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# PATHOLOGY OF PERIPORTAL FIBROSIS INVOLUTION IN HUMAN SCHISTOSOMIASIS

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## SUMMARY

Optical and electron microscopical evidences of focal matrix degradation were frequently seen in liver sections of periportal fibrosis caused by schistosomiasis mansoni in man. The material came from 14 wedge hepatic biopsies taken from patients with chronic advanced hepatosplenic disease and undergoing operations for the relief of portal hypertension. Besides the presence of focal areas of rarefaction, fragmentation and dispersion of collagen fibers, the enlarged portal spaces also showed hyperplasia of elastic tissue and disarray of smooth muscle fibers following destruction of portal vein branches.

Eggs were scanty in the tissue sections, and matrix degradation probably represented involuting changes related to the progressive diminution of parasite-related aggression, which occurs spontaneously with age or after cure by chemotherapy. The changes indicative of matrix degradation now described are probably the basic morphological counterpart of periportal fibrosis involution currently being documented by ultrasonography in hepatosplenic patients submitted to curative chemotherapy.

**KEY WORDS:** Matrix degradation; Fibrosis, Schistosomiasis; Collagen; Chemotherapy.

## INTRODUCTION

The last twenty years have witnessed considerable improvement in the treatment of schistosomiasis due to the introduction of new effective and simple drugs[5]. These are drugs which present high cure-rates (70-95%) after one single dose, administered orally with almost no contraindications and which have permitted large scale treatment programs in endemic areas.

By the middle of last decade a decrease in morbidity began to be appreciated[2] and the new drugs were found to be both curative and preventive of hepatosplenic schistosomiasis[7,22]. Improvement or complete reversibility of hepatosplenic disease has been observed to follow curative chemotherapy with diminution in the size and consistency of the liver and spleen and disappearance of the signs of portal hypertension[7,14,22]. The introduction of portable sonography has not only permitted such observations to be made in the field, but also to actually demonstrate that periportal fibrosis (pipe-stem fibrosis) undergoes considerable resorption months or years after chemotherapy[9,17,18].

Experimental studies have shown morphological evidences of early[3,8,27] and late[4] matrix degradation after cure of schistosomiasis, including the involution of pipe-stem fibrosis in the murine model[1], but no pathological studies related to reversibility of pipe-stem fibrosis in humans have been published. The fact that involution of pipe-stem fibrosis in man takes several months or years to be clinically noticed, makes such studies difficult to be performed. On the other hand, to obtain liver biopsies before and after cure for comparison would involve ethical problems. However, reversion

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of periportal fibrosis in schistosomiasis has been observed to occur even spontaneously[19], which suggests that the process of matrix degradation is not necessarily dependent on chemotherapy.

Present investigation is a histological, immunocytochemical and ultrastructural study of wedge surgical biopsies of the liver in advanced, untreated hepatosplenic schistosomiasis. Signs of connective tissue degradation, similar to those observed in experimental material[3,4], were searched in an attempt to understand and to document morphological features possibly related to periportal fibrosis resorption in schistosomiasis.

## MATERIAL AND METHODS

**General Clinical Data** - Patients were admitted to the Roberto Santos Central Hospital in Salvador, Bahia – Brazil, because of complications of portal hypertension. There were ten males and four females, and their ages varied from 18 to 53 years (average: 32.6). They presented hepato-splenomegaly and esophageal varices for several years and all except one gave a past history of massive gastric bleeding. The one who did not bleed had growth retardation and severe anemia and leukopenia (hypersplenism). Schistosome eggs were present in the stools from all patients and diagnosis of hepatosplenic schistosomiasis was confirmed histologically. Under questioning none of the patients could recall being treated for schistosomiasis before. Pipe-stem fibrosis due to schistosomiasis was the main lesion seen histologically in all the cases. Three patients presented signs of chronic persistent hepatitis and one of chronic active hepatitis of moderate degree. This latter was also proved to be infected with hepatitis B virus. One case proven at histological examination to have schistosomiasis and hepatic cirrhosis was excluded from the present study.

There was no emergency operation. Patients were considered to be in good surgical condition. The operation consisted of splenectomy plus ligation of esophageal varices. No surgical mortality occurred.

The wedge liver biopsies were divided into three portions: one destined for histology, another for electron microscopy and the third for immuno-cytochemical studies.

**Histology** - Tissue was fixed in Bouin's fluid and embedded in paraffin as usual. The sections for light microscope examination were stained with hematoxylin and eosin, Weigert's resorcin-fuchsin method, Gomori's reticulin, Masson's trichrome stain, PAS (with and without previous diastase treatment), Perls' method for iron, and the picrosirius-red technique, these latter sections being examined with and without polarized light.

