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Non-epithelial basement membrane thickening in the urinary tract associated with phenacetin abuse

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Summary. In four cases of capillarosclerosis in the urinary tract associated with analgesic (phenacetin) abuse, the basement membrane (BM) thickening was not confined to the subepithelial capillaries, but was also found around the smooth muscle cells in the luminal part of the tunica muscularis. Electron microscopy confirmed that the changes in the BM around the smooth muscle cells were similar to those seen around capillaries. This non-vascular affection of BM in the urinary tract in patients with phenacetin abuse has not been reported previously. Thus, capillarosclerosis appears to be only part of a BM disorder, that clearly diminishes in intensity with increasing distance from the lumen. It is therefore suggested that the changes are caused by some agent (possibly a metabolite) in the urine diffusing from the lumen into the wall of the urinary tract.

Key words: Urinary tract – phenacetin abuse – capillarosclerosis – smooth muscle cells – basement membrane thickening.

Introduction

The term capillarosclerosis has been introduced recently to describe a lesion of the subepithelial capillaries in the urinary tract seen in patients with a history of analgesic (phenacetin) abuse. The changes appear as a strongly PAS-positive, hyaline thickening of the BM causing narrowing and eventually occlusion of the capillary lumen. (Torhorst 1976 and Abrahams et al. 1976). Ultrastructurally this thickening has been shown to be due to numerous thin lamellae of BM material with empty vacuoles and cell debris interposed between the lamellae (Mihatsch et al. 1978). Later reports claim that capillarosclerosis is specific for a longstanding abuse of phenacetin and thus permits one to diagnose this condition with certainty (Mihatsch et al. 1979; Parpan 1980; Gloor 1982).

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Table 1.

Pt.	Sex	Age (years)	Preoperative renal function		Duration of abuse (years)	Phenacetin consumption (kg)
			GFR[a] (ml/min)	Se-creat[b] (μmol/l)		
1	F	46	13	230	19	1.6
2	F	53	22	204	10	2.3
3	F	54	12	286	23	10.3
4	F	77	22	150	47	2.4

[a] GFR = ^{51}Cr-EDTA clearance (normal lower limit: 50-75 ml/min. (age dependent))
[b] (normal range: 56-114 μmol/l)

The pathogenesis of the lesion is not understood, but the consistent involvement of capillaries just beneath the urothelium points strongly towards diffusion of a causative agent/metabolite from the urine. Only endothelial (and pericytic?) BM have been described as being involved. The present report however documents, that non-vascular BM also may become affected in this condition.

Material and methods

A consecutive material consisting of 59 nephroureterectomy specimens with carcinoma of the renal pelvis or ureter was evaluated for the presence of capillarosclerosis. This was found in nine cases, four of which showed the changes reported in this article.

The clinical data concerning these patients were obtained from hospital records and information about analgesic consumption was obtained from the patients and/or their private practitioners.

Formalin-fixed, paraffin-embedded tissue from all parts of the urinary tract was sectioned and stained with haematoxylin-eosin, van Gieson, periodic acid-Schiff (PAS) with diastase digestion, and Congo red. Cryostat sections were stained with Sudan Black and Oil red O for lipids.

In one case fresh biopsy specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. The biopsies were postfixed for 1 h in 1% osmium tetroxide in the same buffer, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Electron microscopy was performed with a JEOL 100 B transmission microscope (JEOL USA, Electron Optics Division, Peabody, MA, USA).

Results

Relevant clinical data, including the estimated analgesic consumption, are summarized in Table 1. None of the patients were diabetics. One had been treated for arterial hypertension during a two-year period prior to the operation and the other three patients were normotensive. In two cases brownish discoloration of the bladder mucosa had been observed on cystoscopy. In all cases intravenous urography had revealed severe bilateral changes suggestive of analgesic nephropathy.

