Calomys callosus: an Alternative Model to Study Fibrosis in Schistosomiasis Mansoni. The Pathology of the Acute Phase

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Twenty Calomys callosus, *Rengger, 1830 (Rodentia - Cricetidae) were studied in the early stage of the acute schistosomal mansoni infection (42nd day). The same number of Swiss Webster mice were used as a comparative standard. Liver and intestinal sections, fixed in formalin-Millonig and embedded in paraffin, were stained with hematoxilin and eosin, PAS-Alcian Blue, pH=1,0 and 2,5, Lennert's Giemsa, Picrosirius plus polarization microscopy, Periodic acid methanamine silver, Gomori's silver reticulin and resorcin-fuchsin. Immunohistological study (indirect immunofluorescence and peroxidase labeled extravidin-biotin methods) was done with antibodies specific to pro-collagen III, fibronectin, elastin, condroitin-sulfate, tenascin, alpha smooth muscle actin, vimentin and desmin.*

The hepatic granulomas were small, reaching only 27% of the volume of the hepatic Swiss Webster granuloma. They were composed mainly by large immature macrophages, often filled by schistosomal pigment, characterizing an exsudative-macrophage granuloma type. The granulomas were situated in the parenchyma and in the portal space. They were often intravascular, poor of extracellular matrix components, except fibronectin and presented, sometimes alpha smooth muscle actin and vimentin positive cells.

The C. callosus *intestinal granulomas were similar to Swiss Webster, showing predominance of macrophages.*

Therefore, the C. callosus *acquire very well the* Schistosoma mansoni *infection, without developing strong hepatic acute granulomatous reaction, suggesting lack of histopathological signs of hypersensitivity.*

Key words: *Calomys callosus* - *Schistosoma mansoni* - fibrosis - liver - intestine - granuloma - extracellular matrix

Schistosomiasis research lacks an experimental model, particularly in small laboratory animals, that reproduces with accuracy the human disease, justifying the search of new ones.

Calomys callosus, Rengger, 1830 (Rodentia - Cricetidae), a wild mouse-like autochthonous rodent from South America (Argentina, Bolivia, Paraguay), is found also in Brazil in the Northeast, Central-west and Southern Regions (Moojen 1952, Hershkovitz 1962, Massoia & Fornes 1965, Mello 1969, Mello & Moojen 1979).

Petter et al. (1967) introduced *C. callosus* as a new laboratory rodent. Further biological studies have shown that this animal is easy to handle and to adapt to the laboratory conditions, and has high fertility rates during the whole year (Justines & Johnson 1970, Mello 1977, 1978).

Johnson et al. (1965) and Justines and Johnson (1969) have experimentally infected *C. callosus* with Machupo virus, thus establishing its importance as an experimental model.

Borda (1972) and Mello (1979, 1980) have shown experimentally that *C. callosus* is a permissible host for *Schistosoma mansoni* and Coelho et al. (1979) found 7.70 % *C. expulsus* (same as *C. callosus* according to Hershkovitz 1962) to be naturally infected in the region of Caratinga, State of Minas Gerais, Brazil.

In this paper we are presenting the major histopathological changes that occur in the liver and intestines of *C. callosus* during the early stage of the acute schistosomal infection, using Swiss Webster mice as a comparative standard.

MATERIALS AND METHODS

Twenty *C. callosus* and twenty Swiss Webster mice were infected when they were five days old by percutaneous exposure to 70 cercariae of the Belo Horizonte isolate of *S. mansoni*, and were sacrificed on the 42nd day of infection. Tissue samples were taken from the liver and intestines, fixed in formalin-Millonig (Carson et al. 1973), and embedded in paraffin. Intestinal samples were prepared according to Swiss roll procedure (Lenzi & Lenzi 1986). Sections were stained with hematoxilin and eosin; PAS-Alcian Blue, pH = 1,0 and 2,5, Lennert's Giemsa; Picrosirius plus polarization microscopy (Junqueira et al. 1979), Periodic Acid Methanamine Silver (PAMS), Gomori's silver reticulin and resorcin-fuchsin.

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The size of hepatic granulomas was determined in histological section of six animals per group, stained with hematoxilin and eosin. The diameters of ten granulomas containing single egg in the center were measured in each liver using an ocular micrometer. Granuloma volume was calculated assuming a spherical shape (Cheever & Barral-Netto 1985).

