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Papillary Carcinoma of the Urinary Bladder
A Study of Chromosomal and Cytofluorometric DNA Analysis

Ingrid Granberg-Öhman, Bernhard Tribukait, Hans Wijkström, Tomas Berlin and Lars G. Collste

Departments of Morphology and Urology, Huddinge Hospital, and Department of Medical Radiobiology, Karolinska Institute, Stockholm, Sweden

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Summary. The chromosomal constitution and DNA distribution of transitional cell bladder carcinoma were examined by karyotyping and flow cytometry in 16 patients. In tumours of low grade malignancy (grades I and II) the cell population was mainly diploid and marker chromosomes were present only occasionally. Grade III tumours were polyploid in all but one instance and marker chromosomes were constantly found. The DNA distribution was in good agreement with karyotyping data. These two methods may be regarded as complementary in the cytogenetic assessment of bladder tumours.

Key words: Bladder neoplasms - Chromosome aberrations - DNA analysis.

It is generally felt that superficial transitional cell bladder tumours are best treated with conservative therapy such as local excision and fulguration (2). In invasive tumours prognosis is best if early cystectomy (8) or irradiation alone are used. Thus the early recognition of invasiveness is of paramount importance in the choice of therapy.

The correlation between histopathological malignancy (16) and invasiveness has been found to be good but not complete: Grade I tumours are very rarely invasive while the majority of grade III tumours are infiltrative. In the rather large group of grade II lesions, however, both invasive and non invasive lesions occur (6), and histopathological classification of this group must be regarded as difficult. There is a need for improved diagnostic criteria to aid in the classification and subsequent management of such tumours.

Cytogenetic studies have in recent years been used in attempts to further elucidate the biological properties of tumours. Recent findings suggest that DNA distribution is abnormal in most neoplasms (1). A number of reports on DNA measurements and chromosomal modal numbers in urinary bladder neoplasms have been published (3, 5, 7, 10, 12, 13). Generally, the cells in invasive tumours are aneuploid while in tumours of lower malignancy (grade I and II) both diploidy and aneuploidy are found (14).

Structurally abnormal chromosomes, "markers", seem to be a specific indicator of potential malignancy. Recent studies (4, 5, 9) conclude that the presence of marker chromosomes is an important prognostic finding, relevant to the choice of therapy.

In a group of patients with transitional cell tumours of the bladder we have studied chromosomal patterns by karyotyping and DNA distribution by flow cytometry. The following is a report of our findings in the first 16 patients.

MATERIAL AND METHODS

16 patients with newly diagnosed or recurrent papillary bladder carcinoma were examined. 5 of the patients had noninvasive tumours, 2 were found to have superficial invasion into the lamina propria, and in 9 the tumours had invaded the muscular wall (see table).

The histological grading was based on World Health Organization (WHO) recommendations (16). In addition, the grade II tumours were subdivided into groups "a" and "b", based on the polarity of the cells, nuclear atypia and frequency of mitoses (Fig. 1 and 2). Thus grading was based on the cellular picture regardless of whether there was invasiveness or not. Group IIa may include borderline cases between grades I and II,

