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DYNAMICS OF FRIBROSIS PRODUCTION AND RESORPTION IN INTESTINAL SCHISTOSOMIASIS OF MICE

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A histological, morphometric and immunocytochemical study of schistosomal periovular granulomas in the liver and intestines of mice revealed that intestinal granulomas are smaller and contain less collagen than those in the liver. After curative treatment intestinal granulomas undergo a relatively more rapid resorption, although the general pattern of collagen degradation apparently does not differ from that observed in the liver.

Tendency to form scattered, usually isolated granulomas that are only mildly fibrogenic, coupled with a well-balanced process of resorption appear as the explanation why intestinal fibrosis is not an outstanding feature of schistosomiasis as it is in the liver.

Key words: schistosomiasis – intestinal fibrosis – periovular granuloma

Fibrogenesis is outstanding in schistosomal periovular granuloma (Wu et al., 1982; Wyler et al., 1986). Hepatic fibrosis is an important feature of advanced schistosomiasis both in man and in experimental animals and it results from the repeated deposition of schistosome eggs within the portal spaces (Andrade, 1987).

Egg laying occurs mainly in the intestines. It is assumed that a large number of eggs become trapped in the intestinal wall inducing granuloma formation. Methods of egg extraction and counting reveal more schistosomal eggs in intestines than in the liver (Cheever, 1969). However, except for some rare cases of so-called pseudo-neoplastic lesions (Armbrust et al., 1963; Abrantes & Katz, 1964; Andrade & Melo, 1974), intestinal fibrosis is not an impressive feature of schistosomiasis (Cheever & Andrade, 1967; Andrade & Silveira, 1990). Is is rather surprising, since during infection with *Schistosoma mansoni*, which may last for years, eggs are continuously passing through and being deposited in the intestines.

In an attempt to better understand why intestinal fibrosis in schistosomiasis is so scanty as compared to the liver, it was investigated whether a difference exists in these two organs concerning the formation and degradation of fibrosis in schistosomal periovular granulomas.

MATERIALS AND METHODS

Outbred albino mice of both sexes, weighing 18 to 20 g and maintained with water and a commercial diet *ad libitum* were infected with 50 cercariae each, by the transcutaneous route. Ten weeks after infection, with the animals passing viable eggs in the stools, 48 of them were submitted to treatment (1st. group) and 48 were left untreated (2nd. group). Treatment consisted of administration of 100 mg of examniquine and two doses of 200 mg each of praziquantel given on the same day by gastric intubation. Eight animals from each group were killed on the 11th, 12th, 13th, 16th, 22nd and 30th weeks after infection. Within each group, three animals were utilized for parasitologic studies, three for collecting of autopsy data and for histopathologic studies and two for immunocytochemical and biochemical data. During autopsy the animals were submitted to perfusion by the method of Duvall & DeWitt (1967) for the recovery of worms. Eggs were counted in samples from the liver and intestines by Cheever's (1970) method and the

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results expressed in number of eggs per grams of tissue. Smash preparations of portions of liver and intestines were utilized for the oogram according to Prata (1957).

Histology – The intestines were opened through the mesenteric border, gently washed off its contents and turned into a roll before being fixed in 10% neutral formalin. The entire rool was embedded in paraffin and the sections included all parts of the intestines. Fragments of the liver were fixed in Bouin's fluid and embedded in paraffin as usual. Sections of intestines and liver were stained with hematoxylin and eosin, and with picrosirius-red for collagen. These latter sections were observed with and without polarized light.

Morphometry – Sections of the liver and intestines from the 2nd group of animals only were examined by digitized morphometry using an electronic drawing board (Summasketch II, Summagraphics, Connecticut, USA) connected to an IBM-PC-AT compatible computer and the Sigma-Scan Measurement System (Jandel Scientific, San Francisco, USA) and a Zeiss optical microscope in which a camera lucida was attached. For morphometric calculations periovular granulomas were considered as spheres having a normal size distribution. The size of the section was registered and measurements of the granulomas were made with a 10X objective, the system being calibrated for that amplification. The overlayed images of the granulomas on the tablet were traced in their structural contours with the cursor connected to the microprocessor to compute the respective areas in μm2. The following parameters were calculated: size, volume density and numeric density. Size was represented by the surface area of the granulomas in the histological sections. Volume density was calculated as the quotient of the total granuloma profile area to the total section area per animal. The number of periovular granulomas per unit volume studied was calculated by applying the Weibel & Gomez's formula (Weibel, 1969).