To study specific intra and extracellular matrix components, cryostat or trypsinized liver sections were studied by indirect immunofluorescence or indirect peroxidase labeled extravidin-biotin staining (ExtrAvidin-Peroxidase, Sigma E-2886; Anti-mouse polyvalent immunoglobulins biotin conjugate (Sigma, B-2016); Anti-rabbit immunoglobulins biotin conjugate (Sigma, B-3275). Anti-tenascin and anti-mouse IgG, IgA and IgM were tested with immunofluorescence and the other antisera with immunoenzimatic method: Pro-Collagen III (Institut Pasteur, Lyon); Fibronectin (Dakopatts, A-082); Condroitin-sulfate (Sigma, C-8035); Elastin (Sigma, E-4013); Vimentin (Sigma, V-7505); Desmin (Sigma, D-9284) and alpha smooth muscle actin (Sigma, A-2547). Normal sera were used as control, plus secondary antibody-FITC or secondary antibody-biotinylated-extravidin-peroxidase. Diaminobenzidine (DAB) was used as chromogen (DAB: 3mg; TBS: 10 mL; 30% H_2O_2: 10 uL). Sections were coverslipped in buffered glycerin with p-phenylenediamine (Johnson et al. 1982) and examined with a Zeiss microscope equipped with epifluorescence system.

RESULTS

The hepatic granulomas of *C. callosus* were small, reaching only 27 % of the volume of hepatic Swiss Webster granulomas, used as controls (Fig. 1). They were predominantly composed by large and mature macrophages, often filled by schistosomal pigment (Fig. 2), and less number of eosinophils and neutrophils, and scarce lymphocytes. Mast cells were detected only in advanced S.Webster exsudative-productive granulomas. Unlike Swiss Webster hepatic granulomas, they were less zonally configured, and presented very few reticular and birefringent picrosirius fibers (Fig. 4). The smaller granulomas were situated in the parenchyma and the larger ones in the portal space. These were often intravascular, externally demarcated by residual vascular wall elastic and reticular fibers (Fig. 3). The granuloma extracellular matrix components were better developed by immunohistology, showing the presence, in both group of animals, of fibronectin and pro-collagen III, which in the *C. callosus* were usually localized in the periphery of the granulomas (Fig. 5 A-D). The *C. callosus* granulomas never exhibit an external zone with myeloid metaplasia as was seen in Swiss Webster hepatic ones. Periovular diffusion of PAS positive material was more common in Swiss Webster than *C. callosus* granulomas. Focal parenchymal necrosis was found in both group of animals, and portal inflammation and fibrosis was absent or minimal in the *C. callosus* liver. These presented marked Kupffer cell hypertrophy, with cells enlarged and filled with granular schistosomal pigment.

While the Swiss Webster hepatocytes were alpha smooth muscle actin and weakly vimentin positive, the *C. callosus* hepatocytes showed higher amount of alpha-actin and intermediate filaments in the following order of intensity: alpha smooth muscle actin > vimentin > desmin (Fig. 5 E-H). It was impossible to discriminate Ito cells by these antisera due to their striking labeling in the periphery of the hepatic cells. Some portal granulomas in both group of animals exhibited cells positive to alpha smooth muscle actin and vimentin. The antibodies against mouse immunoglobulins did not react with *C. callosus* immunoglobulins. The tenascin and condroitin-sulfate were negative in both group of animals and elastin was detected only in vessel walls.

In *C. callosus* intestines, there was large number of eggs within the mucosa and submucosa vessels. The intestinal granulomas, predominantly located in the mucosa layer, were also rich in macrophages, with less number of eosinophils and neutrophils. In the mucosal lamina propria there was a diffuse infiltration of eosinophils and some mast cells not related to the granulomas. The main hepatic and intestinal histopathological findings are schematized in Tables I-III.

