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An ultrastructural and morphometric study of bladder tumours (III)

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Summary. Quadrant biopsies were taken at cystoscopy from 12 male patients previously diagnosed on light microscopy as having flat carcinoma in situ (CIS) of the urinary bladder. There was also material available from 3 cystectomy specimens with widespread CIS associated with papillary or solid urothelial tumours.

Sections of normal ureter from kidney transplant donors and biopsies from two patients investigated for non-malignant bladder conditions servered as controls.

The biopsies from 4 patients were classified as mild dysplasia of the urothelium, while those from 11 patients were categorised as CIS.

Biopsies categorised as mild dysplasia on light microscopy showed an increase in the number of cells with large nuclei and nucleoli when compared to controls. The number of desmosomes was significantly reduced compared to controls, while the frequency of abnormalities of the basal lamina was increased. These features were more pronounced in the CIS group.

Biopsies from the CIS group could be divided into "classical" and "large cell" CIS, the latter showing a higher frequency of ultrastructural abnormalities than the "classical" type.

The patients diagnosed as having CIS fell into two clinical categories, the "early onset" and the "late onset" group. The five patients in the former had been diagnosed as having CIS with or without urothelial tumours elsewhere within 3 months of presentation. In the remaining four patients CIS was observed after recurring episodes of papillary or solid tumours during the previous 9 months to 20 years.

The biopsies of 3 out of 5 patients with early onset CIS had been classified "large cell" CIS, wheras only one patient out of 4 in the late onset group came into this category. An early appearance of CIS is thought to have a worse prognosis, and it is therefore suggested that "large cell" CIS is a more severe form of the disease.

Key words: Electron microscopy – Bladder neoplasms – Measurement – CIS

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Introduction

Carcinoma in situ of the urinary bladder is a disorder with uncertain prognostic features. There is little or no hyperplasia, yet the cells have an appearance characteristic of high grade tumours. Classification of severe dysplasia tends to be arbitrary. The clinical outcome is also uncertain, some patients developing muscle invasion or metastatic disease relatively early in the course of the disease.

The present study was carried out to assess any features, both qualitative and quantitative, which might distinguish between mild dysplasia and CIS and attempt to correlate the frequency of abnormal morphological characteristics with the eventual outcome of the disease.

Materials and methods

Biopsies were taken at cystoscopy from 12 male patients. Their age range was 44–80 (mean 65.5 years). In 6 instances CIS had been diagnosed, with or without papillary tumours elsewhere in the bladder within 3 months of presentation. In the remaining 6 CIS was only observed following a history of recurring papillary urothelial tumours of 9 months to 20 years. In addition, macroscopically normal samples were taken after cystectomy from 3 bladders with extensive CIS as well as papillary and/or invasive tumours. Most patients had had multiple biopsies and a total of 30 was available for examination.

Sections of normal ureter from 6 kidney donors or transplants served as controls. There were also 2 bladder biopsies from patients with non-malignant urothelial disease (small capacity bladder, exercise related haematuria), which were also included in the control group.

A portion of each biopsy was fixed in formalin for subsequent paraffin embedding, and stained with haematoxylin and eosin. The rest was fixed in glutaraldehyde, postfixed in OsO_4 and after dehydration embedded in Emix (Emscope Laboratories, Ashford, Kent). 1 μm sections were stained with toluidine blue for light microscopy. 70 nm sections were stained with uranyl acetate and lead citrate and examined in a Zeiss 109 transmission electron microscope.

Morphometric analysis was performed on 20.3 × 25.4 cm photographic prints using a MOP semi-automated image analyser (Reichert-Jung UK, Slough).

Results

A. Light microscopy (H and E)

Paraffin-embedded sections were submitted to a histopathologist for classification. 9 of the biosies were categorised as "mild dysplasia", the rest as "moderate to severe dysplasia" or "carcinoma in situ" (CIS).

B. Light microscopy (toluidine blue)

1. Controls. There were 8 biopsies in the group. The epithelium lining the ureters was 4–6 layers thick. There was normal polarity and differentiation, the basal and intermediate cells were cuboidal or low columnar with oval, dark nuclei and a single nucleolus. The superficial cells were typical umbrella cells, large and light, and parallel to the basement membrane (Fig. 1).

