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HYPERPLASIA OF ELASTIC TISSUE IN HEPATIC SCHISTOSOMAL FIBROSIS

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Elastic tissue hyperplasia, revealed by means of histological, immunocytochemical and ultrastructural methods, appeared as a prominent change in surgical liver biopsies taken from 61 patients with schistosomal periportal and septal fibrosis. Such hyperplasia was absent in experimental murine schistosomiasis, including mice with "pipe-stem" fibrosis.

Displaced connective tissue cells in periportal areas, such as smooth muscle cells, more frequently observed in human material, could be the site of excessive elastin synthesis, and could explain the differences observed in human and experimental materials. Elastic tissue, sometimes represented by its microfibrillar components, also appeared to be more condensed in areas of matrix (collagen) degradation, suggesting a participation of this tissue in the remodelling of the extracellular matrix.

By its rectratile properties elastic tissue hyperplasia in hepatic schistosomiasis can cause vascular narrowing and thus play a role in the pathogenesis of portal hypertension.

Key words: elastic tissue – elastin – schistosomiasis – periportal fibrosis – liver

Elastic tissue is scarce in the normal liver (Porto et al., 1990). Except for the vascular walls, there are but a few fibers within the portal spaces and a little more in the liver capsule. However, elastic tissue undergoes variable degree of hyperplasia in the course of several hepatic diseases (Liban & Hungar, 1959). The causes, mechanisms, consequences and the significance of such increase are still poorly understood. Elastic hyperplasia may play a role in the pathogenesis of portal hypertension and because of its strength and elasticity may contribute to the stability of the extracellular matrix, both processes being of considerable importance in schistosomiasis.

Only one previous study refers to elastic tissue in human schistosomal fibrosis (Liban & Hungar, 1959). In this general study on elastic hyperplasia in several chronic liver diseases, two cases of schistosomal periportal fibrosis were included. Rather surprisingly, elastic fibers were found to be absent from schistosomal periovular granulomas observed in experimental material (Andrade & Grimaud, 1986; Junqueira et al., 1986), although oxytalan microfibrils, a presumed component of the elastic system, have been described in such granulomas (Cotta-Pereira et al., 1990).

During examination of surgical liver biopsies taken from patients with hepatosplenic schistosomiasis marked elastic hyperplasia was observed associated with portal, septal and capsular fibrosis. This finding motivated the present investigation which is a histological, ultrastructural and immunocytochemical search of elastic tissue in the hepatic lesions of advanced schistosomiasis. It is aimed at further characterizing the participation of this tissue in the hepatic lesions caused by *Schistosoma mansoni* in man and in experimental animals, a first step toward understanding its significance.

MATERIALS AND METHODS

Clinical data – Biopsies were taken from the livers of 61 patients with hepatosplenic schistosomiasis at the moment of laparotomy for splenectomy and ligation of esophageal varices. The operation was performed for the relief of serious complications of portal hypertension (esophageal bleeding and hypersplenism). There

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were 51 males and 15 females and their ages varied from 16 to 61 years (average: 30.4 years).

Histology – Biopsy materials were fixed either in Bouin's fluid or buffered 10% formalin and embedded in paraffin. Sections were stained for elastic tissue with orcein (Shikata) or resorcin-fucsin (Weigert). Hematoxylin-eosin, PAS, Gomori's reticulum and Picrosirius-red methods were also used.

Electron microscopy and immunocytochemical methods – For the last 18 specimens a different procedure was used: besides the routine histological preparations above described, one part of the material was processed for electron microscopy and another for immunocytochemical studies. For the former, small fragments of the liver were immediately fixed in a mixture of 0.25% tannic acid and 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 h. After washing in buffer, the fragments were post-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer, pH 7.4. The fragments were dehydrated in alcohol and embedded in Epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Zeiss-EM 109 electron microscope at 50 mv.

For immunocytochemical study the liver tissue was covered with Tissue-Tek (Milles Inc., USA) and snap frozen in liquid nitrogen. Blocks were kept in air-tight plastic boxes at $-70\,^{o}C$ until the moment they were cut in a cryotome at $-20\,^{o}C$. Sections were submited to an indirect immunofluorescence method for the demonstration of elastin and actin. The primary antibodies used were: a) polyclonal rabbit anti-human elastin (Lethias et al., 1987) and b) polyclonal anti-rabbit chicken actin (Sigma A 2668), obtained through courtesy of Dr Jean-Alexis Grimaud, Institut Pasteur de Lyon, France.