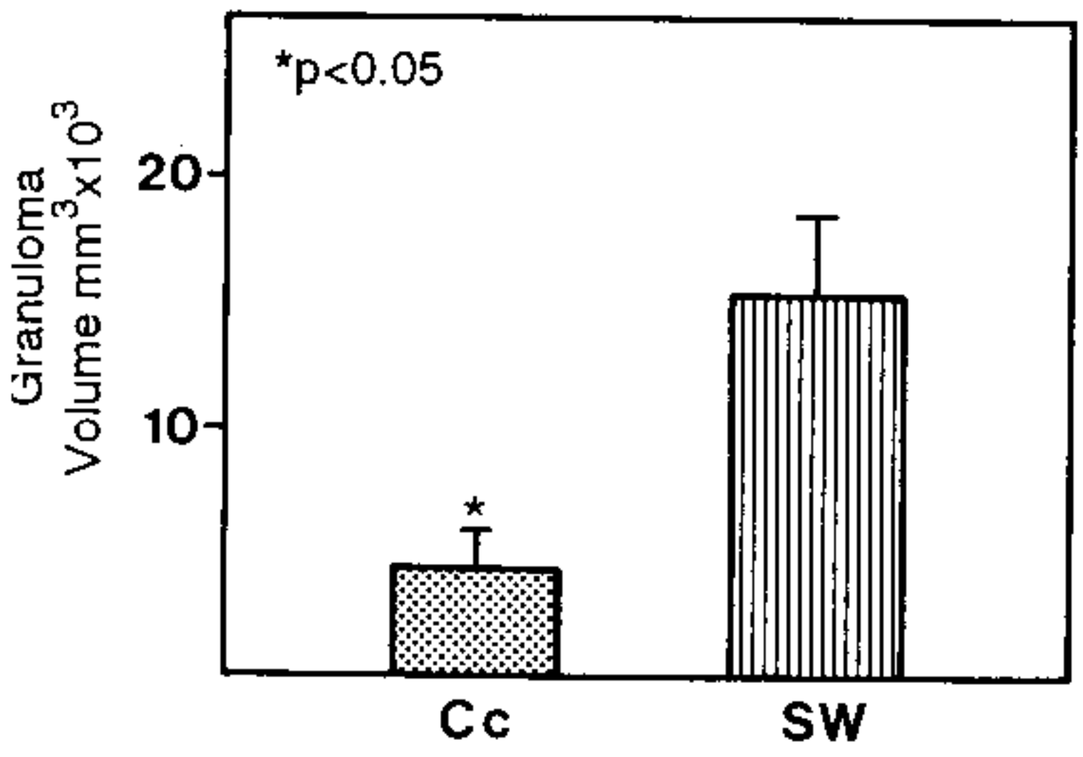

Fig. 1: comparative hepatic granulomas volume between *Calomys callosus* and Swiss Webster mice in acute phase of infection (42nd day).

DISCUSSION

The Swiss Webster hepatic granulomas, at this time of infection (42nd day) showed the classical aspects described by Hsu et al. (1972), with pre-