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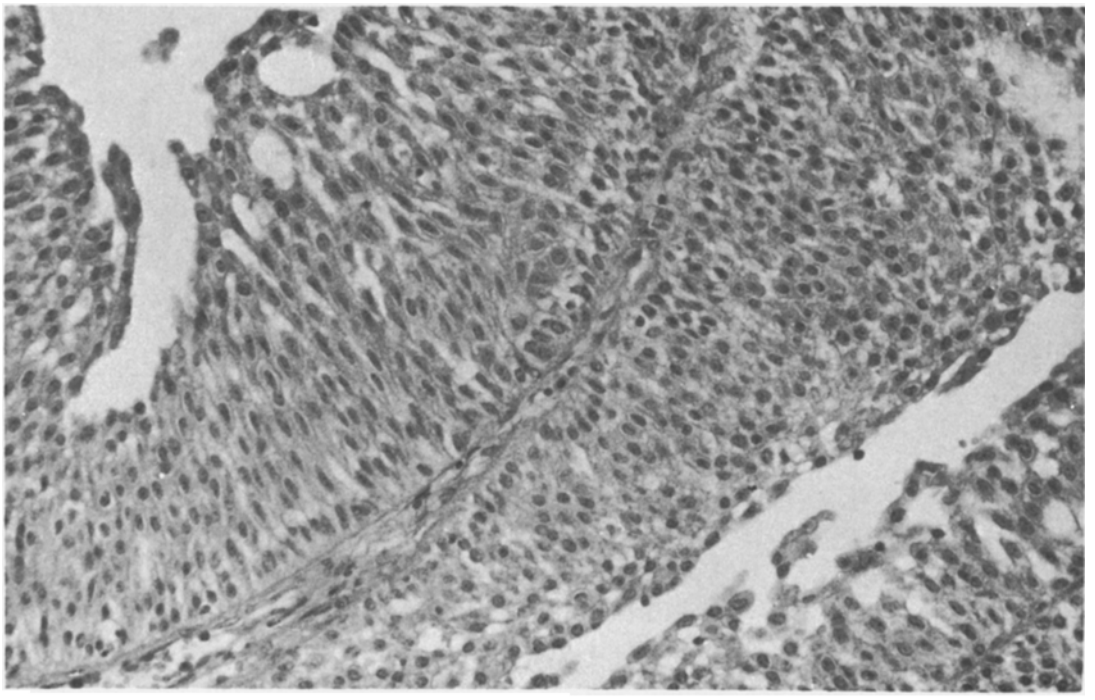

Fig. 1. Papillary carcinoma grade IIa (H & E x 125). Patient 1

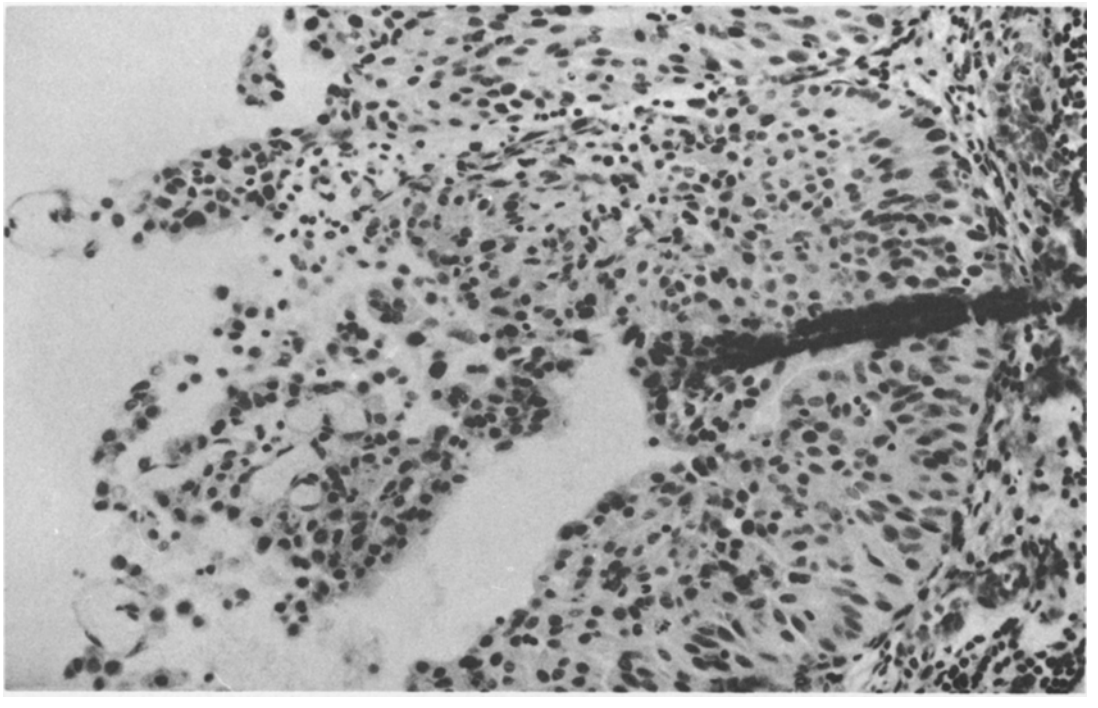

Fig. 2. Papillary carcinoma grade IIb (H & E x 200). Patient 6

and group IIb, borderline cases between grades II and III. All of the microscopic sections were coded and evaluated by one of us (I. G. -Ö.).

