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EVOLUTION AND INVOLUTION OF HEPATOSPLENIC SCHISTOSOMIASIS

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Hepatosplenic schistosomiasis is an advanced form of schistosomiasis that, besides hepatosplenomegaly, presents clinical signs of portal hypertension. The condition is pathologically represented by systematized portal fibrosis accompanied by intra-hepatic portal vein obstruction, with preservation of a relatively undisturbed parenchyma. Splenomegaly results from chronic passive congestion and cellular hyperplasia.

The advent of new curative drugs is nowadays teaching us that the severe lesions associated with hepatosplenic schistosomiasis are susceptible of involution when adult worms and their eggs are eliminated. In cases with not yet far advanced lesions, the post therapeutical involution may be relatively rapid. On the other hand, in long standing cases portal lesions may seem irreversible, which is not surprising, since in such cases the number of parasites and their eggs may have already been spontaneously reduced or even totally eliminated.

Also, there are some evidences on record that the spontaneous extinction of the parasitic infection may likewise be followed by the involution of hepatosplenic disease (Katz & Brener, 1966).

In this paper an attempt wil be made to analyze the pathologic events concerned with either formation and involution of the lesions associated with hepatosplenic schistosomiasis.

EVOLUTION OF HEPATOSPLENIC SCHISTOSOMIASIS

Although hepatosplenic disease may occasionally show an accelerated course of development, appearing a few months after the manifestations of acute toxemic schistosomiasis (Neves & Raso, 1965), the development of hepatosplenic schistosomiasis seen in individuals living in endemic areas is actually a much slower process that may take from 4 to 6 years or more to evolve (Prata & Bina, 1968).

There is general agreement that severe hepatic fibrosis, the hallmark of hepatosplenic schistosomiasis, is related to high parasitic load, with continous deposition of numerous eggs. The contribution of dead-worm lesions, of living worms and of putative toxins are of ancillary importance only. Since not all subjects with heavy worm burden develop that form of the disease, probably other factors, such as a failure in immunological modulation of the inflammatory reactions may play a role (Colley et al, 1986).

The so called "pipe-stem fibrosis" has been experimentally reproduced in mice (Warren, 1966) and in monkeys (Lichtenberg & Sadun, 1968).

We have learned from these studies that the eggs are really the main pathogenetic factors and therefore high worm burden is a prerequisite. For the mouse, one pair of worms represent a heavy infection (Cheever, 1969). The heaviest infections reported in man at autopsy have seldon been greater that 5 worm-pairs per kilogram of body weight, whereas in mice the lightest infections are in the order of 50 worm-pairs per kilogram of body weight. Infections usually obtained in the laboratory with 50 to 120 cercariae per mouse will result in considerable distortion of the portal vasculature, but not in "pipe-stem fibrosis". Only mouse with a few pairs of worms and a prolonged time of infection (20-26 weeks) will develop systhematized portal fibrosis (Warren, 1966).

Despite the existence of experimental models, little was known about the anatomic changes that allow the parasite eggs to be deposited, not at random within the liver as it usually

happens, but rather preferentially along the portal spaces, from the larger to the tiny ones, in order to produce the characteristic focal and systhematized portal fibrosis seen in man (Fig. 1).

Recent experimental studies (Andrade, 1987) indicated that this seems to depend on two sorts of events: first, a peripheral partial blocking of the portal vasculature after numerous eggs are embolized into the liver in a relatively short period of time; second, the opening up of thin-walled portal collaterals along the entire portal system, probably as a consequence of a progressive increase in intrahepatic portal vein pressure. These vessels are pre-existing venules and capillaries that serve to irrigate the portal tissue and their small caliber does not normally allow for the lodging of schistosome eggs. Injection-corrosion plastic casts prepared from the portal veins from normal and 10-week infected mice give a striking demonstration of such changes (Fig. 2). Similar plastic casts have been obtained from human cases of hepatosplenic schistosomiasis, where a peri-portal cuffing of thin and anastomosing blood vessels may be so evident as to form a charateristic diagnostic feature to be seen readiologically in contrasted portograms (Bogliolo, 1957). The progressive lodging of newly arrived eggs into these periportal vessels leads to granuloma formation, vascular obstruction and fibrosis along periportal areas.