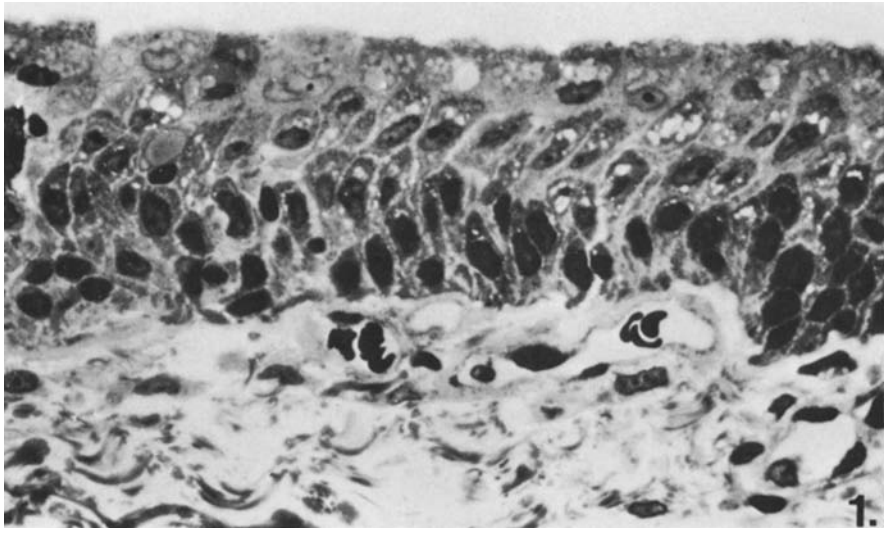

Fig. 1. Normal urothelium – orderly polarity; fully differentiated large superficial cells lie parallel to the basement membrane. Basal and intermediate cells are perpendicular to the basement membrane, smaller, with dark nuclei. ×160. Toluidine blue

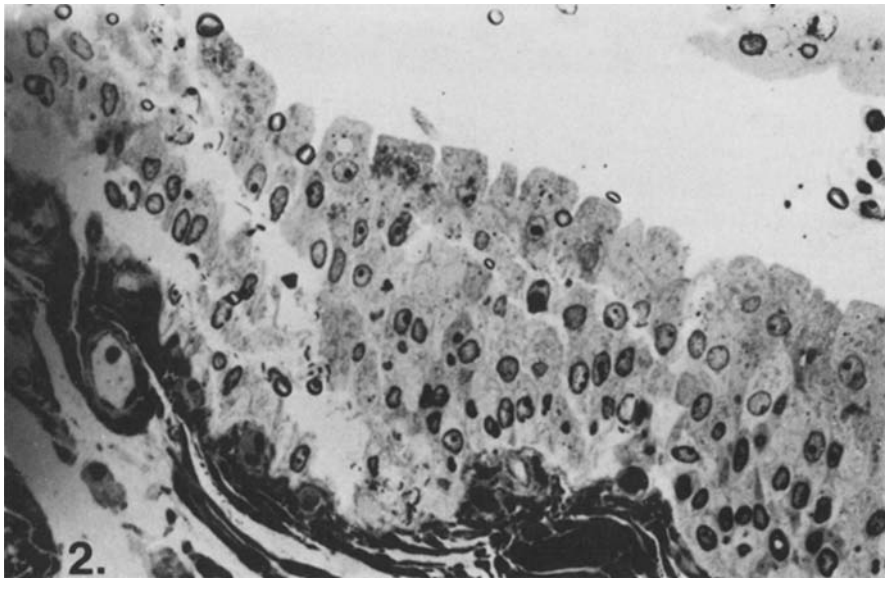

Fig. 2. Mildly dysplastic urothelium – slightly disordered; the superficial cells are smaller. Basal and intermediate cells vary in size and orientation to the basement membrane, their nuclei are heterogeneous in size and staining properties. ×160. Toluidine blue