Light microscopy. In all four cases extensive PAS-positive (diastase resistant) BM thickening was evident not only in the subepithelial capillaries, but also

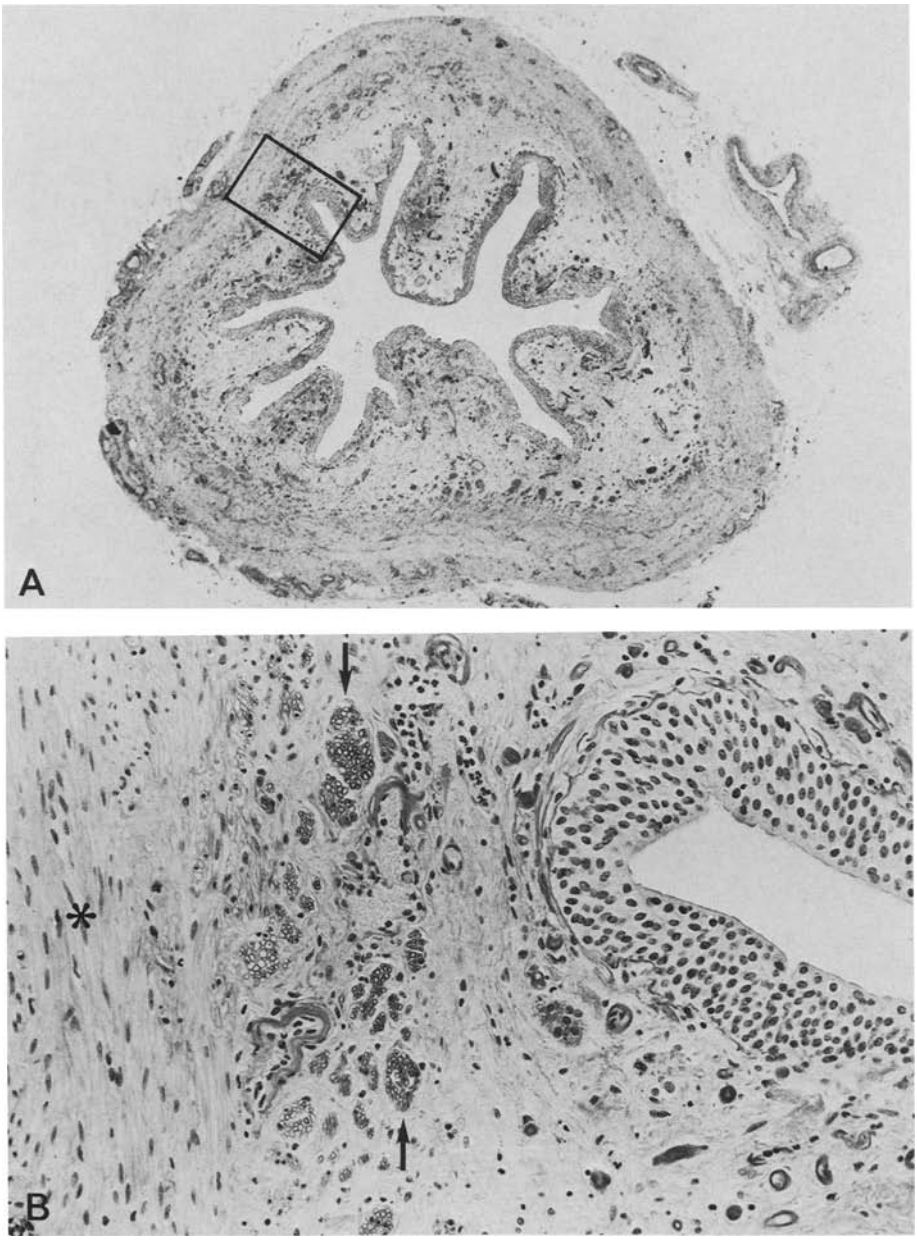


Fig. 1 A Cross section of ureter showing accumulation of PAS-positive material in the luminal part of the wall (PAS, ×21). **B** Framed area in A magnified to show the localization of the PAS-positive material around small subepithelial vessels ("capillarosclerosis") and around smooth muscle cells in the luminal part of tunica muscularis (*arrows*). Vessels and smooth muscle deeper in the wall (*asterisk*) appear normal (PAS, ×180)

