Hepatic Connective Tissue Changes in Hepatosplenic Schistosomiasis

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Destruction of intrahepatic portal vein branches with dispersion of smooth muscle cells into the periportal fibrosis and preservation of arterial and ductal structures were the main characteristic findings seen in 66 surgical liver biopsies from patients with the hepatosplenic form of schistosomiasis. Besides these diagnostic features, the present histologic, immunocytochemical, and ultrastructural study revealed the presence of a complex matrix forming the portal and septal fibrosis in advanced schistosomiasis. There was marked hyperplasia of elastic tissue, presence of several collagen isotypes (I, III, procollagen III, IV, and V), actin, desmin, fibronectin, and laminin in a richly vascularized connective tissue. Signs of multifocal matrix (collagen) degradation were observed both at light and electron microscopic levels, suggesting a predominance of a fibrolytic process, at the time parasite-related lesions had almost disappeared. The latter findings are related to the involution of periportal fibrosis now being observed in patients who have undergone antischistosomal chemotherapy. They exemplify morphologic changes connected with chronic collagen degradation in human schistosomiasis that are similar to those first seen in experimental material. Evidence of either persistent or active chronic hepatitis was seen in several cases but its etiology could not be determined. HUM PATHOL 23: 566–573. Copyright © 1992 by W.B. Saunders Company

Schistosomiasis is one of the most prevalent parasitic diseases in the world. The World Health Organization estimates that more than 200 million people are infected and that 500 to 600 million are exposed to infection.[1] Five schistosome species can infect man but only one, *Schistosoma mansoni*, is found in the New World, especially in Brazil. The paired worms in this species live inside the veins of the mesenteric system and lay eggs in the intestinal mucosa. Eggs are frequently carried into the liver by the blood flow and are trapped in the portal radicles, inducing granulomas.

Advanced hepatic schistosomiasis is clinically represented by the hepatosplenic form of the disease, with periportal fibrosis, intrahepatic portal vein obstruction, and portal hypertension. Its incidence is less than 5% of all infected people in endemic zones of Brazil. Its pathogenesis is related to high worm burden,[2] coupled with a host inability to immune-modulate egg-induced lesions.[3,4] Gross hepatic pathology is highly characteristic and since 1904 has been known as "clay pipestem fibrosis".[5] The histopathology of hepatic schistosomiasis in its advanced form also has been well documented in the literature.[6-8] However, the application of different morphologic techniques to abundant material from human biopsies has revealed new findings that justify the present report. These new aspects are related to the potential for reversibility of the portal changes, to the participation of elastic tissue in schistosomal fibrosis, to associated changes of chronic hepatitis, to the peculiar behavior of the portal vein smooth muscle fibers, and to the dynamics of matrix changes in schistosomal portal and septal fibrosis.

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MATERIALS AND METHODS

General Clinical Data

A total of 66 surgical biopsies of the liver were obtained from hepatosplenic patients undergoing splenectomy and ligation of esophageal varices to relieve the manifestations of portal hypertension (gastroesophageal bleeding and hypersplenism). There were 51 males and 15 females and their ages varied from 16 to 61 years (mean, 30.4 years). Patients were operated upon at different hospitals in the city of Salvador, Bahia, Brazil, but all surgical specimens were examined at the Gonçalo Moniz Research Center. Preoperative diagnoses were made on essentially clinical grounds, but schistosome eggs were demonstrated in the stools from all patients. No information on previous antischistosome chemotherapy could be obtained from the patients. Diagnoses were pathologically confirmed in every case, and portal fibrosis with destruction of the intrahepatic portal vein branches and preservation of arterial and bile ductal structures were the primary, and sometimes the sole, lesion.

Histology

Fragments of liver tissue were fixed in Bouin's fluid or buffered 10% formalin and embedded in paraffin. Sections were routinely stained with hematoxylin-eosin, Weigert's elastic method, periodic acid-Schiff with and without previous diastase digestion, Picrosirius-red collagen stain, Gomori's reticulum method, Perls' iron stain, and the Shikata's orcein stain for hepatitis virus B surface antigen. Material stained with Picrosirius-red was examined with and without polarized light.

Immunocytochemistry

The last 18 biopsies were obtained from Roberto Santos Central Hospital in Salvador and followed a different routine. The liver fragments were divided into three parts. One portion was fixed and processed for histologic study as described above. Another portion was embedded in Tissue Tek (Miles Inc, Elkhart, IN) and snap-frozen in liquid nitrogen. Blocks were kept in air-tight bottles at −70°C until the moment they were cut in a cryotome at −20°C. The sections were then submitted to