Immunocytochemistry – Fragments of intestine and liver were rapidly embedded in Tissue Tek (Miles Co., USA) and frozen in liquid nitrogen. Cryotome sections were submitted to indirect immunofluorescence for the demonstration of type I and III collagens and fibronectin. Specific primary antibodies were obtained by courtesy of Dr Jean-Alexis Grimaud, Institute Pasteur de Lyon, France. Details about preparation, purification and tests of specificity of these anti-sera appear elsewhere (Andrade & Grimaud, 1986).

Biochemistry – Determination of hydroxyproline was performed according to Rojkind & Gonzales (1974) in samples of livers and intestines taken from both experimental groups on the 11th, 16th, 22nd and 30th weeks after infection. Results were analyzed according to a formula that correlates optical density with collagen content (Chehter, 1987) and were expressed as milligrams of collagen per gram of tissue.

Statistical evaluation – Data from egg counting, morphometry and biochemistry were evaluated by Kruskal-Wallis and Mann-Whitney non-parametric tests, with significance level of $p < 0.05$.

RESULTS

Recovery of worms averaged six pairs per each animal with equivalent distribution of males and females. In the treated group only dead worms were seen following the 2nd week of treatment. The counting of eggs in intestines and liver appears in Figs 1 and 2, respectively. The data for the 11th, 12th and 13th weeks after infection were combined to represent early infection. The same for the data related to the 22nd and 30th weeks (late infection). While no significant difference appeared regarding numbers of eggs in liver and intestines in untreated animals during early infection, in late infection there were many more

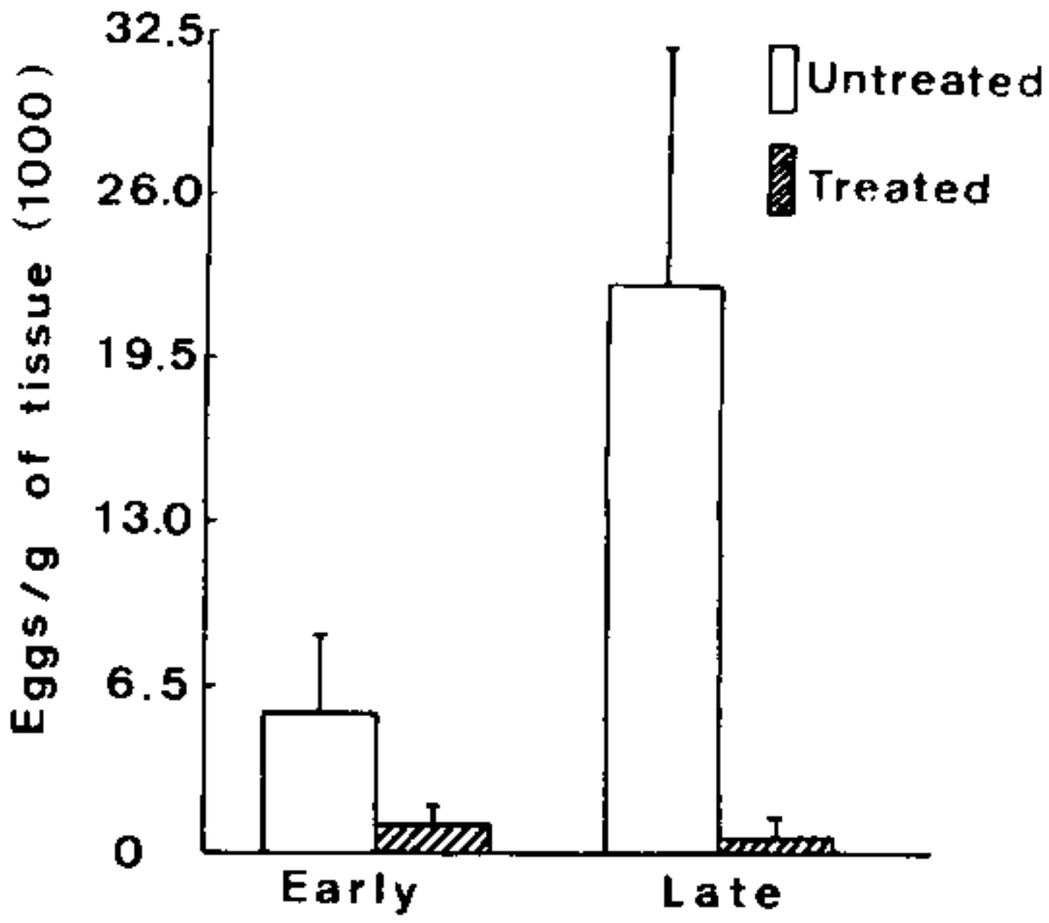

Fig. 1: demonstration of the numbers of *Schistosoma mansoni* eggs in the intestines of mice during early and late infection.