Experimental material – It consisted of material from previous experiments kept as paraffin embedded tissues and which were taken from the files. The material represented mouse livers belonging to different experiments, such as early, (Andrade & Grimaud, 1986) and late (Andrade & Grimaud, 1988) periovular granulomas in treated (praziquantel, oxamniquine) and non-treated animals and material from the experimental production of pipestem fibrosis in mice (Andrade, 1987). By early it was meant 10-12 week old infection, while late was represented by 16-20 week old infection. Representative paraffin blocks were re-cut and the sections stained for the demonstration of elastic and collagen fibers (Weigert-Van Gieson).

RESULTS

Elastic tissue was found in abundance only in the hepatic fibrotic lesions associated with hepatosplenic schistosomiasis in man. A great number of elastic-staining fibers formed a compact matrix in the enlarged and fibrotic portal spaces and along the fine and long septa radiating from them (Fig. 1). Some fibers run in straight lines, but the majority had a wavy appearance. They were more concentrated around bile ducts and vascular canals. At the periphery of altered portal vein branches elastic fibers were abundant around displaced smooth muscle cells. Elastic tissue was also concentrated in areas where the extracellular matrix assumed an amorphous and eosinophilic appearance, with few and fragmented collagen fibers and many thin-walled dilated blood vessels. Such areas were usually seen in sub-capsular zones of the liver. In general, the amount of elastic fibers varied from case to case and even from area to area in a same section. In some cases elastic fibers were compactly arranged throughout the portal and septal fibrous tissue (Fig. 2). Changes suggestive of elastic tissue degradation were also seen. There were instances where the Weigert stained fibers appeared coarse, fragmented, forming small dark clumps (Fig. 3). Some fibers even appeared fragmented to the point of forming a granular material dispersed around other less damaged fibers (Fig. 4).

Sections treated with specific anti-elastin serum and submitted to immunofluorescence showed positive staining for fibers of variable diameters and lengths, disposed in parallel rows or crossing at different angles (Fig. 5A). Roughly, the anti-elastin antibodies recognized the same structures which were stained with orcein and resorcin-fuchsin. In the portal spaces (Fig. 5B) and intraparenchymal septa the fluorescent staining revealed the numerous blood vessels present much better than the histological staining for elastic fibers. Around portal veins there was accumulation of elastic fibers (Fig. 5C), usually in the same areas where smooth muscle fibers were dispersed (Fig. 5D).

Fig. 1: capsular and septal fibrosis in a liver with pipe-stem fibrosis. Elastic fibers are abundant and are stained black by the Weigert's resorcin-fucsin method. 120 X.