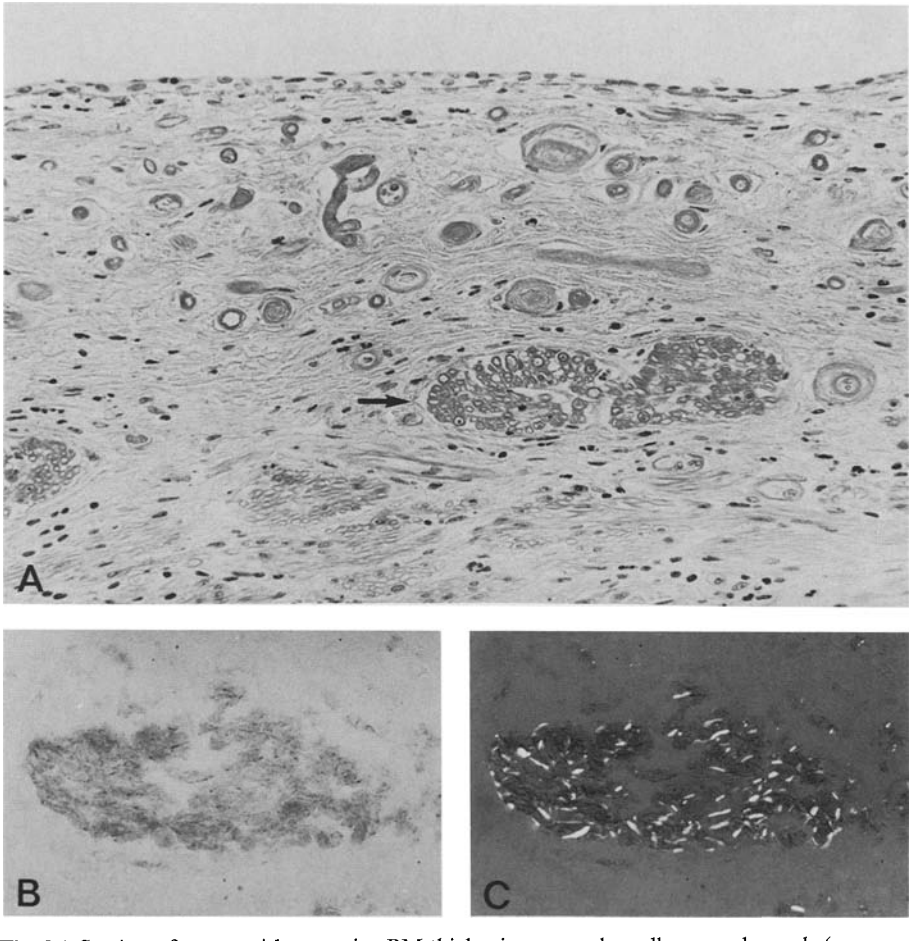
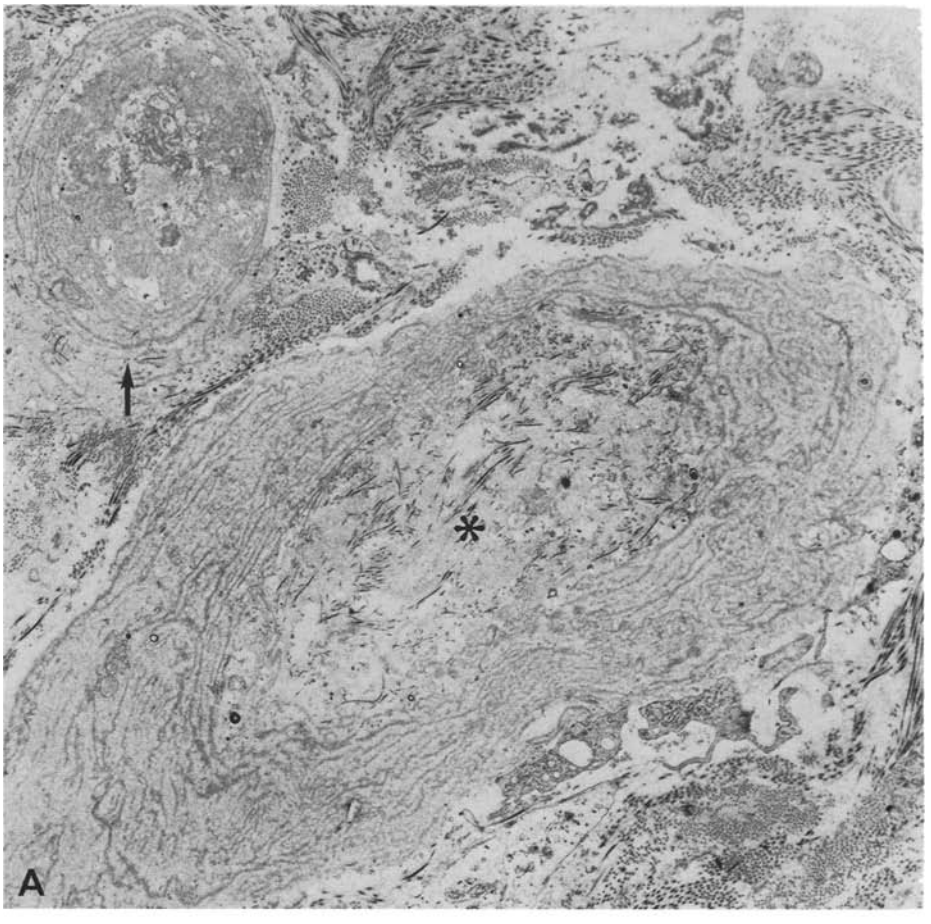


