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Fine-Structural Evidence for Vascular Injury in Patients with Interstitial Cystitis

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Summary. Bladder vessel walls of 20 patients with interstitial cystitis were studied by the electron microscope.

14 (70%) had severe endothelial injury. 10 (50%) showed injured smooth muscle cells. Odd basement membrane proliferations and disruptions were seen. Clusters of microfibrils about 10 nm in diameter and numerous partially membrane-bound vesicles of 100–600 nm with granular or tiny vesicular content ("granulovesicular bodies") were also seen. Intercellular junctions of endothelial cells were open and there was emigration of polymorphonuclear leucocytes and platelets.

The findings show pronounced vascular injury to have taken place, with neoformation of elastic tissue. It is suggested that the injury is immunologically mediated and that particularly those clusters of connective tissue microfibrils not yet covered by an amorphous elastin component may be involved in the pathogenesis of this disease.

Key words: Cystitis – Electron microscopy – Vasculitis – Elastic tissue – Autoimmune diseases

Interstitial cystitis is a condition of unknown aetiology and its pathogenesis has remained obscure. Some investigators have found evidence in favour of an autoimmune origin and have stressed the resemblance to connective tissue disease (Oravisto, review 1980). Immunoglobulin and complement deposits have been found in bladder vessel walls in patients with interstitial cystitis (Boye et al. 1979; Weisman et al. 1981; de la Serna and Alarcón-Segovia 1981; Mattila 1982), pointing to an immunological vasculitis. Few earlier papers include the results of electron-microscopy of bladder mucosa, particularly in its blood vessel walls. Skoluda et al. (1974) found lymphocyte and plasma cell infiltrates and fragmentation of plasma cells pointing to a mild immunological process. Collan et al. (1975) reported subepithelial capillaries with strongly thickened basement membrane. Boye et al. (1979) found multilayered basal lamina in small blood vessels and widened subendothelial connective tissue compartments with amorphous material of variable electron density.

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The purpose of this study was to examine the fine structure, particularly of medium-sized vessel walls, in bladder mucous membrane in patients with interstitial cystitis and to assess the importance of vessel wall alterations in the pathogenesis of the disease.

Material and Methods

Patients. The diagnosis of interstitial cystitis was based on criteria previously described (Oravisto and Alfthan 1976). Out of 20 patients 18 were women and two men. The mean age was 49 years. Biopsies were performed between two months and seven years from onset of the disease.

Control Patients. Eighteen patients, both women and men, with other urological disease than interstitial cystitis served as controls. (7 prostatic hyperplasia, 3 control patients fully recovered from urinary bladder papillary tumor, one ureter stone, one urinary bladder stone with cystitis, two microscopic haematuria, one carcinoma of the prostate gland, one urethral stricture, one neurogenic bladder, one urethral condyloma)

Electron Microscopy. One-millimeter cubes of bladder biopsy tissue were fixed in 1.5% glutaraldehyde in 0.1 M phosphate buffer and postfixed with 2% OsO_4 in phosphate buffer. Dehydration was carried out in acetone. Specimens were embedded in Epon 812 and sectioned on an LKB ultrotome. 1 μ sections stained with alkaline toluide blue were screened by light microscope and representative specimens with medium-sized blood vessels were selected for further studies. The sections were stained with uranyl acetate and lead citrate and examined under a JEM 100 C electron microscope.

Results

Endothelium. Fourteen out of 20 patients showed severe endothelial injury (Fig. 1). Medium-sized vessels were most heavily affected. These vessels had endothelium and basement membrane and collagen and elastic fibers in the subendothelial and intermuscular spaces. Focally, endothelial cells were swollen and disrupted, or even absent in places. Cells adjoining the damaged ones were often morphologically intact. Sloughing of endothelial cells into the microcirculation could be seen. There was also emigration of polymorphonuclear cells (Fig. 2) and platelets were apparently cleaving endothelial cells apart (Fig. 3). Lipid droplets and myelin-like figures could be seen in endothelial cells. Pinocytotic activity was increased.

