

MORPHOLOGICAL FEATURES OF COLLAGEN DEGRADATION IN ADVANCED HEPATIC SCHISTOSOMIASIS OF MAN

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Optical and electron microscopical evidences of focal matrix degradation were frequently seen in liver sections taken from patients with periportal ("pipe-stem") fibrosis caused by schistosomiasis mansoni.

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 the destruction of portal vein branches.

Ultrastructural changes represented by focal lytic and/or electron dense alterations of collagen fibrils were similar to those first seen in experimental material and designated as "chronic collagen degradation". Elastin and related microfibrils were also affected by focal condensation, fragmentation, distortion and dissolution. Schistosome eggs were scanty in the tissue sections examined. Matrix degradation represented involuting changes related to the progressive diminution of parasite aggression, which occurs spontaneously with age or after cure by chemotherapy. Changes of focal matrix degradation now being described represent the basic morphological counterpart of periportal fibrosis involution documented clinically, especially by ultrasonography, in patients with hepatosplenic schistosomiasis submitted to curative chemotherapy.

Key words: hepatic schistosomiasis – collagen degradation

Some years ago it would have seemed a heresy to say that schistosomal periportal fibrosis (pipe-stem fibrosis) could undergo complete reversion. Fibrosis was then considered, and is still considered by some, to be irreversible.

Pipe-stem fibrosis is a dense periportal scar within which intrahepatic portal vein branches are distorted, narrowed or occluded (Lichtenberg, 1955). At the same time, hepatic artery undergoes progressive compensatory hypertrophy (Andrade & Cheever, 1971), increasing the complexity of the vascular pathology. Therefore, reversibility of pipe-stem fibrosis would imply not only resorption of fibrosis, but also re-arrangement of intrahepatic vasculature. Clinically, it means regression of hepatosplenomegaly and disappearance of esophageal varices. It is amazing that such complex and advanced lesion can be completely repaired.

However, it has been suggested that such reversion may even occur spontaneously. In 1966, Katz & Brener reported on the clinical

status of schistosomiasis patients who remained in the endemic area and were examined after an interval of 10 years. Seven out of 91 hepatointestinal patients evolved to hepatosplenic disease. From 21 patients considered as hepatosplenic, 6 had their disease aggravated, 7 were unaltered and 8 reversed from the hepatosplenic form to mild intestinal or hepatointestinal schistosomiasis. Only three of the latter patients received specific treatment.

Later, Lees (1968) observed regression of schistosomal hepatosplenic disease in two children 12-18 months after treatment with lucanthone hydrochloride and corticoids. He considered corticoid effect as further evidence that an abnormal immunity reaction was responsible for the development and maintenance of hepatosplenic schistosomiasis.

The advent of new curative and highly effective drugs, given by mouth in one single dose and with no serious side-effects, permitted large scale treatment programs in regions endemic for schistosomiasis.

TABLE I

Clinical observations on post-therapeutical periportal fibrosis involution in schistosomiasis

Authors	Year	Period	No. patients	Involution	Drug
Bina & Prata	1983	2-4 yrs	23	20.0%	Oxamniquine
Dietze & Prata ^a	1986	6, 18, 24 mos	70	40.0%	Oxamniquine
Domingues	1986	1 yr	46	18.5%	Praziquantel
Mohamed-Ali et al.	1991	7 mos	420	20.0%	Praziquantel

a: includes only patients with recently developed hepatosplenic disease.

After the introduction of these new drugs in Brazil it became clear that specific chemotherapy was both curative and preventive of hepatosplenic disease. Bina & Prata (1983) observed that six years after treatment of 115 patients with schistosomiasis who continued to live in area of transmission of *Schistosoma mansoni* and were re-infected, none had their disease aggravated, while 4 out of 14 reversed the hepatosplenic form. On the contrary, of the 115 non-treated paired controls several had their clinical conditions worsened and 22 evolved from the mild hepato-intestinal form to hepatosplenic disease.

Several other reports followed and demonstrated that in some patients curative treatment of schistosomiasis lead to progressive resorption of periportal fibrosis, disappearance of esophageal varices and return of liver and spleen sizes back to normal. Table I lists the main data from recent literature. With the introduction of ultrasonographic methodology, a non-invasive tool that can be taken to field research, diagnosis and post-chemotherapy follow-up (Homeida et al., 1988; Coutinho, 1990; Mohamed-Ali et al., 1991) of hepatosplenic schistosomiasis are now largely being used and are confirming the earlier clinical data on post-chemotherapy periportal fibrosis involution (Bina & Prata, 1983; Dietze & Prata, 1986; Domingues, 1986).

MORPHOLOGICAL FEATURES

We searched for morphological evidences of matrix degradation in 66 surgical liver biopsies taken from patients with hepatosplenic schistosomiasis. There were 51 males and 15 females, with ages varying from 16 to 61 years (average: 30.4). No clear history of previous treatment was obtained from those patients and at least the majority of them had never been treated for schistosomiasis. Evidences of focal matrix degradation were found in all cases, although their degree varied from mild to

marked from case to case. Histological, immuno-cito-chemical and ultrastructural methods were applied.