Fig. 2 A Section of ureter with extensive BM thickening around small mucosal vessels (some are totally obliterated) as well as around smooth muscle cells in the luminal part of tunica muscularis (*arrow*) (PAS, × 240). **B, C** Cryostat section showing a fascicle of smooth muscle cells (like that marked with an *arrow* in **A**) with thickened BM containing Sudanophilic (**B,** *ordinary light*) and birefringent (**C,** *polarized light*) material. No Sudanophilia or birefringence was seen in the corresponding paraffin section (Sudan black, × 170)

in some larger vessels throughout the mucosa. A similar BM thickening was seen around smooth muscle cells in the luminal part of the tunica muscularis (Fig. 1 and 2). In all cases the intensity of the BM thickening clearly diminished with increasing distance from the lumen. In the majority of the capillaries the lumen was greatly narrowed, and in many capillaries the endothelial cells had disappeared. In some capillaries the lumen was

Fig. 3 A Obliterated subepithelial vessels consisting of multilamellated BM material surrounding cellular debris (*arrow*) and collagen fibers (*asterisk*) (EM, × 5200). **B** Concentric lamellae of BM material with cross-striated collagen fibers present centrally. Small empty vacuoles (probably fat) are seen between the lamellae (EM, × 5700).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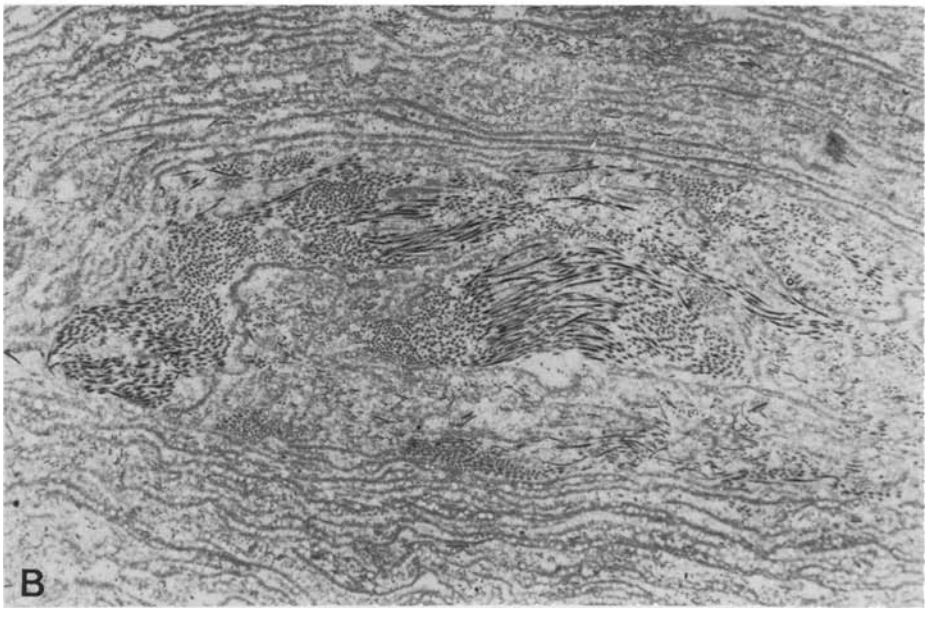
