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An Ultrastructural and Morphometric Study of Bladder Tumours (I)

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Summary. The ultrastructure of urothelium from 4 patients with no evidence of tumour was compared with that taken from 22 patients with bladder neoplasms of different grade and stage. Two features were quantified: the percentage of reduplication of the basal lamina and the percentage of discontinuous basal lamina.

Tumours showed a lower percentage of reduplicated basal laminae than normal tissues, the difference being significant in 3 out of 4 types of tumours. The difference in frequency between normal tissues and non-recurring tumours was not significant, but there was a significant difference between normals and recurring tumours.

All types of tumours showed discontinuities in the basal lamina, including 80% of those staged non-invasive by light microscopy. None of the normal tissues showed these. The percentage of discontinuities seen in non-recurrent tumours was half that seen in recurrent ones, but both groups were significantly higher than normals.

Loss of continuity of the basement membrane distinguishes invasive from non-invasive tumours. However, there is a high probability of these being missed by light microscopy alone. Therefore, electron microscopic studies on recurrent bladder tumours would increase the accuracy of staging and prognosis.

Key words: Electron microscopy – Bladder neoplasms – Measurement.

Introduction

The successful treatment of the patient with bladder cancer depends on accurate staging of the tumour, but the earliest histological evidence of invasion may be difficult to determine with the light microscope. The present study has been

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undertaken to see if there are any ultrastructural characteristics which might increase the accuracy of pathological staging.

Materials and Methods

The normal tissues were taken from 4 patients. Two of these had non-malignant prostatic disease and the bladder was biopsied at the time of perurethral resection. The remaining two were female kidney transplant donors, from whom ureteric tissue was taken.

Tumours of the bladder constitute a clinico-pathological entity, and may affect any part of the transitional epithelial lining of the urinary tract (Pugh 1973). Ureters and bladder also have the same ultrastructure (Hicks 1965, 1975).

Tumour tissue from 22 patients was available for study; 10 were papillary non-invasive tumours, 2 flat in situ, 7 differentiated invasive, 3 were intermediate papillary invasive (Pugh 1973). In 13 patients the biopsy was taken at the initial pre-treatment cystoscopy; the rest had had recurrent tumours diathermised over periods ranging from 3 months to 10 years before entering this study.

The original electron microscopic findings were reassessed 18 months after the completion of the study, so that the morphology of the tissues could be correlated with the subsequent behaviour of the tumours.

There were 2 patients who died 10 and 12 months respectively after the biopsy reported on in the present study without any further biopsies being done. Of the remaining 20, 7 patients had no recurrence of the tumour; in 8 others the recurrence was of the same stage and grade, while in 5 the tumour had become more invasive or of a higher grade.

Due to the deaths of the 2 patients the total numbers in the tables are not the same as those in the recurrence categories. Tissue from one additional patient was inadequate for quantification.

Tissue from ureters was cut into 1 mm blocks. Bladder tissue was examined under the dissecting microscope and 1 mm cubes of epithelium removed using a scalpel. The blocks were placed in buffered osmium tetroxide, dehydrated using a series of graded alcohols and embedded in TAAB resin (TAAB Laboratories, Reading). Thin sections were stained with uranyl acetate and lead citrate and examined in an AEI 6 M electron microscope.

Frei's method (1978) was used to measure discontinuities and reduplication of the basal lamina. Sections were photographed at a magnification of 2,500 and 6–12 enlarged photographs were taken from each specimen. A transparent sheet with parallel lines one cm apart was laid over the photograph so that the lines were perpendicular to the basal lamina. At each intercept the basal lamina was characterised as "normal", "reduplicated" or "absent". 130–300 observations were made in each tumour category, as in Table 1.

Results

In each specimen a number of features was examined. The basal lamina; the nuclear shape, chromatin distribution, nucleoli, and the morphology and distribution of cytoplasmic organelles. Luminal and lateral specialisations are being studied and will be dealt with in a subsequent paper.

Table 1 and Figs. 1–3 b summarise and illustrate the morphological features.

Two ultrastructural features – the percentage of reduplication and of discontinuity of the basal lamina were quantified in order to assess more accurately differences between normal urothelium and various stages and grades of bladder tumours.

The percentage reduplication of the basal lamina is higher in the normals than in any of the tumours. The difference is significant for all but papillary intermediate tumours, and highly significant when normal tissues are compared to differentiated invasive tumours (Table 2).