**Electron Microscopy** - Tiny liver fragments were immediately fixed in the operating room with cold 0.2% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4, for two hours and post-fixed in 1% osmium tetroxide. They were embedded in epon resin, cut in a Reichert ultramicrotome with a diamond knife and ultra-thin sections were contrasted with lead nitrate and uranyl acetate. Examination was performed in a Zeiss EM-109 electron microscope at 50 Kw.

**Immuno-cytochemical Studies** - Small pieces of liver were covered with Tissue Tek (Miles Inc., USA) and snap frozen in liquid nitrogen (-196°) for 5-10 minutes. Blocks were kept in air-tight plastic boxes at -70°C until the moment of sectioning in a cryotome at -20°C. Sections were treated by indirect immuno-fluorescence with monospecific antibodies against collagen isotypes I, III, IV and V, laminin, fibronectin, actin, desmin and elastin. All these antibodies were obtained from and utilized at the Institute Pasteur de Lyon, France.

Anti-collagen, anti-laminin, anti-fibronectin antibodies were raised in rabbits and prepared at the Pasteur Institute. The details about their preparation and tests of monospecificity are referred to elsewhere[3,4]. The other reagents were commercially obtained: polyclonal chicken anti-rabbit actin[21], monoclonal anti-mouse alpha actin from smooth muscle (Synthetic decapeptide, Sigma A 2547), monoclonal anti-mouse desmin (Amersham RPN 1101) and rabbit anti-human elastin[21]. Optima dilution for the primary antibodies was 1:5, except for anti-type I collagen (1:2) and for monoclonal antibodies (1:200 or 1:300). The fluoresceinated conjugates were diluted at 1:40 or 1:80.

## RESULTS

So-called "pipe stem" fibrosis was present in all cases, with variable degrees of septal fibrosis and general preservation of the acinar

architecture of the hepatic parenchyma. Portal fibrous tissue presented variable densities under the light microscope, especially when the sections were stained with picrosirius red and observed under polarized light. There was no present evidence of fibroblast or vascular proliferation. Periovular granulomas were rare, small, fibrotic and discrete. Coarse clumps of material stained by the Weigert method or appearing with positive fluorescence for elastin were also found. Collagen fibers were disposed in compact parallel rows, alternating with focal clear areas where the fibers appeared fragmented and loosely arranged (Fig. 1). Some areas contained sparse fiber fragments within a ground amorphous eosinophilic matrix. These focal and less dense areas contained many small well-differentiated blood vessels, as did the rest of the enlarged portal spaces (Fig. 2). Frequently, a more or less thick band of less dense matrix, with a finely granular appearance was seen along the limits between the parenchyma and the portal space. Intrahepatic branches of the portal veins were frequently altered with phlebosclerosis, thrombosis, narrowing and retraction, with parts of their muscular walls being "burried" into the portal fibrous tissue (Fig. 3). Sometimes muscular fibers appeared dispersed in several directions (Fig. 4). This dispersion of smooth muscle fibers was observed in every case.

Type I and Type III collagen fibers were present together in areas of portal and septal fibrosis, including the areas where collagen tissue appeared rarefied. (Fig. 5A). Elastic fibers, either stained by the Weigert's method or demonstrated with the anti-elastin antibodies, were present, sometimes abundantly, in the extracellular matrix of portal and septal areas (Fig. 5B). When only a few elastic fibers were present, they were located around partially destroyed portal veins in the center of the portal space or in the sub-capsular zone. Numerous small thin-walled blood vessels were also always present, forming the well-known angiomatoid pattern. Antibodies against type IV collagen and against laminin as well marked the walls of these blood vessels (Fig. 5C). Type V collagen formed a network of fine fibrils throughout the fibrous tissue, especially evident in areas with rich vascularization. As has been observed by others[16,17], desmin marked the endothelial cells

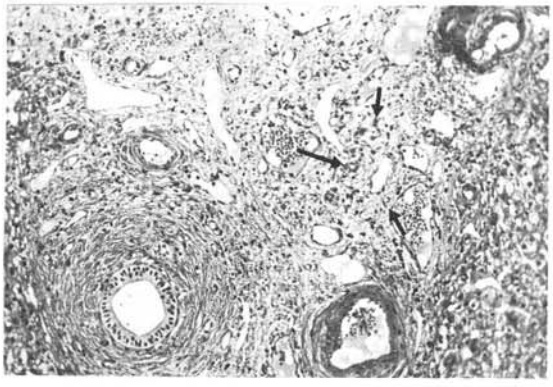

Fig. 1 – Periportal fibrosis. Fibrous tissue is dense around the bile duct (bottom left), but appears with loosely arranged and fragmented fibers and dispersed smooth muscle fibers (arrows) in between the numerous thin-walled blood vessels. Hematoxylin & Eosin, 100 X.