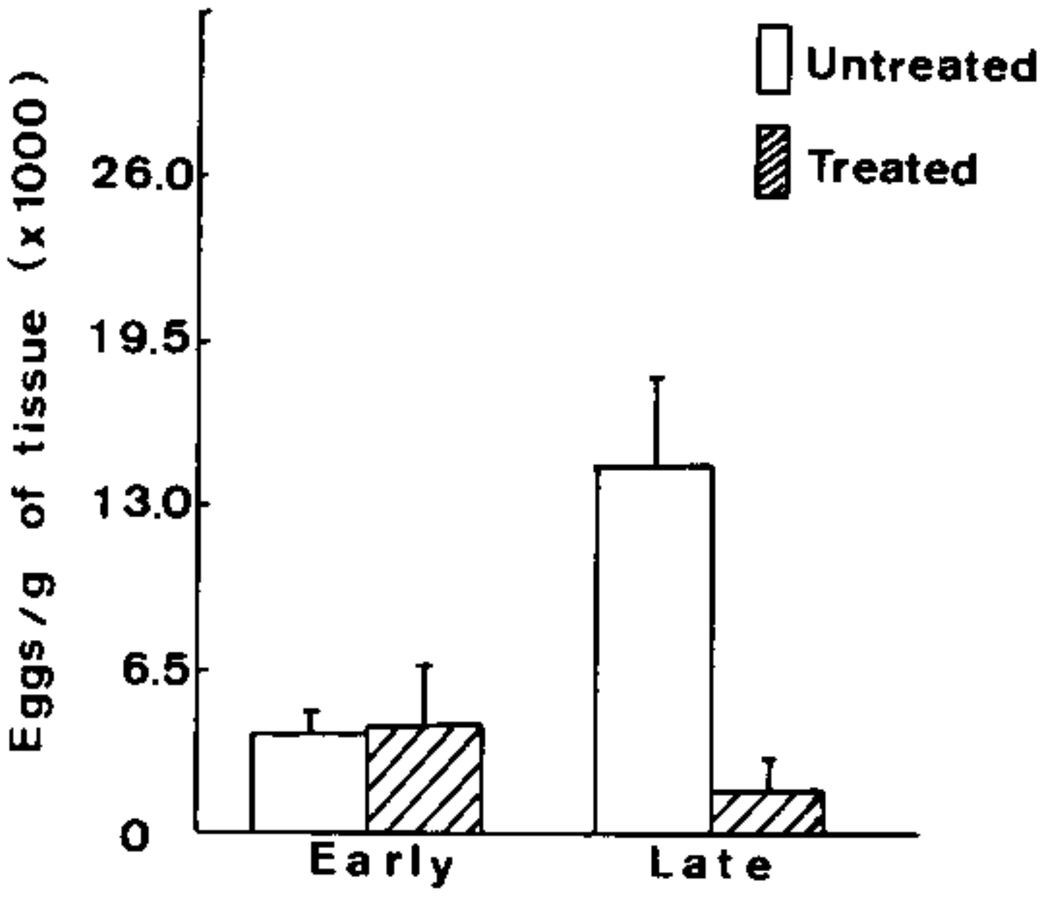

Fig. 2: results of the counting of *Schistosoma mansoni* eggs in the livers of mice during two periods of infection.

eggs in the intestines than in the liver. However, in the treated group, both in early and late infection, the intestines revealed less eggs than the liver. The oogram showed active infection (immature and mature eggs), but after the 2nd week of treatment only non-viable eggs were found in treated animals.

Histological examination revealed periovular granulomas along the entire intestinal length, preferentially located in the submucosa. They were usually scattered, but sometimes were aggregated in clusters. Compared to the hepatic granulomas (Fig. 3) in the same period of infection, intestinal granulomas were smaller, more discrete, less exudative and contained less collagen fibers as seen in picrosirius-red stained sections (Fig. 4). After treatment progressively involuting granulomas were seen in the intestines and liver. Although their number gradually decreased in the intestines, some of them remained for the entire experimental period. The presence of fibronectin and collagens of types I and III was documented in intestinal and hepatic granulomas, but the staining was weaker in the former. This remained true for the treated group as well.