Table 1. Ultrastructural features associated with normal urothelium and bladder tumours (number of cases in brackets)

Type of tissue		Nuclei	Chromatin	Nucleoli	Nuclear pores	Mitochondria	Endoplasmic reticulum	Tonofibrils	Autophagic vacuoles	Dark cells	Lateral microvilli
Normal (4)		Oval invaginated	Marginated	Single	Not prominent	Large light	In short stretches	Absent	Present	Absent	Absent
Carcinoma	Papillary differentiated non-invasive (10)	Oval invaginated	Dispersed	Large, a few multiple	Prominent in 50%	Mostly large light	In short stretches rarely in long filaments	In one case	Rare	In a few cases	In 22%
	Flat in situ (2)	Oval invaginated	Mainly dispersed	Rare multiple	Not prominent	Mostly large light	In short stretches	Absent	Rare	Absent	In 50%
	Papillary Differentiated invasive (7)	Similar to papillary non-invasive				Numerous, some small dark	"as in situ"	In one case	Rare	Frequent	In 90%
	Papillary Intermediate invasive (3)	Deeply invaginated	Dispersed	Many multiple	Prominent in 50%	Reduced in number	Often in long filaments and whorls	Absent	Very rare	Rare	Absent

There were no discontinuities in the basal lamina of any normal tissues, but they were seen in all the tumours. The differences were all significant, and highly so when differentiated invasive and flat in situ ones were compared with the normal tissues (Table 2).

The percentage loss of continuity in the basal lamina is significantly higher than normal in all recurring tumours, and also in the non-recurring differentiated invasive ones.

Table 3 shows the relationship of all non-recurring and recurring tumours to the normal tissues in terms of reduplication and discontinuity in the basal lamina. The average percentage of reduplication seen in non-recurring tumours is not significantly different from the normal tissue, while there is a significant difference between the normal and the recurring tumours. The difference in the average number of discontinuities in the basal lamina between normal tissue and non-recurring tumours is significant with both types of tumours, but more highly significant in the case of recurring ones.

Several of the ultrastructural features did not lend themselves to reliable quantification. However, correlation coefficients could be calculated which relat-