Basal Lamina. Proliferation and multilayering of basement membrane could also be seen in small capillaries. The basement membrane was sometimes disrupted by escaping leucocytes. Ominous proliferations with invaginations into the subendothelial space were seen (Fig. 4).

Subendothelial and Intermuscular Space. The subendothelial space was widened and oedematous. 7 out of 20 patients (35%) had clusters of microfibrils 10–12 nm in diameter without matrix component in the vicinity of more mature-appearing elastic elements (Fig. 5). Normally microfibrils are found only at the peripheral mantle of elastic fibers and laminae (Greenlee et al. 1966).

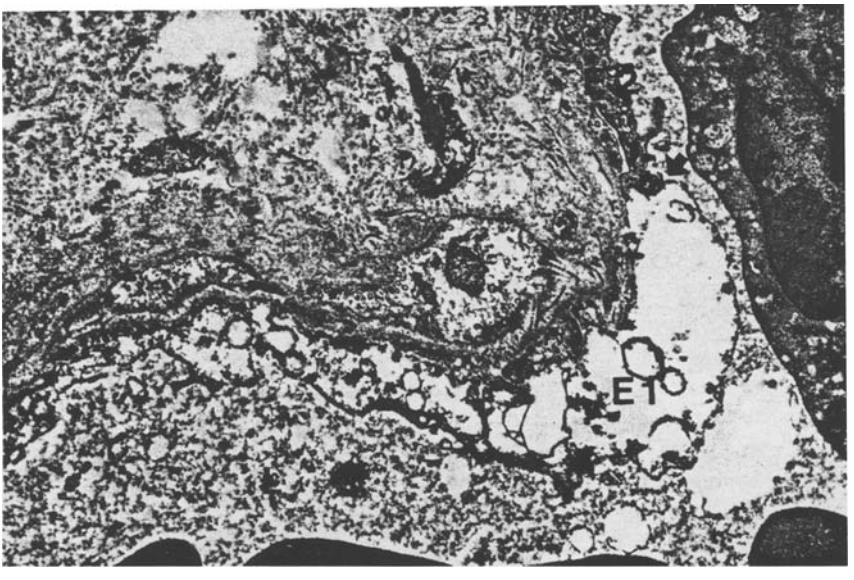

Fig. 1. Severely damaged endothelial cell (*E1*). Note the intact neighbouring endothelial cell (*E2*). (*arrow*) intercellular junction, (*L*) indicates lumen. ×9,900

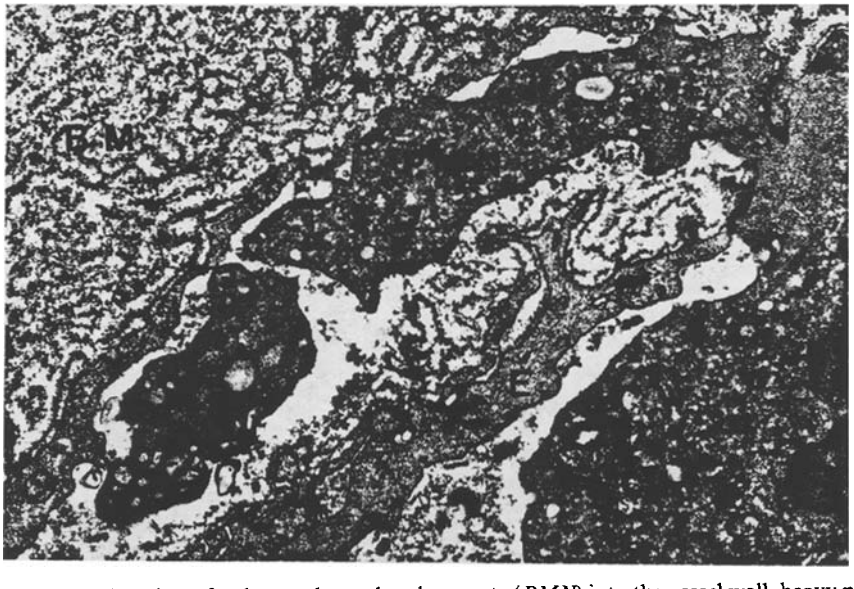

Fig. 2. Emigration of polymorphonuclear leucocyte (*PMN*) into the vessel wall, heavy proliferation of basement membrane (*BM*). One neutrophil lies in direct apposition to the basement membrane (*arrow*). (*E*) endothelial cell, (*L*) lumen. ×12,000