From the enlarged and fibrosed portal spaces, long and thin fibrous septa take origen and dissect the parenchyma in several directions. Thin septal fibrosis is also a dominating feature in advanced schistosomiasis, but its pathogenesis has not been well determined. It may result from lines of forces generated at the portal fibrous tissue, where contractile elements such as elastic fibrils and myofibroblasts are known to be present (Grimaud & Borojevic, 1977) or may be due to vascular obstruction followed by atrophy and reticulum collapse of the related parenchymal trabeculae. Periovular granulomas are not usually found in these thin septa, but mononuclear-cell infiltration is common.

Since pipe-stem fibrosis is an essentially portal lesions, associated with intrahepatic portal vein obstruction and a pre-sinusoidal type of portal hypertension, hepatic parenchyma is not primarily compromised (Fig. 3). The latter maintains its

normal lobular arrangement for prolonged time, even when portal and septal fibrosis are well developed. This accounts for the good hepatic function presented by most of the patients. However, parenchyma involvement may appear later on, due to the progressive infiltration by fibrous tissue, to focal necrosis after periods of esophageal bleeding, which are followed by post necrotic scarring, to associated diseases such as chronic viral hepatitis and to interstitial changes involving the sinusoidal territory, which are probably induced by compensatory hepatic artery hypertrophy (Andrade, 1986).

INVOLUTION OF HEPATOSPLENIC SCHISTOSOMIASIS

Several experimental studies have demonstrated that the lesions caused by schistosomiasis can be reversed following curative treatment. The fibrous tissue formed can be removed , and even the complex and severe hepatic vascular changes can return to normal (Andrade & Brito, 1981).

However, there has always been a firm belief that the lesions causing hepatosplenic schistosomiasis are irrever sible. This was apparently supported by experimental data which indicated that fibrosis in older periovular granulomas did reach a stage of irreversibility (Grimaud et al., 1987) and by those data showing that although the lesions resulting from severe early infection in mice can disappear under chemotherapy, those caused by a light and prolonged infection cannot (Warren & Klein, 1969).

A great challenge to the above assumption came as a result of modern chemotherapy, when extensive use of new highly efficient curative drugs begun to change the patterns of morbi- dity in schistosomiais . As a result of mass treatment campaigns, hepatosplenic schistosomiasis is becoming rare in endemic areas where such advanced form of the disease used to be highly pre- valent (Menezes Netto, 1987; Andrade & Bina, 1985). Also, clini cal observations of series of cases have documented the benefi- cial results of chemotherapy, with total or partial regression of hepatosplenic schistosomiais (Bina & Prata, 1983; Coutinho et al., 1984; Lees, 1968).

Involution of hepatosplenic schistosomiasis means

total or partial disappearance of hepatosplenomegaly due to resorption of fibrous tissue from the portal spaces, remodelling of the portal vasculature with consequent return of portal pressure to normal. Documentation of these events in humans has been so far indirect. Treated patients have presented a return of the spleen and liver sizes to normal and disappearance of esophageal varices several months following treatment. The utilization of ultrasonography is now permitting the observations of large numbers of treated patients in the field and adding important data on the involution of hepatosplenic schistosomiasis (Honeida et al., 1988).

More direct data have been experimentally obtained, with the demonstration of the diminution of hepatic collagen concentration in the livers of mice infected with *S.mansoni* and submitted to curative treatment (Cameron, 1964; Warren, 1972), of collagen degradation in periovular granulomas (Andrade & Grimaud, 1986), of the complete remodelling of portal vasculature (Andrade & Brito, 1981) and normalization of portal pressure (Warren, 1972).

The few experimental studies on post-therapeutical involution of schistosomiasis regarded the periovular granuloma only, but recently the model of pipe-stem fibrosis of the mouse was also submitted to similar observations (Andrade & Grimaud, 1988).