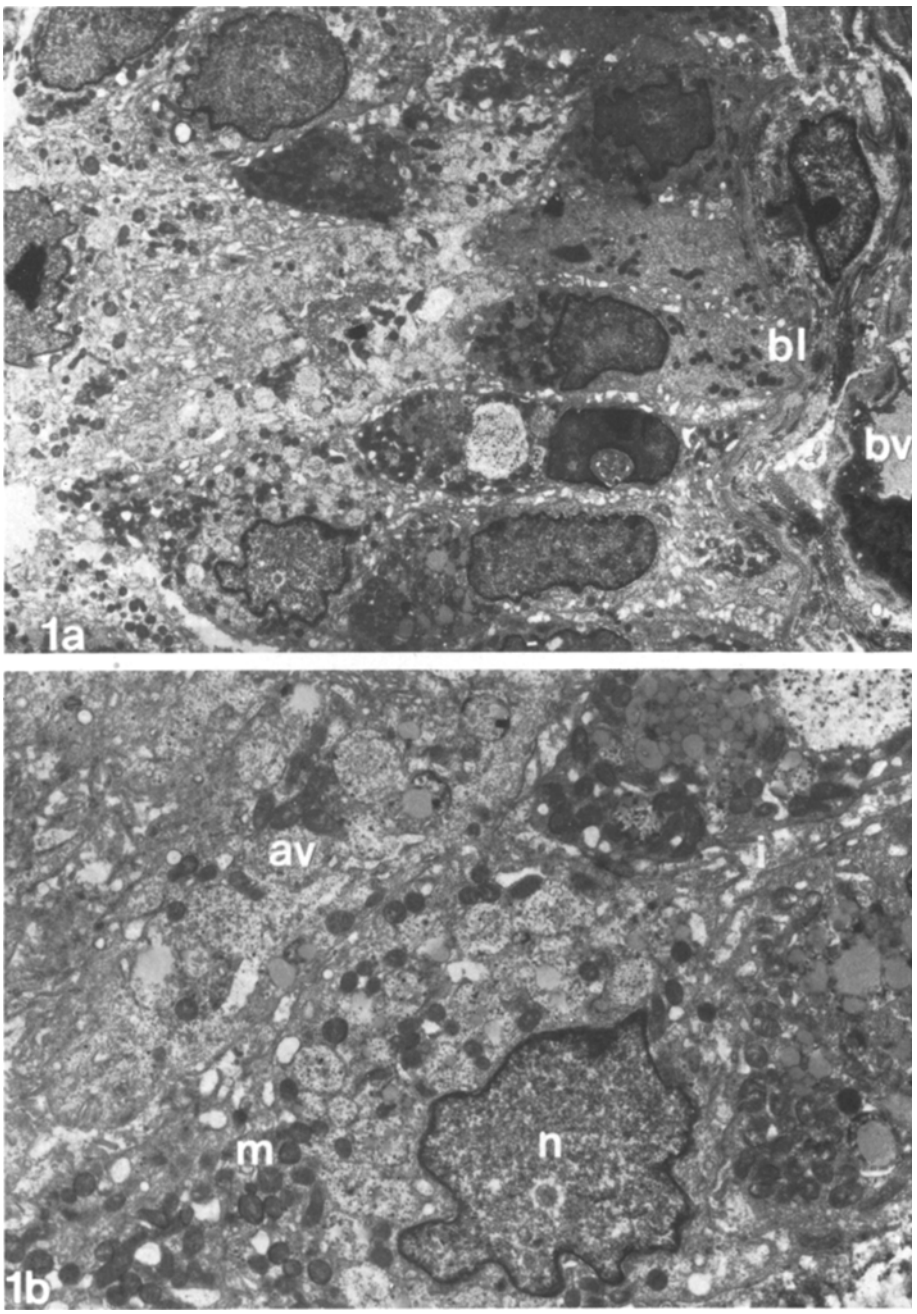

Fig. 1. a "Normal" epithelium, intermediate and basal cells. Slightly invaginated nuclei, marginated chromatin. Autophagic vacuoles are occasionally seen, mitochondria have variable densities. Lateral interdigitations and a continuous basal lamina are present. ×4,000. b Details as above. ×10,000. *n* nucleus, *av* autophagic vacuole, *m* mitochondria, *i* interdigitations, *bl* basal lamina, *bv* blood vessel