Fig. 2 – Periportal fibrosis. Detail of the previous picture to show the fragmented collagen fibers in a well-vascularized edematous looking connective tissue. Collagen fibers are specifically stained by the Picrosirius-red method, 400 X.

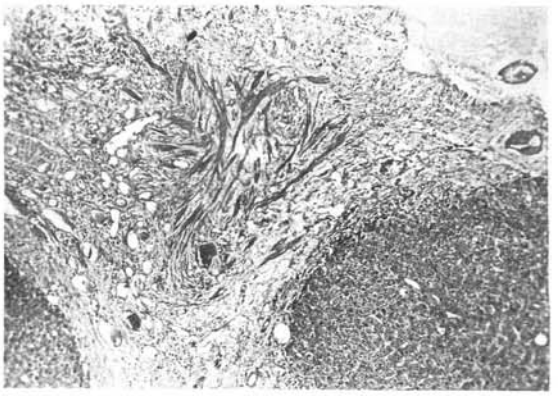

Fig. 3 – Enlarged and fibrosed portal space with many blood vessels and disarray of smooth muscle fibers. These muscle cells probably originated following destruction of the intrahepatic portal vein walls. Hematoxylin and Eosin, 100 X.

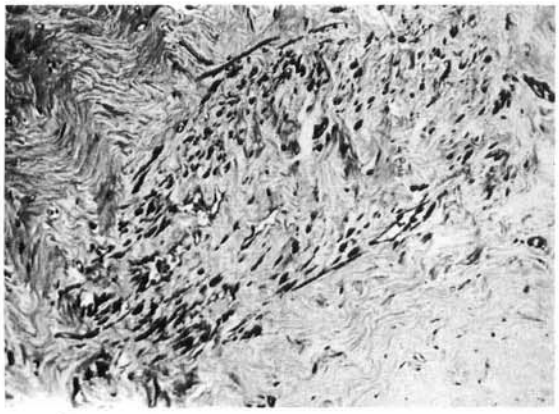

Fig. 4 – Portal fibrosis. The original portal vein branch is replaced by vascular slits while the smooth muscle fibers appear "buried" into the fibrous tissue. Masson's trichrome stain, 250 X.

present in the portal spaces (Fig. 5D). Fusiform cells along the sinusoidal walls, probably fat-storing cells, were also desmin-positive. The remnants of smooth muscle were better revealed by the immuno-fluorescence technique with anti-actin antibodies. Then, numerous small fragments of actin-positive material appeared around portal veins as well as far from these vessels (Fig. 5E). The positive correlation of these fragments of muscle with elastic fibers was quite apparent.

Fibronectin was prominent, forming a network of fibrils that became more dense in the rare periovular granulomas, as well as within zones of focal chronic inflammation, also seen in a few cases. In foci of collagen degradation fibronectin also disappeared (Fig. 5F).

At the ultrastructural level portal tissue was represented by densely packed collagen fibrils forming thick fibers separated one from the other by cellular processes and by an amorphous ground substance containing numerous and thin interwoven microfibrils and variable amount of elastin (Fig. 6). The long and thin cytoplasmic processes were associated with fibroblasts, but some such prolongations were from myofibroblasts, since they contained submembranous dark contractile structures and were surrounded by thin basement membrane-like material. The presence of polymorphonuclear neutrophils and eosinophils was noted in some sections, but their presence apparently did not modify the general appearance of the matrix. From time to time focal clear areas appeared in the middle of the collagen fibers disrupting their continuity. In these areas collagen fibers were dissociated and fragmented, exhibiting variation in thickness and in electron density (Fig. 7). In larger areas, the empty space was occupied by collagen fragments of different sizes and by finely granular electron-dense material as well as by concentrated amount of elastin and/or microfibrils (Fig. 8). Transitional features between fragmented collagen and the electron-dense granular deposits were noted in focal areas (Fig. 9). These focal "degenerative" changes were also present in collagen fibers forming intra-parenchymal septa.

