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An Ultrastructural and Morphometric Study of Bladder Tumours (II)

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Summary. Bladder tissues from 3 groups of patients were examined, using the light and electron microscopes (LM and TEM). One group of patients had a history of well-differentiated papillary transitional cell carcinomas and specimens were taken from cystoscopically normal areas. In a second group frank papillary carcinoma was biopsied. Finally, patients with no history of urothelial tumours and a normal cystoscopic appearance were biopsied during investigations for various benign conditions and these served as controls.

In tissues from the first two groups certain differences were seen when these were compared to the controls and the frequency of these was significant. Light microscopic examination of 0.5 μ toluidine blue stained sections revealed an increased number of immature, small dark cells in the superficial layer of the epithelium ($P < 0.001$). Electron microscopic examination showed that in place of the characteristic asymmetric unit membrane of mature superficial cells, the surface was frequently covered with microvilli and the junctional complexes were often atypical. There was an increased number of abnormalities in the basal lamina ($P < 0.001$).

These features were seen in the absence of cystoscopic and light microscopic changes in three out of eight patients with a history of tumours. It is, therefore, suggested that these are the earliest detectable morphological abnormalities in the pre-neoplastic urothelium.

Key words: Electron microscopy – Bladder neoplasms – Measurement

Recurrence of papillary tumours of the bladder is an unpredictable phenomenon. It has been established from light microscopic studies that hyperplasia and atypia can be found in association with recurrent bladder carcinomas (Koss et al. 1974). Electron microscopic studies in experimental animals have revealed

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further abnormalities which precede malignancy (Koss 1977), but ultrastructural studies of human neoplasia have been mainly limited to examination of the established tumour. The aim of the present investigation was to see if there were any ultrastructural features in the human urothelium which were associated with susceptibility to papillary tumour formation.

Materials and Methods

Group 1. Normal tissues were obtained from 7 patients. Two of these were from female transplant donors, from whom ureteric tissue was taken. Two were from patients with non-malignant prostatic disease, the bladder having been biopsied at the time of perurethral resection. Two patients were biopsied for recurrent haematuria and one for small capacity injected bladder. The observed changes were not indicative of malignancy.

Group 2. Cystoscopically normal tissues, generally from the vicinity of the ureteric orifices, were obtained from 8 patients who had a history of recurrent well-differentiated papillary transitional carcinomas of the bladder.

Three biopsies were taken from patients with a history of recurrent tumour over a period of 5–19 years, the remaining patients had had tumours of more recent onset.

Group 3. Papillae from bladder tumours (well-differentiated papillary transitional carcinomas) of 12 patients from the previous study were re-examined for comparison.

Specimens were obtained by biopsy forceps in sterile water. One to four biopsies were taken from each patient and at least two blocks from each biopsy were examined.