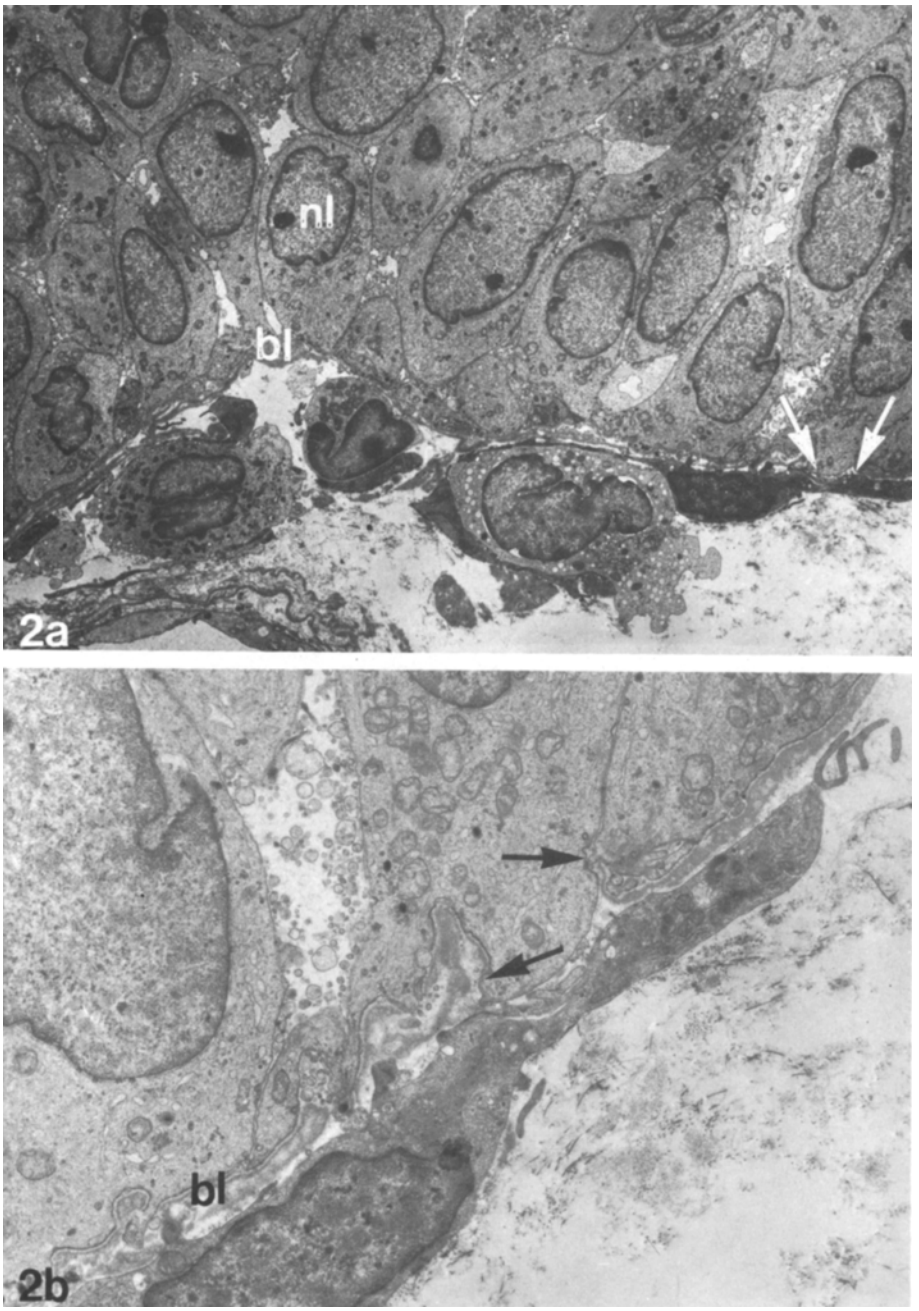

Fig. 2. a Tumour classified P1S differentiated non-invasive, intermediate and basal cells. Nuclei similar to normal in this section, with prominent sometimes multiple nucleoli. Rare autophagic vacuoles are seen. Lateral membranes usually interdigitate. Though classified as non-invasive by light microscopy, areas of discontinuous basal lamina are present. ×3,000. *nl* nucleolus, arrows on either side of discontinuity. b Detail showing epithelial cell cytoplasm protruding into the stroma where basal lamina is absent. ×9,000