## DISCUSSION

Pipe-stem schistosomal fibrosis may qualify as an appropriate model for the study of "chronic" matrix degradation. First, post-therapeutic involution of periportal fibrosis can be followed non invasively in living patients thanks to ultrasonography[9,17]. Second, it is a localized fibrosis that enlarges the portal spaces but usually does not disturb the hepatic parenchymal architecture, such as occurs in cirrhosis. Third, it is a complex matrix. Almost all components of the extra-cellular matrix are well represented in the areas of portal and septal fibrosis. Type I and III collagens are abundant. The angiomatoid lesion contributes for the prominence of type IV collagen and laminin. Elastin and proteoglycans appear in large amounts. There is displacement of smooth muscle fibers into the fibrous tissue following destruction of intrahepatic portal vein walls by schistosomiasis. The relationship of these "ecologically" displaced muscle fibers with myofibroblasts and with the elastic fiber hyperplasia seen so prominently in pipe-stem schistosomiasis deserves further investigation. Besides its basic biological implication, displaced smooth muscle cells, myofibroblasts and elastic fibers may contribute to tissue contraction and vascular narrowing and thus may play a role in the pathogenesis of portal hypertension. Displaced smooth muscle results from portal vein obstruction and destruction. Therefore, it has diagnostic importance, since schistosomal periportal fibrosis is the only hepatic disease in our area that presents portal fibrosis with portal vein alterations and preservation of bile