It was clear to us that matrix degradation in periportal fibrosis was not a new change that just started after treatment and cure of schistosomiasis. Our findings are in keeping with the concept that matrix formation and degradation occur side by side, accompanied by changes of chronic inflammation and granulation tissue formation (Perez-Tamayo, 1965, 1982). Probably the effect of specific treatment on periportal fibrosis involution is to suppress the cause of chronic inflammation and thus allow the forces of matrix resorption to surpass those leading to matrix synthesis. Probably, being our cases representative of old advanced periportal fibrosis, the parasite stimuli had already decreased to post treatment levels when liver biopsies were taken.

When observed under the optical microscope, periportal fibrosis of schistosomiasis is seen to exhibit variable densities. There are areas where the collagen appears compact and homogeneous. But, within or at the periphery of such areas, there are foci where the collagen fibers are less numerous, fragmented, with variable caliber, forming irregular masses. The whole area presents a loose pattern, but neither cellularity nor the amount of amorphous extracellular ground substance is increased. These latter aspects serve to differentiate areas of focal resorption with those areas of new formation of matrix (Fig. 1) where amorphous ground material is abundant, fibroblast proliferation is prominent and the small blood vessels exhibit plump endothelial cells. The vessels in the portal spaces are probably pre-existent, belonging to the well-known "angioma-toid lesion" of advanced schistosomiasis, as seen in our cases. They are thin-walled, dilated blood and lymphatic vessels, with flattened endothelial cells.

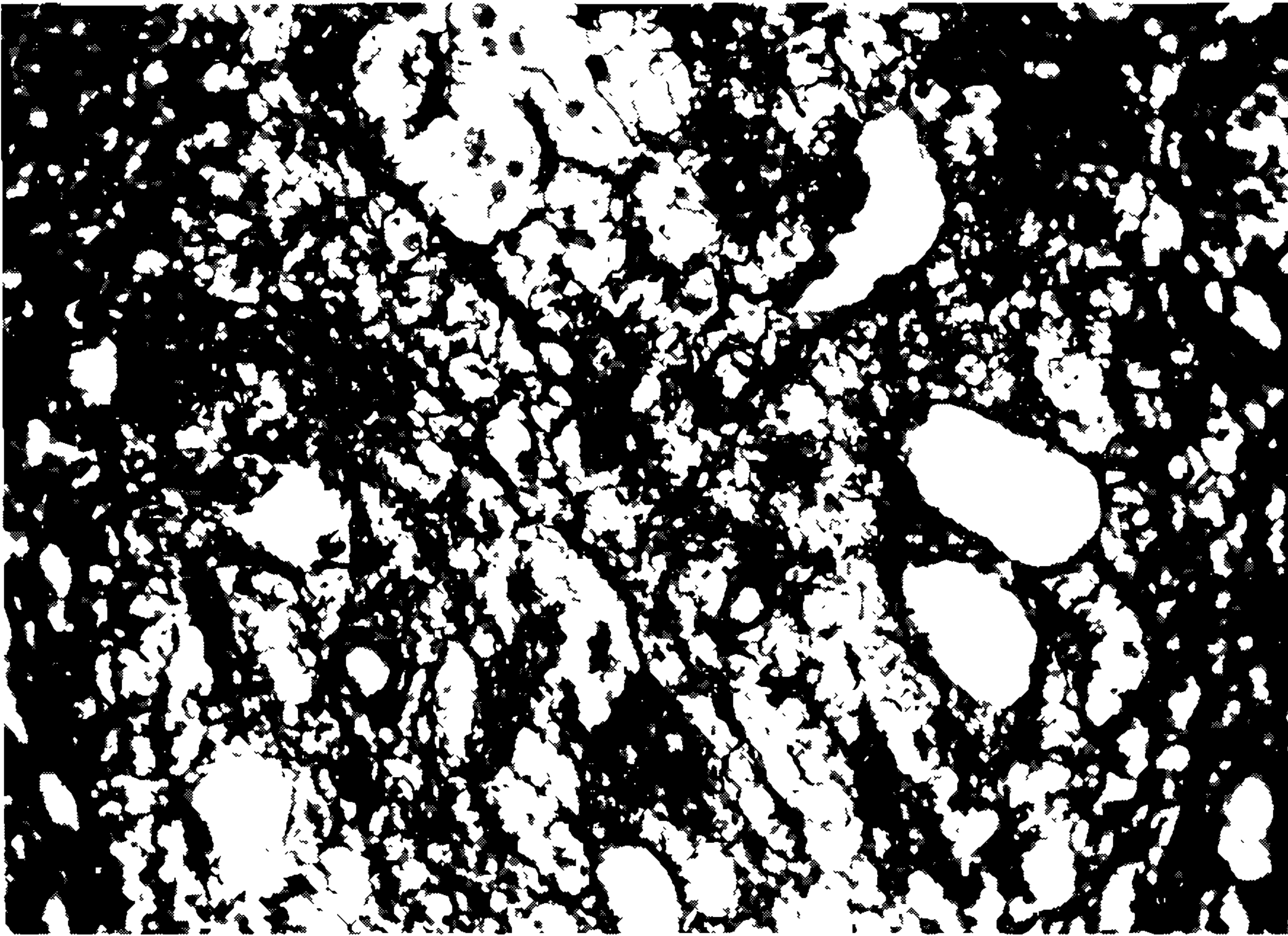


Fig. 1: collagen fibers are specifically stained and appear forming a network of variable densities. Within clear, rarefied zones collagens fibers are thin, fragmented, sometimes clumped. Picosirius-red method, 400 X.