Morphometric study revealed that both granulomas in the intestines and liver of non-treated animals gradually diminished in size from the 11th up to the 16th week of infection (Fig. 5). Hepatic granulomas in the 11th, 12th and 13th weeks were larger than those in the intestines ($p < 0.05$). Such difference ceased to be observed from the 16th week on. Data from volume density and numerical density appear in Figs 6 and 7, respectively.

The volume density of hepatic granulomas was found to be significantly greater than those in the small and large intestines. On the other hand, the difference between the volume density of the granulomas found in the small and large intestines becomes statistically significant by the 30th week only.

Only after the 30th week of infection, the numerical density of the granulomas in either the small or large intestines, was greater than that for the liver ($p < 0.05$).

Hydroxyproline content of the liver dropped after treatment (See Table).

COMMENTS

Apparently the liver and intestines have not the same potential to develop fibrosis from schistosomal periovular granulomas. Our studies show that eggs are more numerous in the intestines than in the liver of non-treated mice. However, in the intestines many counted eggs at a given time are presumably going to be eliminated into the feces and others will drift back toward the liver. When eggs are concentrated in a limited area marked fibrosis can appear in the intestines, forming polyps (Mohamed et al., 1990) or masses that may simulate cancer (Abrantes & Katz, 1964). In experimental infection of mice, intestinal fibrosis, sometimes accompanied by intestinal obstruction (Warren, 1969), can occur in *S. japonicum* infection, since in this latter species worm pairs have a tendency to remain in place longer than the more constantly migrating *S. mansoni*.

On the other hand, our data also reveal that volume density of granulomas is greater for the liver, which means that in this organ the granulomas occupy a larger volume than in the intestines.

During early infection hepatic granulomas are larger than the intestinal ones. This difference is no longer maintained by the 16th week on, when immunological modulation is knwon to decrease granuloma size in the liver (Andrade & Warren, 1964; Chensue & Boros, 1979). Intestinal granulomas seem to be already modulated from the start (Weinstock &