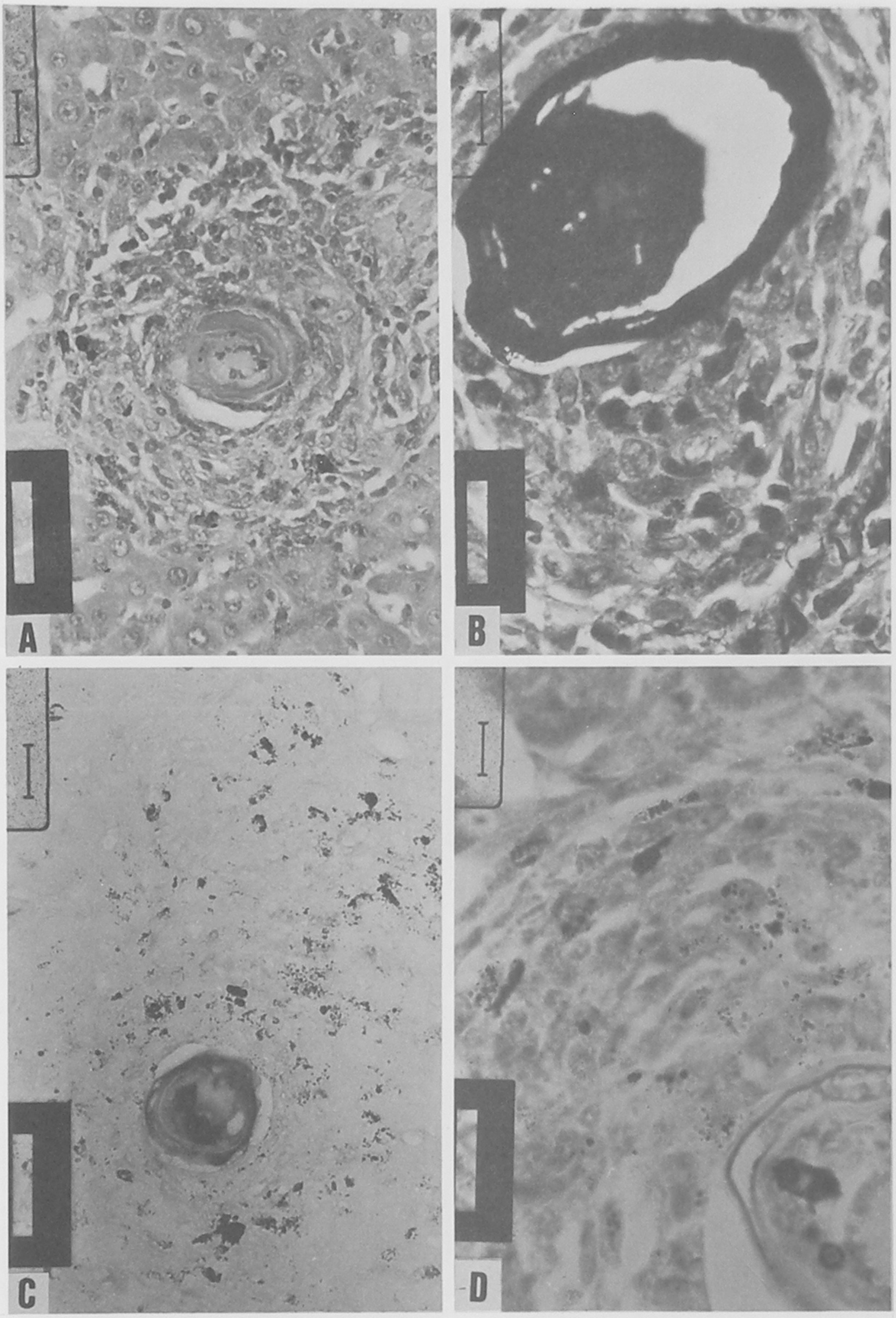

Fig. 2: small intraparenchymal hepatic granulomas in *Calomys callosus*, composed essentially by large macrophages, often containing schistosomal pigment even close to eggs. (A: H&E. X 400. B: PAMS. X 1000. C: AB-PAS pH=2,5. X 400. D: Lennert's Giemsa. X 1000).

dominance of initial exsudative-productive type. The *C. callosus* hepatic granulomas, on the contrary, presented peculiar aspects characterized by prevailing intravascular location, development of scarce or null fibrosis, and predominance of matures and large macrophages, many of them containing schistosomal pigment, characterizing a peculiar exsudative-macrophage granuloma type. This small granuloma pattern is unlike the mouse down-regulated granulomas, which exhibit more fibrosis, suggesting different mechanisms involved in their functions. The small parenchymal granu-