All patients were given 1.8 mg Vinblastine intravenously four hours prior to cystoscopy in order to increase the number of metaphases (by permission of the ethical committee of the Karolinska Institute). Transurethral resection biopsies were performed. After mincing, part of the fresh tumour material was immersed in 0.075 M KCl for 20 minutes. After centrifugation, the cells were fixed in a solution of concentrated acetic acid and methanol (1:3). Air-dried specimens were prepared after 24 hours during

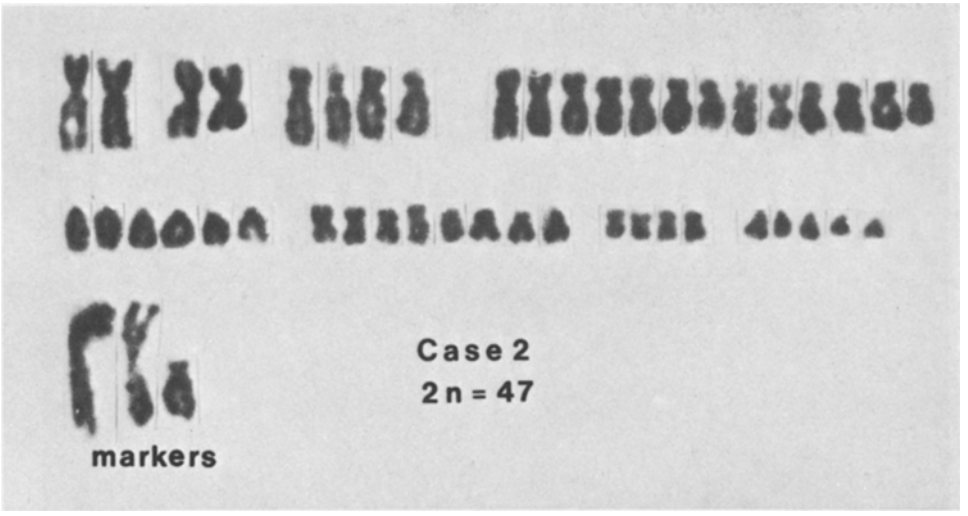

Fig. 3. Deviant metaphase with two missing A 1 and C chromosomes, excess of E chromosomes. Three unidentified marker chromosomes are present. Patient 2

which time the fixative was changed at least twice. The analysable metaphases were counted and whenever possible karyotyped. The average number of metaphases was 17, but in some specimens only single cells could be analysed. These tumours are included because of their characteristic chromosome patterns, e.g. distinct aneuploidy and easily discernible marker chromosomes.

The relative amount of DNA in individual cells of the same cell suspension was determined by flow cytometry as described earlier (15). The DNA was stained with ethidium bromide and the cells were counted in a PHYWE ICP 11 Flow Cytometer (Gottingen, FRG). Ordinarily 35, 000 to 50, 000 cells from each specimen were analysed.

RESULTS

The grade IIa tumour cells were predominantly in the diploid region, the majority of the cells having 46 chromosomes (see table). In 2 cases occasional marker chromosomes were observed (Fig. 3). Most tumours were found to have normal diploid metaphases, but occasional pseudodiploid metaphases and a single periploid, without any clear deviant trend in any specific chromosome, were observed.

In patient number 1 two separate papillary tumours were found. In one of these, which was noninvasive, DNA analysis showed normal diploid distribution (Fig. 4). In the superficially invasive larger tumour a few cells exceeded the normal value for G2 cells (4% ± 1.8, S.D.) by 3 times the standard deviation (14.4%), and this tumour was therefore classified as aneuploid (Fig. 5). The 46 metaphases analysed by karyotyping were pooled from the two separate bladder lesions since all of the chromosome counts were in the diploid region. One cell, in the invasive tumour, had a marker chromsome (Fig. 6).