Generally, fibrosis is considered to be a stable tissue not susceptible to be removed once well formed. However, many investigators are now considering that fibrosis can undergo extensive resorption and even hepatic cirrhosis may be viewed as a reversible process (Perez-Tamayo, 1979). Collagen resorption in cirrhosis seems to pose problems which differs from those in schistosomiasis. In cirrhosis there is distortion of liver architecture with displacement of cells from their normal environment. In schistosomiasis there is a localizated fibrous enlargement of the portal spaces, the cause of which is susceptible to be removed by drug treatment.

Actually, likewise any other living tissue, fibrous tissue has also an essentially dynamic behavior, passing through phases of growth, maturation and involution. There are evidences that nature try to preserve a balanced relationship between pa-

renchyma and stroma, which is of fundamental importance for normal organ physiology. The increase in matrix components that results from abnormal stimulations, especially during chronic inflammation, is promptly followed by removal of the excess of fibrous tissue as soon as the evoking stimulations are interrupted. Formation and degradation of collagen occur simultaneously during matricial reactions, the end results depending on whether fibrogenesis or fibrolysis predominates over one another.

When excess collagen fibers scape degradation and remain in place for relatively long time, their genetic types may change, with Type I collagen predominating over Type III , and their molecules stablishing inter and intra-molecular cross-linkings that turns the fibers more resilient to degradation by collagenase. This means that collagen may become more difficult to undergo digestion, but does not mean that it did become totally irreversible.

These aspect can be well illustrated by the observations on schistosomal granuloma formation and its degradation following chemotherapy in experimental animals. Periovular granuloma in schistosomiasis is an essentially fibrosing lesion.

Miracidium-derived antigens can act directly or indirectly (through the immune system) to stimulate the cells involved in fibrogenesis during granuloma formation, but the *in vivo* mobilized macrophages and fibroblasts are at the same time induced to secrete collagenase (Wyler, 1987).

At the 8th week of infection with 50 *S.mansoni* cercariae in the mouse, the amount of Type III collagen increased 22 times against 11 times for Type I collagen (Wu et al., 1982). Type II collagen is supposed to be more easily degraded , since it is arranged in a loose texture of fibers and can be attacked by many types of proteases, while Type I collagen appears as thick compact fibers that can only be degraded by a specific collagenase. It has been suggested that the reversibility of fibrosis in early schistosomiasis is due to the predominating presence of Type III collagen in the granulomas (Biempica et al., 1983). Immunofluorescent techniques reveal a predominance of Type III collagen in periovular granulomas, but 39 days after treatment, both Type III and I are present in similar amounts in the shrunken granulomas. Apparently there is a fraction of Type

III collagen that is soon removed, but there is another (more mature?) that remains for a more prolonged time (Andarde & Grimaud, 1986). When the period of observation is extended for four months and half after treatment, the majority of hepatic granulomas produced by a 10-week infection with 50 cercariae have disappeared, but the remaining tiny scars still present are composed of both types of collagen fibers. Therefore, the genetic collagen isotypes seem to be less important for collagen degradation than the degree of collagen maturation, which may bear a relationship with time of deposition and the occurrence of molecular cross-linkings.

Curative treatment of schistosomiais does not induce something qualitatively new to the fibrous tissue in periovular granulomas. During the spontaneous evolution of the granulomas in the liver, it passes through passes through phases of inflammation, increased fibrogenesis, death of the miracidium, and total or partial resorption of fibrosis. Chemotherapy turns these changes synchronized to all granulomas.