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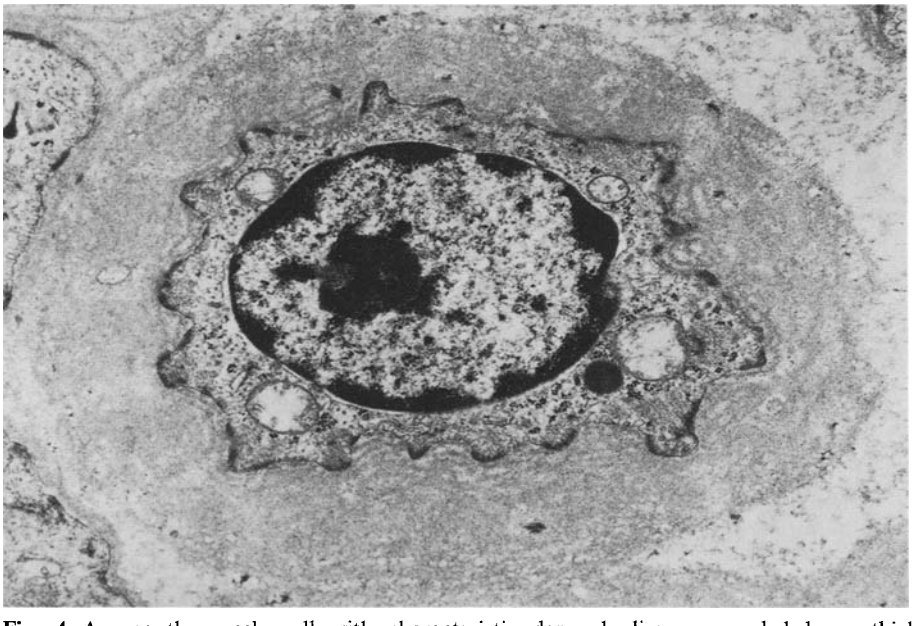

Fig. 4 A smooth muscle cell with characteristic dense bodies surrounded by a thick multilamellated BM (EM, ×15000)

completely obliterated, converting the vessel into a massive cylinder. The smooth muscle fascicles in the luminal part of the tunica muscularis had a reduced number of nuclei. Often the smooth muscle cells had disappeared completely, leaving the thickened BM like empty tubes.

In many of the capillaries with obliterated lumena the van Gieson stain revealed a small amount of red staining material located centrally, indicating the presence of collagen. This was not observed in the capillaries still containing endothelial cells.

The Congo red stain was negative in all cases.

In one case inflammatory cells were completely absent, while a light or moderate round cell infiltration was seen in the other cases. Minimal fibrosis of the connective tissue stroma could be detected in one case only.

The subepithelial PAS-positive lamina did not appear thickened in any of these cases.

The above mentioned changes were present throughout the urinary tract. Although the BM thickening was most pronounced in the renal pelvis and ureter, the same changes, including thickening of the smooth muscle BM, were evident in the bladder.

Cryostat sections showed a strong Oil red O and Sudan black positivity of the thickened BM around capillaries as well as around smooth muscle cells (Fig. 2B, C).