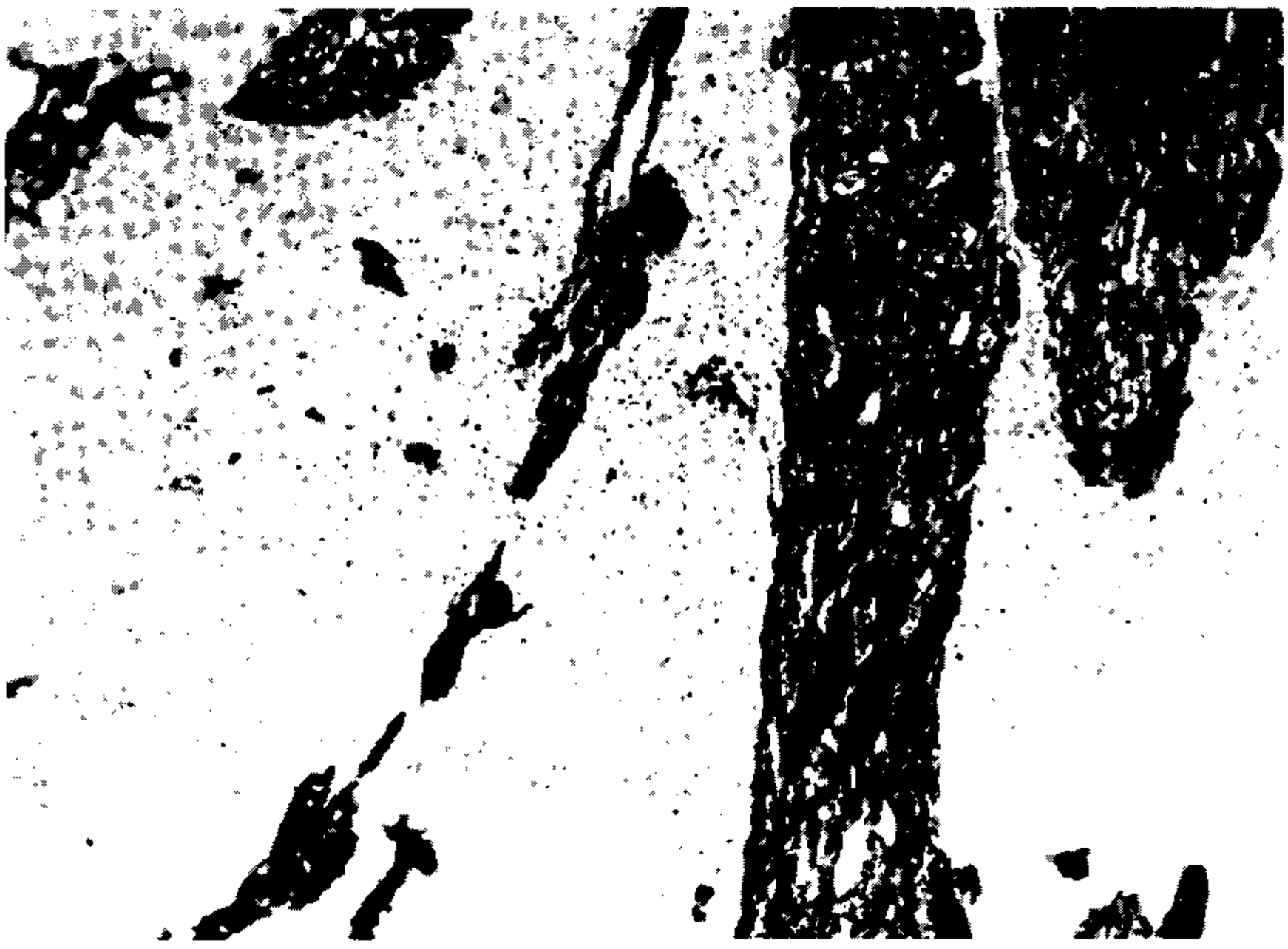

Fig. 2: abundant elastic fibers in portal and septal areas of the liver. They make a sharp contrast with the relatively undisturbed liver parenchyma which appears pale. Weigert's stain. 100 X.