Fig. 2: fibrotic portal space. Blood vessels present hyalinized walls. Variability in the appearance of fibrosis is evident: dense areas around blood vessels alternate with areas of rarefaction of the extracellular matrix. Hematoxylin & Eosin, 200 X.

Changes in collagen fibers are not readily seen in sections stained with hematoxylin & eosin, Masson's trichrome or Weigert-Van Gieson, although some degree of atrophy and fragmentation of fibers can be observed (Fig. 1) in routine material. When collagen fibers

are specifically stained with picosirius-red or picosirius-blue, the areas of rarefaction are clearly shown (Fig. 3). There is no particular distribution for the areas of focal collagen clearing. They may occur around blood vessels or away from them, but usually the periductal

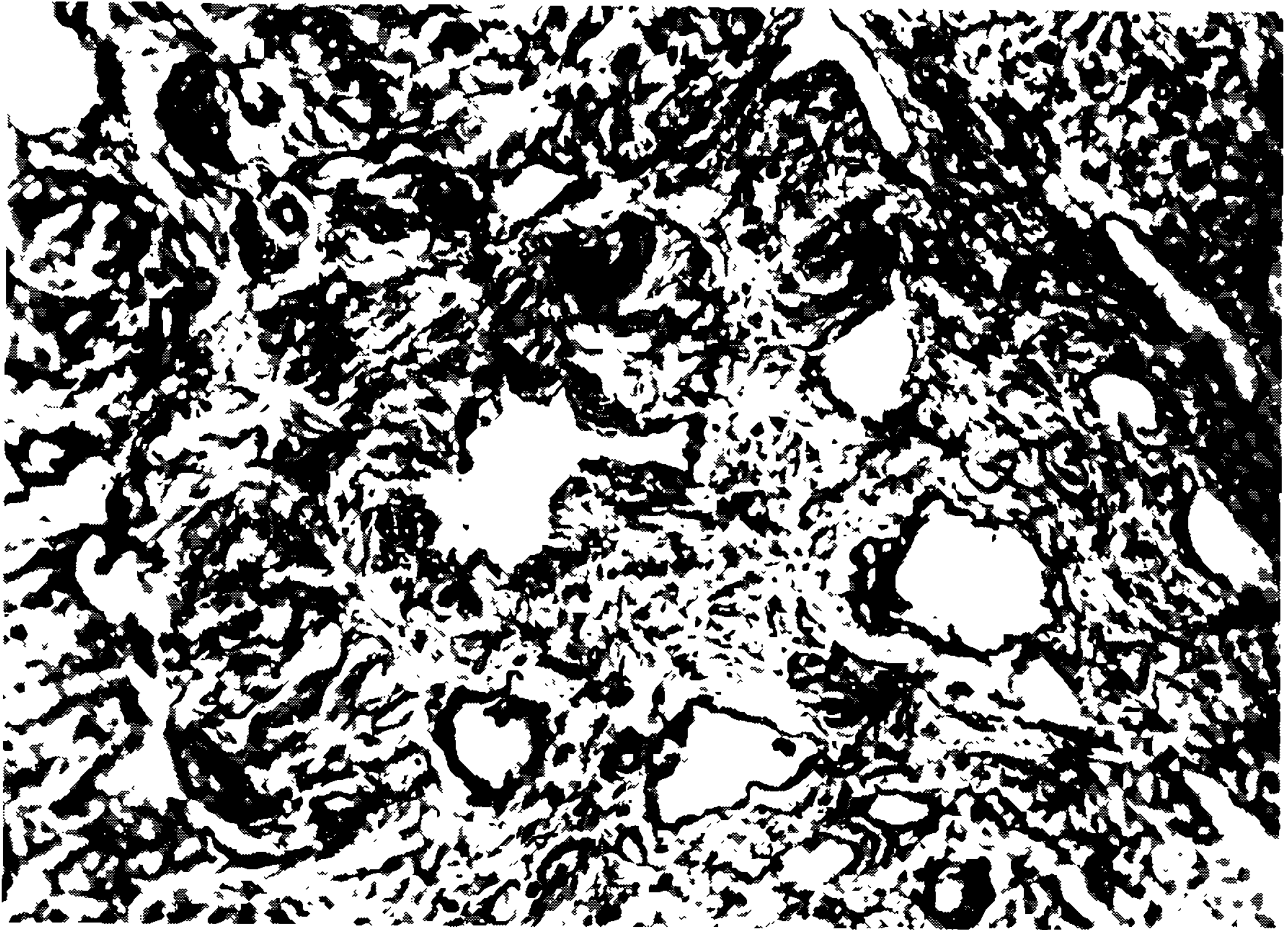


Fig. 3: portal fibrosis and angiomatoid lesion. Collagen fibers vary in size and caliber, some being hyalinized and others are fragmented. A routine trichrome stain would give a uniform appearance to this area, because it would also stain the amorphous ground substance. Picrosirius-blue method, 400 X.