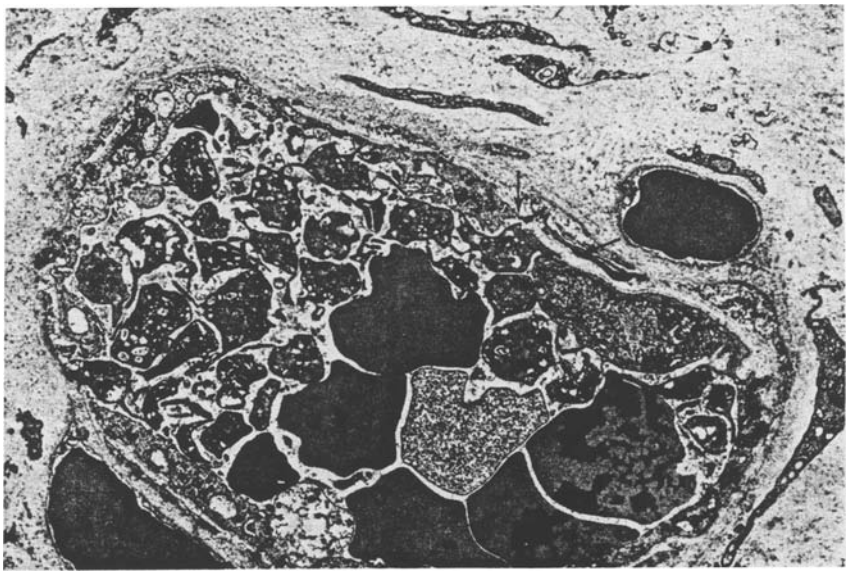

Fig. 3. Increased numbers of platelets (*PL*). One platelet is making its way through an endothelial gap (*arrows*) and adheres to the basement membrane. ×4,900

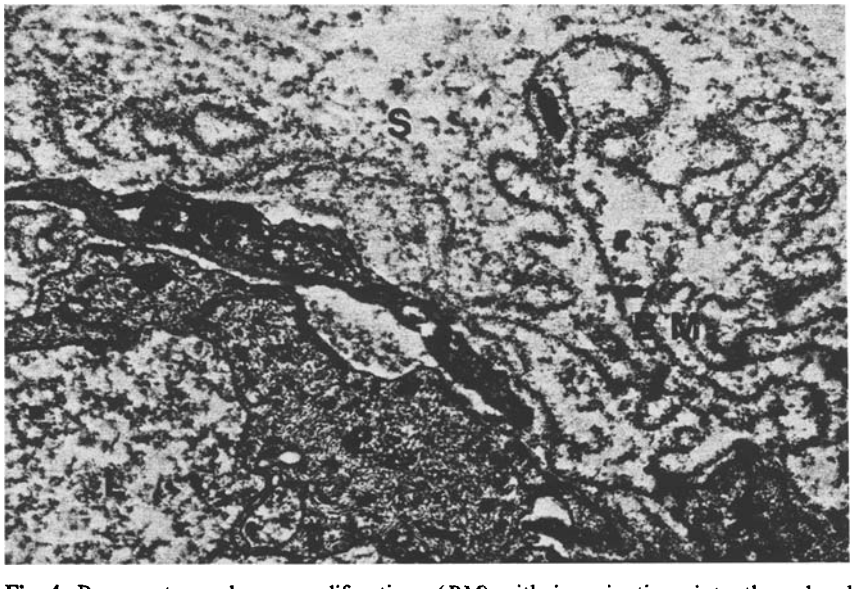

Fig. 4. Basement membrane proliferations (*BM*) with invaginations into the subendothelial space. (*L*) lumen, (*E*) endothelial cell, (*S*) subendothelial space. ×12.400