Electron microscopy. Ultrastructurally the subepithelial capillaries showed BM alterations characteristic of capillarosclerosis as described by Mihatsch

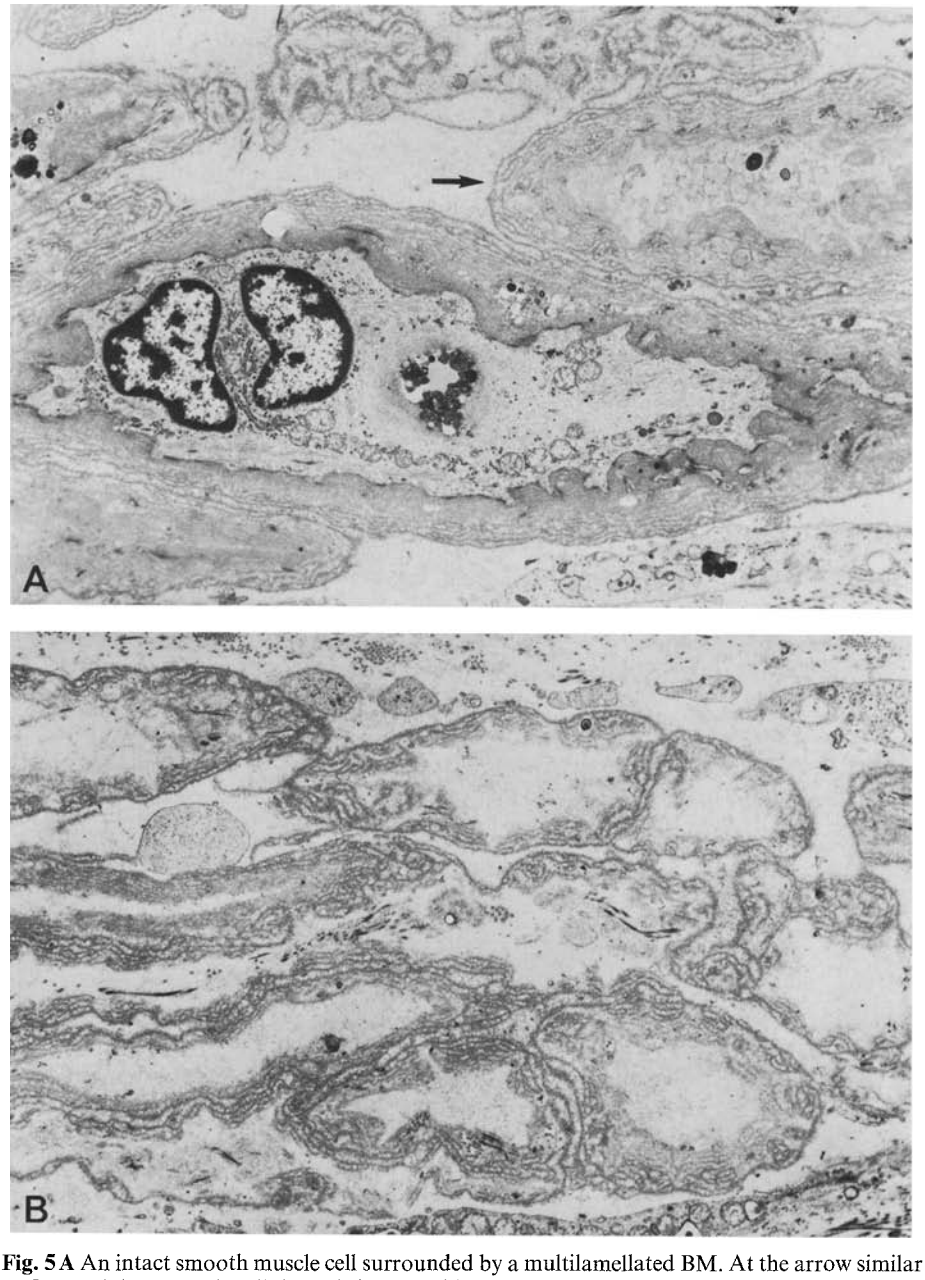

Fig. 5 A An intact smooth muscle cell surrounded by a multilamellated BM. At the arrow similar BM material surrounds cellular debris, probably a destroyed smooth muscle cell (EM, × 5700). **B** Empty tubes formed by concentric lamellae of BM material. Cellular debris is no longer identifiable centrally (EM, × 6000)

et al. (1978) (Fig. 3). A similar multilamellation of the BM was seen around smooth muscle cells (Fig. 4). In both locations empty small vacuoles (probably fat) and cellular debris were found between the lamellae of BM material. Some capillaries contained well preserved endothelial cells, but in others the endothelial cells showed signs of degeneration. Some capillaries contained only cellular debris and others were completely obliterated, often with cross-striated collagen fibers present centrally (Fig. 3 B).