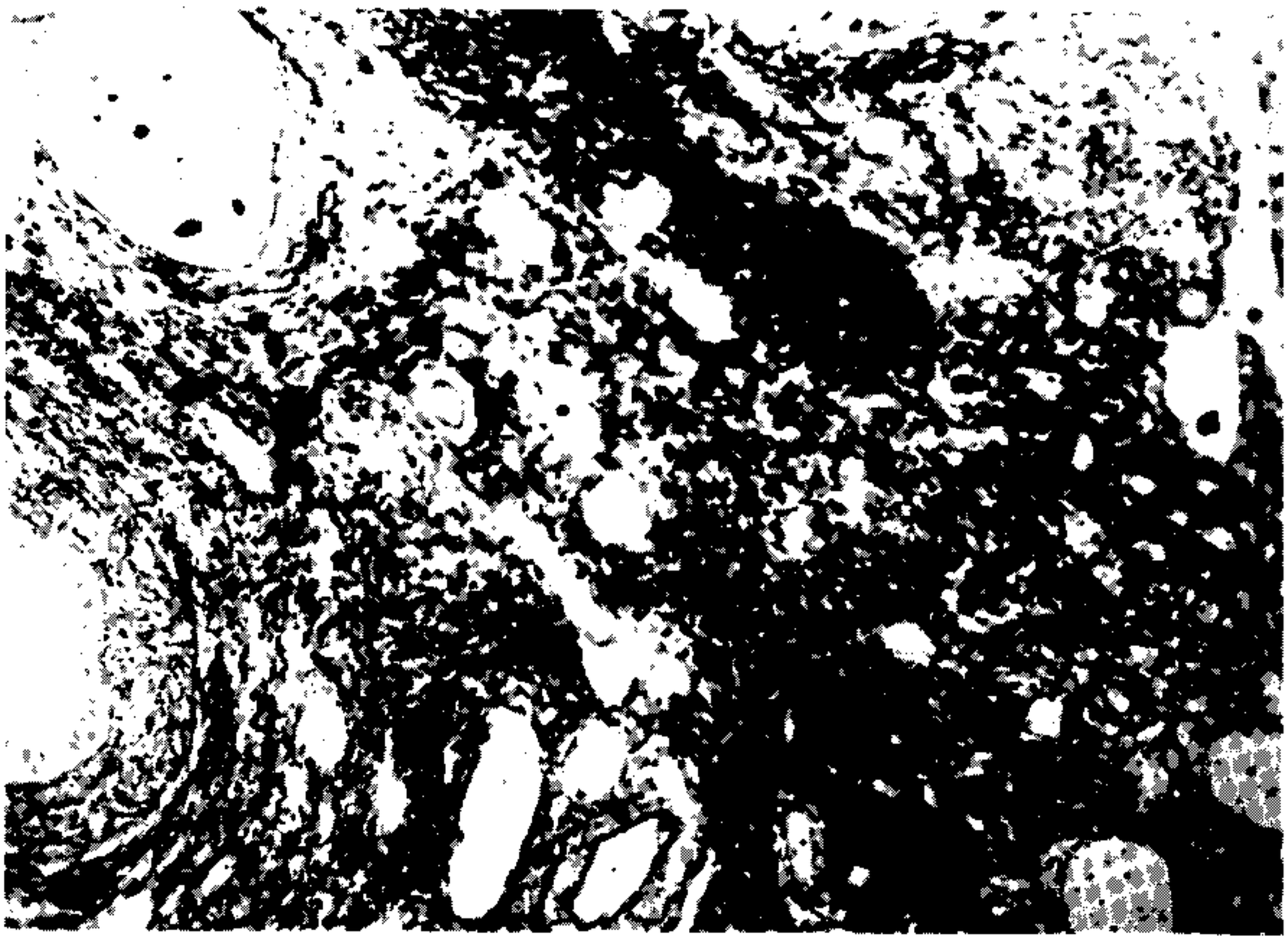

Fig. 3: well vascularized portal space (angiomatoid) showing evidence of degradation of elastic fibers. Some of them are fragmented, compacted or loosely arranged, with variable density from area to area. Weigert' stain. 200 X.

Fig. 4: around a portal vein branch, which shows increased elastic tissue in its walls, there is deposition of partially degraded elastic fibers. These dark stained fibers appear fragmented and sometimes transformed into a granular material. Weigert's stain. 200 X.