Indeed, there is a differential speed for collagen removal in different granulomas, and even in a same granuloma part of the collagen can disappear more rapidly than another. Collagens mature with time and become more resistant to degradation. When the ultrastructural changes affecting the hepatic periovular granulomas after chemotherapy are sequentially followed for a sufficiently prolonged period of time, a dual phase of collagen degradation becomes apparent (Andrade & Grimaud, 1988). On the first days and weeks following curative treatment collagen fibers appear fragmented, forming portions of different sizes and thickness dispersed into a rather edematous matrix, while some fragments may be seen within smooth-membrane-bound vesicles within the cytoplasm of fibroblasts, myofibroblasts and occasionally macrophages (Fig. 4). These features representing extracellular collagen breakdown and internalization of collagen fragments are commonly observed during collagen degradation occurring in several models (post partum involution of the rat uterus, reversal of cirrhotic changes in rats after discontinuation of carbon tetrachloride administration, involution of carrageenin granuloma, metamorphosing involution of the tadpole tail, etc), which are all examples of acute degradation

occuring in less than a month period.

After four and four and half weeks following chemotherapy, collagen in the remaining involuting granulomas shows different patterns of degradation, which suggests that after a period of acute changes, degradation of collagen proceeds at a slow rate. There are two main focal changes seen at ultrastrural level in densely packed collagen fibers: dissolution of groups of fibers leaving empty spaces or holes (lytic changes) and the transformation of the fibers into a finely granular or fibrilar electron dense material (electron-dense changes) (Fig. 5).

The concept of chronic collagen degradation seems important for two main reasons: first, for the studies on the biology of collagen, as a feature that needs investigation on its functional aspects; second, on the practical level, as an indication that after removal of the causative agents, degradation of fibrosis should be expected in a matter of months or even years . Patients with hepatosplenic schistosomiasis submitted to treatment (Domingues, 1986, Bina & Prata, 1983) were seen to improved their clinical conditions in different degrees one and two years after treatment. In some cases such improvement progressed still further when the patients were again examined five years afterwards.

Experimentally, pipe-stem fibrosis produced in mice , could be easily reversed three months after specific treatment (Andrade, 1987), indicating that periportal fibrosis can undergo degradation in a way similar to that of periovular granulomas (Fig. 6).

Effective drugs now available against schistosomiasis can prevent and cure the advanced forms and thus are contributing to change the pattern of morbidity for these parasitic diseases and emerging as an important tool for control measures.

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LEGENDS

FIG. 1 - Gross appearance of pipe-stem fibrosis. Cut surface of the liver showing the caracteristic fibrous enlargement of the portal spaces against a background of normal-looking parenchyma.

FIG. 2 - Injection-corrosion plastic casts of the portal system of mice:

A - normal liver. The vasculature presents abundant and regular ramifications and no fine collaterals are seen emerging directly from the main portal branches.

B - liver from a mouse with a 10-week old S.mansoni infection. Due to extensive vascular destruction the portal system appears without the fine normal branches and the main remifications have a hairy aspect, because all the portal branches are giving off small dilated collaterals. The further lodging of schistosome eggs into these latter vessels leads to periportal inflammation and pipe-stem fibrosis. Enlargement: approximately 4 X.

FIG. 3 - Typical representation of pipe-stem fibrosis at the microscopic level: a small portal space is fibrotic and enlarged, with preservation of arterial and ductal structures, but with destruction of the portal vein branch, the muscular coat of which is fragmented and buried within the connective tissue (arrows). Surgical biopsy from a patients with hepatosplenic schistosomiasis. Hematoxylin and Eosin, 120 X.

FIG. 4 - Acute collagen degradation. Nine days after curative treatment of schistosomiasis this granuloma present in the liver of a mouse shows extracellular collagen breakdown (above) and internalization of collagen fragments (arrows). Electron Microscopy, 7.000 X.

FIG. 5 - Chronic collagen degradation - Four and a half months after treatment, collagen fibers in an involuting granuloma present focal lytic changes (short arrows) and an accumulation of dark granular and fibrilar material (electron dense changes). Electron Microscopy, 20.000 X.

FIG. 6 - Experimental pipe-stem fibrosis in long standing murine schistosomiasis. A - Portal spaces are enlarged due to the presence of many periovular granulomas, while the hepatic parenchyma appears preserved.
B - Only small fibrous tracts and involuting granulomas are present in the mouse liver with previous pipe-stem fibrosis three months following curative treatment. Picrosirius red staining for collagen, 100 X.