In the tunica muscularis a similar range of changes was noticed from intact smooth muscle cells surrounded by a multilamellated BM to more or less damaged cells (Fig. 5). Often only cellular debris was left inside otherwise empty tubes of BM material. However, collagen fibers were scarcely ever seen in these, and total obliteration of the tubes was not observed.

Discussion

Torhorst (1976) and Abrahams et al. (1976) were the first to describe the occurence of PAS-positive, hyalinized capillaries just beneath the urothelium in patients with analgesic (phenacetin) abuse. This lesion, which later became known by the term capillarosclerosis, has been seen in all parts of the urinary tract including the bladder (Hesse et al. 1976 and Furie et al. 1982). Capillarosclerosis seems to be specific for a longstanding abuse of analgesic compounds containing phenacetin. The changes are confined to the urinary tract.

The present study shows for the first time that this hyaline thickening of BM in the urinary tract, previously thought only to involve suburothelial capillaries, also involves non-vascular BM. In the cases presented here multilamellated BM thickening was seen around muscle cells in the tunica muscularis as well as around subepithelial capillaries. The urothelial BM, however, always appeared to be normal.

The consistent affection of capillaries just beneath the urothelium has been suggested to result from damage by direct contact through urine containing concentrated metabolites of analgesics (Gloor 1978). The involvement of the luminal part of the tunica mucularies in our cases with clearly diminishing changes with increasing distance from the lumen points strongly towards diffusion from the urine of a causative agent/metabolite. In very severe and longstanding abuse the damaging substance seems able to reach deeper parts of the wall of the urinary tract and affect not only BM of vessels in the mucosa, but also BM of smooth muscle cells in the tunica muscularis.

Ultrastructurally, BM reduplication or lamellation was seen around capillaries as well as around smooth muscle cells. Reduplication of BM is also frequently found around capillaries in patients with diabetic micro-angiopathy (Vracko 1974; Fisher et al. 1982). In diabetes BM changes have been reported not only in capillaries throughout the body, but also in skeletal muscle and peripheral nerves. BM changes around smooth muscle cells have not, however, to our knowledge, been described in diabetes. In both diabetes

and phenacetin abuse totally obliterated capillaries in the form of massive cylinders composed entirely of concentric lamellae of BM material with empty vacuoles located centrally and between the lamellae are found. Deposits of cross-striated collagen fibers centrally in the obliterated capillaries have not, however, been described in diabetic micro-angiopathy, and not previously in capillarosclerosis. This seems puzzling as endothelial cells do not normally produce collagen fibers.

It is well known that many cells respond to sublethal injury by increasing the production of BM material (Martinez-Hernandez and Amenta 1983). During the past two decades an extensive study of BM has taken place to reveal the composition and function of these structures. BM are found in all organs and consist of extracellular matrix with a specific ultrastructure. They are made from the cells resting upon them, separating the cells from the surrounding connective tissue stroma. Alterations in BM can produce disease states such as diabetic micro-angiopathy. Despite much research the mechanism leading to alterations in BM in different disease states has not been clarified. Whether the changes are brought about by a direct effect on the BM or the cells producing them is not know. It is also unknown whether the thickening is due to excessive production or decreased destruction of BM material. Further biochemical studies may provide a clue to this question by determining the ratio of components in the altered BM.

The BM thickening in diabetes is generalized and thought to be secondary to metabolic disturbances although the precise pathogenesis remains unknown (Martinez-Hernandez and Amenta 1983). The occurrence of a similar lesion in phenacetin abuse limited to the urinary tract and probably caused by a yet unknown agent/metabolite in the urine seems very interesting and deserves further study. A better understanding of this local form of BM disease could probably shed more light on the mechanism regulating the production, destruction and turnover of BM material in general and in disease states in particular, such as in diabetic micro-angiopathy.

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