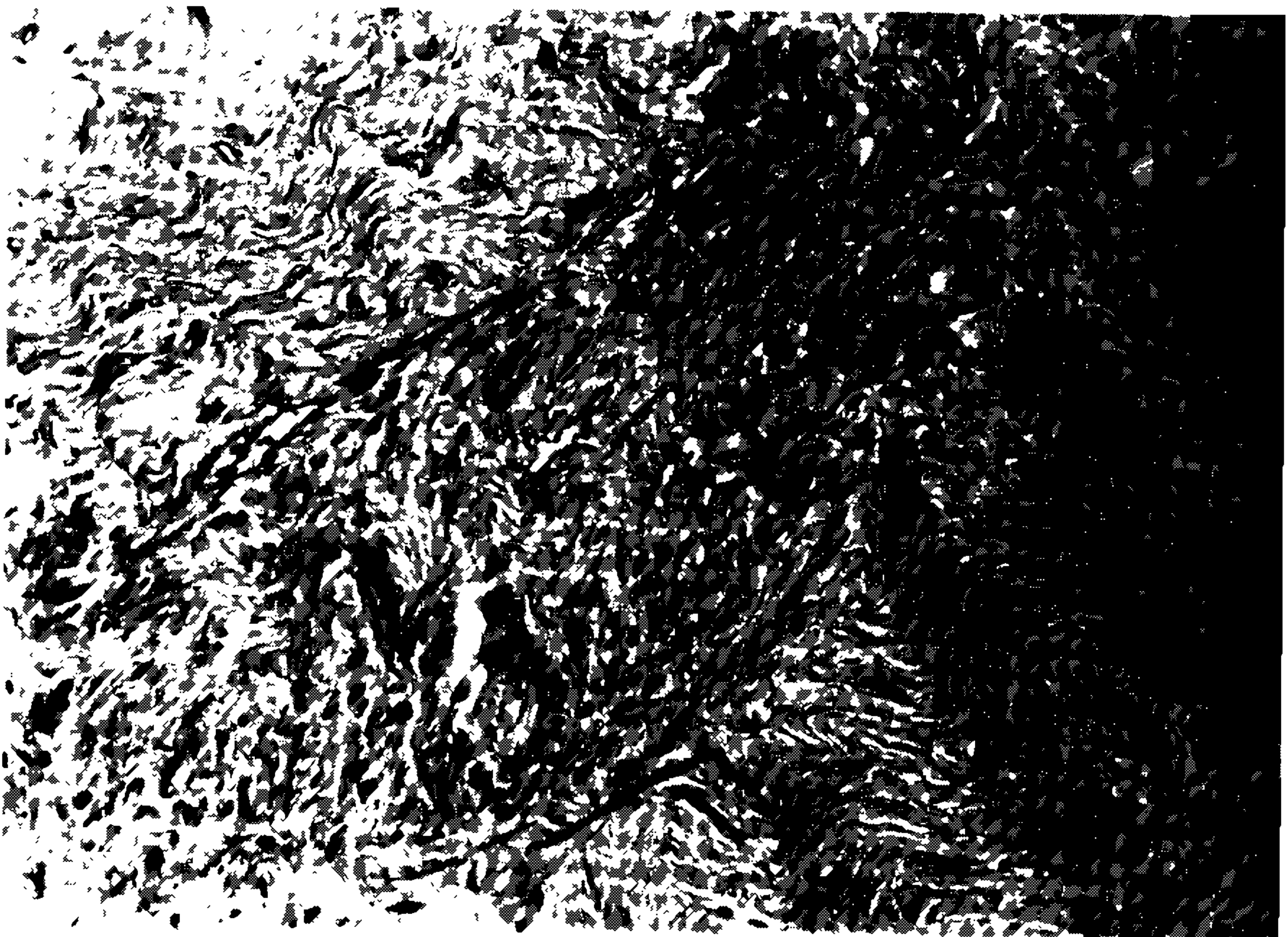


Fig. 4: dispersion of smooth muscle fibers into the fibrous tissue of the portal space in pipe-stem fibrosis of schistosomiasis. The main branch of the portal vein has been destroyed and replaced by fibrosis. Masson's trichrome, 200 X.

tissue remains compact. No correlation with any kind of cellular changes was detected by light microscopy.