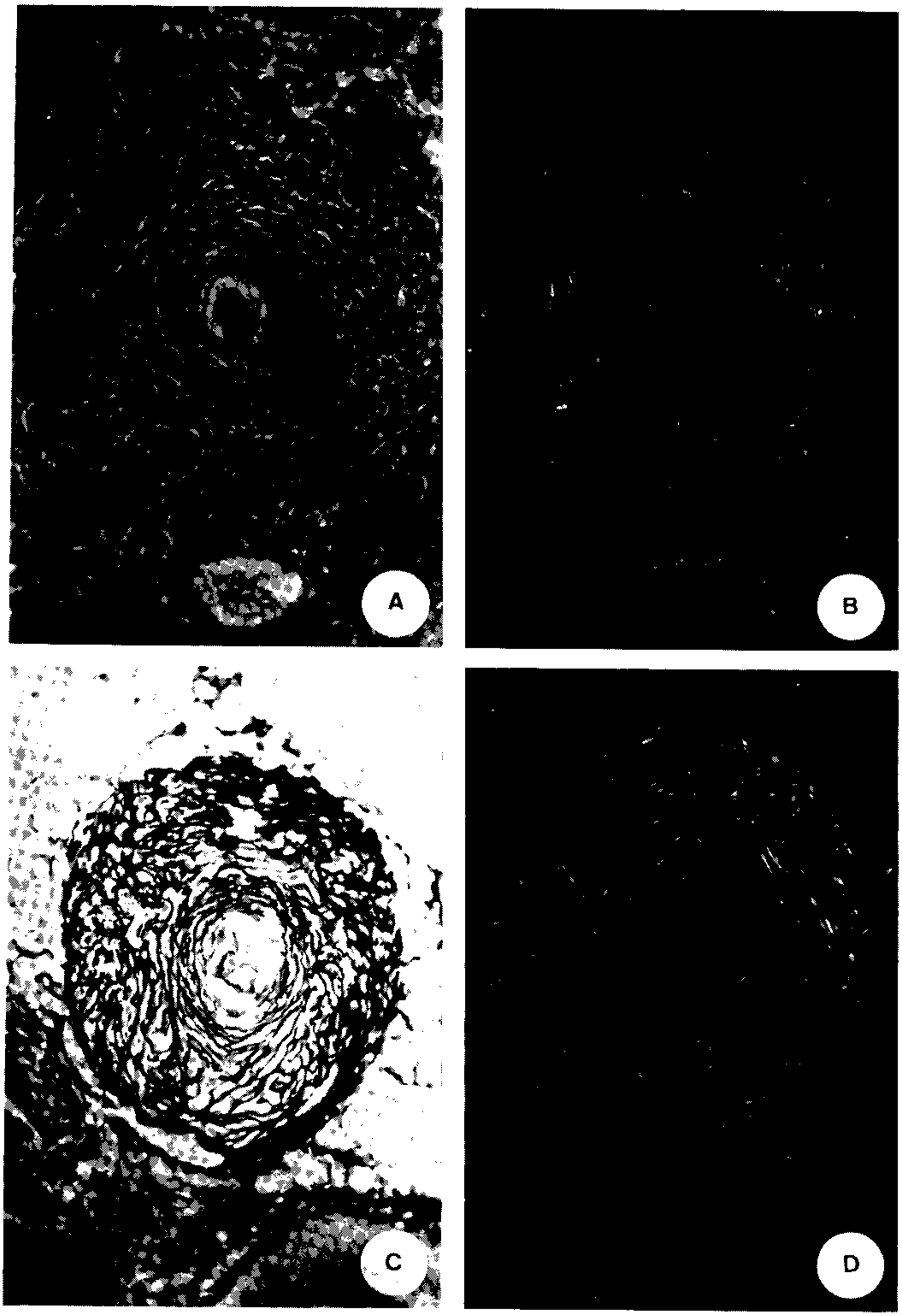

Fig. 3: hepatic periovular granulomas during early infection showing collagen irregularly deposited at the periphery. Picrosirius-red staining without (A) and with polarized light (B). In later granulomas the collagen appears more compact and discrete. Picro-sirius-red staining (C) and as seen after polarization microscopy (D). All microphotographs taken at 250 X.