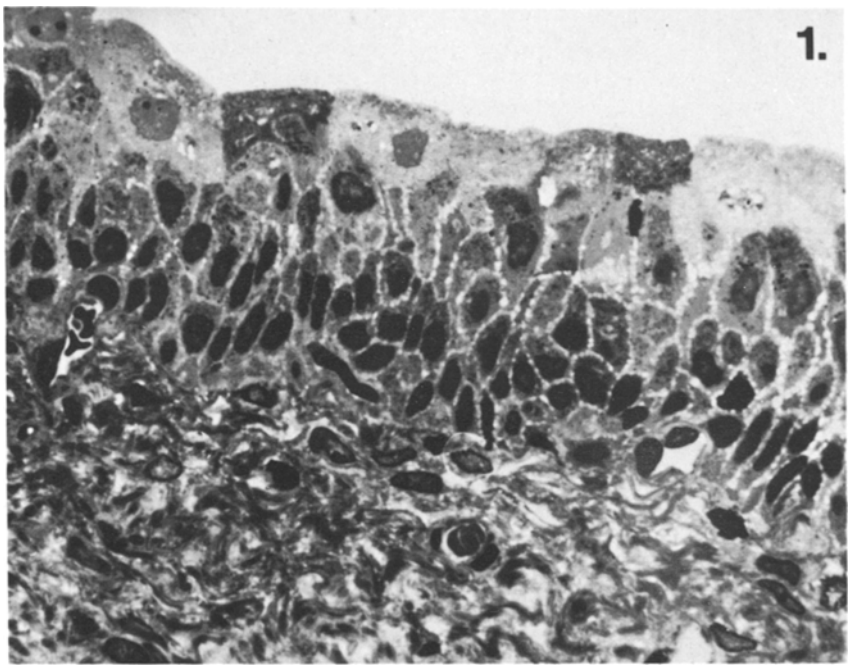

Fig. 1. Normal urothelium, 5–7 orderly layers. Large, light superficial cells with round nuclei. Smaller darker intermediate and basal cells with oval nuclei. 0.5 μ section stained with toluidine blue (× 160)

Processing of the tissues has been described in the previous paper (Smith 1981). The light microscopic observations were made on resin embedded tissues 0.25 mm^2 cut at 0.5 μ, stained with toluidine blue. A total of 1,100 cells was counted for quantification of immature cells. The quantitative assessment of basement membrane abnormalities involved 6,300 contact points, the point counting method having been described previously (Smith 1981).

Results

Morphology

Group 1. Normal Urothelium

a) Light Microscopy. The epithelium consisted of 3–7 layers except in tissue from one patient where there were areas of hyperplasia with up to 20 layers. The superficial layer consisted of umbrella cells which were light and large with round nuclei. A few small dark cells with oval nuclei were seen amongst the typical umbrella cells. The basal and intermediate cells were orderly, darkly stained and columnar with oval nuclei. The basement membrane was intact, wavy or tortuous (Fig. 1).

b) Electron Microscopy. The umbrella cells showed the characteristic asymmetric unit membrane, (AUM) with crescentic invaginations (Fig. 2). Occasionally short, knob-like protrusions and microvilli were present. Adjacent cells were

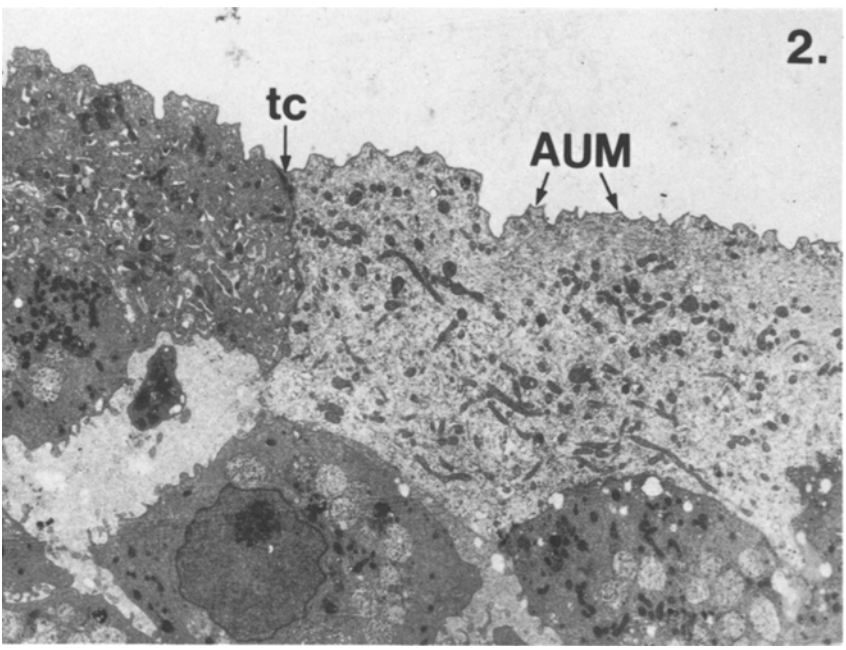

Fig. 2. Normal urothelium, superficial and intermediate cells. Luminal asymmetric unit membrane. The cells are laterally joined by a terminal complex. *AUM*, asymmetric unit membrane; *tc*, terminal complex (×4,000)