Dispersion of smooth muscle fibers into the

periportal tissue was a constant finding (Fig. 3). This change is so constant that it can be considered as a valuable histopathological diagnostic sign. It is related to partial or total destruction of portal vein branches. This smooth muscle

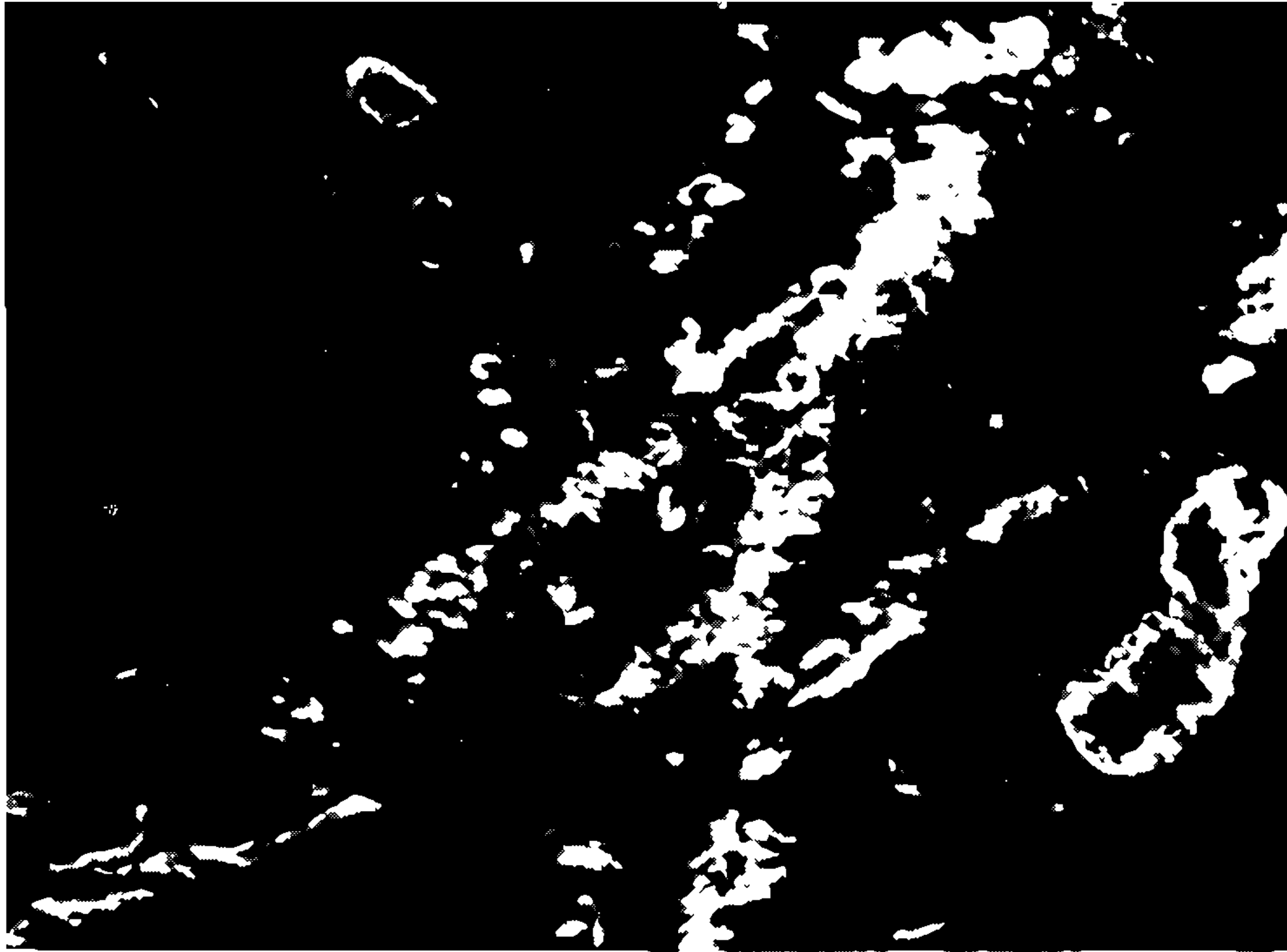


Fig. 5: immunofluorescent staining of dispersed smooth muscle fibers in schistosomal portal fibrosis. Besides main groups of fibers there are also small isolated collection of muscle cells. Cryostat section, fluorescent anti-actin staining, 400 X.



Fig. 6: abundant dark staining elastic fibers in schistosomal periportal fibrosis. Fibers appear concentrated in some areas and dispersed and rarefied in the clear zones, a disposition similar to that of the collagen fibers. Orcein stain, 150 X.

cell dispersion can be better appreciated in cryostat sections prepared for the immunofluorescent demonstration of actin (Fig. 5). Then, the smooth muscle cells are seen throughout the periportal fibrous tissue, as a normal component of the media of blood vessel walls or dispersed as isolated cells, groups of cells or fibers of various sizes and thickness. The apparent functional importance of this finding is that

dislodged smooth muscle cells are able to synthesize elastin (Davidson, 1987). Elastin is an important component of the extracellular matrix and it frequently formed in excess within the periportal spaces in schistosomiasis (Andrade & Freitas, 1991). Likewise collagen, evidences of elastic tissue degradation can also be found in schistosomal periportal fibrosis. In orcein stained slides dense elastic tissue can be seen



Fig. 7: a detail of the previous picture showing clumped elastic fibers in an area of rarefaction. Orcein stain, 400 X.