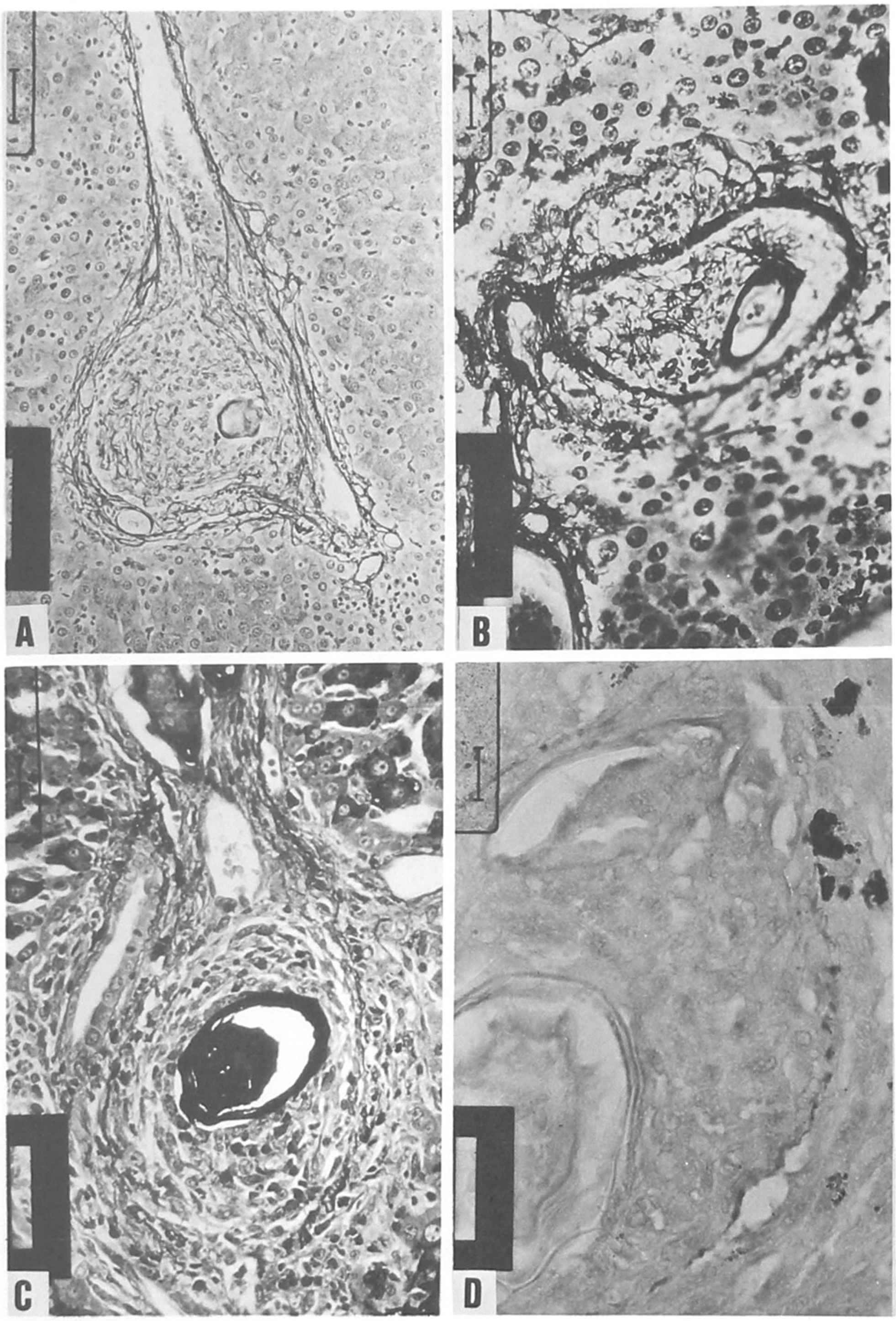

Fig. 3: hepatic intravascular granulomas in portal vessels of *Calomys callosus* with partial (A, B) or almost total destruction of the vascular wall (C, D), showing only collagenic (C) or elastic (D) residual fibers. Reticular fibers are formed in the periphery of the granulomas, close to the vascular wall (A) or next to the eggs (B). (A: Gomori's reticulum. X 200. B: X 400. C: PAMS. X 400. D: Weigert's resorcin-fuchsin. X 1000).

lomas are similar to BALB/C nude mice granulomas as was described by Byram and Lichtenberg (1977). They observed that the hepatic lesions in the nude mice were much smaller and the lacked epithelioid macrophage, with lesions about mature eggs typically consisting of monocytes and macrophages filled with pigment, occasional neutrophils, and rarely one or more eosinophils or giant cells. However, the *C. callosus* appear to be efficient in circumscribing the egg antigens, because there was very limited periovular diffusion of PAS positive material. The *C. callosus* hepatic lesions