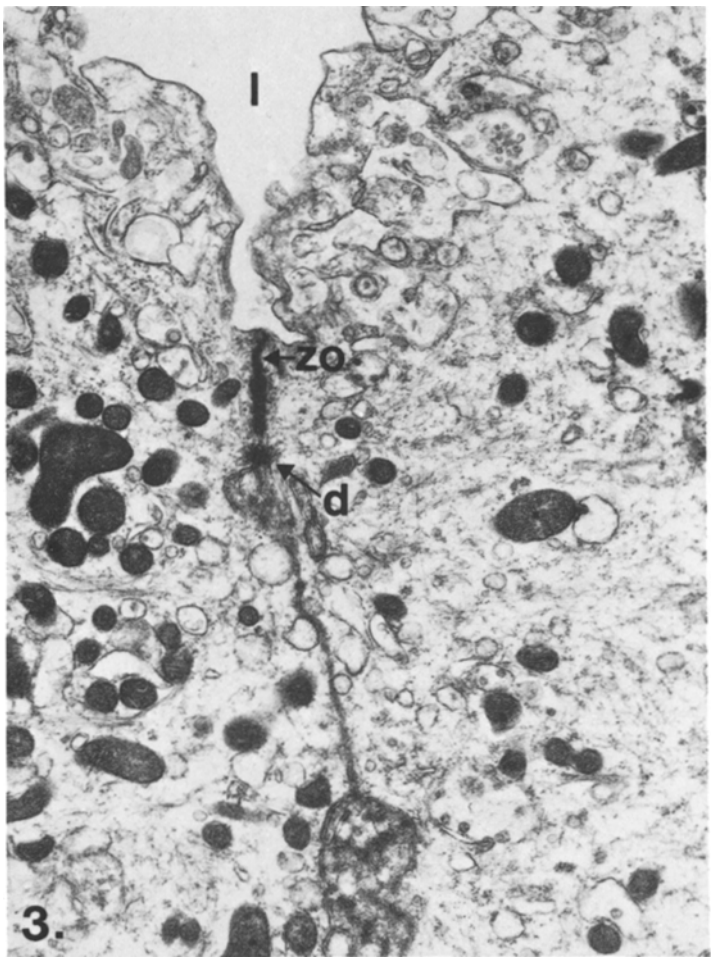

Fig. 3. Detail of terminal complex. *l*, lumen; *zo*, zonula occludens; *d*, desmosome (×26,000)

connected by the terminal complex, consisting of a fused region, the zonula occludens, below which there was a zonula adherens and occasional desmosomes deep to it (Fig. 3). The smaller cells which had stained dark with toluidine blue were electron-dense and the AUM with its crescentic invaginations was replaced by sparse microvilli (Fig. 4). There was no terminal complex seen between the 'light' and 'dark' cells except in a few instances, though desmosomes were present. The basal and intermediate cells have been described in a previous publication (Smith 1981). The basal lamina was thin, wavy or occasionally tortuous, with widespread reduplication. Though no discontinuities were seen, there were areas where the 3 layers consisting of the cell's limiting membrane, the lamina lucida and the lamina densa of the basal lamina were indistinct. This was not a cutting artefact (Fig. 5).