either direct or indirect immunofluorescence techniques for the demonstration of type I, III, pro-III, IV, and V collagens, as well as for fibronectin, laminin, desmin, elastin, and actin. Collagen types I, III, pro-collagen III, and IV were prepared from fibrotic human liver, calf skin, and bovine lens capsule, respectively, after limited pepsin digestion and fractional precipitation with sodium chloride, according to Rhodes and Miller,[9] as modified for human liver by Chevalier et al.[10] Polyclonal monospecific antibodies against type V collagen were obtained from Institut Pasteur, Paris, France. The purity of the collagen fractions was verified by SDS-polyacrylamide gel electrophoresis. Human plasma fibronectin was prepared by affinity chromatography using gelatin-sepharose 4b according to Envall and Rouslahti,[11] purified by DE cellulose chromatography, and verified by SDS-polyacrylamide electrophoresis. Laminin was provided by Dr G. Martin (National Institutes of Health, Bethesda, MD.). Antibodies to the antigens listed above were raised in rabbits or goats. Antibodies cross-reacting with common determinants of the different collagen types were eliminated by absorption after repeated passages through the different collagens bound to CNBr-activated sepharose. Other antibodies were commercially obtained: mouse monoclonal anti-alpha actin from smooth muscle (Sigma A 2547, synthetic decapeptide), polyclonal chicken anti-rabbit actin (Sigma A 2668, Sigma Chemical Co. St Louis, MO), mouse monoclonal anti-desmin (Amersham RPN 1101, Arlington, IN), and human polyclonal anti-rabbit elastin[12] (Institut Pasteur, Paris, France). Optimal dilution for the primary antibodies varied from 1:2 for anti-type I collagen to 1:5 for all the other antisera, and 1:200 for the monoclonal antibodies (anti-actin and anti-desmin). The fluoresceinated secondary antibodies were diluted 1:40 or 1:80. The presence of *S. mansoni* antigen(s) in the inflammatory foci was investigated in eight cases with the use of cryotome sections and immunofluorescence microscopy with mouse monoclonal antibodies against circulating anodic antigen and circulating cathodic antigen, as well as goat polyclonal antisoluble egg antigen.

Electron Microscopy

The third portion was cut into tiny pieces and immediately immersed into iced 0.2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4, for 2 hours. Fragments were washed in the buffer solution, post-fixed for 1 hour in 1% osmium tetroxide, and embedded in Epon resin. Blocks were cut in a Reichert Ultra Cut automatic ultramicrotome (Reichert, Vienna, Austria). Semithin sections were stained with toluidine blue and used for selecting areas for ultrathin sectioning. Ultrathin sections were made with a diamond knife, contrasted with lead nitrate and uranyl acetate, and examined with a Zeiss EM-9 electron microscope at 50 kV.

RESULTS

General Data

Portal fibrous enlargement, with partial or total destruction of the main portal vein branch and preservation of the ductal and arterial structures, could be demonstrated in every case. The parenchyma usually maintained its normal lobular or acinar architecture, although dissected by long and thin fibrous septa. Periovular granulomas were not considered essential for the histopathologic diagnosis of hepatosplenic schistosomiasis, but were searched for and registered, their presence being rare, as can be seen in Table 1.

TABLE 1. Main Microscopic Findings in Surgical Liver Biopsies From 63 Patients With Hepatosplenic Manson's Schistosomiasis

Findings	No.	%
Granulomas		
With eggs	21	31.8
Without eggs	2	3.0
Chronic hepatitis*		
Active (mild 5, moderate 6)	11	16.6
Persistent (mild 26, moderate 8, marked 2)	36	54.5
Septal fibrosis	35	53.0
Nodular regeneration	7	10.6
Cholestasis	1	1.5

* Classification of chronic hepatitis was made according to the criteria of DeGroote et al.[13]

Extracellular Matrix

Fibrous tissue in portal spaces and in septal bands exhibited variable densities (Fig 1). In zones of dense collagenous tissue one could see areas in which the matrix appeared rarefied with loosely arranged and dispersed fibers in the middle of amorphous cosinophilic ground substance. Sometimes a relatively extensive area of the portal tissue had a fibrillar appearance, with numerous dilated thin-walled blood vessels (angiomatoid change) in the absence of inflammatory changes and fibroblast or endothelial cell proliferation. Picrosirius-red staining showed collagen fibers to be fragmented and to exhibit different thicknesses and variable sizes and forms in areas of rarefaction (Fig 2). The same was also detected in sections examined under polarized light.

Fibrous tissue in portal and septal areas showed specific fluorescence for type I collagen. The fluorescence was brighter for type III collagen and, particularly, for pro-collagen III. Usually type I collagen was better seen in areas of more compact fibrosis. Type III collagen had a more diffuse distribution and was the predominant type in septal fibrosis. In areas of portal fibrosis exhibiting focal rarefaction both collagen types could be identified inside and outside the rarefied zones. A quantitative estimation of which collagen type was more abundant seemed impossible with the techniques used. There were variations not only from case to case, but also from area to area. Antibodies against type III and pro-III collagens are probably more reactive and usually yield brighter fluorescence. Type V collagen was seen as thin and dispersed fibers, especially within highly vascularized areas, forming a reticular network. Type IV collagen was scanty within the fibrous tissue but gave good staining for the basement membranes of blood vessels and biliary ducts. The blood vessels were distinctly demonstrated with anti-type IV collagen antibodies and so the angiomatoid lesion of schistosomiasis became more evident. The same happened with anti-laminin. Anti-elastin antibodies not only showed the inner walls of blood vessels, but recognized numerous hyperplastic fibers in the portal spaces and fibrous septa (Fig 3A). The amount of elastic tissue shown by elastic stain (Weigert's) or by immunofluorescence with anti-elastin antibodies was markedly increased. Elastin appeared more concentrated in areas where the portal vein