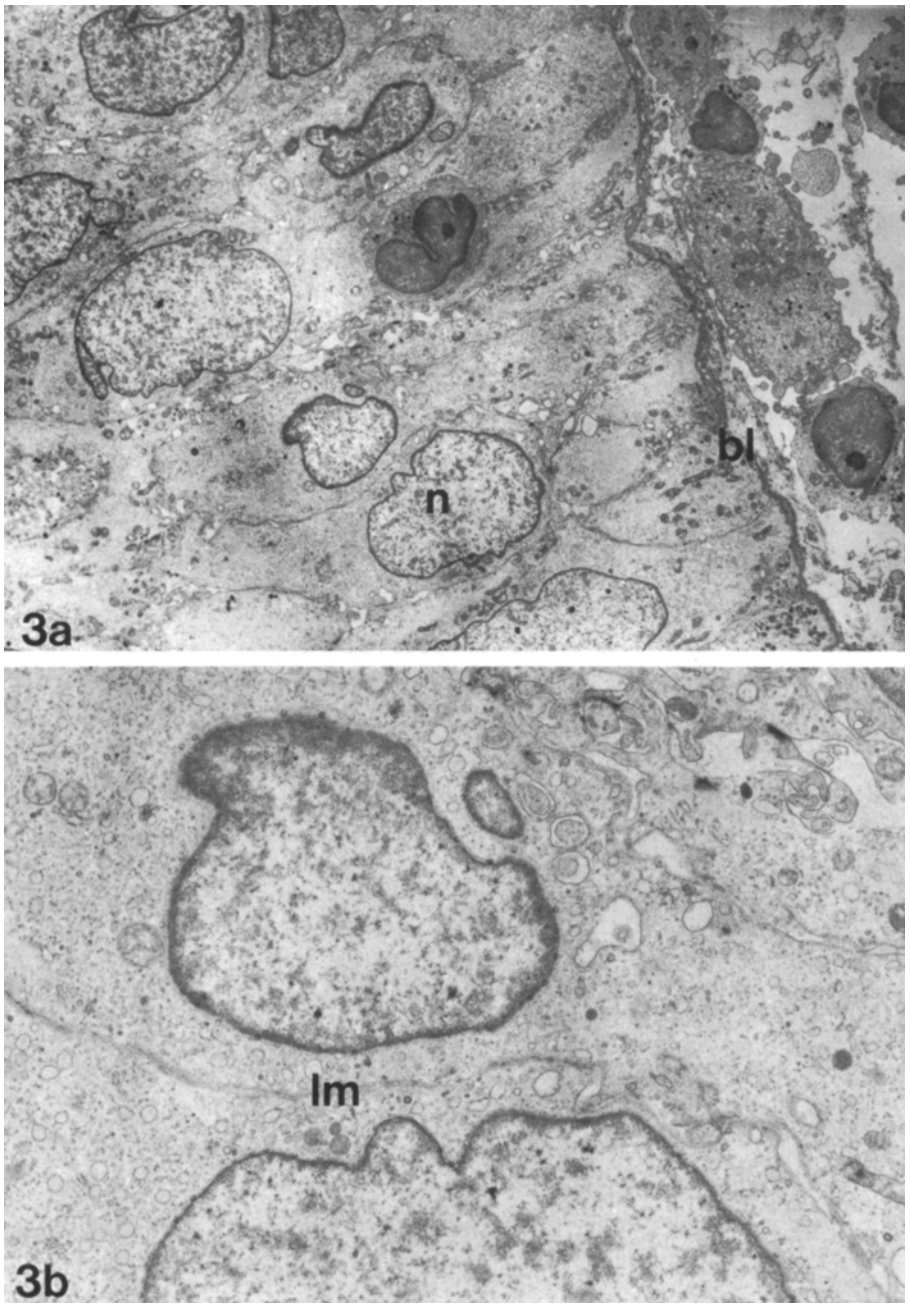

Fig. 3. **a** Tumour classified P1a intermediate invasive, intermediate and basal cells. Nuclei with deep invagination or blebs, dispersed chromatin. There are few cell organelles present, and no autophagic vacuoles are seen. Lateral membranes are parallel. The basal lamina is continuous in this section. ×2,000. *lm* lateral membranes. **b** Detail from above. ×9,000

Table 2. Percentage of reduplication and discontinuities of the basal lamina related to stage, grade and recurrence of tumour (Number of cases in brackets)

Stage and Grade	Reduplication				Discontinuity			
	Individual means	Weighted means			Individual means	Weighted Means		
		All cases	Non-recurrent	Recurrent		All cases	Non-recurrent	Recurrent
Normal (4)	35 30 59 32	39% (4)	–	–	0 0 0 0	0% (4)	–	–
Papillary Differentiated Non-invasive (10)	34 14 22 30 38 0 5 2 0 35	19%[b] (10)	26% (4)	16%[a] (6)	3 11 3 10 24 0 10 1 0 0	7%[a] (10)	5% (4)	8%[b] (6)
Papillary Differentiated Invasive (6)	22 11 39 13 9 3	16%[c] (6)	22%[a] (1)	15%[c] (5)	11 43 8 5 0 14	12%[c] (6)	11%[c] (1)	12%[c] (5)
Papillary Intermediate Invasive (3)	52 31 20	27% (3)	52% (1)	31% (1)	3 12 15	8%[b] (3)	3% (1)	12%[c] (1)
Flat in situ (2)	5 50	19%[b] (2)	– (0)	50% (1)	15 9	13%[c] (2)	– (0)	9%[b] (1)

Point counting according to the method of Frei (1978) x^2 significantly different from normal

[a] $P<0.025$; [b] $P<0.005$; [c] $P<0.001$

ed their presence or absence in the tissues to recurrence, stage and grade. Though none of these reached significance, the highest correlations were between recurrence and lateral gaps with microvilli; recurrence and the presence of electron-dense cells; and multiple nucleoli and intermediate grade tumours. In the latter group of 3 cases, 80% contained cells with multiple nucleoli, compared to 62% in differentiated invasive tumours and only 37% in non-invasive tumours.

Of the 12 tumours staged papillary non-invasive or flat in situ carcinoma with the light microscope, 10 showed submicroscopic discontinuities in the basal lamina. The possible significance of this finding will be referred to in the discussion.