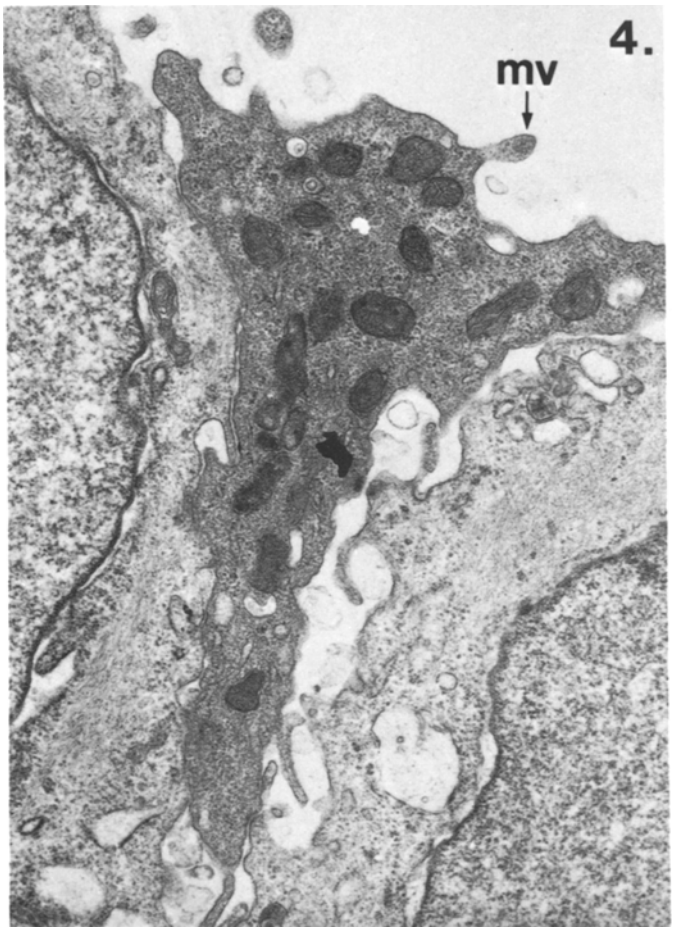

Fig. 4. *'Dark'* superficial cell between two 'light' ones. The AUM has been replaced by microvilli and the terminal complex is missing. *mv,* microvilli (×23,000)

Group 2. Cystoscopically Normal Bladder from Patients with Previous Bladder Tumours

a) Light Microscopy. Tissue from 3 patients had 3–4 epithelial layers (Group 2a), whereas in the rest there were stretches of epithelium with up to 20 layers (Group 2b). The umbrella cells were similar to those in Group 1, with single smaller dark cells among them, apparently more often than in normal tissue. The basal and intermediate cells also resembled those of normal tissue. The basement membrane was continuous (Fig. 6).

b) Electron Microscopy. The umbrella cells showed some differences from those seen in the control specimens. Though most cells had AUM, some cells were

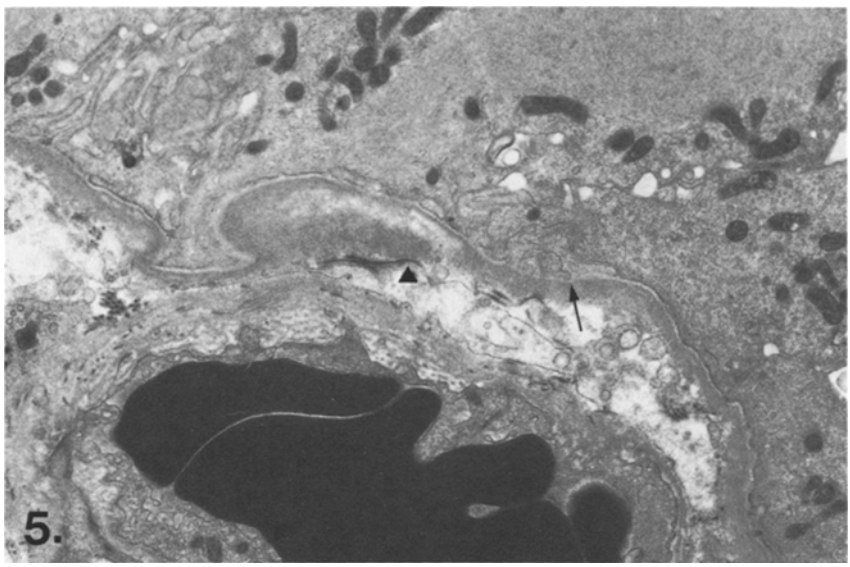

Fig. 5. Basal lamina showing reduplication and abnormal areas. *Arrow head:* reduplication; *Small arrow:* indistinct region (×6,400)