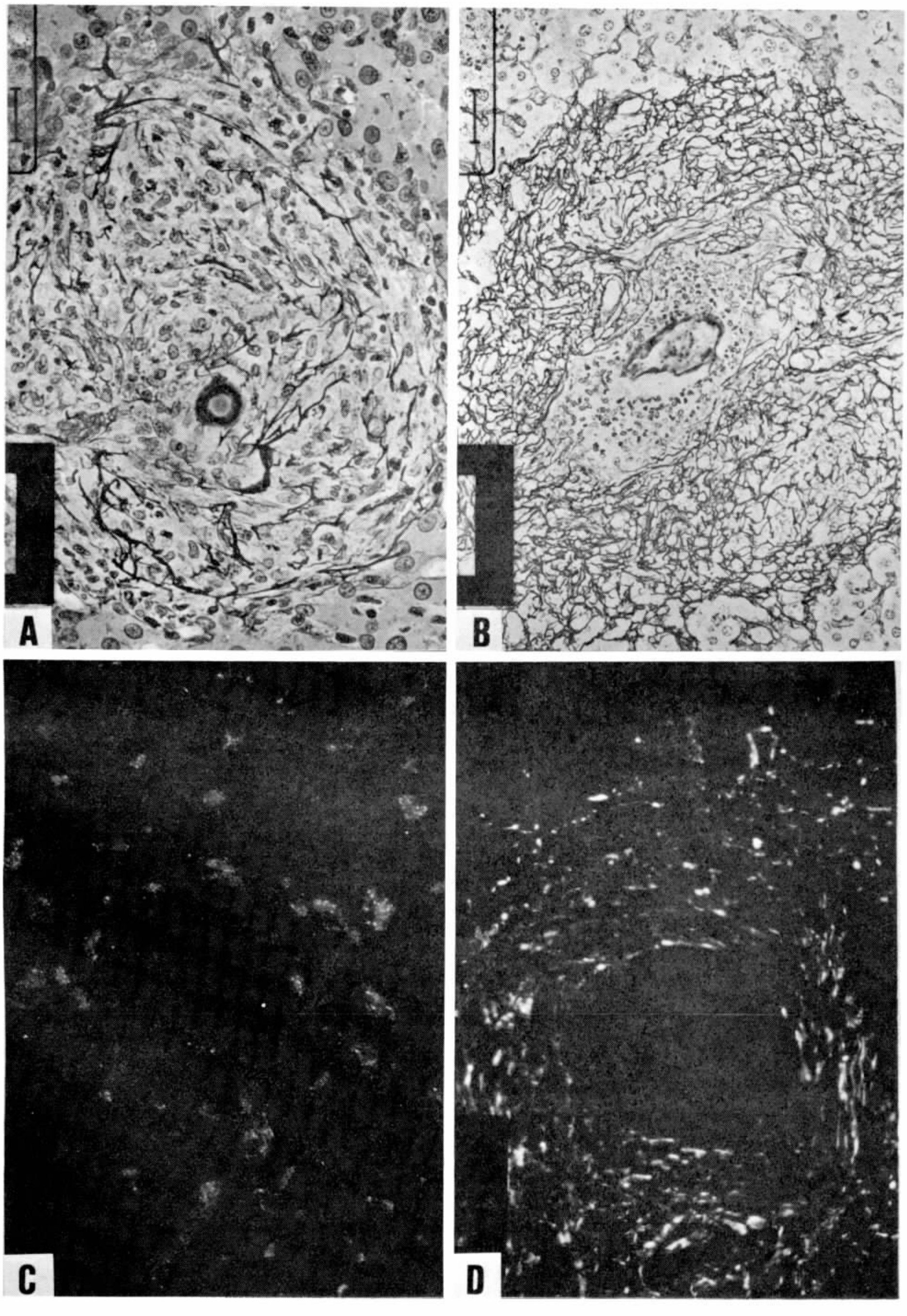

Fig: 4: comparison between hepatic granulomas of *Calomys callosus* (A, C) and Swiss Webster (B, D). The *Calomys callosus* showed few reticular fibers (A) and absence of birefringent picrosirius fibers (C), while the Swiss Webster presented a rich mesh of collagenic (D) and reticular fibers (B), which are organized in paracentral and peripheric zones. Large macrophage in the *C. callosus* granulomas were full of birefringent schistosomal pigment. (A: Gomori's reticulum. X 400. B: X 200. C.D: Picrosirius plus polarization. X 400.)

were also more or less like to those described in *Nectomys squamipes* by Rodrigues-e-Silva (1989), which is also a cricetidae.

It is important to stress the absence of perigranulomatous myeloid metaplasia concomitant with absence of an external zone, which was usually present in Swiss Webster hepatic granulomas, presenting a peripheric mesh of reticular fibers, rich in pro-collagen III and fibronectin, that functions as a medullary stroma (Fig. 4-B).