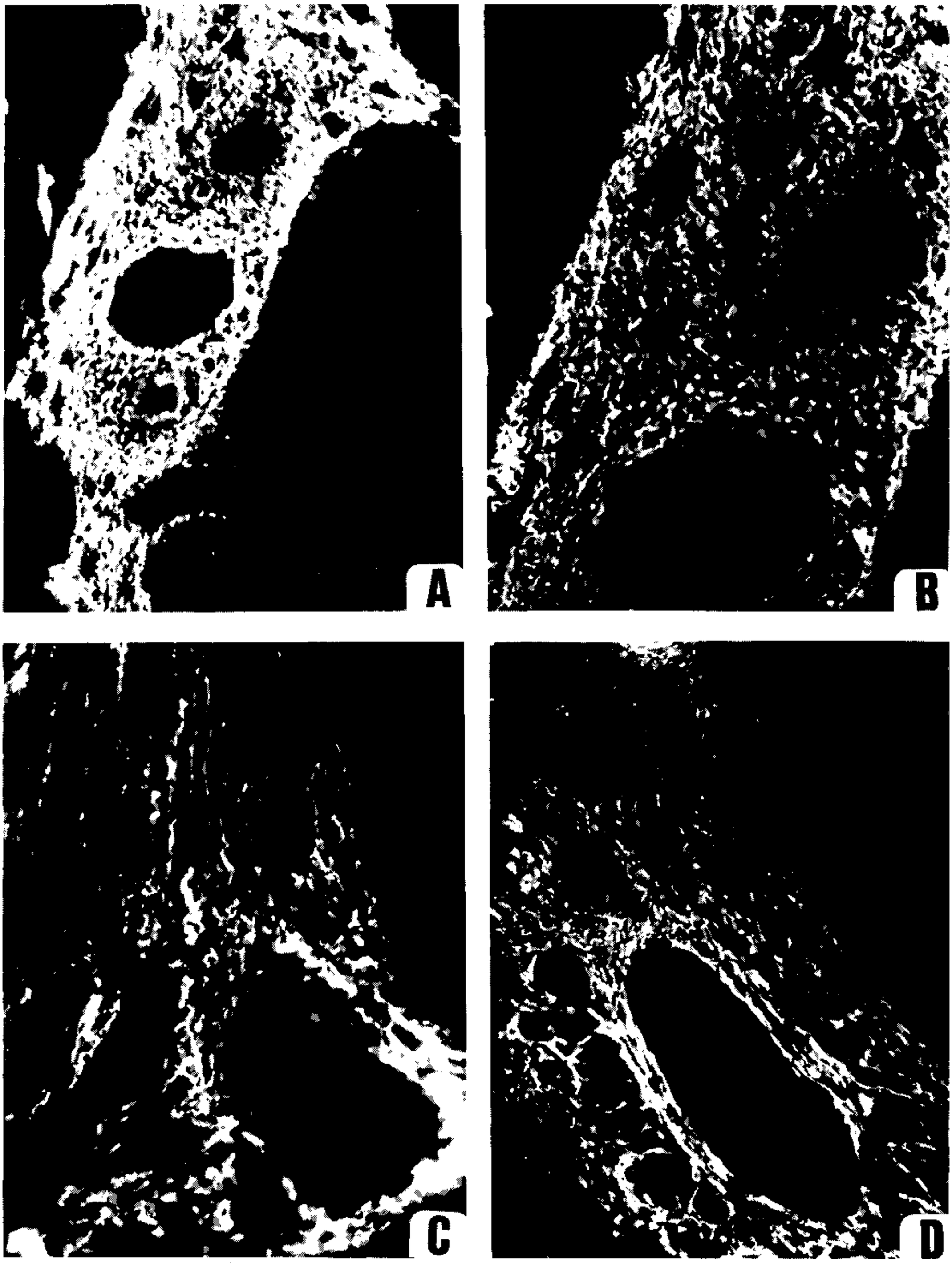

Fig. 5: immunofluorescence microscopy showing the presence of elastin in some enlarged portal spaces (A, B and C). The rich vascularization of the tissue can be better appreciated in A and B. In C the elastic fibers maintain a relationship to the portal vein similar to the smooth muscle fibers depicted in D, which were stained with anti-actin antibodies. A = 200 X; B, C and D = 400 X.

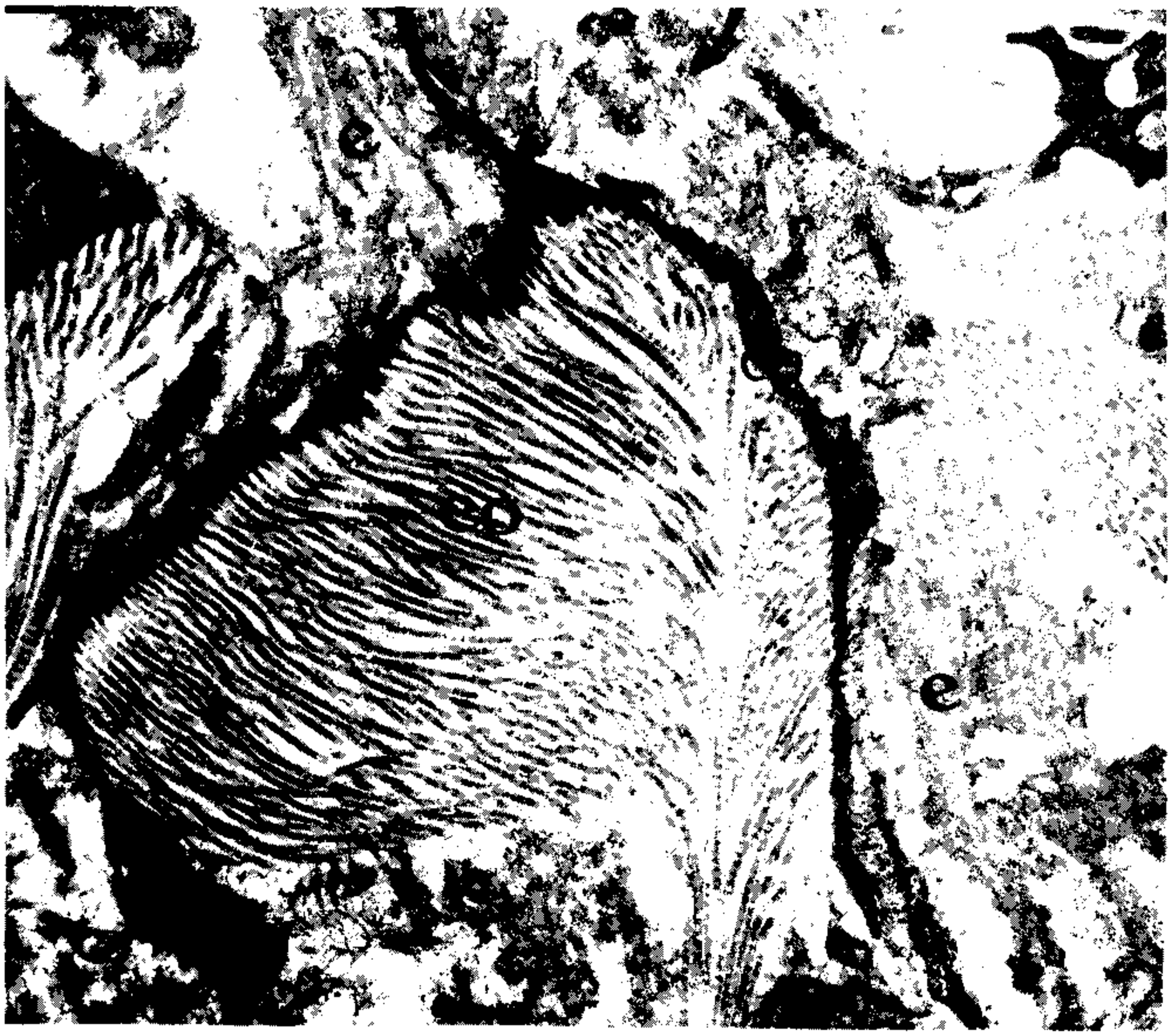

Fig. 6: connective tissue of the portal space in schistosomal "pipe-stem" fibrosis exhibiting collagen fibers (co), cytoplasmic prolongations (cp), elastin deposits (e) and focus of matrix (collagen) degradation. Electron Micrograph, 7,700 X.