Table 3. Percentage of reduplication and discontinuities of the basal lamina. Comparison of tissues from "normal" urothelium with those from recurring and non-recurring tumours

Patient Groups	Reduplication		Discontinuity	
	Individual mean	Weighted mean	Individual mean	Weighted mean
Normal (4)	35		0	
	30	39%	0	0%
	59		0	
	32		0	
No further tumours within 18/12 (6)	34		3	
	14		11	
	22	28%	2	5%[a]
	35		0	
	52		3	
	22		11	
Recurrent tumours within 18/12 (13)	30		10	
	38		24	
	0		0	
	5		10	
	2		1	
	0		0	
	31	16%[b]	12	11%[b]
	5		15	
	11		43	
	39		8	
	13		5	
	9		0	
	3		14	

x^2 significantly different from normal
[a] $P<0.025$ [b] $P<0.001$

Discussion

The ultrastructure of normal human urothelium has been described by Battifora et al. (1964), Fulker et al. (1971), Cooper (1972), Haynes et al. (1975), Hicks (1975). Our observations substantially agree with theirs.

The findings summarised in Table 1 confirm those made by others in human urothelial tumours (Battifora et al. 1965; Fulker et al. 1971; Cooper 1972; Koss 1977). There have also been numerous studies of ultrastructural changes in experimentally induced bladder neoplasms in animals where it was possible to study the progression of the tumours under standard conditions (reviewed by Koss 1977).

Reduplication of the basal lamina as a sign of healthy cell turnover is borne out to some extent by the present findings. The average percentage is lower in all tumours than in normal tissues, though only 2 of these reach

significance. Gould et al. (1972) in their study of papillary and sclerosing thyroid carcinomas comment on the fact that multiple basal laminae usually characterise tumours of low malignancy. Their view is that the basal lamina plays a role in orderly growth and may be indicative of some feature of cell machinery which restricts growth. Though the findings of the present study do not bear out their assertion that multiple basal laminae might be predictors of low level malignancy, the results show that widespread reduplication correlates highly with non-recurrence of the tumour, at least during the period under study.

Discontinuities in the basal lamina were observed in all types of tumours, including those staged in situ light microscopically. Though the percentages varied, all were significantly different from the normal.

It is now well established that the basal lamina is a product of the epithelial cell, but the reasons for its frequently observed absence in tumours are still unclear. It has been shown that most carcinomas are capable of producing basal laminae, but some carcinomas are characterised by patchy deposition of the basal lamina, while it seems to be completely absent in others (Gould and Battifora 1976). These authors comment that its absence may indicate a degree of cell dedifferentiation, while others have speculated that it is disrupted enzymatically or mechanically (Fasske 1966; Tarin 1967; Cohen et al. 1971 and others). Frithiof (1969) has suggested that the variations seen in the basal laminae of malignant tissues might be related to the variability of their immunologic status.

Whatever the reasons for the patchy absence of the basal lamina, it is in the areas free of it that small portions of the epithelial bytoplasm protrude into the underlying stroma. Once the epithelium is exposed to the lamina propria contact between these two tissues is often followed by rapid growth and proliferation of the epithelial cell population. This has been shown in regenerating epithelium (Saltpeter and Singer 1960) and in embryonic development (Mathan et al. 1972). It seems equally true of urothelial tumours and it is possible that this micro-invasion is the first morphological sign of tumour formation, as has been suggested by Sugar (1968) who observed this phenomenon not only in early tumours but in precancers of the larynx and of skin tumours.

Table 3 shows a highly significant relationship between frequency of absence of the basal lamina and recurrence of the tumours during the following 18 months, and the present findings of discontinuities in 80% of tumours staged papillary non-invasive or flat in situ thus take on added significance. These results are similar to those of Frithiof (1969) who studied tumours of the oral epithelium on an ultrastructural level. He observed disruptions in 85% staged in situ with the light microscope. In an electron microscopic study Tannenbaum et al. (1970) found that in 50% of human bladder tumours staged A or B (pathologic stage P1, P2 or P3) there were clusters of tumour cells present which had penetrated 1–2 stages deeper than the light microscopic classification had indicated. Figs. 4a, b illustrate a similar finding.