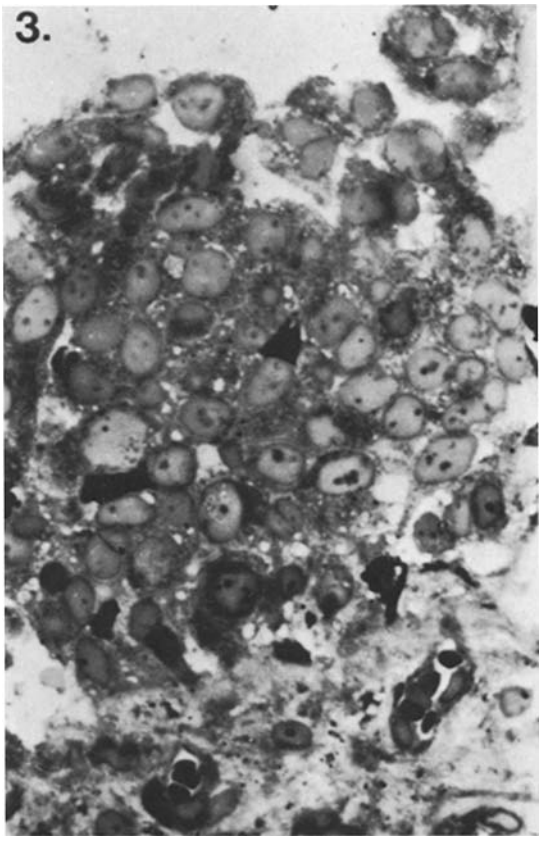

Fig. 3. Severely dysplastic/CIS urothelium – total loss of polarity; no superficial cells seen, basal and intermediate cells are varied in size with mainly large, light nuclei and prominent, often multiple nucleoli. × 160. Toluidine blue

2. Mild dysplasia. The were 9 biopsies in this category. The epithelium was 4–9 layers thick. 3 biopsies showed some loss of polarity. There was a marked increase in the number of cells with large nuclei and prominent nucleoli. There was evidence of normal differentiation, and some superficial cells were seen in all the biopsies (Fig. 2).

3. Severe dysplasia/CIS. 21 biopsies had been allocated to this category. The epithelium was 4–7 layers thick, though there were a few hyperplastic areas with 10–20 cell layers. Varying degrees of disordered polarity were seen in all biopsies and little evidence of differentiation, with no superficial cells. There was heterogeneity of nuclear size and shape at all levels of the epithelium. 12 of the biopsies had a preponderance of cells with very large, light nuclei and almost no normal basal cells.

Multiple nucleoli and occasional mitotic figures were seen. In 4 biopsies there were isolated patches of cells which appeared to have undergone partial differentiation. These islands of more normal cells were from 1–10 cells wide (Fig. 3).

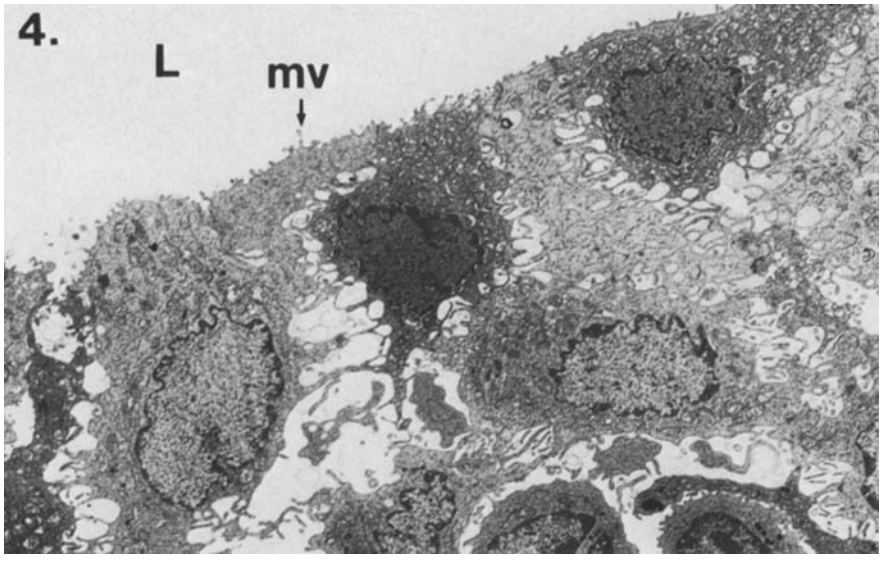

Fig. 4. Mild dysplasia – incompletely differentiated superficial cells with microvilli. ×4,400; *L*, Lumen; *MV*, Microvilli

C. Transmission electron microscopy

1. Controls. These have been described in a previous paper (Smith, 1982). An additional observation was the presence of occasional interchromatic nuclear granules, electron-dense particles 15–20 nm in diameter; large nucleoli were occasionally seen.

2. Mild dysplasia. The basal and intermediate cells resembled the normal controls. Some umbrella cells were also seen, though most of them had a microvillous surface and the zonulae occludentes were attenuated. There were few desmosomes seen. The basal lamina was often reduplicated, usually wavy or tortuous, with few apparent defects (Fig. 4).