Ultrastructurally, the extracellular matrix exhibited variable densities. Some areas presented collagen fibrils and fibers densily packed, forming parallel rows with interstitial spaces filled with cytoplasmic prolongations derived from fibroblasts and myofibroblasts and with elastin deposits in between (Fig. 6). In other areas the collagens fibrils appeared fragmented, leaving empty zones and areas with deposition of a pale amorphous material with some dark granules in its periphery (elastin material, Fig. 7). Usually elastin was associated with a network of microfibrils. That network of microfibrils sometimes appeared alone, non-associated with elastin or collagen fibers (Fig. 8). Those were areas in which the collagen fibers appeared apparently lysed. In such areas was also common the deposition of dark clumps of elastin (Fig. 9). The fragmented collagen fibers exhibited variable diameters. These dissociated collagen fibrils appeared mixed with coarser and darker tannic-acid impregnated elastin fibers, sometimes in the presence of smooth muscle cells (Fig. 10).

Material from murine livers with schistosomiasis failed to show the prominent elastic tissue that was seen in the human material. Elastic fibers were practically absent in periovular granuloma regardless the duration of infection (8, 10, 16 or 20 weeks). Only a thin elastic-staining tread was seen within some granulomas, probably derived from the destroyed blood vessel walls. Involuting granulomas observed 10, 15 days or 4 months after treatment of schistosomiasis with oxamniquine and/or praziquantel did not present increase of elastic tissue. Also, the fusing granulomas and the portal fibrosis of experimental "pipe-stem" lesions were devoid of elastic hyperplasia.

Fig. 7: the left side of the picture shows disapearance of collagen fibrils with their replacement by deposits of elastin material. In the left lower part of the picture collagen fibrils present dark granular foci of chronic (electron dense) degradation. Collagen fibrils = co; Elastin = e. 7,000 X.