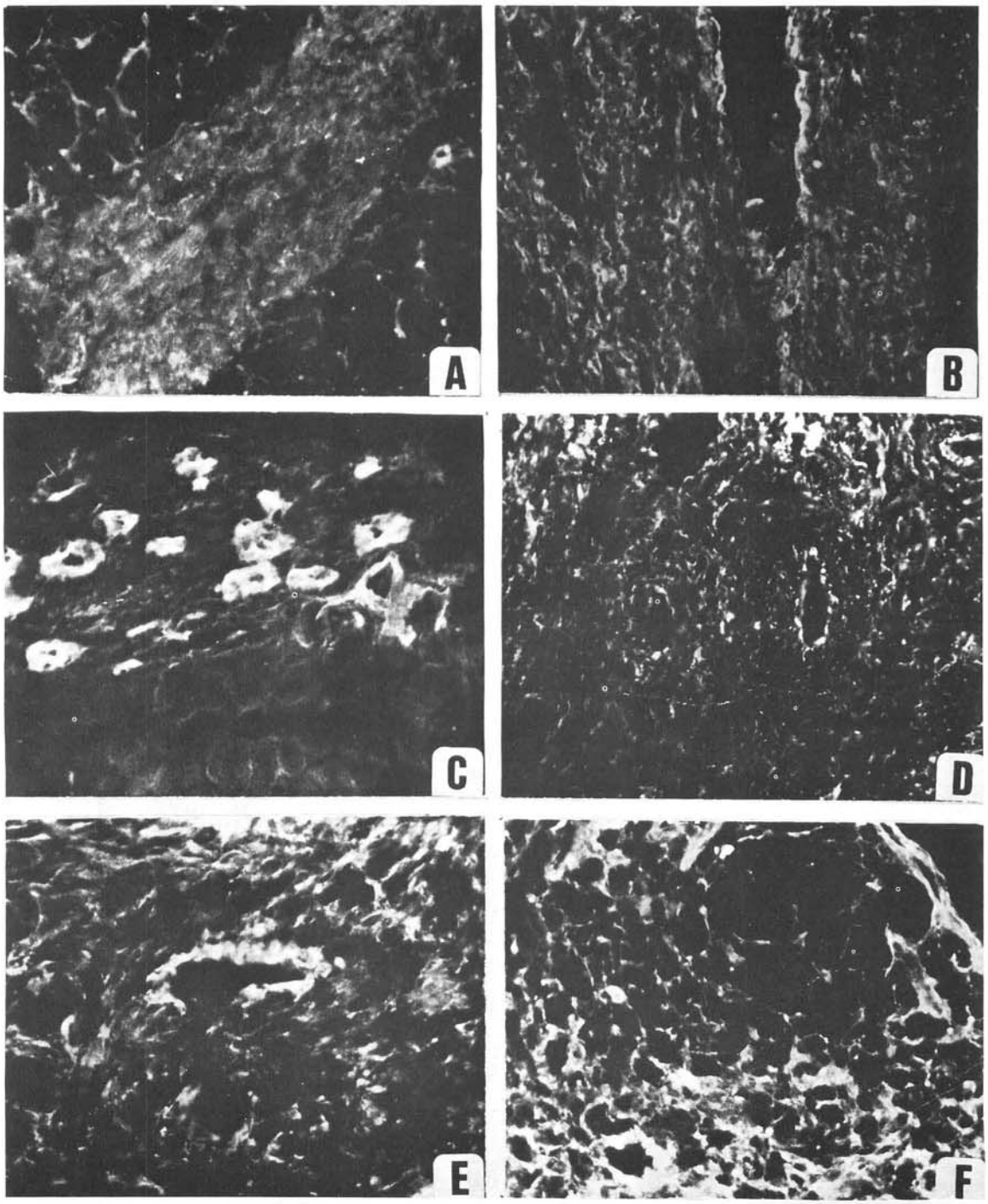


Fig. 5 – A: Type I collagen. The variable density of the area occupied by the collagen fibers can be appreciated; B: Elastin fibers appearing in increased amount in a fibrotic portal space; C: Staining for type IV collagen reveals collection of blood vessels and their thickened basement membranes; D: Desmin marks the endothelial cells in portal space; E: Actin antibodies recognize muscular wall of a partially destroyed blood vessels and fibers scattered in the fibrosed portal space; F: Fibronectin in portal fibrosis is abundant, but shows areas of rarefaction and increased concentration. Immunofluorescence Microscopy, 400 X.

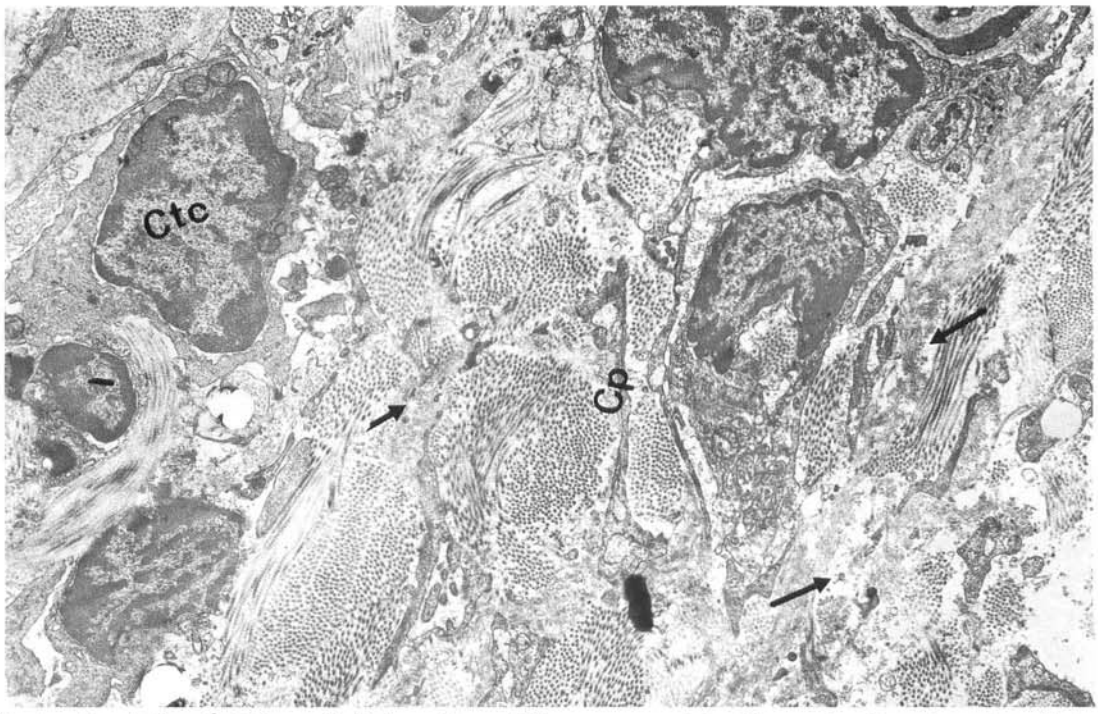


Fig. 6 – Portal fibrosis. Collagen fibrils form fibers which are separated by thin cytoplasmic prolongations (Cp) and connective tissue cells (Ctc). At the lower left of the picture there are features of matricial degradation (arrows), which can also be seen in other areas. Electron Microscopy, 4,400 X.