3. Severe dysplasia/CIS. The basal and intermediate cells showed varying morphology. In some biopsies oval nuclei and marginated chromatin were present, the nuclei often deeply invaginated and of irregular shape. In others the nuclei were large and round with dispersed chromatin and large or multiple nucleoli which were occasionally marginated (Fig. 5). Sometimes the nuclei had large protrusions or blebs (Fig. 6). Inter and parachromatic granules were frequent. No umbrella cells were seen. The desmosomes were scarce, though sometimes there were prominent hemidesmosomes. The basal lamina was thin, with little reduplication and usually straight or wavy. Sometimes it was frayed or broken, with an occasional cell apparently penetrating the lamina propria (Fig. 7).

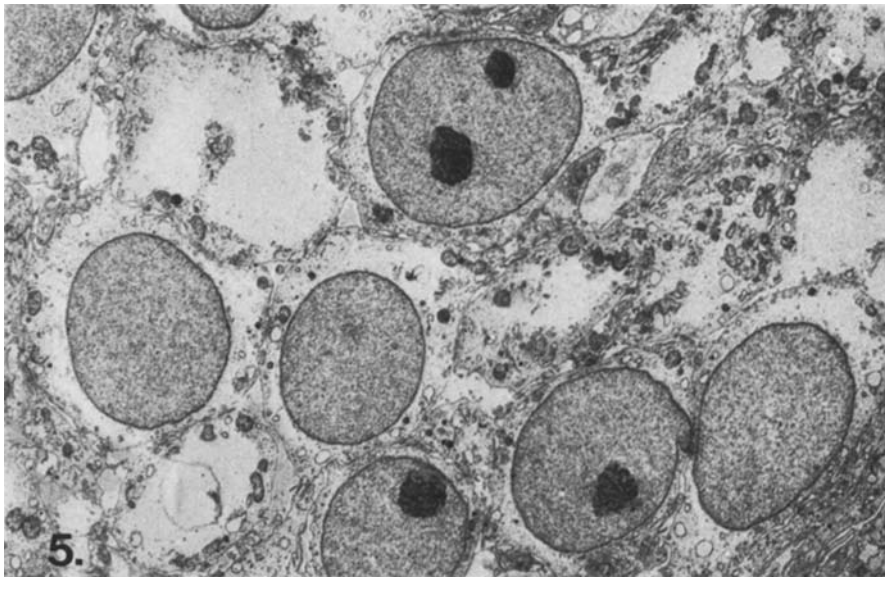

Fig. 5. Severe dysplasia/CIS – cellular detail. Very large nuclei with dispersed chromatin and prominent or multiple nuclei. ×4,100