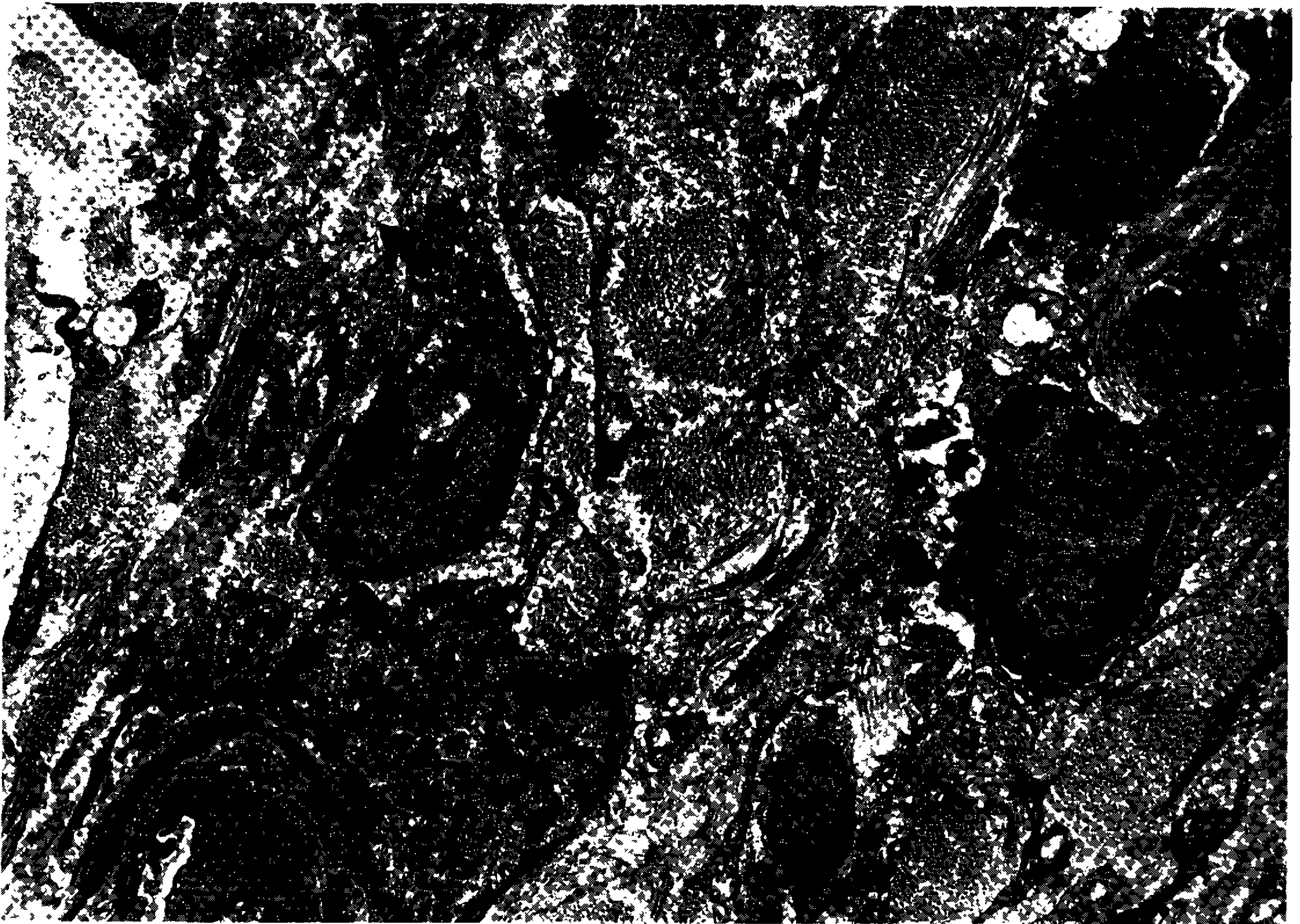


Fig. 8: general ultrastructural appearance of portal fibrosis. This area is little altered by degradative changes and shows compacted bunches of collagen fibrils separated by connective cells and cytoplasmic processes. Areas of degradation are signaled by arrows. Electron micrograph, 4,400 X.



Fig. 9: detail of ultrastructural degradative changes of the matrix in schistosomal portal fibrosis. Collagen fibrils are disrupted in an area where elastin deposits (e), accumulation of dark granular material (g) and fragmented fibers (f) can be seen. Electron micrograph, 7,000 X.



Fig. 10: "electro-dense" change interrupting the continuity of the collagen fibrils. Electron micrograph, 20,000 X.