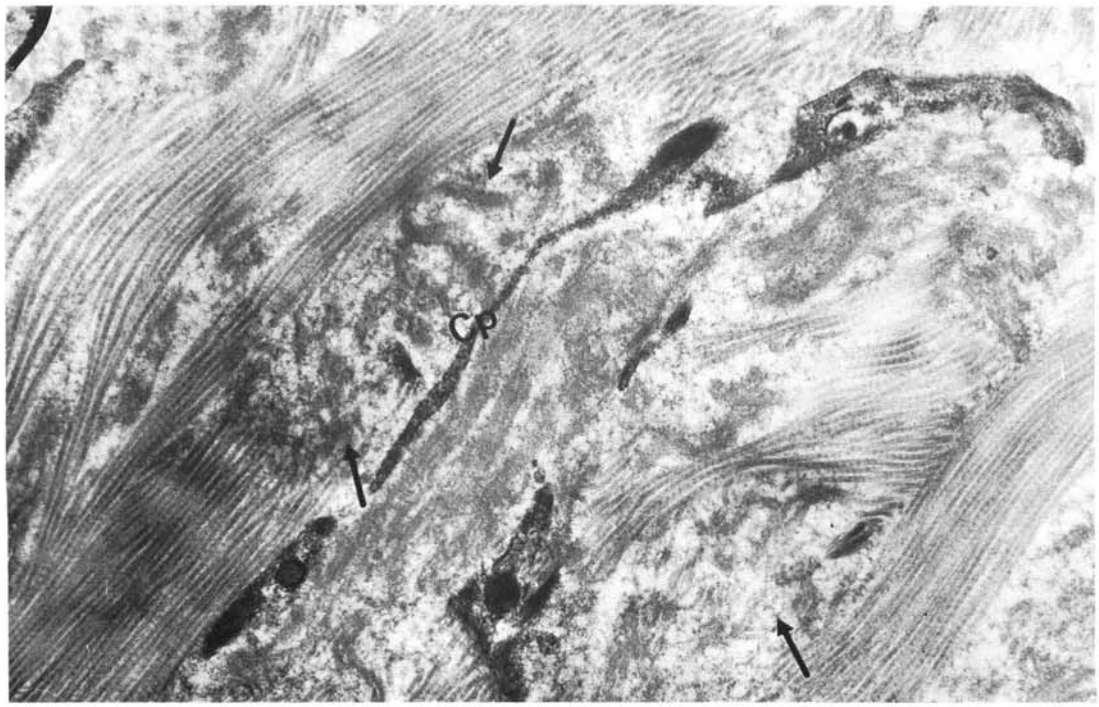


Fig. 7 – Focal collagen degradation. The course of the collagen fibrils is interrupted with formation of dark clumps of granular and amorphous materials ("Electron-dense changes", arrows). Cp = cytoplasmic prolongations. Electron Microscopy, 7,000 X.

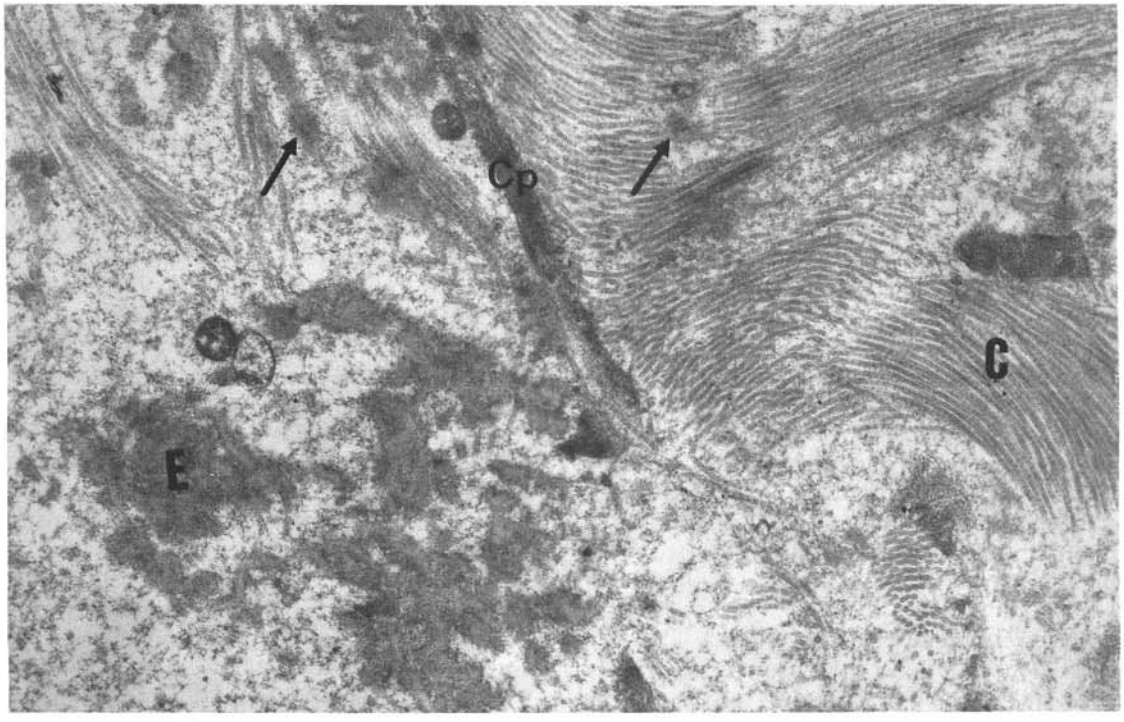


Fig. 8 – Area where collagen fibers appear replaced by abundant deposits of elastin (E), Cp = cytoplasmic prolongation, C = collagen. "Electron-dense changes" of collagen fibrils (arrows). Electron Microscopy, 12,000 X.