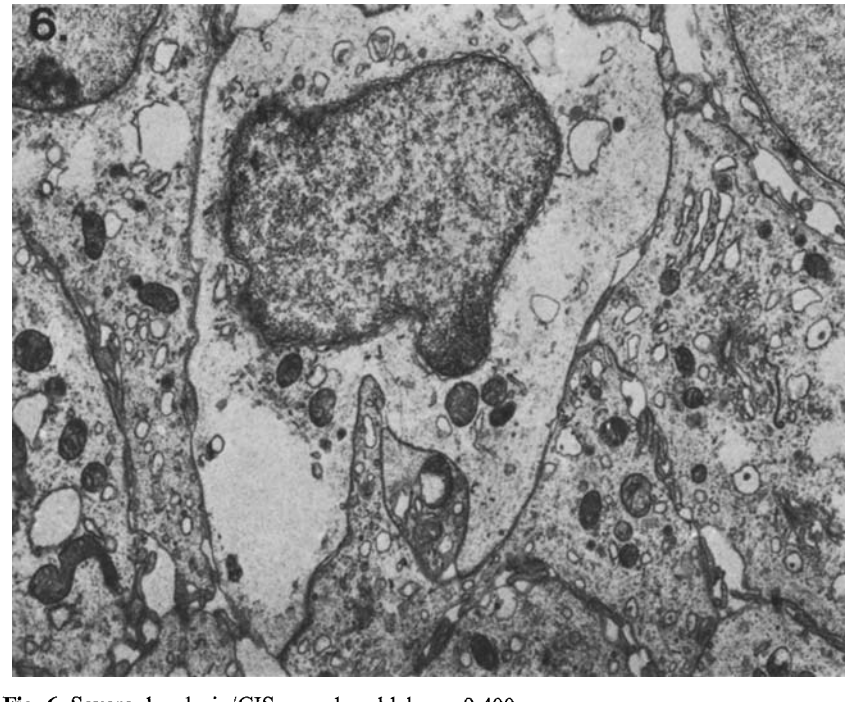

Fig. 6. Severe dysplasia/CIS – nuclear blebs. ×9,400

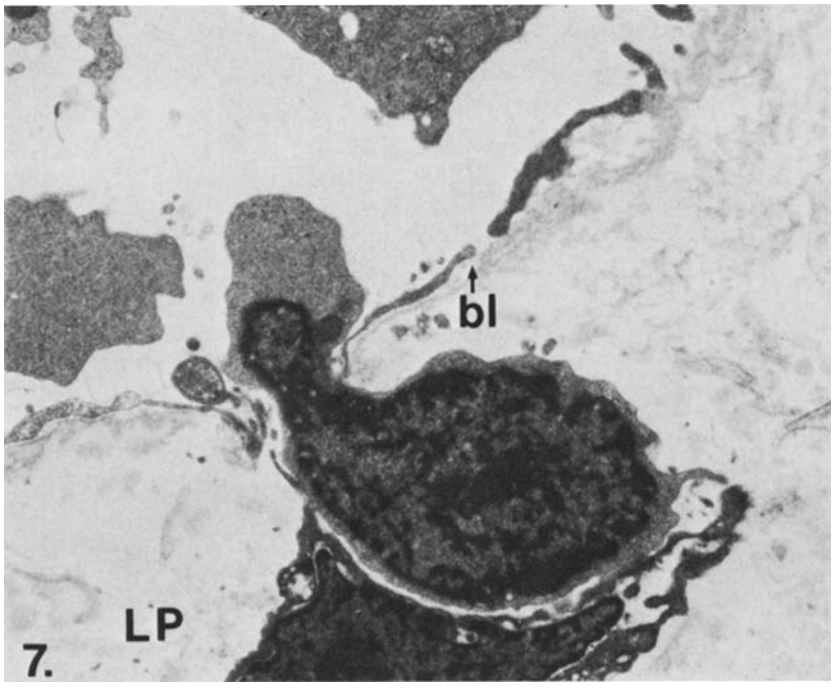

Fig. 7. Severe dysplasia/CIS – urothelial cell apparently penetrating into the lamina propria. ×10,100. *BL*, Basal lamina; *LP*, Lamina Propria

Table 1. Comparison of abnormalities between normal controls and dysplasia/CIS patients

Patient group	No. of biopsies	% biopsies with loss of polarity	Abnormal cell/total cell no. (%)	Abnormal b.l./total basal lamina (%)	Number of desmosomes/ 100 μm
Controls $N=8$	8	0/8 (0%)	25/270 (9%)	176/2365 (7%)	1.77
Patients $N=15$	30	18/30 (60%)	1350/3036 (44%)	1637/8565 (19%)	0.84

D. Morphometry

Three features were quantified:

(1) Percentage of abnormal cells with large, round nuclei and dispersed chromatin as seen in 1 μm sections stained with toluidine blue.

(2) Abnormalities of the basal lamina, such as discontinuities, absence of the lamina lucida or of the basal limiting membrane of the overlying cells, expressed in percentage of total length.

(3) Number of desmosomes per 100 μm cell perimeter. See Tables 1–3.

Table 2. Comparison of abnormalities between biopsies staged mild dysplasia or CIS

Stage	No. of biopsies	Abnormal cell/ total cell no. (%)	Abnormal b.l./ total basal lamina (%)	Number of desmosomes/ 100 μm
Mild dysplasia $N=4$	9	106/641 (16%)	234/2341 (10%)	0.83
Severe dysplasia/CIS $N=11$	21	1244/2395 (52%)	1403/6227 (22%)	0.84

Table 3. Comparison of biopsies showing thin or reduplicated basal laminae

Type of basal lamina	No. of biopsies	% biopsies with disordered layers	% abnormal cells	% abnormal basal lamina	No. desmosomes/ 100 μm
Thin	12	83%	57%	22%*	0.58
Reduplicated	16	38%	33%	17%*	0.60

* $P<0.001$

Discussion

CIS of the urothelium is a challenging phenomenon. There is little hyperplasia and no invasion of the lamina propria, yet the cells themselves resemble those seen in high-grade invasive tumours, with loss of polarity, a high nuclear:cytoplasmic ratio, bizarre nuclear blebs and large or multiple nucleoli.