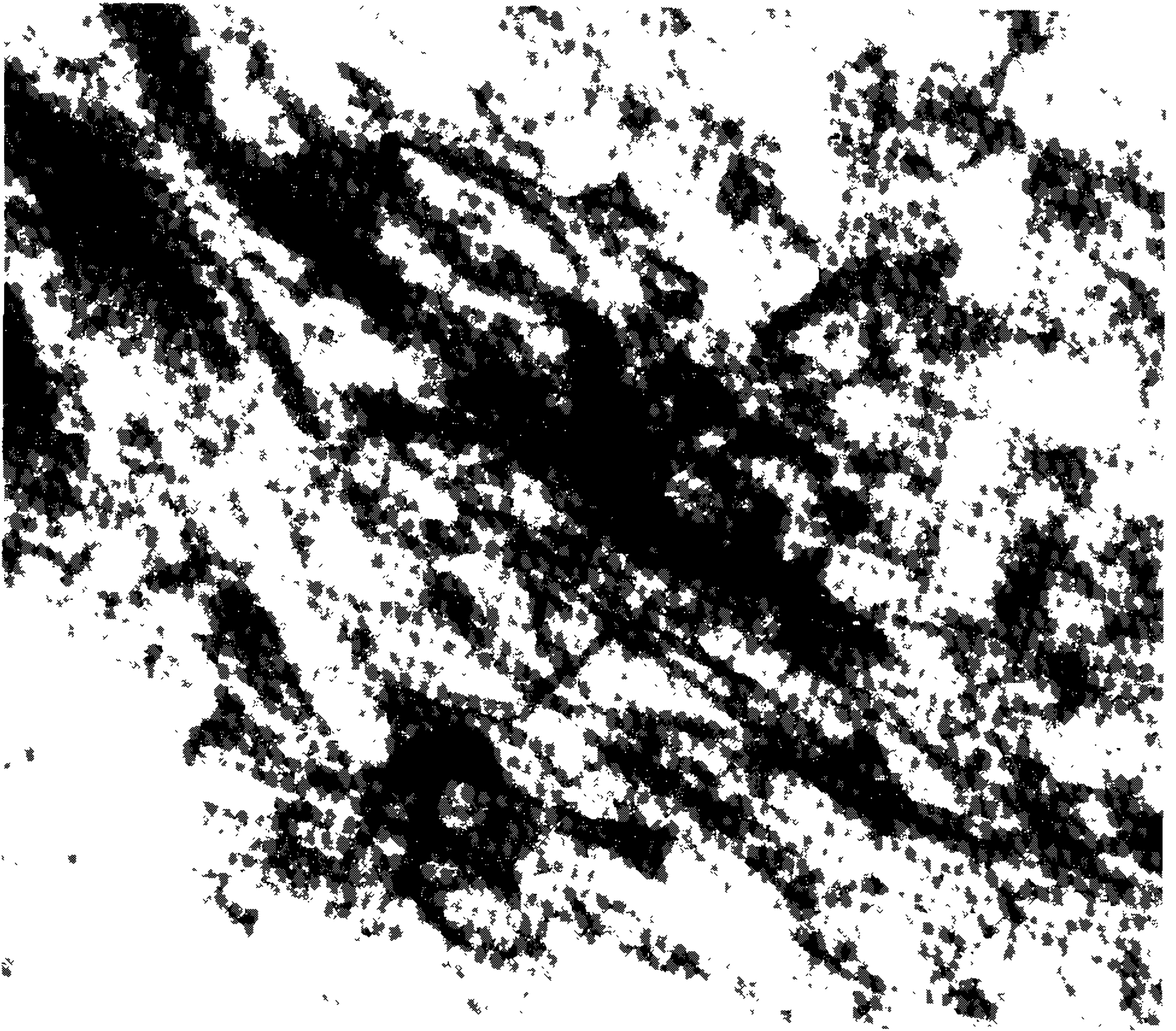


Fig. 11: space where the collagen fibrils were replaced by microfibrils associated with some tannic acid-contrasted elastin material (arrows). Electron micrograph, 20,000 X.

concentrated in one area while appearing as scattered fragmented fibers in another areas (Fig. 6). Sometimes only debris or small aggregation of fibers are seen, strongly stained by orcein (Fig. 7). These changes were also documented by immunofluorescence using specific antibodies against elastin (Andrade et al., 1991; Andrade & Freitas, 1991).

When viewed under the electron microscope the fibrotic portal tissue exhibited dense packed collagen fibrils forming thick fibers separated by thin and long cytoplasmic processes. Interruptions of the continuity of such fibers were frequently seen, with replacement by: elastin deposits, fragmented collagen fibrils, dark collections of amorphous or granular material or the appearance of empty and irregular spaces. Areas of collagen fibril alterations sometimes appeared in close proximity to connective tissue cells (Fig. 8). The areas appearing as empty spaces that interrupt the continuity of collagen fibrils ("lytic changes") or as fine granular deposits ("electron-dense changes") (Figs 9, 10) mimic those found in the late stages of

periovular granuloma involution in the liver of mice and that were described as "chronic collagen degradation" (Andrade & Grimaud, 1988). In some areas devoid of collagen fibrils the spaces are sometimes replaced by numerous microfibrils (Fig. 11). They were considered to represent oxytalanic and/or elauninic microfibrils since they were frequently seen to be associated with elastin deposits.