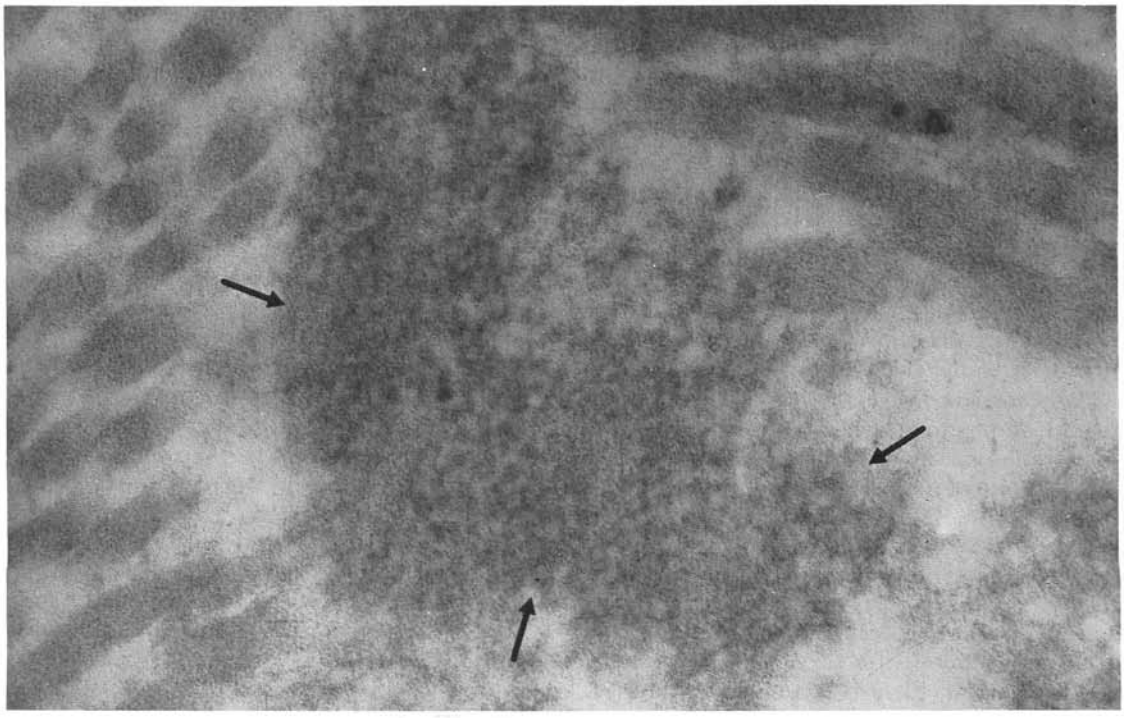

Fig. 9 – High magnification showing an "electron dense" transformation of collagen. In the middle of the collagen fibrils there is the deposition of finely granular electron dense material (arrows). Electron Microscopy, 80.000 X.

duct and hepatic artery structures. The importance of myofibroblasts for the contraction of portal tissue in schistosomiasis has already been emphasized[16]. On the other hand, smooth muscle as well as endothelial cells are capable of synthesizing elastin[12].

Portal fibrosis in schistosomiasis results from the massive deposition of **S. mansoni** eggs in the periportal space[1]. A periovular granuloma forms around each mature egg producing a highly fibrogenic lesion. The dynamics of cellular and extra-cellular matrix formation have been well studied with biochemical and morphological methods[6,14] but its degradation is far less studied and understood. Periovular granulomas pass through a cycle of changes, with fibrogenesis predominating while the miracidium is alive and eliminating its secretions. The miracidium life-span lasts for 14-17 days within the tissues of a susceptible host. When the miracidium dies, matrix degradation predominates and eventually leads to complete resorption of the granuloma. Treatment causing the death of adult worms is soon accompanied by the destruction of all the miracidia and considerable resorption of fibrosis. Early fibrosis, such as that present in the liver of a mouse with 8-10 week old schistosome infection can be rapidly reabsorved following treatment[3,8,27], but old fibrosis (20-26 week old infection) takes more time to be degraded and has even been considered as irreversible fibrosis[28].