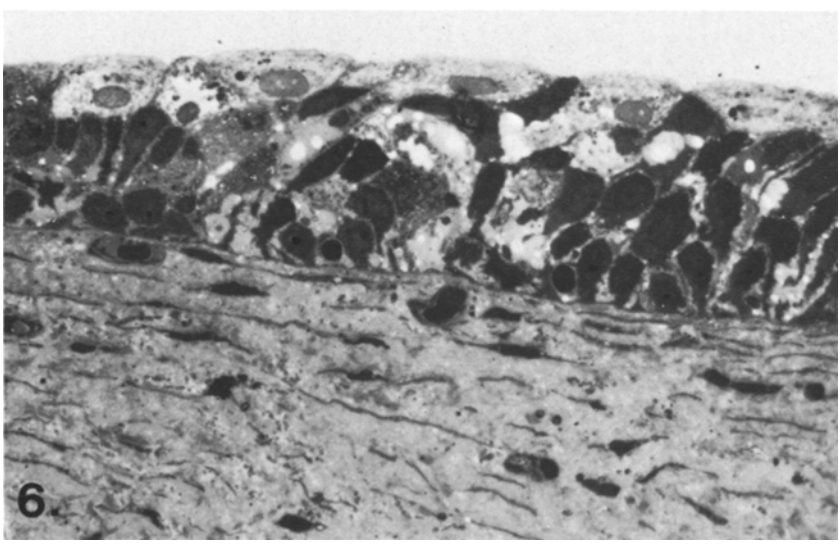

Fig. 6. Urothelium from Group 2 patient. 5–6 orderly layers. Small dark cells can be seen among the large superficial cells. The basement membrane is intact. 0.5 μ section stained with toluidine blue. (×160)

covered in microvilli and occasional knob-like protrusions could also be seen. In others the cell membrane appeared flexible and thinner than in the normal superficial cells. The zonula occludens of the terminal complex was often attenuated or absent (Fig. 7). The basal and intermediate cells resembled those seen in the control group. The basal lamina was variable, usually thin, but at some sites thick and fluffy-looking. Its course was wavy or tortuous and reduplication

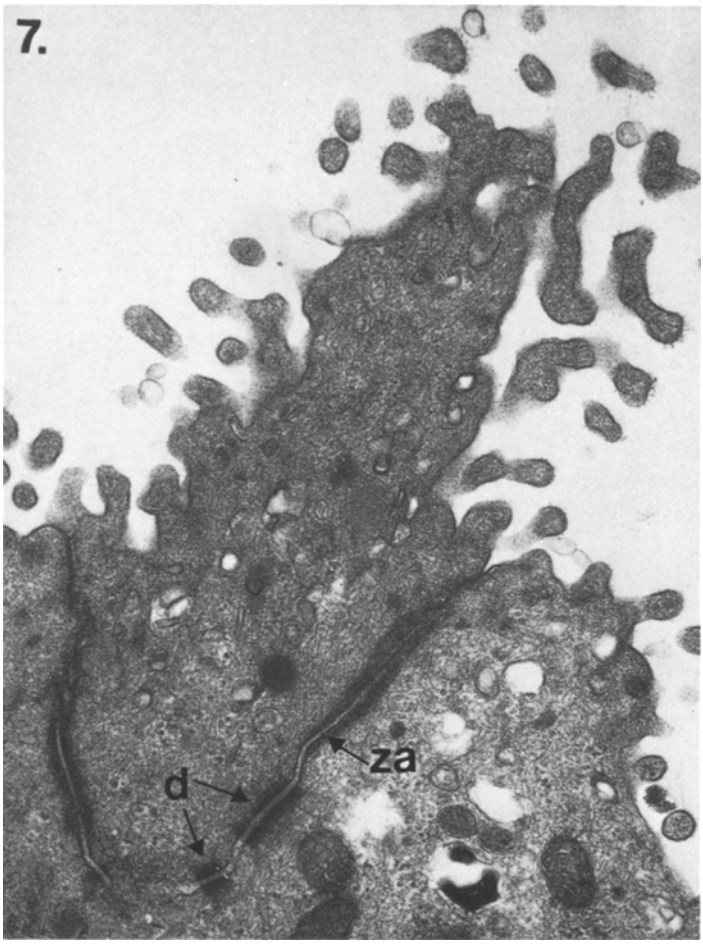

Fig. 7. Detail of 2 superficial cells from Group 2 patient. Cells lack the AUM which is replaced by microvilli. The zonula occludens is attenuated, but the zonula adherens and desmosomes are normal. *za*, zonula adherens (× 26,000)

was present in only about half the tissues examined. The absence of 3 distinct layers was more frequent than in the normal controls.