The breaching of the light microscopic basement membrane is the criterion which differentiates non-invasive tumours from invasive ones. As the results of the present study show, there is a very great probability ($P<0.001$) that a urothelial tumour will be "misstaged" by light microscopy alone and as

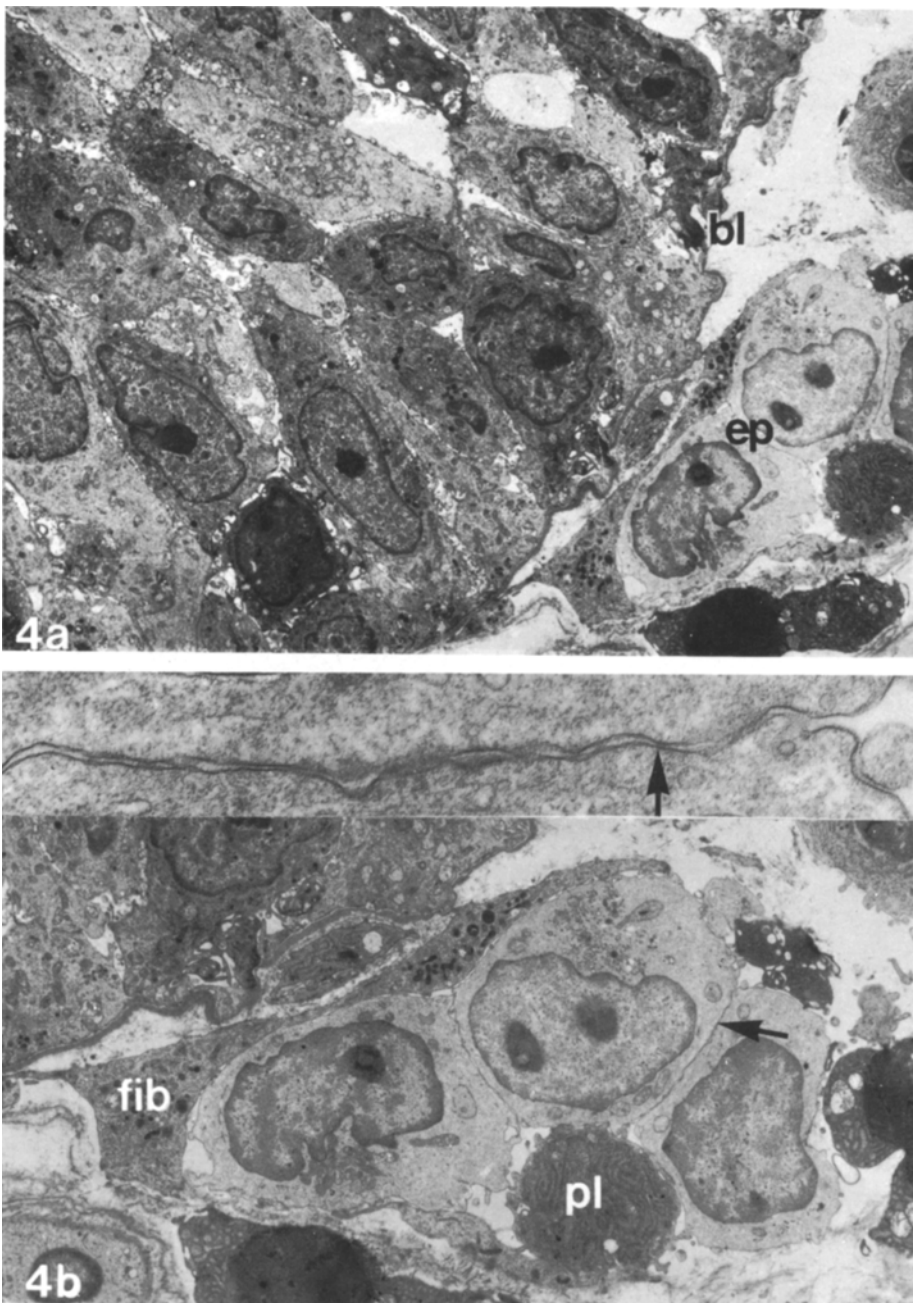

Fig. 4. a From the same tumour as in Fig. 2. Though the basal lamina is uninterrupted in this section, there are cells resembling epithelial cells in the stroma. (c.f., cells in the epithelial layer). ×3,000. b Detail from above. Three epithelial-like cells, a plasma cell and portions of a fibroblast can be seen in the stroma. ×4,000. *ep* epithelial-like cells, *fib* fibroblast, *pl* plasma cell, *arrow* desmosome. *Inset:* Desmosome-like thickening can be seen between adjacent cell membranes. Desmosome formation is a characteristic of epithelial cells. ×38,000

we have shown, a highly significant relationship exists between the frequency of breaches and recurrence. Therefore, ultrastructural examination of early tumours might increase both the accuracy of the staging and the prognosis.

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