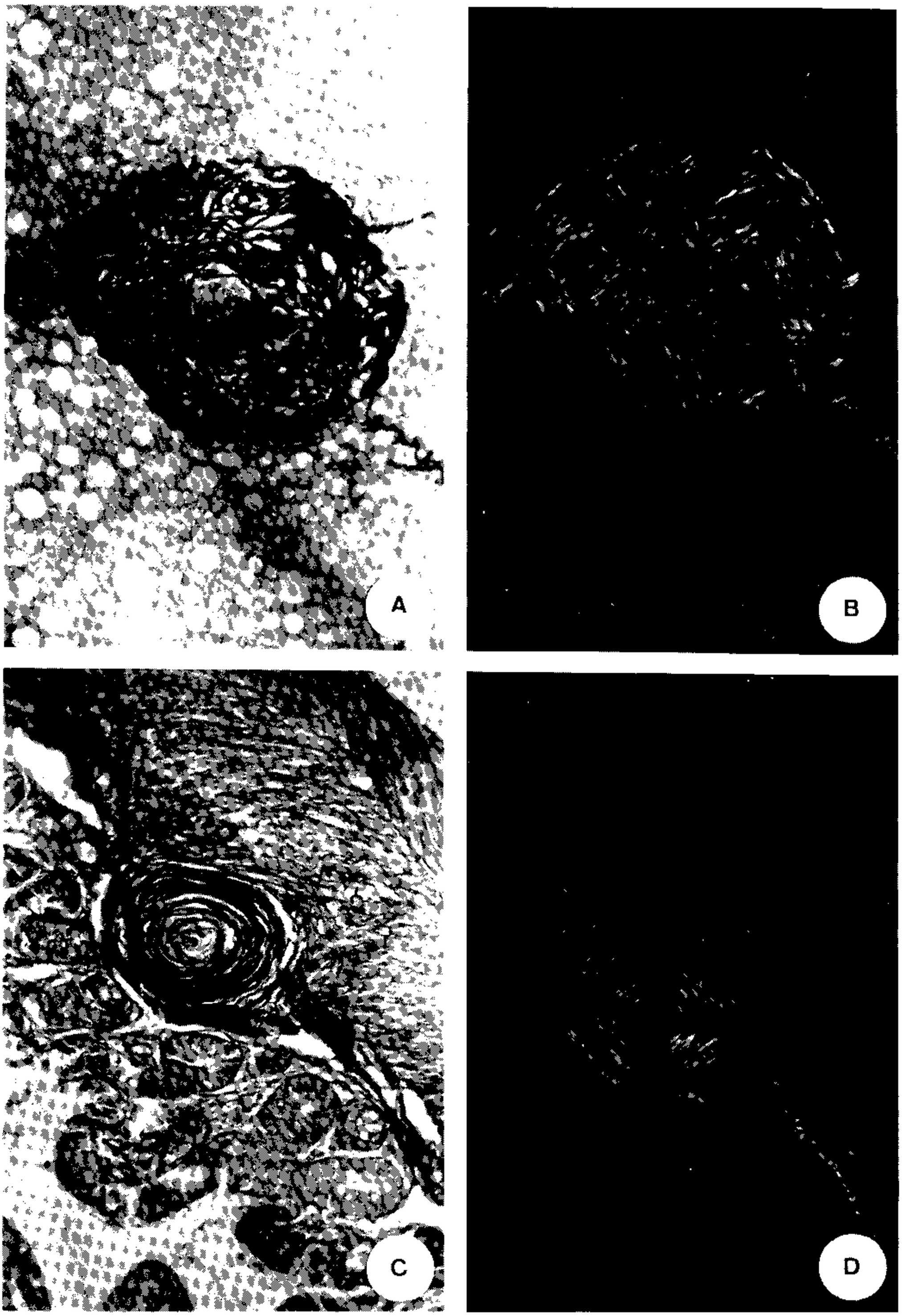

Fig. 4: intestinal granulomas in early phase of infection, in picro-sirius-red staining (A) and after polarization microscopy (B). Smaller granulomas are observed during the late phase of infection. Picro-sirius-red (C) and polarized light (D). All magnifications: 250 X.

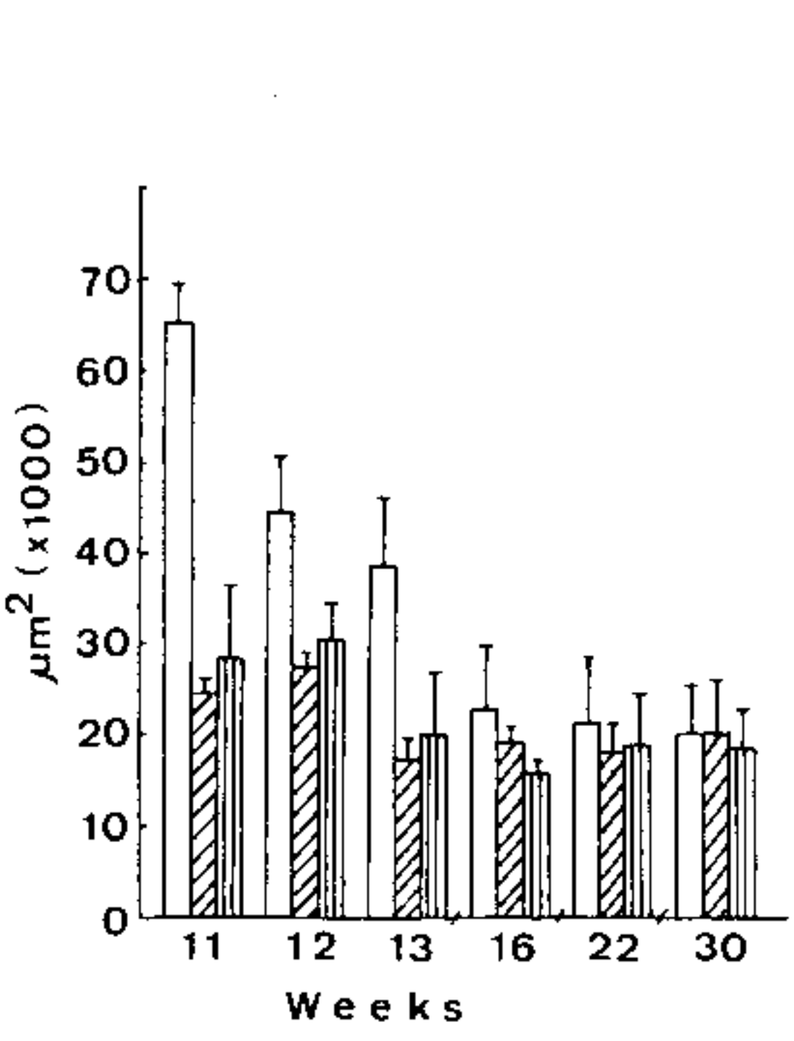

Fig. 5: variations of sectional areas of hepatic and intestinal *Schistosoma mansoni* granulomas during the 11th up to the 30th week of infection. Animals in this group were infected, but not treated.