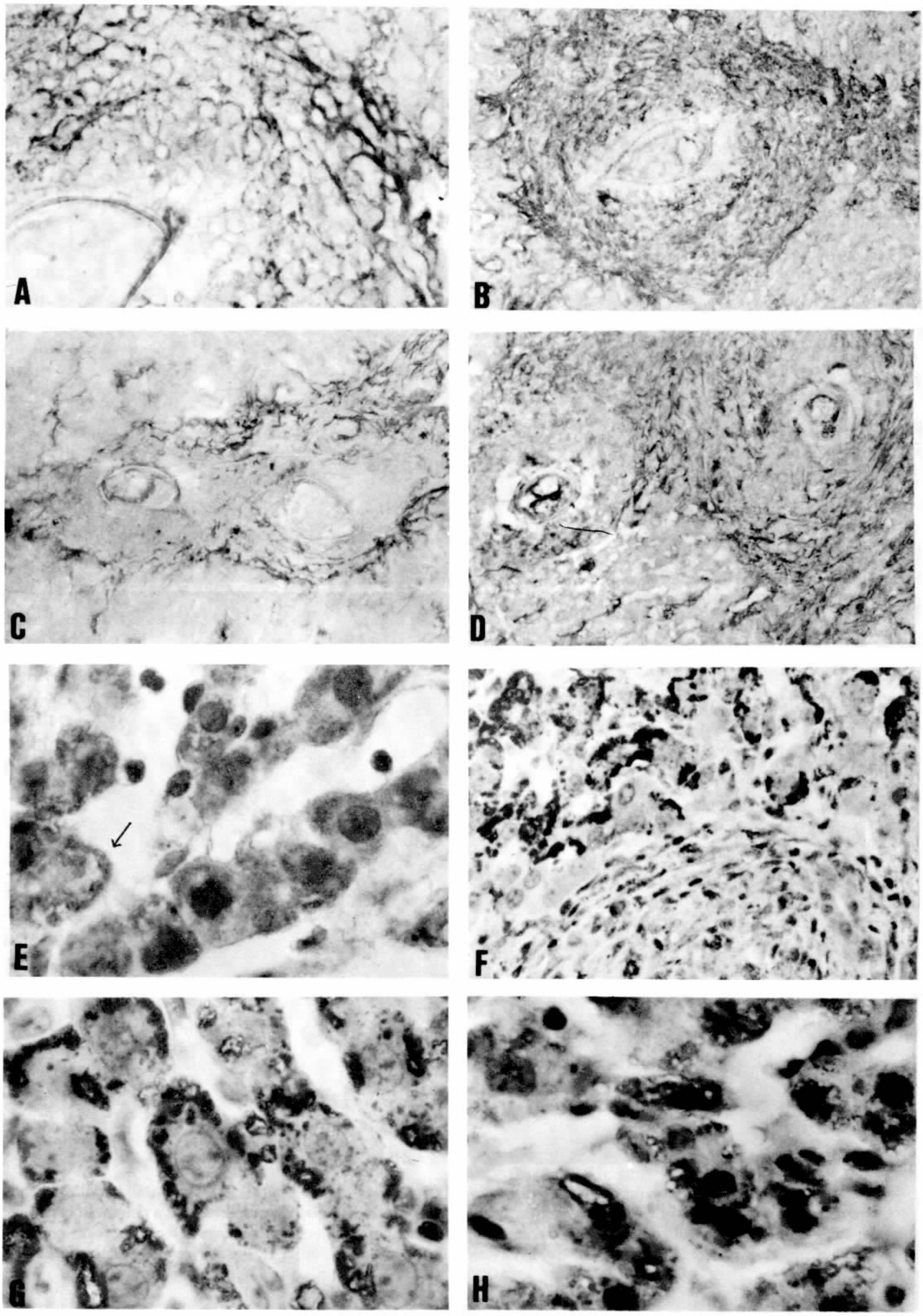

Fig. 5: the distribution of fibronectin (A,B) and pro-collagen III fibers (C,D) in *Calomys callosus* hepatic granulomas. Periphery of hepatocytes (and or possible Ito cells) positive to vimentin (arrow) (E) and alpha smooth muscle actin (F,H). Sometimes, the labeled areas are vacuolated (G,H), appearing to be Ito cells. Lymphocytes (Pit cells ?) in contact with sinusoidal endothelial cells (E). Granuloma rich in large macrophages, without positivity to alpha smooth muscle actin (F). (B.D:Immunoperoxidase. X 200. A.F: X 500. E.G.H: X 1250).