FIG. 1

FIG. 2

FIG. 3

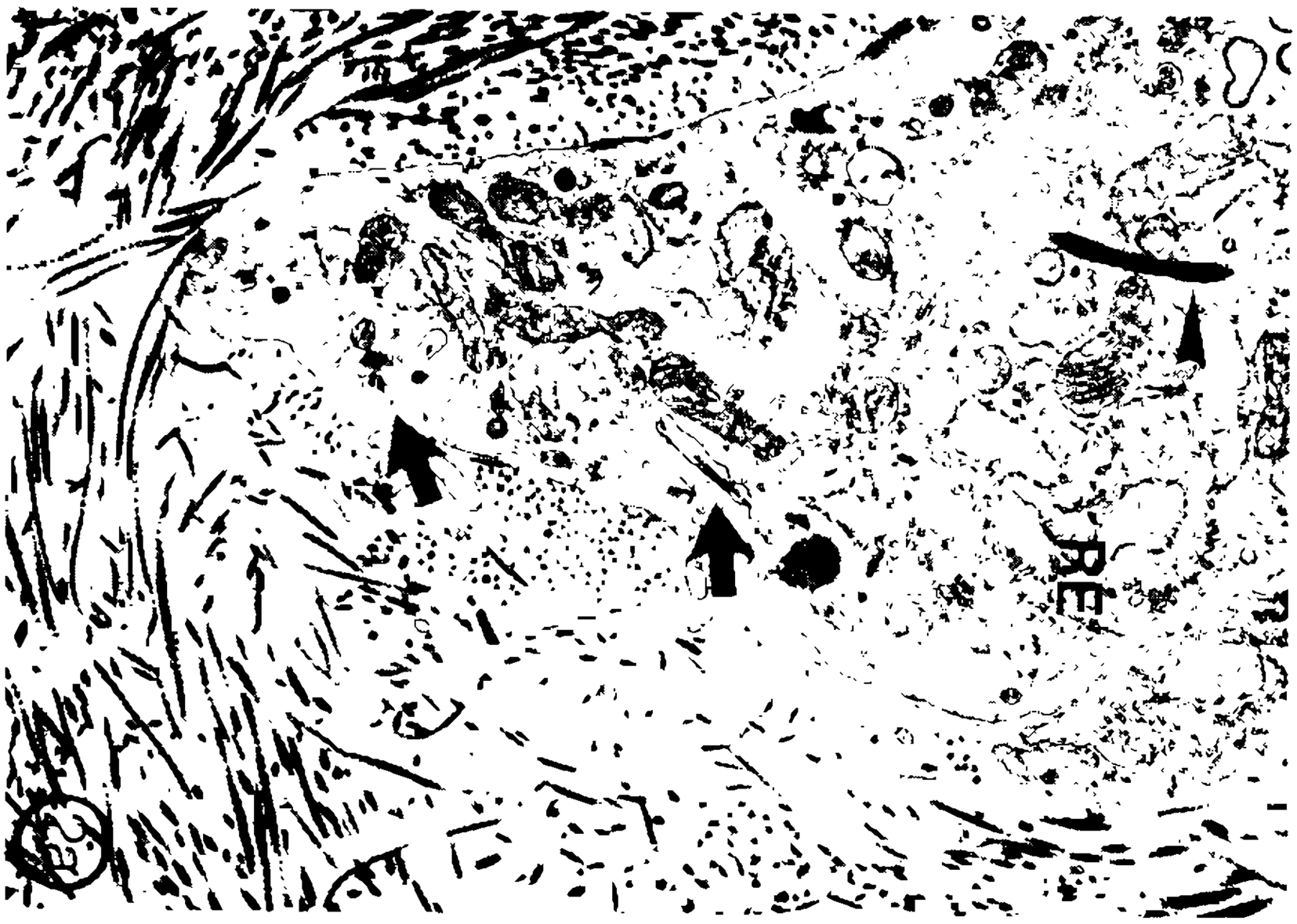