Matrix degradation sometimes occurs as a swift change. Current models used in the study of matrix degradation (involution of the pregnant rat uterus, of carrageenin granuloma, the metamorphosing tad-pole tail, involution of CC14-induced cirrhosis in the rat after discontinuation of the drug) represent instances in which excessive fibrous tissue disappears in a matter of days to less than a month. Schistosomal granuloma involution in the murine liver may take 4 to 6 months to be completed. After a period of weeks of rapid changes, with extracellular collagen breakdown and internalization of collagen fragments seen ultrastructurally[3] the process resorts to a slow pace and may be called "chronic" collagen degradation[4]. Little is known about this process besides that it has a peculiar ultrastructural morphology. Such morphology was observed in the present material as foci of collagen fragmentation, dissolution, transformation into granular material and focal lysis. But evidence of matrix degradation was already present at the ordinary light microscopic level. Fibrosis in portal spaces showed variable densities and also foci of fragmentation and dissolution. These are not new changes, but its significance can now be better appreciated. Post chemotherapy periportal fibrosis involution is an impressive demonstration of the potential for reversibility of hepatic fibrosis in human disease. Probably this involuting process is related to but basically different from remodelling. Remodelling occurs when the matrix is being formed and degraded at the same time, as so often happens during fibrogenesis. Chronic degradation referes to the slow progressive and predominating removal of matrix as observed in the liver following curative treatment of schistosomiasis[3,4]. Then, degradation occurs in the absence of signs of fibroblast and vascular proliferation, ultrastructural features of functional activation of cells or increased accumulation of basophilic amorphous ground substance (proteoglycans).

Our patients presented advanced hepatosplenic disease and few eggs in the tissues. Infection in long standing cases may evolve toward self-cure. In 1966 KATZ & BRENER[19] observed reversion of schistosomal hepatosplenic disease in non-treated patients. They reported on patients who remained in the endemic area for a period of 10 years. Seven of them evolved from mild to severe clinical forms, while of 21 hepatosplenic patients, 6 had their disease aggravated, 7 were unaltered and 8 reversed to mild hepato-intestinal form. Only three of the latter received specific treatment. With the advent of new drugs, the potential for reversibility of hepatosplenic schistosomiasis became more evident[7,10,13,17].

Foci of matrix degradation were detected at light and ultrastructural levels in the present cases. One cannot be sure that these patients had not been treated previously. In any case, we are considering the involuting matrix changes as spontaneous and one may doubt whether material from treated patients would show much more. Our present findings suggest that when parasitic stimuli decrease fibrolysis predominates over fibrogenesis leading to the gradual removal of fibrosis. This can be morphologically appreciated with ultrastructural and even optical microscopy if special demonstration of collagen (picrosirius-red and polarized light) is performed.

Our findings are in keeping with the concept that matrix formation and degradation are balanced processes dependent on the same cell types. Fibrosis occurs when formation exceeds degradation[24]. Cells of chronic inflammation secrete cytokines[20] that are stimulators of matrix synthesis. When inflammation subsides, degradation predominates and tends to re-establish a normal or near normal stroma-parenchyma ratio[25]. The process in human schistosomiasis is a slow one and may pass unnoticed by the clinician or the patient, but in some special cases (younger fibrosis?) can lead to complete disappearance of hepatosplenic disease after months or even years have ellapsed.

## RESUMO

**Patologia da involução da fibrose periportal na esquistossomose humana**

Um estudo feito com microscopia ótica e eletrônica permitiu verificar sinais de degradação da matriz extracelular na fibrose periportal humana causada pela esquistossomose avançada. O material de estudo proveio de 14 biópsias hepáticas em cunha feitas em portadores de esquistossomose hepato-esplênica, no momento em que eles foram submetidos a cirurgia para o alívio das manifestações de hipertensão porta. Além da presença de áreas focais de rarefação, fragmentação e dispersão dos feixes colgenos, os espaços porta alargados e fibrosados também mostraram hiperplasia do tecido elástico e dissociação de fibras musculares lisas em seguida à destruição de ramos da veia porta. Os ovos do parasito eram escassos nas secções histológicas. A degradação da matriz provavelmente representa processo involutivo relacionado com a progressiva diminuição da agressão parasitária, o que pode ocorrer espontaneamente em casos de longa duração ou após a cura pela quimioterapia. As alterações indicativas de degradação da matriz aqui descritas representam provavelmente a face morfológica da involução da fibrose periportal que vem sendo atualmente documentada pela ultrasonografia em pacientes hepato-esplênicos submetidos à quimioterapia curativa.

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