TABLE I
Liver: comparative histopathologic lesions between *Calomys callosus* and Swiss Webster mice infected with *Schistosoma mansoni* (42 days of infection)

Findings	*C. callosus*	Swiss Webster
Granuloma:		
- stage	"E" (macropha-	E - P
- size	ge) Small	Large
Granuloma composition:		
- Macrophages	++	++++
- Epithelioid cells	+/-	-
- Giant cells	-	-
- Neutrophils	+/-	+/-
- Eosinophils	+/++	++++
- Fibroblastoid cells	-/+	+
- Mast cells	-	-/+
- Lymphocytes	+/-	+/-
- Plasmocytes	-	-

E = Exsudative; E-P = Exsudative-Productive; (+ to ++++) = Levels of intensity;
(-) = Negative; (+/-) = Weakly positive; (-/+) = Negative or positive.

TABLE II
Liver: comparative histopathologic lesions between *Calomys callosus* and Swiss Webster mice infected with *Schistosoma mansoni* (42 days of infection)

Findings	*C. callosus*	S. Webster
Granuloma ECM:		
- Reticulin Fibers	+/-	+/+++
- Collagen Type I	-/+	+
- Collagen Type III	+	++
- Fibronectin	++	++
- Tenascin	-	-
- Elastin	-	-
- Condroitin-sulfate	-	-
- Alpha-smooth muscle Actin	-/+	-/+
- Vimentin	-/+	-/+
- Desmin	-	-
- Pigmented Macrophages	+++	+/-
Extramedullary metaplasia	-	++
Extragranulomatous changes:		
Portal inflammation	+/-	++
Portal fibrosis	-	+
Kupffer hypertrophy with pigment	+++	+
Focal necrosis	+	+
Adult worms	++	+/-

ECM = Extracellular Matrix; (+ to +++) = Levels of intensity;
(-) = Negative; (+/-) = Weakly positive; (-/+) = Negative or positive.

TABLE III
Intestine: comparative histopathologic lesions between *Calomys callosus* and Swiss Webster mice infected with *Schistosoma mansoni* (42 days of infection)

Findings	*C.callosus*	Swiss Webster
Granuloma:		
- stage	Exsudative	Exsudative
- size	Small	Large
- location	Mucosa	Mucosa
Granuloma composition:		
- Macrophages	++	++
- Eosinophils	++	+++
- Neutrophils	+	-
Mucosa:		
- Eosinophil infiltration	Diffuse	Localized
- Mast cells	+/-	-

(+ to +++) = Levels of intensity; (-) = Negative; (+/-) = Weakly positive; (-/+) = Negative or positive.

The presence of alpha smooth muscle actin and vimentin positive cells in some portal granulomas of both group of animals, suggests the participation of myofibroblasts in the granuloma composition.

The *C. callosus* intestinal lesions, except the diffuse eosinophil infiltration in the mucosal lamina propria and predominance of macrophages in the granulomas, was similar to those observed in mice.

The frequent presence of intravascular granulomas in the *C. callosus* liver may be due to a more limited periovular inflammatory reaction than in Swiss Webster. We suggested that the passage of the eggs from the vessels to the tissues, as the passage of the eggs to the intestinal lumen, is dependent on inflammatory cells surrounding the eggs (Lenzi et al. 1991). Indeed, Raso et al. (1983) observed in the liver of thymectomised and *S. mansoni* infected mice a marked inhibition of the development of granulomas, scarce inflammatory exsudation around the parasite eggs and many intravascular eggs.

It appears that *C. callosus* acquire very well the schistosomal infection, as was demonstrated by Borda (1972), Mello (1979) and Coelho et al. (1979) without developing strong acute granulomatous response. Therefore, we detected an excellent model to study low acute responsiveness to egg antigens in the liver, which forms granulomas that, in contrast to Swiss Webster, lack histopathological signs of hypersensivity, without being an athymic animal.

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