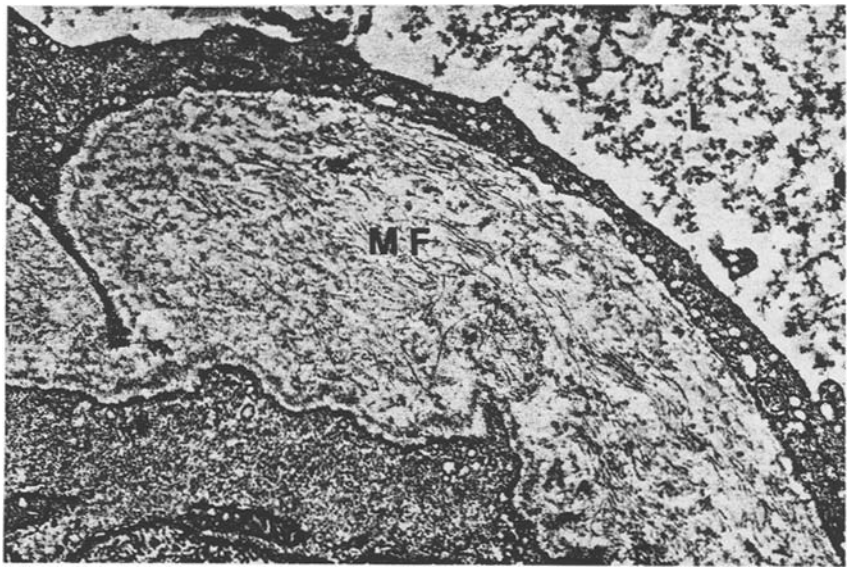

Fig. 5. Clusters of microfibrils (*MF*) in subendothelial space. (*E*) endothelium, (*L*) lumen. ×15,500

15 out of 20 patients (75%) had round vesicles with granular or tiny vesicular content (Fig. 6). These vesicles had a normal trilaminar membrane and measured 100–600 nm in diameter. Sometimes limiting membrane was partially or totally absent, especially near the elastic elements. Elastic tissue was often oedematous and ill-defined. There seemed to be fewer than normal microfibrils at the periphery of elastic fibers. The amount of collagen seemed to be decreased in the region immediately beneath the endothelium. Occasionally electron-dense deposits were seen in the subendothelial space (Fig. 7).

Smooth Muscle Cells. Ten out of 20 patients (50%) had injured smooth muscle cells. Damaged cells displayed a variety of degenerated lesions, i.e. clumping of nuclear chromatin, swelling of mitochondria. In the cytoplasma there were huge vacuoles with protein-like material. Lipid droplets and myelin-like figures were also seen. There were detached cytoplasmic fragments or long cytoplasmic processes connected to the cell body by a narrow stalk. Pinocytosis was increased especially near the clumps of microfibrils.

Controls

None of the control patients had damaged endothelial cells. Occasionally some small lipid droplets were seen in the cytoplasma of endothelial cells. No clumps of microfibrils were seen in subendothelial or intermuscular space. Elastic tissue was more easily identifiable in controls due to the peripheral mantle of microfibrils. Collagen fibers were increased especially