Group 3. Well-Differentiated Transitional Cell Carcinoma

a) Light Microscopy. The epithelium of the papillae consisted of 6–27 layers. The relatively few umbrella cells which were present appeared normal.

Single small dark cells were present among the superficial cells. The intermediate and basal cells resembled those in Groups 1 and 2. The basement membrane was normal (Fig. 8).

b) Electron Microscopy. The superficial cells showed AUM in about half the cells, elsewhere the luminal membrane looked flexible and there were numerous microvilli and knobs on the cell surface. As these cells were electron lucent

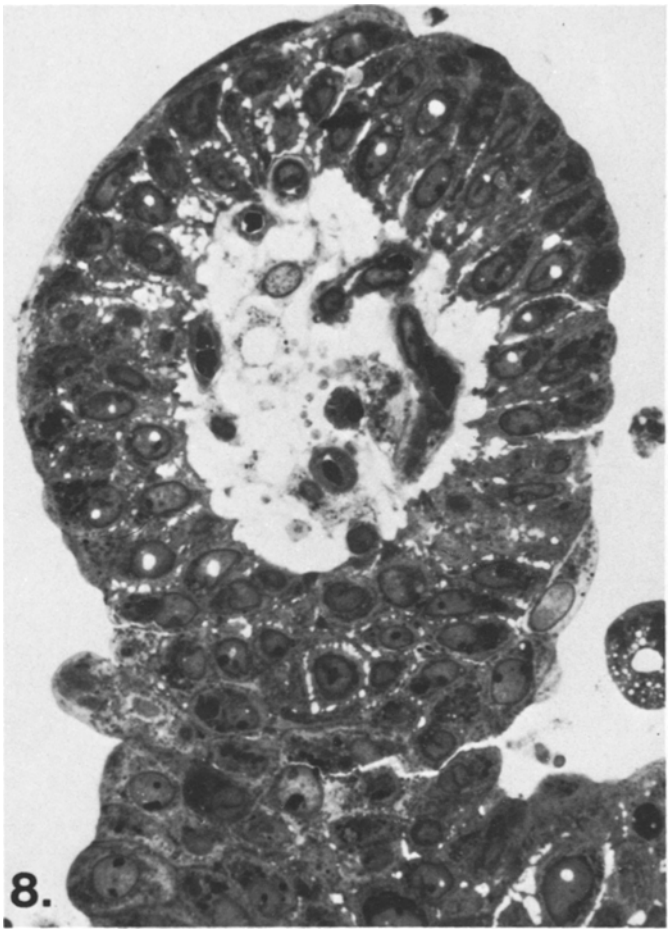

Fig. 8. Transverse section of papilla from Group 3 patient. Only a few superficial cells are present. The basement membrane is irregular but intact (× 160)

yet showed microvilli and incomplete terminal complexes they appeared to be intermediate between normal superficial cells and intermediate cells (Fig. 9). The basal and intermediate cells were similar to those already described (Smith 1981). The basal lamina was variable in thickness, wavy, rarely tortuous, and there was little reduplication. The three layers consisting of the cell membrane and the two layers of the basal lamina were often indistinct and at times there was complete absence of the basal lamina.