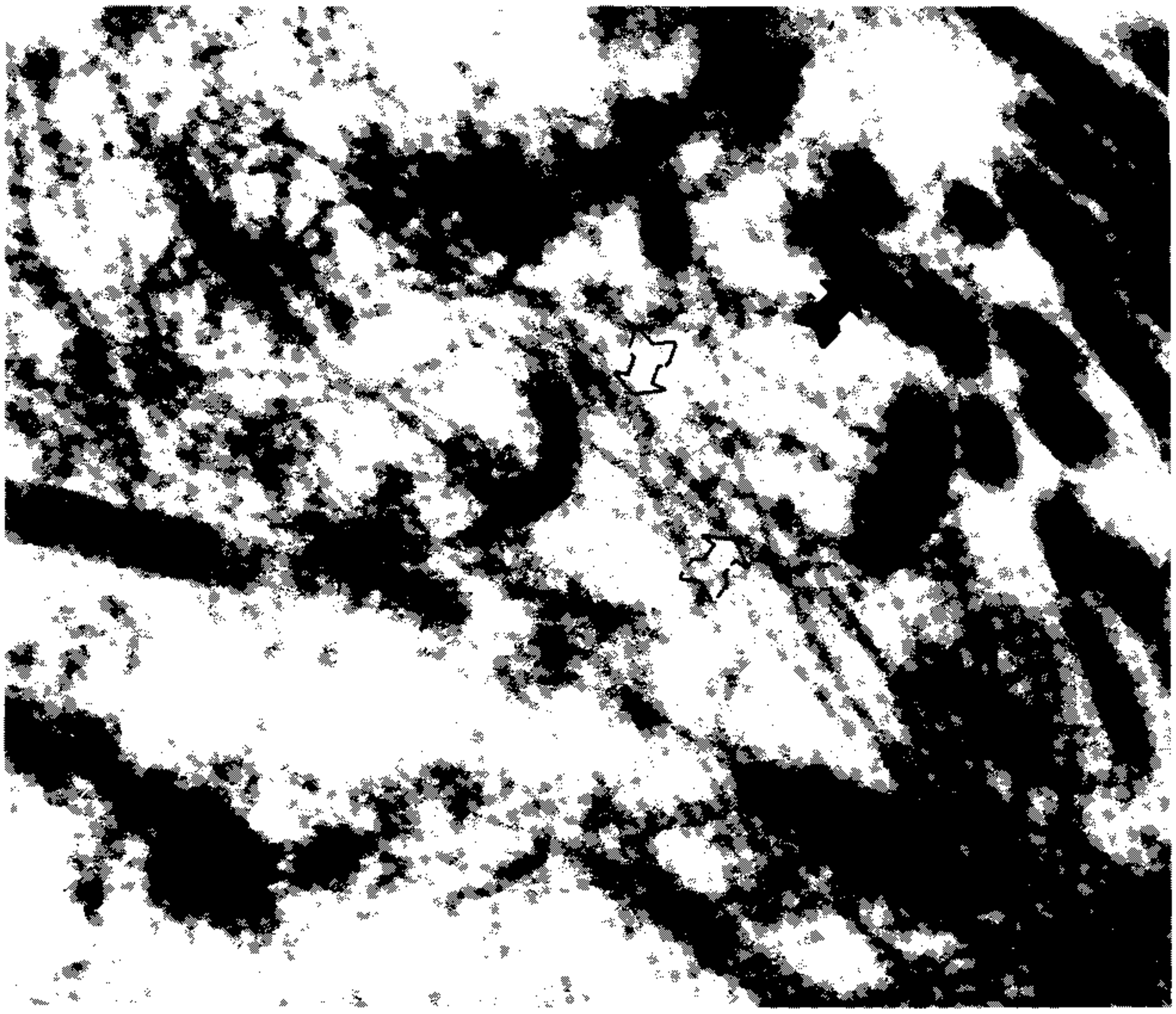

Fig. 8: fragmented collagen fibrils (full arrow) appear irregular and dispersed, leaving an empty space filled by microfibrils (empty arrows). Electron micrograph, 20,000 X.

Fig. 9: area devoid of collagen fibrils and filled with deposits of an amorphous dark material, probably altered elastin. Electron micrograph, 20,000 X.