While cellular morphology is highly abnormal, this not always true of the cellular enzymes which have been studied, for example LDH isozymes (Bredin et al 1975). It was shown that LDH isozymic ratios of urothelial CIS resemble those of normal or atypical urothelium. An immuno-cytochemical similarity between CIS and non-invasive tumours is shown by the distribution of epithelial membrane antigen (EMA) which is similar in these two conditions (Pocock et al, 1983).

Many of the cellular abnormalities seen in biopsies from patients in this series, classified on light microscopy as CIS or dysplasia, have been observed in other tumours. Ghadially (1978) has described a number of these. They include:

a) Incomplete differentiation as typified by the microvillous surface also seen with the scanning electron microscope by Price et al. (1980).

b) Nuclear protrusions or blebs, also described by Koss (1979) and associated with large abnormal chromosomes in CIS of the cervix and other tumours (Atkins and Baker 1979).

c) Inter and parachromatic granules, usually seen only in tumours.

d) Reduction in the frequency of desmosomes similar to that seen by Alroy et al. (1983), which may affect cellular cohesion and cause shedding of a large number of cells into the urine, a common cytological finding in urothelial CIS (Koss 1979).

Table 4. Comparison of "classical" and "large cell" carcinoma in situ (see text)

Type of biopsy	No. of biopsies	Nuclear blebs	No. of desmosomes/ 100 μm	% large cells	% abnormal basal lamina
"Classical" in situ ($N=5$)	10	0/10	1.1	17%	19%*
"Large cell" in situ ($N=4$)	7	5/7	0.75	89%	30%*

* $P<0.001$

e) A significant increase in abnormalities of the basal lamina, shown also by Kakizoe et al. (1983) who associated them with microinvasion.

f) A significant reduction in reduplication of the basal lamina. Reduplication of the basal lamina is characteristic of normal tissues and tumours of low malignancy (Gould et al. 1972). The view that reduplication is indicative of a cellular mechanism which controls orderly growth is confirmed in the present study, where only 38% of the biopsies with reduplicated basal lamina showed disordered polarity, compared to 83% with a single-layered, basal lamina (Table 3). Frequencies of other abnormalities followed a similar pattern.

The frequency of abnormalities in the biopsies classified as CIS is significantly greater than in those classified as mild dysplasia (Table 2), indicating that the histopathological classification has a quantitative as well as a qualitative basis.

Examination of the toluidine blue sections showed marked differences in the biopsies from the group classified as CIS. Some biopsies consisted of cells with predominantly large, light-staining nuclei in the basal and intermediate layer, in others the majority of the cells was of more normal appearance.

Koss (1975) has distinguished between these two types of intraurothelial carcinoma, calling the former "large cell" and the latter "classical" CIS.

The high number of large, light-staining cells is characteristic of metabolically active tissues (Ghadially 1978) and as urothelial cell turnover (normally very low) is greatly increased in these tumours (Dlhoš et al. 1979), their increased number is probably an expression of heightened cellular activity. However, these cells, with primarily euchromatin-bearing nuclei, showed a number of abnormal ultrastructural features not seen in cells with smaller, darker, primarily heterochromatin-containing nuclei.

Three out of five patients with early onset urothelial CIS had biopsies of the "large cell" variety, while only one out of four patients of the late onset group fell into this category. As shown in Table 4, "large cell" CIS biopsies show a significantly higher level of abnormalities than "classical" CIS.

Early presentation also has a worse prognosis, as shown by Prout et al. (1983) who found that such patients had a significantly higher rate of subsequent muscle invasion, metastases and clinical indication for cystectomy

than patients in whom CIS had been found subsequent to initial diagnosis of transitional cell carcinoma.

Weinstein et al. (1980) have made the suggestion, based on their clinical findings, that there may be two types of morphologically similar CIS of the urothelium, one of which will go on to invasion, the other remaining intra-epithelial.

In the present study there are two types of morphologically dissimilar urothelial CIS, easily distinguishable in transmission electron microscopy and in resin-embedded sections, but not on H and E. A clinical difference is also apparent between these morphological groups. "Large cell" CIS was more commonly diagnosed early in the disease in contrast with "classical" CIS which usually became apparent after recurrent papillary tumours.

As it is known that early onset CIS is associated with a worse prognosis than that appearing later the disease course it is suggested that the ultrastructural findings in this study provide morphological evidence for this statement.

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