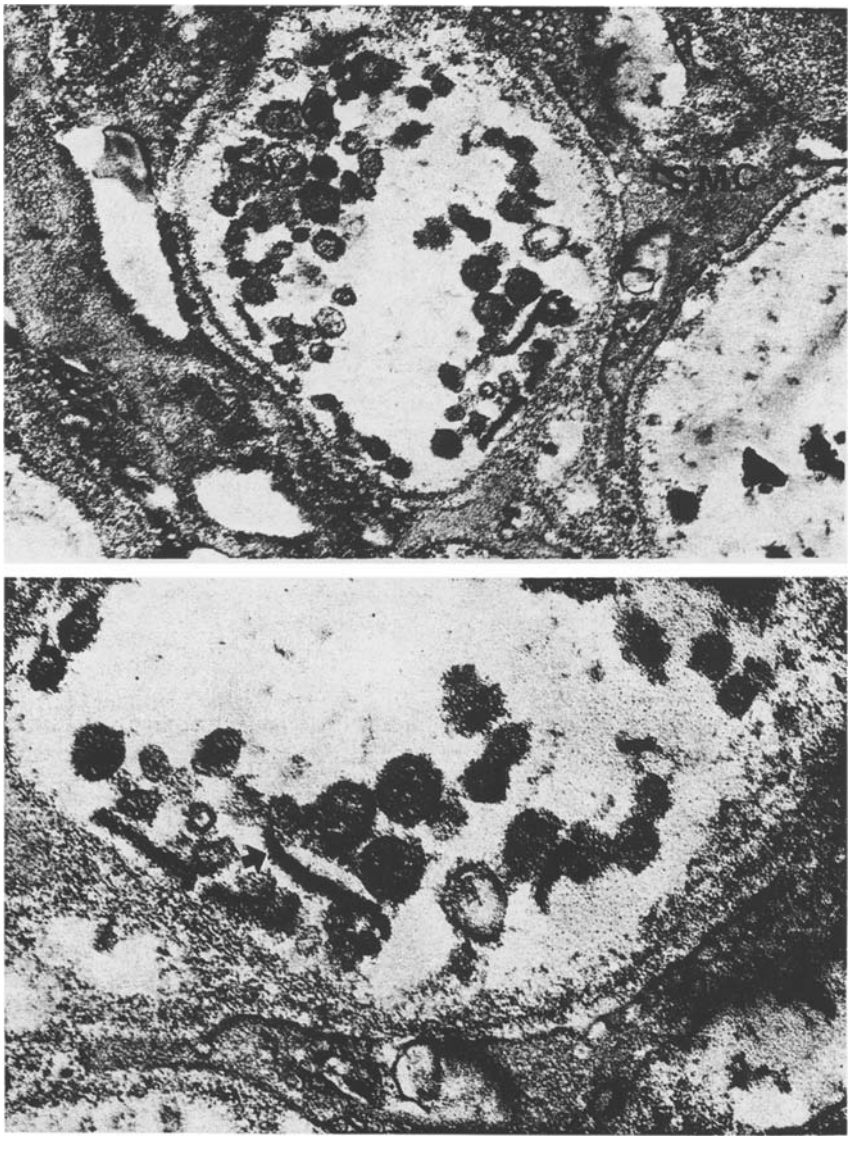

Fig. 6. **A** Membrane-bound vesicles (*V*) in intermuscular space. They are very numerous. (*SMC*) smooth muscle cell. ×30,000. **B** Higher magnification of the vesicles. Note the trilaminar membrane. Membrane envelopes can be discerned around most of the particles. To some extent membranes have "deflated" with the exit of vesicles (*arrow*). ×54,000

in the subendothelial space. Half of the controls showed a very small number of granular, membrane-bound bodies. These seldom had a complete membrane and they were located in the vicinity of elastic fibers.

Pinocytotic vesicles were present only to a moderate degree. They were slightly more numerous in smooth muscle cells than in endothelial cells.