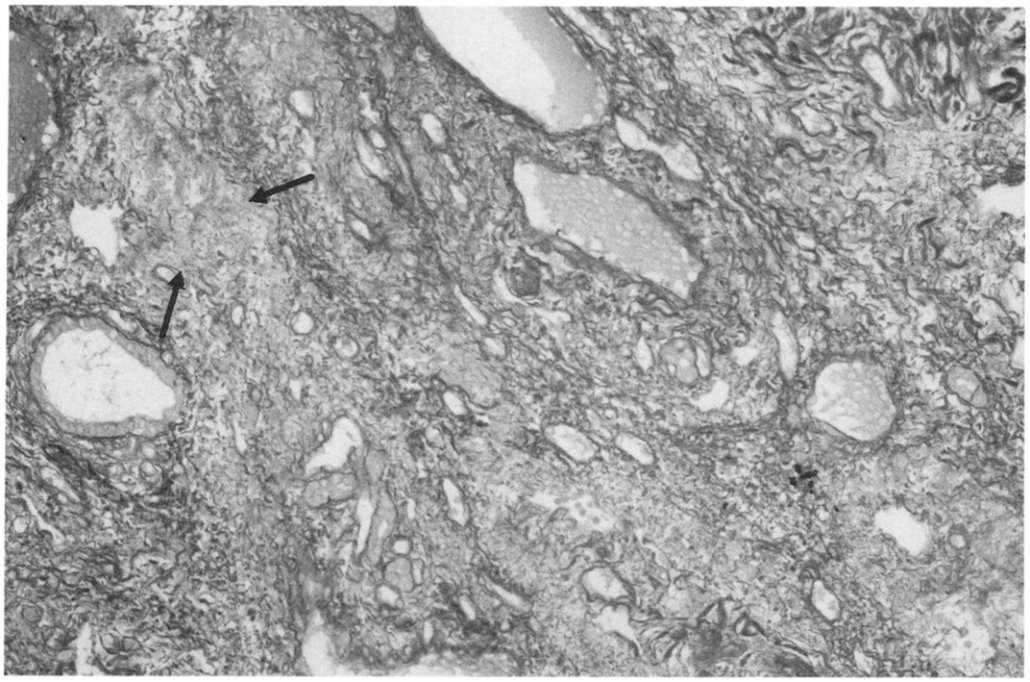

FIGURE 1. A fibrotic and well-vascularized portal space showing focal areas where the collagen fibers have almost disappeared (arrows) and zones of fragmentation of coarse fibers (upper left corner). (Picrosirius-red stain; magnification ×90.)

branches were destroyed, especially around dispersed smooth muscle cells. It was also abundant in subcapsular areas, in zones where the matrix appeared rarefied, and in areas of angiomatoid transformation. Sometimes dark clumps and wavy and fragmented fibers of elastic staining material were observed, suggesting areas of elastic tissue degradation (Fig 4). Fibronectin was abundant in portal spaces, forming a dense network, but sometimes showing areas of rarefaction (Fig 3B). The anti-actin antibodies reacted with the muscular wall of blood vessels and with several structures dispersed within the portal fibrous tissue, identified as dispersed smooth muscle fibers (Fig 3C). Desmin antibodies were a good marker of endothelial cells in the portal vessels (Fig 3D), but at the parenchymal level they stained elongated and plump cells along the sinusoidal walls that probably represented fat-storing cells (Fig 3E).

At the ultrastructural level the matrix usually contained abundant normal-looking, cross-striated collagen fibrils, forming dense and parallel bundles separated by spaces containing long and thin cellular cytoplasmic prolongations or amorphous material, within which a network of fine and straight microfibrils and elastin deposits was seen. No clear-cut correlation was obtained

