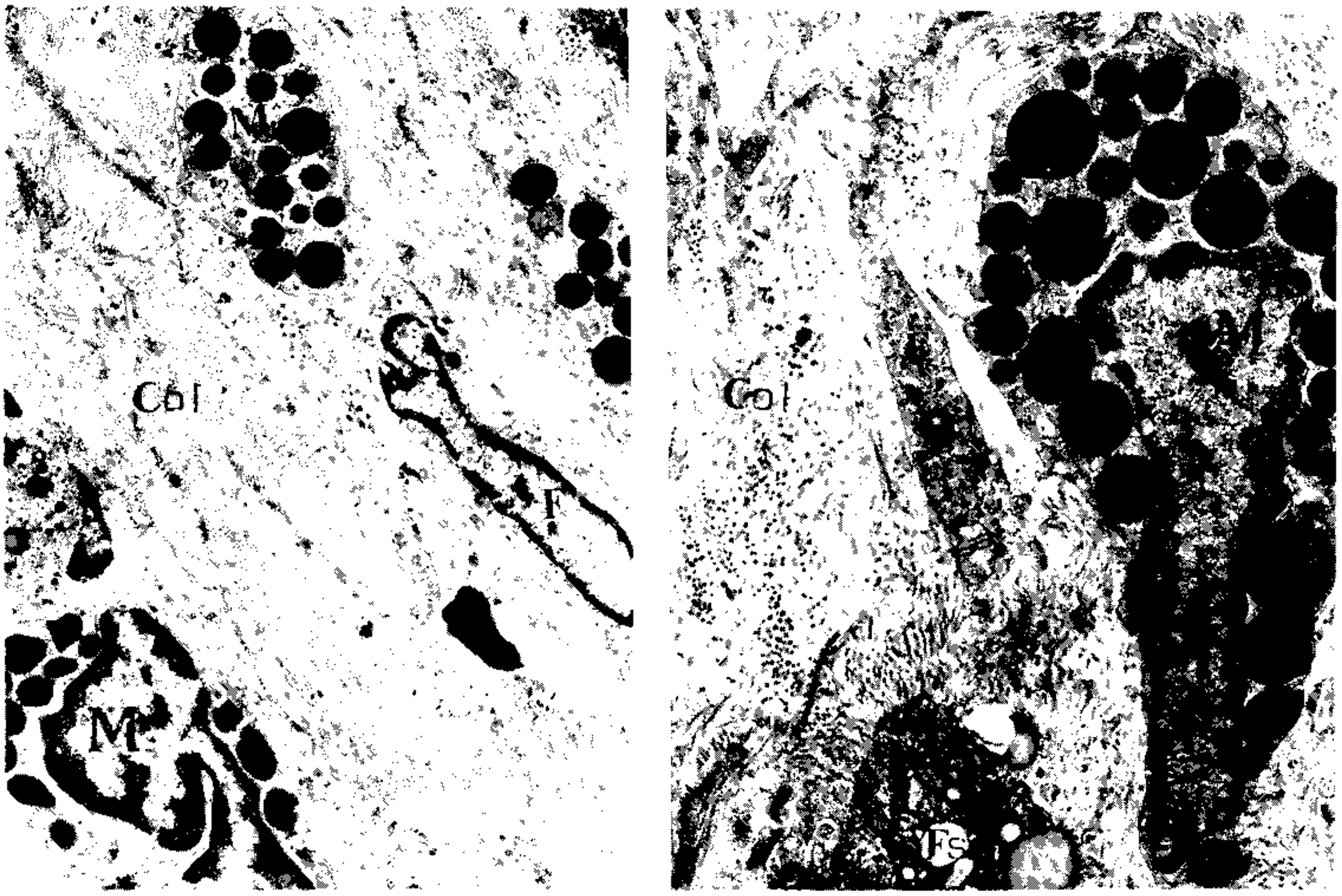

Fig. 4: ultrastructure of fibrous septum showing abundant collagen fibrils (Col), fibroblast (F), fat-storing cells (Fsc) and mast-cells (M). E.M., Right, 4,400 X, Left, 7,000 X.

mic fat droplets and presented hypertrophic endoplasmic reticulum and dilated Golgi vesicles and appeared in the vicinity of collagen fibrils and fibers. Away from the fibrous septa the hepatic sinusoid and its cells showed only mild changes, represented by an increased amount of lipofuscin granules within some littoral cells and a slight increase in the amount of collagen fibrils in the perisinusoidal space.