FIG. 4

FIG. 5

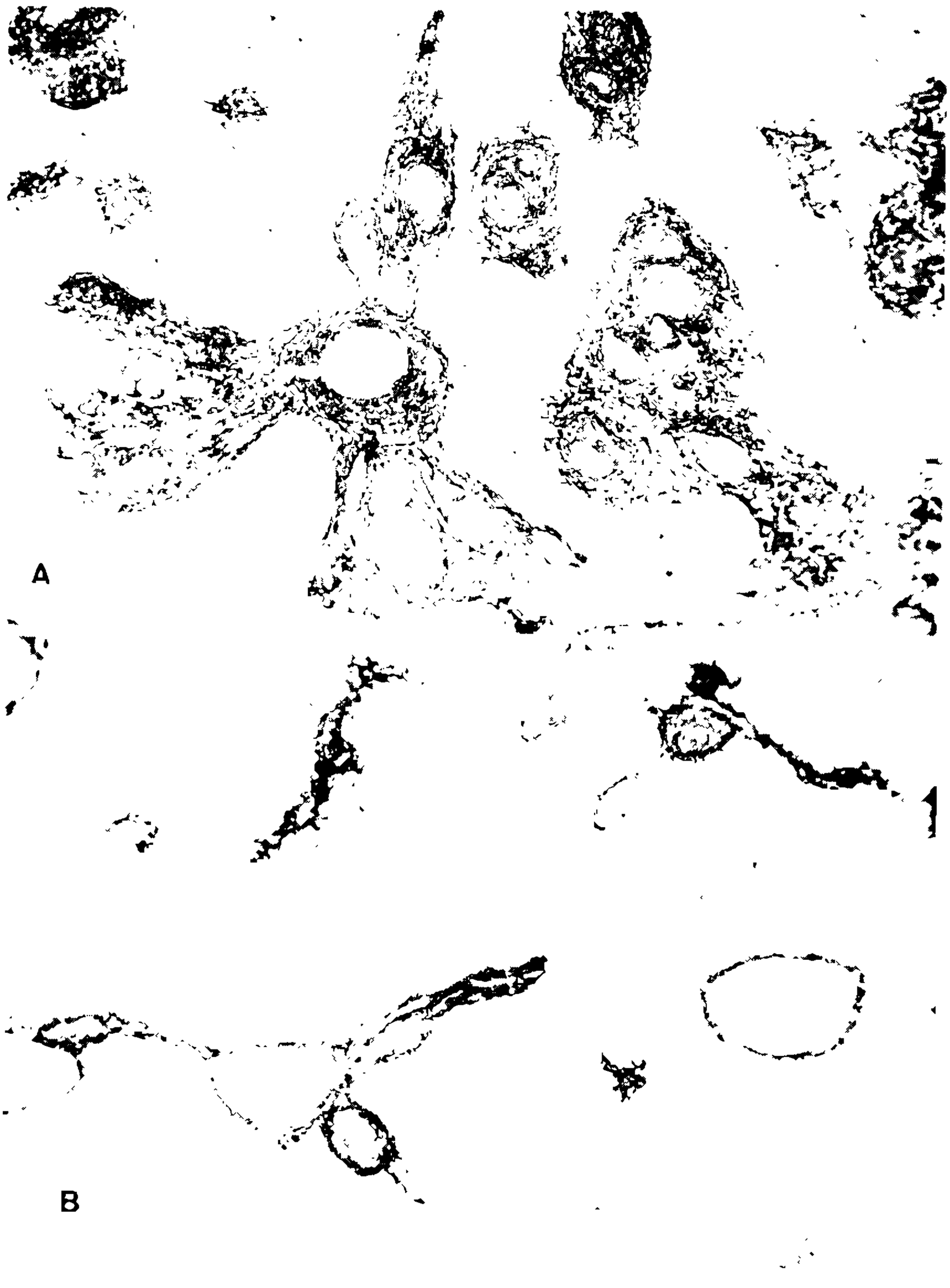

FIG. 6