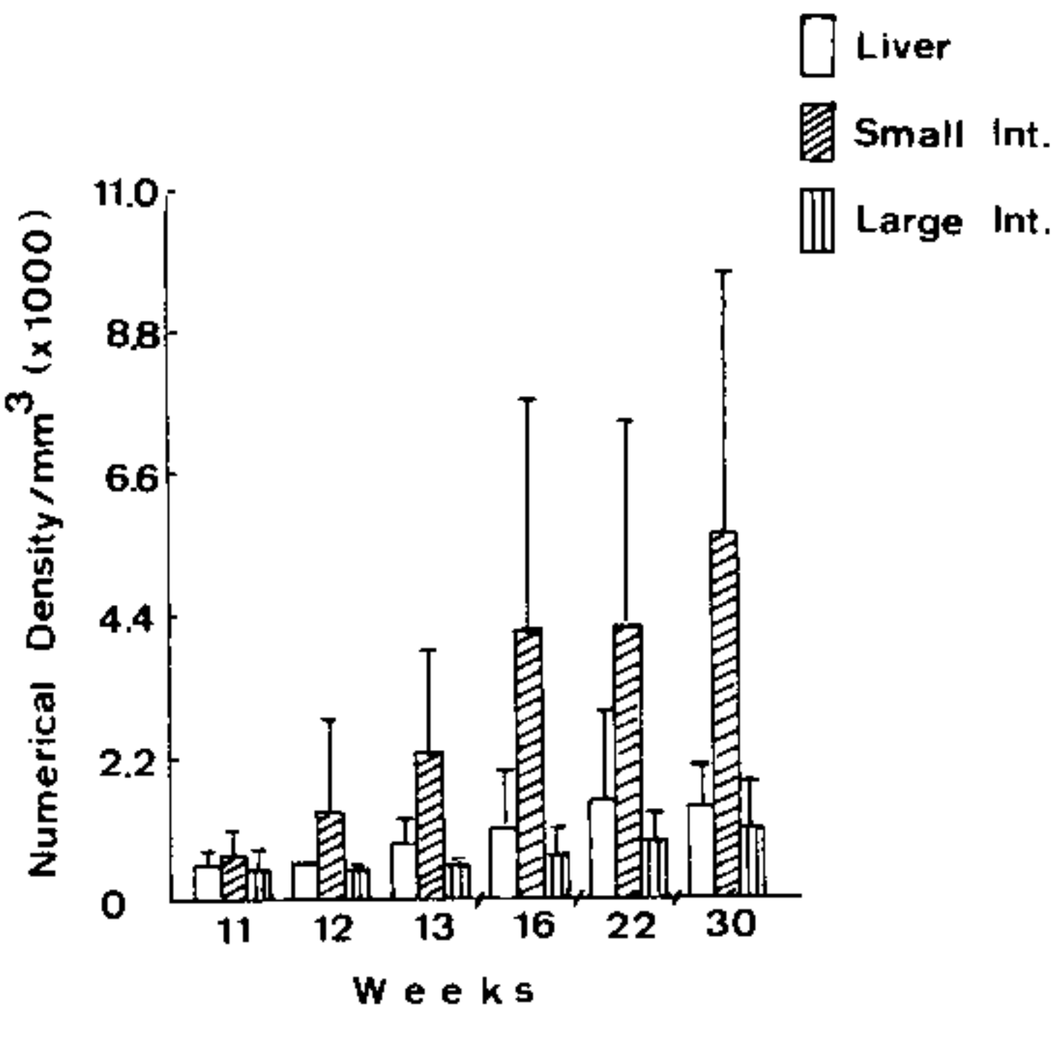

Fig. 7: numerical density of schistosomal periovular granulomas in the liver and intestines (small and large) of mice along several weeks of infection. Animals were not treated.