DISCUSSION

Hepatic fibrosis and cirrhosis have been described in association with *C. hepatica* infection in several hosts, including man (Ewing & Tilden, 1956; Cochrane et al., 1957; Ward & Dent, 1959). However, septal fibrosis of the liver has not been previously described as a manifestation of capillariasis.

Recognition of hepatic fibrosis as belonging to a septal type has more than an academic or semantic connotation. It has pathogenetic implications.

Several experiments in which foreign proteins were repeatedly injected into different animal species have resulted in septal fibrosis of the liver (Blackwell, 1965; Paronetto & Popper, 1966; Ballardini et al., 1985; Andrade, 1991). In some experiments the injected proteins (antigens) have been demonstrated inside sinusoidal phagocytic cells of the liver (Kupffer cells), months or years after the last injection (Garvey & Campbell, 1957; Fennel, 1965). In some cases septal fibrosis following foreign protein injections was preceeded by shock and liver-cell necrosis (Fennel, 1965). However, pure septal fibrosis without liver cell degeneration or regeneration has been obtained in rats repeatedly injected with pig serum (Rubin et al., 1968). In all these experiments the common denominator seems to be a chronic or repeated challenge with foreign proteins during a relatively prolonged period. Such conditions can be assumed to be present in our rats infected with *C. hepatica*. The encapsulated focal lesions within the liver contained eggs and parasite debris that reacted *in vitro* with antibodies raised against *C. hepatica*. Besides the dormant eggs, capillaria antigens appear to be associated with worm debris inside the involuting parasitic nodules. As such debris is being progressively removed, probably the antigens are slowly and constantly being released. The eggs of *C. hepatica* do not differentiate within the liver and are apparently metabolically inactive. However their ability to sen-

sitize the host has been demonstrated and found to be similar in this regard to that of *Schistosoma mansoni* eggs (Solomon & Soulsby, 1973). In addition, antigenic material can be demonstrated within parasitic nodules at least 170 days after inoculation.

Some foreign materials, probably antigen-antibody complexes, can be taken up by Kupffer cells and other phagocytic cells in the hepatic sinusoids. There is evidence that such cells can then be stimulated to release fibrogenic cytokines such as transforming growth factor-beta, interleukin-1, fibroblast growth factor, etc (Gressner & Zerbe, 1987; Arenson et al., 1988; Kovacs, 1991; Manthey et al., 1992). The interactions of these cytokines with fat-storing cells (Ito cells) can cause the latter to be activated, to differentiate into myofibroblasts and fibroblasts and to secrete various matrix components, especially collagens (Friedman et al., 1985; Davies & Vucic, 1989; Gressner & Bachem, 1990). Several matrix components were found within the fibrous septa in the present study. Fibronectin and type III collagen appeared predominating with the immunofluorescent techniques applied. However, the study was merely qualitative, and besides the notion that variable types of collagens and associated proteins were present within the septa, little more can be said on this regard.

The acinar zone III where septal fibrosis is especially formed and located is a zone of low oxygen tension where collagen formation is assumed to be enhanced (Popper & Undenfriend, 1970).

Septal fibrosis is a prominent microscopic finding of some parasitic diseases. It is an outstanding finding in human hepatosplenic schistosomiasis (Cheever & Andrade, 1970). In hepatic fascioliasis, fibrosis of the septal type has been described as "monolobular fibrosis", and is a major change during chronic infection that can progress to cirrhosis (Dargie et al., 1974). To these two examples of septal fibrosis associated with parasitic diseases affecting the liver can now be added a third: hepatic capillariasis. In all of these there is antigenic parasitic material present in the liver. These antigens can apparently be sequestered inside the liver for prolonged period of time, even when the infection is no longer active. Present findings strongly suggest that this can be an important pathway to septal fibrosis formation. Of course further investigation is necessary to better substantiate this point.

C. hepatica infection in rats may be a good model for the study of septal fibrosis of the liver. In other models, especially in the pig-serum model (Andrade, 1991), not all treated animals develop septal fibrosis. In the present investigation all *C. hepatica* infected rats developed septal fibrosis by the 40th day following inoculation.

Two important human diseases of unknown etiology, namely chronic septal hepatitis (Gerber & Vernace, 1974) and incomplete septal cirrhosis (Sciot et al., 1988) present thin and long fibrous septa connecting portal spaces to portal spaces and to central canals as a main histopathological feature. Septal fibrosis of milder degrees is a common and apparently non-specific finding of several chronic liver diseases. If not for other reasons, these aspects would justify hepatic septal fibrosis as a subject worthy of investigation.

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