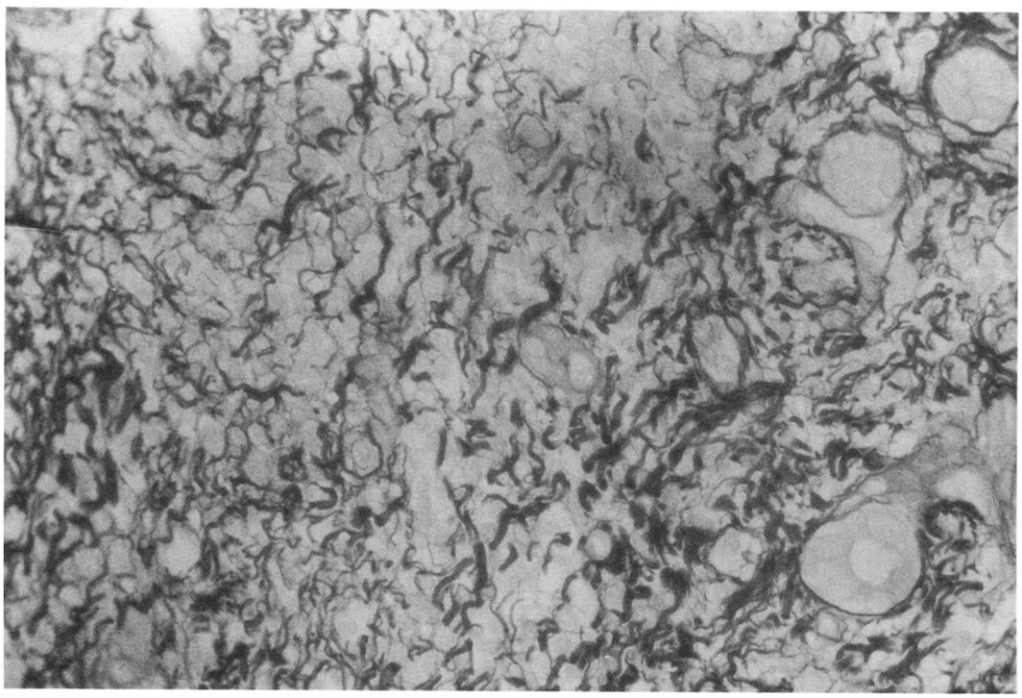

FIGURE 2. Specifically stained collagen fibers present fragmentation, dissociation, and variation in thickness. (Picrosirius-red stain; magnification ×356.)