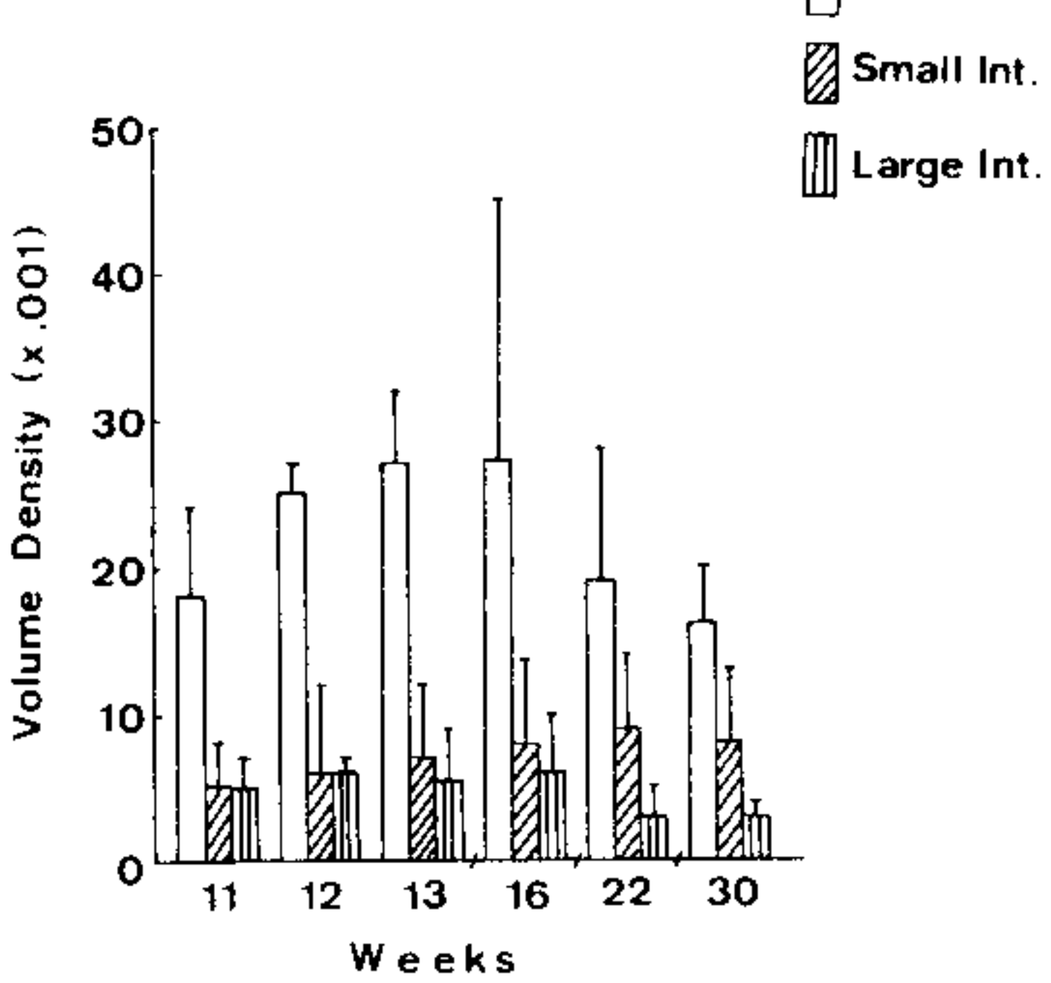

Fig. 6: volume density of schistosomal granulomas in the liver, small and large intestines in mice infected and not treated, and sacrificed along several weeks.

Boros, 1981), and as such they are small, discrete and weakly fibrogenic.

Immunochemical characterization of the main extracellular matrix components did not give any clue to differentiate hepatic and intestinal granulomas. Both type I and III collagens and fibronectin were present and remained until the last stages of post-therapeutic granuloma involution, such as has been observed in the liver (Andrade & Grimaud, 1988).

TABLE

Hydroxyproline content of the liver of mice infected with *Schistosoma mansoni*, treated or not. (Results expressed as milligrams of collagen per gram of wet-liver tissue, with SD)

Duration of infection (weeks)	Non-treated	Treated
11	12.48 ± 2.2	11.03 ± 3.13
16	9.71 ± 2.67	7.48 ± 3.36
22	12.95 ± 0.7	6.18 ± 1.43
30	9.86 ± 4.03	3.58 ± 0.46

Another possibility to explain the paucity of fibrosis in intestinal schistosomiasis is that fibrolytic processes could be more active in the intestines. This was investigated by following the effect of curative treatment of schistosomiasis. By suppressing parasite stimuli, such treatment leads schistosomal lesions to involute, which is accompanied by considerable degree of collagen degradation (Andrade & Grimaud, 1986, 1988). Even in human patients resorption of fibrosis in periportal ("pipe-stem") lesions (Bina & Prata, 1983; Homeida et al., 1988), in intestinal polyps (Farid et al., 1974) and in intestinal pesudoneoplastic lesion (Coutinho et al., 1984) has been documented.

A sequential follow up of our animals failed to demonstrate any significant difference in

the pace of connective tissue matrix degradation in intestinal and hepatic granulomas after specific chemotherapy and cure of schistosomiasis. Therefore, there was no evidence that a more rapid matrix degradation in the intestines could explain the mild fibrosis usually seen in intestines.

In conclusion, intestinal periovular granulomas in schistosomiasis are small, form little matrix (including collagens) and are usually scattered in a large area, allowing a well balanced equilibrium between the formation of new lesions and the resorption of old ones, thus preventing the accumulation of an excess of connective tissue (fibrosis).

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