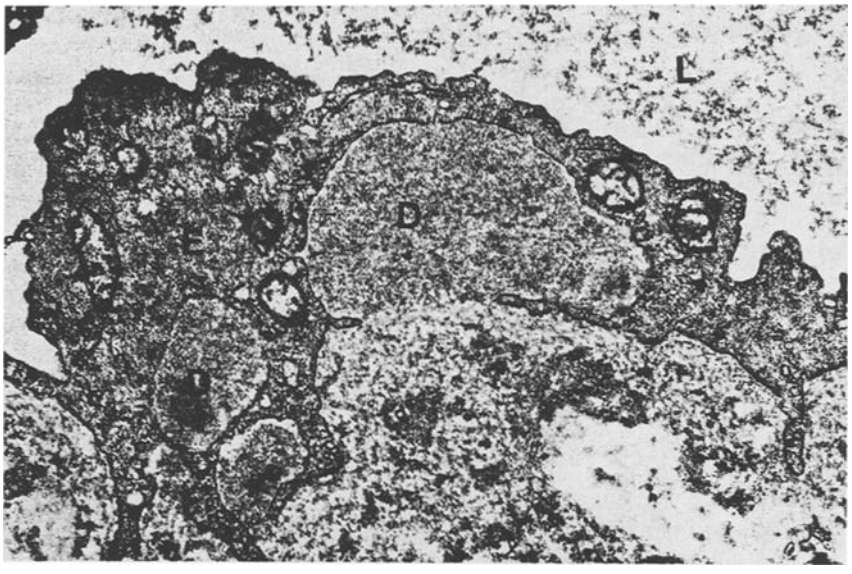

Fig. 7. Subendothelial electron-dense deposits (*D*). (*E*) endothelium, (*L*) lumen. × 9,900

Basement membrane sometimes had a parallel multilayering, especially in venous-type blood vessels.

Discussion

This study disclosed severe vascular damage and fine-structural alterations of great interest in bladder vessel walls in patients with interstitial cystitis. Medium-sized vessels were most heavily affected. They are known to be most sensitive to vasoactive amines (Rhodin 1968). Endothelial and, partly, smooth muscle cells were seriously injured. Basement membrane layering, proliferation and disruptions were seen pointing to cell necrosis and uncontrolled restoration and growth (Wracko 1974).

To our knowledge this is the first time ultrastructurally small round membrane-bound vesicles have been seen in the vicinity of elastic elements in the vascular walls in man. In the literature such vesicles are referred to as granulovesicular bodies in experimental conditions in animals (Trillo and Haust 1972). In our study the number of these granulovesicular bodies was considerably increased in patients compared with controls. Similar findings have been made in animals under pathological conditions. They are believed to be associated with both regenerating and newly developing elastic elements (Trillo and Haust 1972; Trillo and Haust 1975). They might be associated with elastin matrix transport. Extracellular vesicles have however not been observed in transit during elastogenesis. Membrane-bound partially or totally detached cytoplasmic processes were seen in the subendothelium. They are reported in the literature as "ghost bodies" in animals under experimental conditions (Trillo 1981).

Trillo suggested that certain kinds of toxic substances, e.g. immunoglobulins, could be eliminated by this mechanism.

Paucity and clumps of presumably elastic microfibrils were observed only in the patients. Clusters of microfibrils without an amorphous component of elastic tissue point to newly synthesized material (Haust 1979). Microfibrils form during fetal development of the first elements of elastin. With maturation of the fibers the amorphous elastin component becomes more prominent, so that the developing elastic fiber consists of a central amorphous core surrounded by an envelope of microfibrils (Greenlee et a. 1966).

It is of great interest that autoantibodies against these microfibrils have been found in sera from patients suffering from chronic inflammatory conditions (Linder and Lehto 1978; Linder et al. 1979). We suggest that in particular this microfibrillar part uncovered by amorphous matrix may be involved in the pathogenetic process as antigens. Vessel walls may be injured by autoantibodies or immune complexes formed in them. Fluorescence evidence has been presented favouring the concept of immunological damage (Boye et al. 1979; Weisman et al. 1981; de la Serna and Alarcon-Segovia 1981; Mattila 1982). Occasionally electron-dense deposits were seen in the subendothelial space. They correspondend to the findings in the immunological studies mentioned above. They may represent immune complexes containing autoantibody and autologous antigens. The paucity of such deposits may be explained by the focal nature of the deposits, or they may have been lost in the embedding procedure. We cannot exclude the possibility that the electron-dense deposits were condensed serum proteins.

Microbial infections, especially virus infections, have been regarded as a potential triggering mechanism for autoimmunisation causing chronic types of injury (Glynn 1975; Allison 1973). Consequent inflammation may liberate connective tissue microfibrils, with subsequent autoimmune response. A variety of virus infections are associated with autoantibodies and are followed by autoallergic manifestations (Allison 1973). Autoimmunisation probably depends on genetic constitution as in other autoimmune diseases (Glynn 1975). Cell injuries in vessel walls may be secondary to the immunological damage in the subendothelial space, or the same autoantibodies against connective tissue microfibrils react with intermediate filaments in cell cytoplasma as reported in other chronic inflammatory lesions (Linder et al. 1981).

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