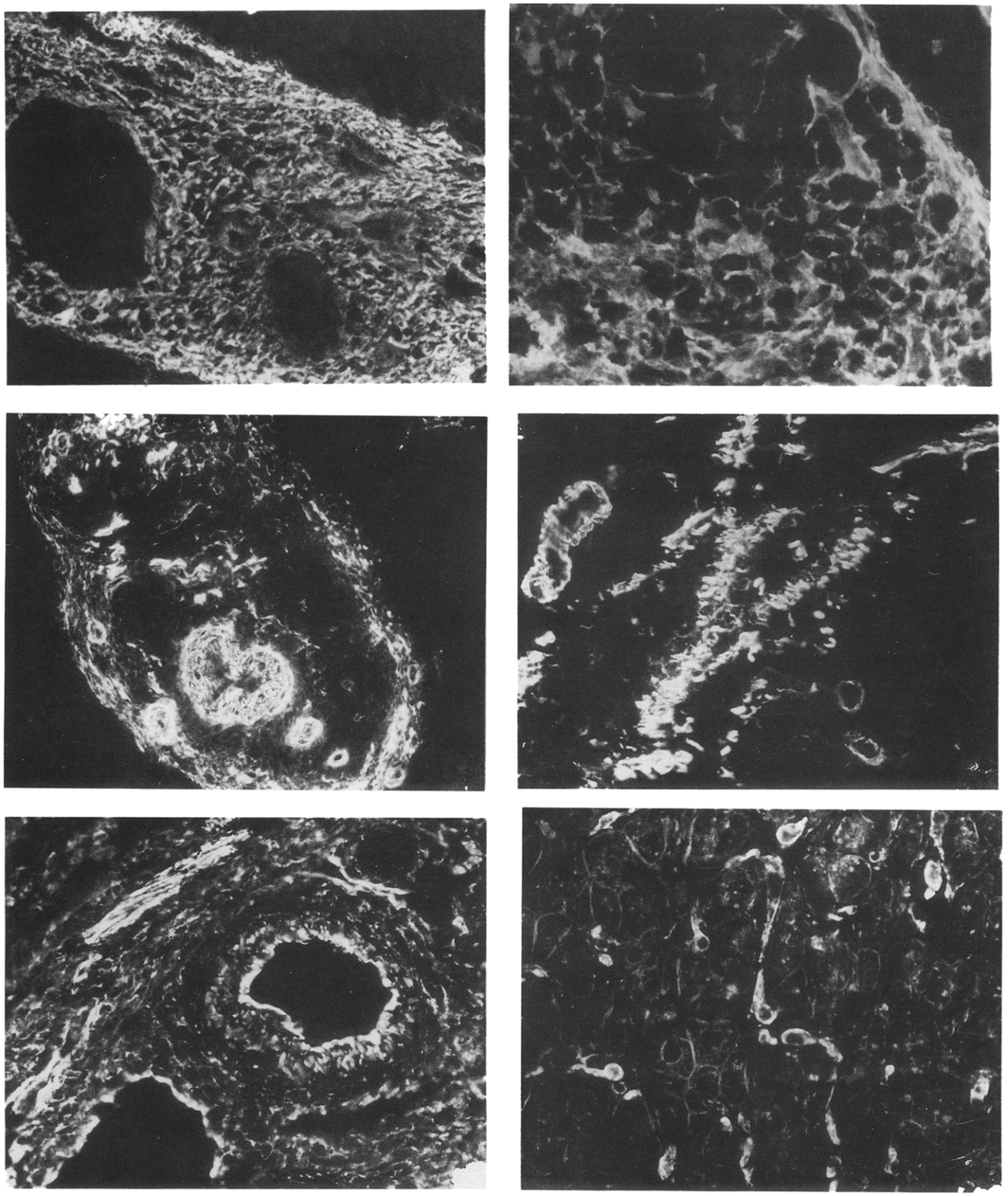

FIGURE 3. (Top left) Presence of increased elastin content in an enlarged fibrotic portal space. (Immunofluorescence; magnification ×182.) (Top right) Fibronectin appears abundantly in schistosomal periportal fibrosis, but with irregular concentration from place to place. The photograph shows a focus of rarefaction. (Immunofluorescence; magnification ×364.) (Center left and right) Portal space stained with specific fluoresceinated antibodies against actin. One can see the wall of blood vessels, but also dispersed fragments of muscular tissue and groups of isolated muscular fibers. (Immunofluorescence: Magnifications: center left, ×182; center right, ×364.) (Bottom left and right) Fluorescent staining for desmin. The endothelial cells in the portal space as well as other connective tissue cells dispersed within the fibrous tissue appear brightly positive. (Immunofluorescence. Magnifications: bottom left, ×364; bottom right, ×376.) (Bottom left) Within the parenchyma the positive cells are elongated and have a parasinusoidal location, probably representing fat-storing cells (Ito cells), while the sinusoidal endothelial cells are negative (bottom right).

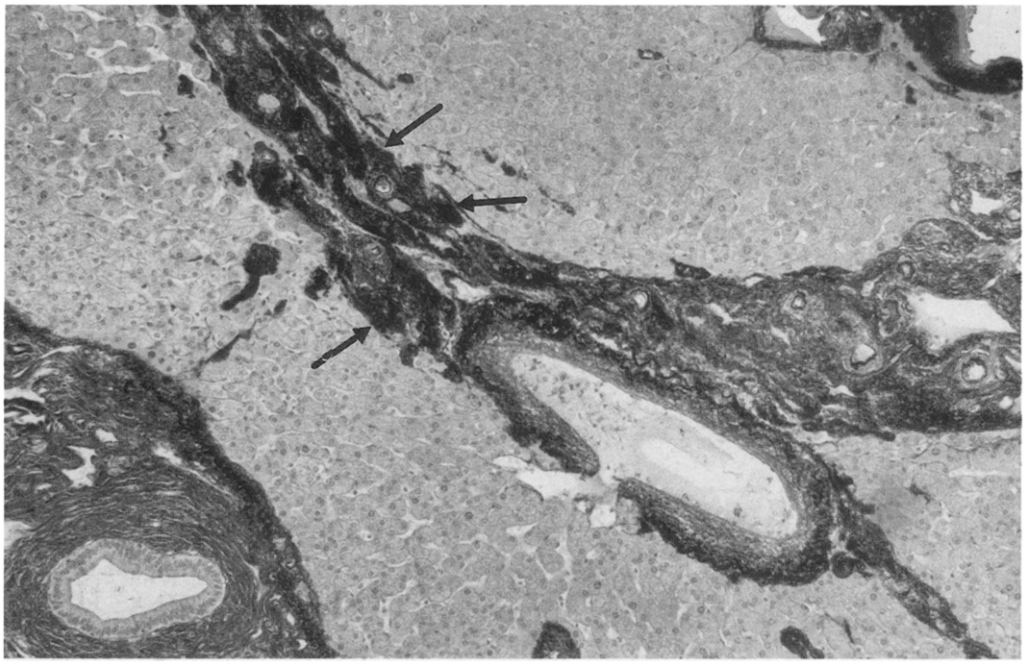

FIGURE 4. Elastic fibers are hyperplastic in the enlarged portal space, sometimes forming dark and structureless clumps (arrows). (Weigert's stain for elastic tissue; magnification ×108.)

between these focal areas of matrix dissolution and the presence of cells, inflammatory or otherwise, although several cellular types could sometimes be identified in the sections (Fig 5). Besides fibroblasts and a few myofibroblasts, eosinophils in different stages of degranulation, neutrophil polymorphonuclear leukocytes, lymphocytes, macrophages, and even mast cells were found in variable numbers within the portal and septal tissues. In the middle of well-differentiated collagen bundles the formation of empty spaces of variable sizes and shapes was often observed. In the vicinity of such spaces collagen fibrils exhibited fragmentation and variations in caliber. In other focal areas of degradation the collagen fibrils were replaced by finely granular electron-dense material (Fig 6) with focal collagen fragmentation, dissociation, and the presence of microfibrils.

Vascular Lesions

Larger portal vein branches frequently showed phlebosclerosis, focal or diffuse intimal thickening, thrombosis in different stages of organization, and re-