Quantitative Analysis

The percentage of dark cells in the superficial layer and the percentage points of abnormal intercepts of the basal lamina in the three groups of patients are seen in Table 1. Statistical analysis has shown that there are significantly more small dark cells present in the superficial layer of tissues from Groups

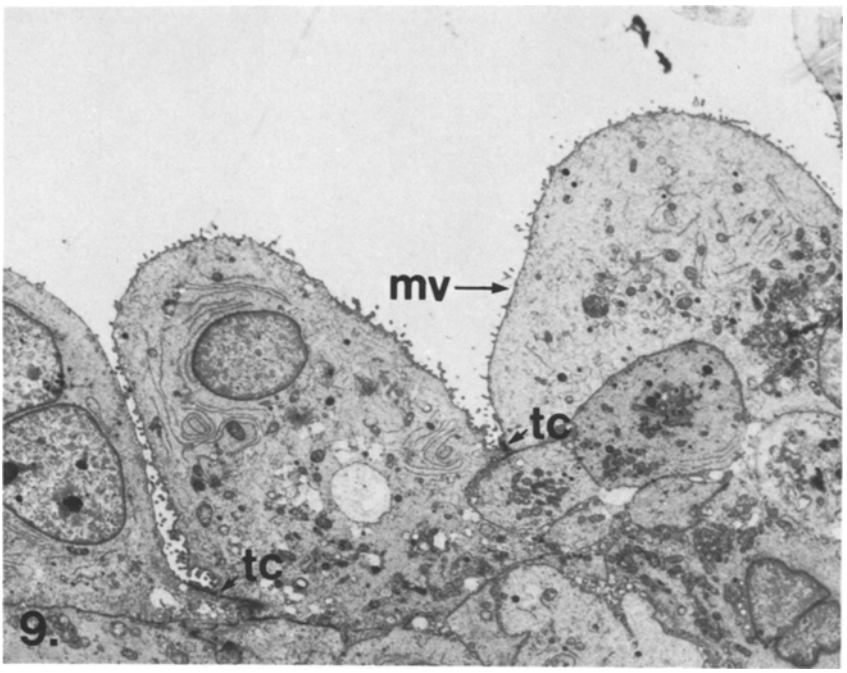

Fig. 9. Superficial cells from a papilla. Electron lucent, but the AUM is replaced by microvilli. The terminal complex shows an attenuated zonula occludens (×2,400)

Table 1. Percentage of immature superficial cells and percentage points of abnormal basal lamina in all 3 groups of patients

Patient groups	% immature cells (LM)	% points abnormal basal lamina (EM)
Group 1 Patients with no history of tumours ($N=7$)	6%	5%
Group 2 Patients with a history of tumours but normal cystoscopy ($N=8$)	14%*	13%*
Group 3 Patients with frank papillary tumours ($N=12$)	17%*	28%*

* $P<0.001$ (when compared to Group 1)

2 and 3 than from Group 1. These differences are highly significant ($P<0.001$). The difference between Group 2 and Group 3 is significant but to a lesser extent ($P<0.05$).

Similarly the frequency of abnormalities of the basal lamina showed a gradation increasing from Group 1 to Group 3 ($P<0.001$).

It was not possible to quantify the percentage of incomplete terminal junc-

Table 2. Percentage of immature superficial cells and percentage points of abnormal basal lamina in Group 1 and Group 2a and 2b patients

	% immature cells	% points abnormal basal lamina
Group 1 Patients with no history of tumours ($N=7$)	6%	5%
2a No hyperplasia ($N=3$)	14%*	15%*
2b Hyperplasia ($N=5$)	13%*	12%*

* $P<0.001$ (when compared to Group 1)

tions as the number per case was relatively small. However, an estimate indicated that the percentage of abnormalities is approximately three times as high in Group 2 patients as in Group 1, and higher still when Group 3 patients are compared with Group 1.

In Table 2 the control group (Group 1) was compared with Group 2, divided into non-hyperplastic (2a) and hyperplastic (2b) categories. The percentage of abnormalities was similar in both categories, but significantly different when compared to those in Group 1 ($P<0.001$).

Discussion

A gradation of ultrastructural changes was seen in human urothelium taken from normal bladder mucosa, "normal" areas within a tumour-forming bladder and papillary carcinomas. These changes included electron-dense cells with microvilli in the superficial layer, incomplete terminal complexes and abnormalities of the basal lamina.