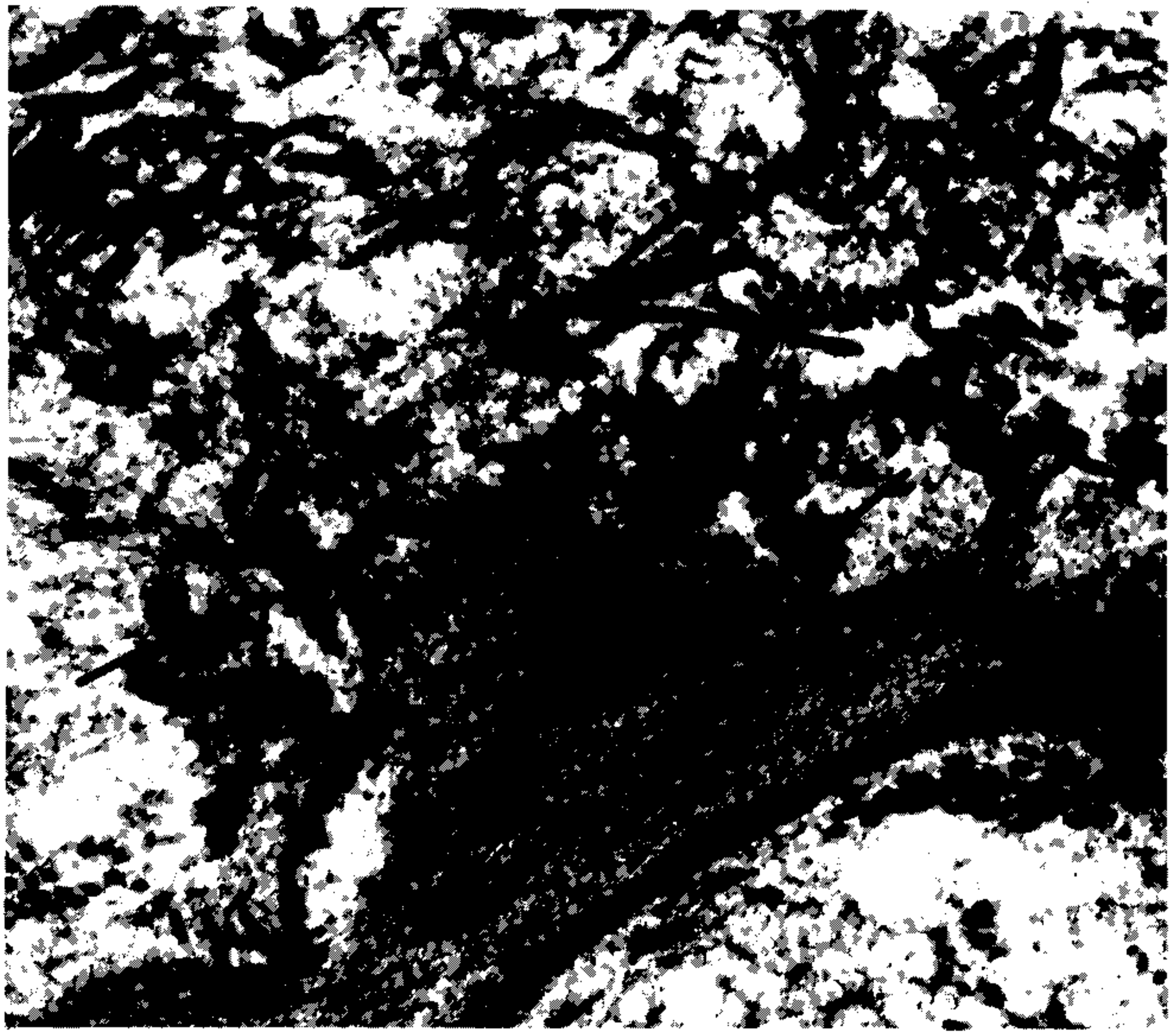

Fig. 10: zone of matrix degradation exhibiting fragmented collagen fibrils (co), filamentous elastin material (arrows) and a smooth muscle cell (smc). Electron micrograph, 20,000 X.

In periportal fibrosis there was accumulation of a few elastic fibers around portal veins which were affected by phlebosclerosis and partial muscular wall disintegration. These focal changes represented the maximum of elastic tissue changes seen in the experimental material examined.

DISCUSSION

Excess elastic tissue in the liver is related to the chronicity of the pathological processes (Thung & Gerber, 1982). In hepatic necrosis, the longer the history, the greater the amount of elastic tissue accumulated, a situation that can be used for the differential diagnosis between the septa from acute necrotizing hepatitis and chronic active hepatitis (Scheuer & Maggi, 1980).

Thung & Gerber (1982) estimate that at the end of 3 weeks fibers first appear around proliferated bile ducts and in centeracinar areas, but, after 4 weeks or more, abundant fibers are present in the septa and sinusoids. Probably the relative transient nature of periovular granuloma in schistosomiasis would explain the lack of elastic fibers in it. However, elastic tissue is also scarce in experimental schistosomiasis of longer durantion. So-called "pipe-stem" fibrosis needs more that 16 weeks to develop in mice (Warren, 1962; Andrade, 1987) but , as documented in the present study, elastic hyperplasia is not present in such lesion. Periportal fibrosis in the mouse may be somewhat different from "pipe-stem" fibrosis of man. This latter usually takes 5 years or more to develop in people living in endemic areas (Prata & Bina, 1968). On the morphological side, the impressive disarray of smooth muscle cells that occurs in human "pipe-stem" fibrosis (Andrade, 1989) has not been observed in the correspondent experimental material. This could be an important point, since displaced muscle cells are able to synthesize elastin (Davidson, 1987). Another difference, actually of a quantitative rather than of a qualitative nature, is the outstanding presence of "angiomatoid lesion" in human schistosomal periportal fibrosis. This lesion contains numerous vascular canals lined with prominent ("activated") endothelial cells. Likewise smooth muscle cells, endothelial cells can also produce elastin (Davidson, 1987). Species differences could be implicated, but apparently there is no indication that elastin production is more vigorous in man than in the mouse.

Elastic hyperplasia in schistosomiasis probably results from displaced or otherwise altered connective tissue cells present in a long standing inflammatory lesion. Excess elastic fibers in portal and septal fibrosis could give tensile strength and cause retraction of these tissues. The importance of this change in leading to vascular narrowing and thus contributing to the pathogenesis of portal hypertension seems obvious, although it needs to be demonstrated.

Elastic tissue is formed by the amorphous substance elastin which is supported by a meshwork of elauninic and oxytalanic microfibrils. Our findings reveal concentration of elastic fibers in areas where signs of extracellular matrix degradation are also present. Ultrastructurally a rich network of microfibrils, either isolated or associated with elastin usually appears coincidentally with evidences of collagen breakdown. Of course the microfibrils here observed could represent different materials, such as fibronectin, fibrillin or elastin-associated fibrils, but their presence in areas devoid of collagen fibers could be interpreted as a replacement phenomenon.

These findings suggest that elactic tissue is formed in old fibrotic lesion at the proportion collagens are degraded and resorbed. On the other hand, elastic tissue is also susceptible to degradation, since signs of elastic fiber fragmentation, condensation and dissolution can be seen in old fibrotic lesions, as observed in this present study.

The dynamics of elastic tissue formation, remodelling and degradation are far from clear, but further investigations on schistosomal fibrosis, where a remarkable elastic hyperplasia occurs, can certainly help in understanding this subject.

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