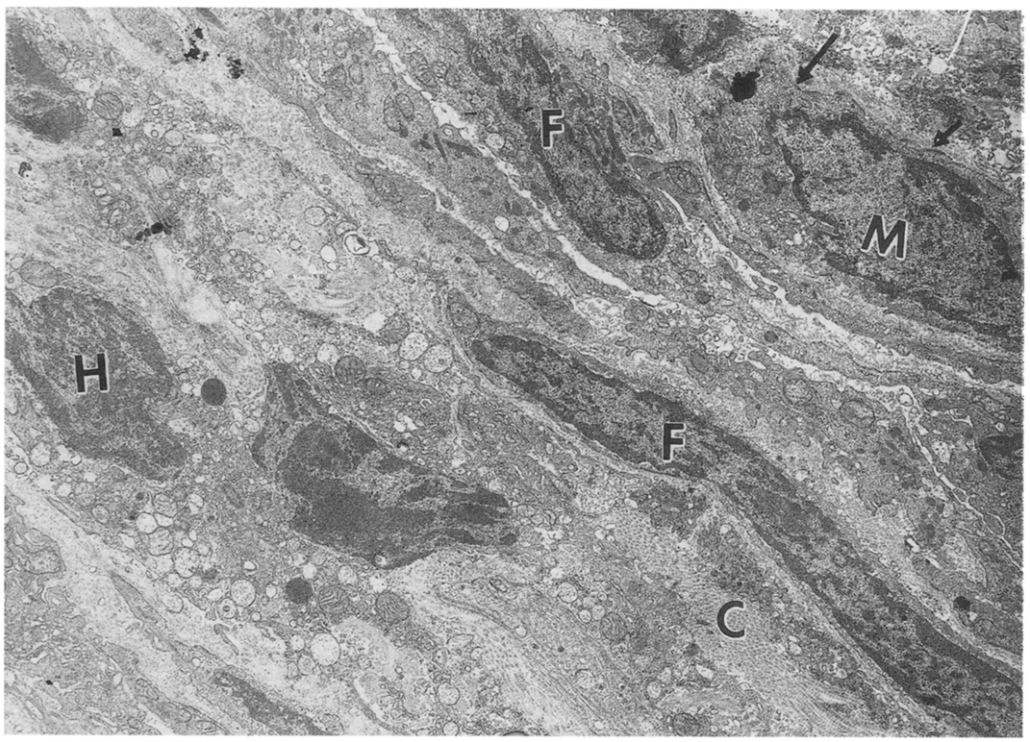

FIGURE 5. Representative area of the portal space in hepatosplenic schistosomiasis. There are parallel rows of collagen fibrils (C), separated by fusiform cells, cytoplasmic prolongations, and areas of amorphous substance. M, a myofibroblast with a prominent endoplasmic reticulum, a dark, discontinuous submembranous contractil apparatus (arrows), and basement membrane-like material around the cell; H, hepatocyte; F, fibroblasts. (Electron micrograph; magnification ×2,610.)

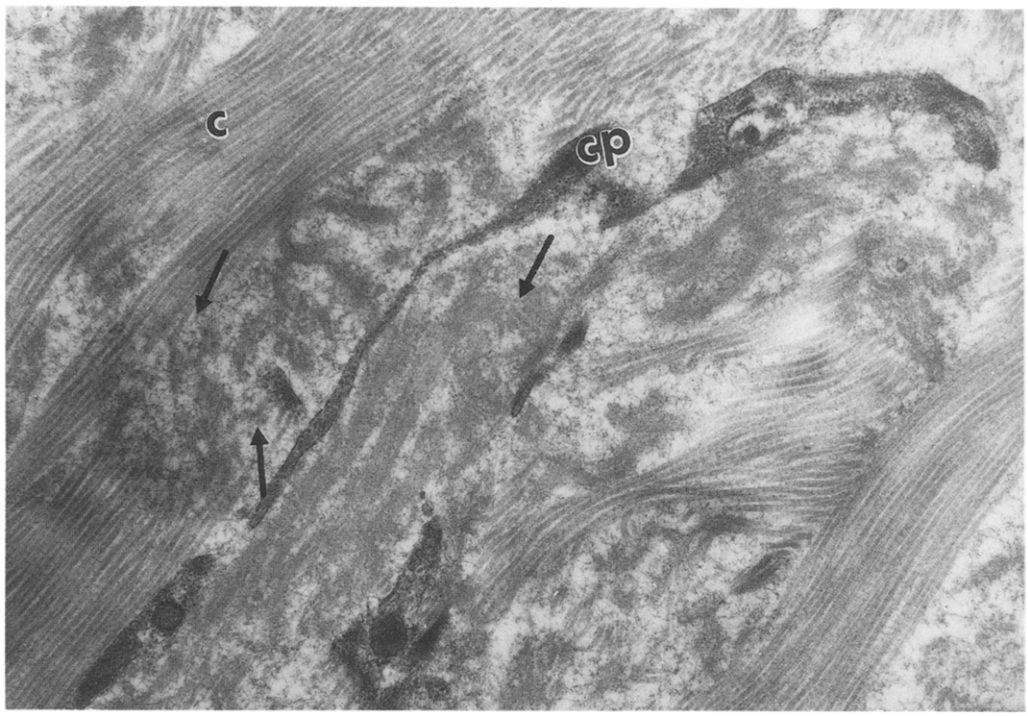

FIGURE 6. Zones where the collagen fibrils appear interrupted and replaced by dark granular material: "electron-dense changes" (arrows). c, collagen fibrils; cp, cytoplasmic prolongations. (Electron micrograph; magnification × 40,800.)

canalization, narrowing, and retraction. In the presence of such alterations, the muscular wall of the portal vein appeared disrupted with smooth muscle cells and groups of muscular fibers becoming dispersed in the fibrous portal tissue, a constant finding in the present material (Fig 7). The use of fluorescent antibodies against actin helped demonstrate muscular tissue throughout the portal space (Figs 3C and 3D). Schistosomal pigment was frequently noticed in the portal spaces, especially around old periovular granulomas. No increase or decrease in fibrosis, inflammation, or any other feature was found in relation to the presence of pigment.