DISCUSSION

There are several experimental studies on the morphological and functional aspects of post-chemotherapeutical involution of the schistosomal lesions in the liver (Andrade & Grimaud, 1986; 1988; Cameron & Ganguly, 1964; Warren, 1962), lungs (Almeida & Andrade, 1983) and intestines (Santos et al., 1991), but with the exception of one article (Andrade et al., 1992) no similar studies in humans are available. This is surprising considering the amount of clinical data being accumulated on an important subject such as the involution of hepatic fibrosis.

TABLE II

Main factors involved in the formation of connective tissues

Cells	Extra-cellular matrix	Mediators
Fibroblasts	Collagens	TGF-B
Myofibroblasts	Proteoglycans	IL-1
Smooth Muscle Cells	Elastin	FGF
Lipocytes	Structural Glycoproteins	etc
Mast cells		

TGF-B = Transforming growth factor -beta; IL-1 = Interleukin 1; FGF = Fibroblast growth factor.

Difficulties to perform human studies in this area probably stem from the belief that one would need tissue taken before and months or years after chemotherapy in order to make comparative studies. Ethical reasons would rule out hepatic biopsies in patients cured by chemotherapy. Inasmuch as such biopsies should be wedged surgical, not needle biopsies, since the portal tissue is to be well represented. But, one might question whether it is absolutely essential to have tissue prior and after chemotherapy. Perhaps not, since it seems highly improbable that anti-schistosome drugs have a direct action on fibrous resorption. Curative drugs act by destroying or decreasing parasite-induced stimuli (i.e. chronic inflammation) thus allowing degradation of the excess of connective tissue to predominate over its formation. Matrix formation and degradation are balanced processes dependent on the same cell types. Table II lists the cell types, the extra-cellular

components and the cytokines involved in matrix formation. Table III refers to degradative enzymes belonging to the family of metalloproteinases that are responsible for matrix degradation. There are stimulators and inhibitors in action, regulated by molecular signs originated in connective-tissue cells and/or inflammatory cells. A delicate modulation is involved, but the factors necessary for the formation and degradation are paradoxically produced at the same time. Accumulation of matrix (fibrosis) occurs when formation exceeds degradation. Cells of chronic inflammation secrete cytokines (Kovacs, 1991) that are stimulators of matrix synthesis. When inflammation subsides degradation predominates and tends to reestablish a normal or near-normal stroma/parenchyma ratio (Perez-Tamayo, 1965, 1982). Then, the younger the fibrosis, the faster its degradation, but the process also occurs in long standing fibrosis, although under a slow pace and with a peculiar morphology (Andrade & Grimaud, 1988). Of course, such a degradative process may be relatively inefficient to remove a large amount of fibrosis to the point it can become clinically evident. The degree of maturation (cross-linking of collagen) may be far advanced blocking the sites of action of metallo-proteinases. The action of proteinase inhibitors may be quite effective and several other factors can operate to slow down matrix degradation. Chronic degradation of scar tissue has been little studied and needs further investigation. Actually, patients with advanced hepatosplenic disease may not improve at all after treatment.

On the other hand, it seems that the common pathologist does not work with the con-

TABLE III

Synopsis of the main factors involved in the degradation of the extracellular matrix

MMP	Substratum	Mediators	Activators	Inhibitors
MMP1 Interstitial Collagenase	Coll. I, II, III, etc.	TGF-B PDGF INF A, B, G IL-1	Plasmin Trypsin Cathepsin B, D.	TIMP A2 MG A1 PI
MMP2 Gelatinase	Denatured coll. Coll. IV	TNF		
MMP3 Stromelysin	Proteoglycans			
MMP4 Telopeptidase	BM			
	Telopeptides			

MMP = Matrix Metallo-Proteinase; TGF-B = Transforming growth factor - Beta; PDGF = Platelet derived growth factor; INF = Interferon; A1 PI = Alpha 2 proteinase inhibitor; BM = Basement membrane; IL-1 = Interleukin 1; TNF = Tumor necrosis factor; TIMP = Tissue inhibitor of metallo-proteinase; A2MG = Alpha 2 macro globulin.

cept that fibrosis is reversible. Therefore, he does not usually search for histological changes representative of matrix degradation. During many years we used to see the changes in portal connective tissue that are above described without understanding its pathogenesis and significance. But, even pathologists engaged in experimental research on reversibility of fibrosis, when it comes to morphological features, they refer to changes observed at the ultrastructural level only (Hennel et al., 1983; Perez-Tamayo, 1970). Also, their models of "acute" collagen degradation give little help for the problems involved in "chronic" matrix degradation such as observed in hepatic schistosomiasis.

The observations on post-chemotherapeutic periportal fibrosis involution in schistosomiasis, especially when made *in vivo* with ultrasonographic methodology, are impressive and constitute the best demonstration on reversibility of fibrosis in human disease. The important significance of this fact to basic research on connective tissue, as well as to the control of schistosomiasis cannot be overemphasized.

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