The origin of the dark cells has been debated in the past; Fulker et al. (1971) assumed them to be injured or dead cells, a view not shared by other investigators. Jacobs et al. (1976); Hicks and Wakefield (1976) saw similar cells in rat urothelium, regenerating after exposure to carcinogens. These cells, sometimes bearing a mixture of AUM and microvilli, disappeared with time in reversibly injured bladders.

Similar cells have been described in a scanning electron microscopic study by Price et al. (1980) who examined normal and cystoscopically "normal" tissues, the latter taken from bladders with tumours elsewhere in the urothelium. The Type 3 cells they describe probably correspond to the dark cells in the present study, with a smaller proportion similar to the Type 2 cells. There seems to be a general agreement that the dark cells are immature superficial cells, their dense cytoplasm being due, at least in part, to PAS (Periodic Acid-Schiff) positive material in the ground substance, while fully mature umbrella cells contain only a few PAS positive granules. Similarly, the intermediate and basal cells are dense when seen with the light microscope and the TEM and also have a PAS positive ground substance. The immature nature of the dense cells is also indicated by the frequently observed abnormal zonula occludens

of the terminal complex. Such attenuated zonulae occludentes were seen when freeze-fractured junctions were examined in tissues from rat bladder bearing experimentally induced tumours and in human material from Grade I transitional cell carcinomas (Merk et al. 1977).

An explanation for the increased frequency of the dark superficial cells and their role in tumour formation can be derived from the experimental work of Hicks (1969); Koss (1977). Their view was that carcinogens in the urine damaged the Golgi apparatus of the immature cells, where the components of the AUM are assembled; the cells were thus unable to complete maturation. Lacking the protection of the AUM the urothelium becomes permeable to urine and the subsequent injury provokes a hyperplastic response. This sequence may become self-perpetuating as increased cell turnover results in yet more immature cells reaching the superficial layer and hyperplasia becomes irreversible, leading to papilla formation.

As present results show, there is an increased number of immature cells in non-hyperplastic urothelium in patients with a history of tumours (Group 2a). The frequency is similar to that seen in hyperplastic tissues (Group 2b) and is not much lower than that seen in frank papillary tumours.

The increased percentage of abnormalities in the basal lamina follows a similar pattern and has been found to be associated with tumour recurrence (Smith 1981).

It seems therefore that the tumour-prone urothelium, though cystoscopically normal and of normal thickness by light microscopy, shows a quantifiable increase in certain abnormal features, which, while not characteristic of malignant tissues only, may nevertheless be associated with premalignant change.

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References

Fulker MJ, Cooper EH, Tanaka T (1971) Proliferation and ultrastructure of papillary transitional cell carcinoma of the human bladder. Cancer, 27:71–82

Hicks RM (1969) Early changes in the fine structure of rat bladder epithelium induced by 4-ethylsulphonylnaphthalene-1-sulphonamide. Chem Biol Interact 1:49–71

Hicks RM, Wakefield JStJ (1976) Membrane changes during urothelial hyperplasia and neoplasia. Cancer Res 36:2502–07

Jacobs JB, Arai M, Cohen SM, Friedell GH (1976) Early lesions in experimental bladder cancer. Scanning electron microscopy of cell surface markers. Cancer Res 36:2512–17

Koss LG (1974) Precancerous lesions of the urothelium pp. 62–70 in Tumors of the urinary bladder. Atlas of tumor pathology. Second series fascicle 11, Armed Forces Institute of Pathology, Washington, DC

Koss LG (1977) Some ultrastructural aspects of experimental and human carcinoma of the bladder. Cancer Res 37:2824–2831

Merk FB, Pauli BU, Jacobs JB, Alroy J, Friedell GH, Weinstein RS (1977) Malignant transformation of urinary bladder in humans and in FANFT-exposed Fischer rats. Ultrastructure of the major components of the permeability barrier. Cancer Res 37:2843–2853

Price DA. Morley R, Hall RR (1980) Scanning electron microscopy in the study of normal, inflamed and neoplastic human urothelium. Br J Urol 52:370–376

Smith A (1981) An ultrastructural and morphometric study of bladder tumours (I). Virchows Arch [Pathol Anat] 390:11–21

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