Chronic Hepatitis

The presence of inflammatory changes in the portal spaces, other than those around the schistosome eggs, was detected in several cases. One case disclosed a positive Shikata test. This case presented chronic active hepatitis and partial nodular transformation of the hepatic parenchyma. All the other cases were negative.

As for inflammatory changes, there were focal accumulations of macrophages and lymphocytes in the middle of the larger portal areas, but not in the smaller portal tracts. The presence of eosinophils varied from

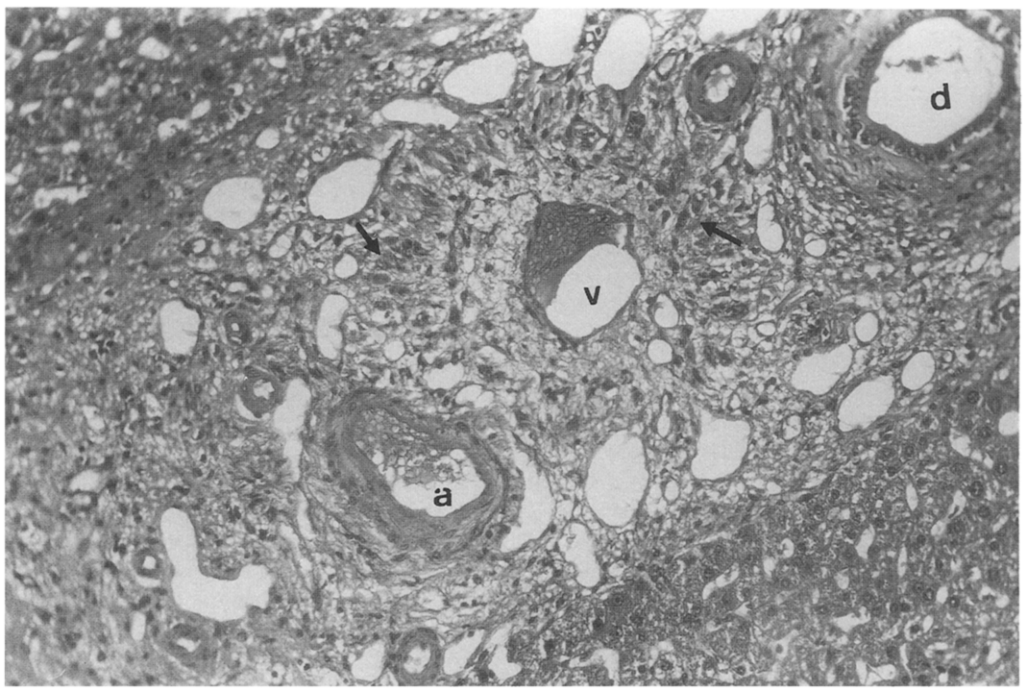

FIGURE 7. Portal changes in advanced schistosomiasis. The portal space is enlarged and fibrosed, with preservation of the arterial (a) and ductal (d) structures, but with partial destruction of the main portal vein branch (v), which shows phlebosclerosis and dispersion of the muscular fibers (arrows). Many thin-walled dilated blood vessels also appear throughout the portal area (angiomatoid change). (Hematoxylin-eosin stain; magnification × 210.)

moderate to mild. Accumulation of mononuclear inflammatory cells also occurred between the parenchymal border and the portal fibrous tissue, especially in the larger portal spaces, but still maintained a focal pattern (Fig 8). Infiltration and dissociation of the liver cell cords at the parenchymal border ("piecemeal" necrosis) were frequently observed. No correlation could be established between the presence of nonspecific hepatitis and the periovular granulomas.

The presence of schistosome antigen(s) was tested in eight cases. Five cases exhibited hepatitis and three did not. None had schistosome eggs in the sections examined. Results were essentially negative in all cases.

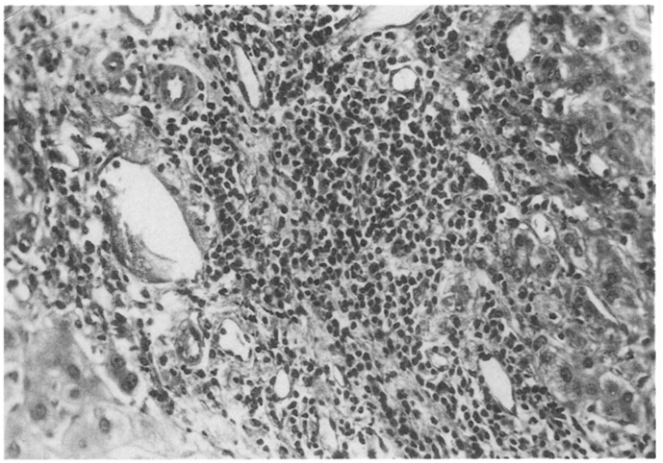

FIGURE 8. Chronic hepatitis with a mild degree of activity in schistosomiasis. Small portal spaces show infiltration by inflammatory mononuclear cells with mild extension into the parenchymal border, a picture representative of the type of chronic hepatitis associated with advanced *Schistosomiasis mansoni*. (Hematoxylin-eosin stain; magnification ×164.)

DISCUSSION

The diagnosis of advanced hepatic schistosomiasis in surgical biopsy material does not constitute a great problem. The presence of portal enlargement due to fibrosis, the partial or total destruction of portal vein branches, the preservation of ductal and arterial structures, and the maintenance of parenchymal lobular or acinar architecture are usually sufficient for a positive diagnosis, since these combined changes are usually not to be found in other conditions. However, when examining material from endemic areas, sometimes one sees focal fibrosis with vascular proliferation and ectasia (angiomatoid formation), especially in the presence of cirrhosis. In such cases the differential diagnosis with schistosomiasis can be crucial. The search for muscular cells or fibers "buried" in the fibrous tissue will frequently offer a key for the diagnosis of schistosomiasis. This finding is an indication that a small or medium-sized branch of the portal vein has been destroyed, an indispensable feature to characterize the periportal fibrosis as being of schistosomal origin. The presence of isolated smooth muscle fibers within the portal tissue was observed in every case of the present series. Although immunofluorescence for actin can make this finding more impressive, light microscopy with conventional staining is entirely adequate. Some degree of phlebosclerosis with dissociation of the portal vein muscular coat may appear in cirrhosis and in other fibrosing hepatic processes, but cannot be compared with the profound disarray usually present in advanced schistosomiasis.

Dislodged muscle cells in portal tissue remain viable. Therefore, they may be important for the contractility of the fibrous tissue and may play a role in the pathogenesis of portal hypertension. They may differentiate into myofibroblasts, which have been recognized as an important cell type in periportal schistosomal fibrosis.[14] Studies in vitro have shown that connective tissue cells isolated from human schistosomal fibrosis exhibited a modified phenotype of smooth muscle cell.[15] Smooth muscle cells also may be engaged in the synthesis of elastin,[16] another matrix component abundant in pipestem fibrosis. Morphologically, there was good correlation between the presence of elastin or elastic fibers with signs of portal vein destruction and muscular dissociation. Endothelial cells may also synthesize elastin.[16] The presence of numerous endothelial cells in the angiomatoid lesion and the dislodgement and proliferation of smooth muscle cells may explain the prominent elastic hyperplasia in advanced hepatic schistosomiasis. However, elastic fibers are conspicuously absent from periovular granulomas.[17,18] It has been suggested that elastic tissue in liver disease is related to chronicity of the lesions.[19] Perhaps an isolated periovular granuloma may not survive the necessary time for excess elastin synthesis to take place.

Matricial changes in schistosomiasis are complex and dynamic. There are different genetic types of collagens, associated proteins, and abundant proteoglycans.[18] Collagen fibers show focal ultrastructural changes compatible with chronic degradation of the same morphologic types as those found in experimental periovular granuloma after chemotherapy.[20] Most of the patients in the present series could not recall being treated for schistosomiasis, which means that degradation of collagen may occur spontaneously after partial or total self-cure of the infection. Spontaneous regression of the hepatosplenic form of schistosomiasis has been recorded,[21] as well as involution following chemotherapy.[22,23] We observed that morphologic evidence of collagen degradation can be detected by light microscopy. It is the counterpart for the involution of periportal fibrosis that can now be followed by ultrasonography in treated patients.[23,24] Excess fibrous tissue in portal spaces is far from having a homogeneous appearance. It shows areas of rarefaction and of actual fragmentation of fibers. These changes are not always properly evaluated by the pathologist. It is expected that the whole subject of periportal schistosomal involution now being described in humans may stimulate a closer microscopic observation of collagen changes within the concept of chronic matrix degradation.[20]

Actually, fibrosis results when synthesis of extracellular matrix exceeds degradation, both processes oc-

curring side by side.[25] The natural tendency is toward a balanced parenchyma/stroma ratio.[26] Today, the use of large-scale chemotherapy for schistosomiasis and portable ultrasound technology has permitted the documentation of slow resorption of portal fibrosis. In a certain proportion of cases, degradation of the fibrous matrix is probably followed by remodeling of the obstructive, intrahepatic vascular changes, which are primarily responsible for portal hypertension, with complete reversion of hepatosplenic disease.[22] However, the tendency of the fibrous tissue to undergo resorption probably occurs in every case in which the schistosomal infection has become inactivated. Matrix degradation is probably more vigorous in early treated infections, but no data are available to substantiate this claim. Our patients did not provide a history of previous treatment. The cases of posttherapeutic periportal fibrosis involution so far reported have not included pathologic data.

The changes of chronic hepatitis seen in hepatosplenic schistosomiasis remain obscure in their pathogenesis and significance. When there are chronic active hepatitis and progression to cirrhosis, an associated infection with the B hepatitis virus can usually be demonstrated.[27] However, such an association is difficult to prove in those more frequent cases of chronic persistent or mildly active hepatitis. These latter cases had some peculiarities, such as the presence of focal accumulation of mononuclear cells in the middle of the fibrotic area and the presence of invasion of the parenchymal border by inflammatory cells in larger rather than smaller portal spaces. These aspects seem related to the schistosome infection, since no other cause was clinically or morphologically apparent in the material studied. However, it was not possible to demonstrate schistosomal antigen(s) in relation to these inflammatory changes. Techniques of immunoelectron microscopy may be necessary in future studies to help clarify this point.

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