The 3 tumours of histopathological grade IIb had peridiploid chromosome counts. Flow cytometry done on 2 of them showed diploidy (see Table 1). Thus in these 2 instances karyotyping but not cytometry disclosed the abnormality.

In patients 5 and 7 early recurrences were detected. In patient 7 the new lesion was histologically more malignant - grade III. Marker chromosomes were present in some metaphases in patient 5 and in all cells in patient 6 while no marker chromosomes could be detected in patient 7.

In summary, the grade II lesions could be separated into subgroups "a" and "b" on the basis of slight but constant DNA abnormalities, indicated either by peridiploid modal numbers or the presence of marker chromosomes.

Grade III tumours were polyploid in all but one instance, but numerical values varied widely even for the same tumour, and there were no evident stem lines (see Table). However, by analysis of a large number of cells by flow cytometry more or less distinct modes could be detected (Fig. 8). All of the grade III tumours displayed marker chromosomes (Fig. 7), frequently at the periphery of the metaphases and identifiable even in metaphases with poor spreading (Fig. 9). Marker chromosomes were found in the majority of the cells analysed.

DISCUSSION

Although specific marker chromosomes such as the Ph^1 chromosome in chronic myeloid leukaemia have so far not been identified in bladder cancer (11), the connection between certain marker chromosomes and a tendency for tumour recurrence has clearly been shown (4, 5, 9).

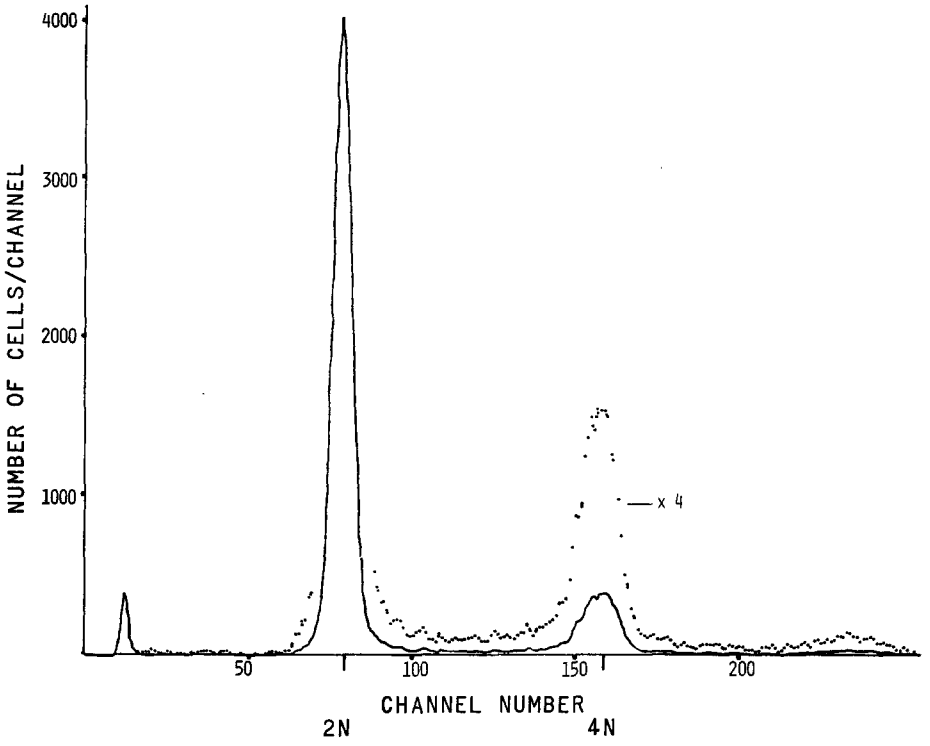

Fig. 4. DNA histogram. The highest peak represents the diploid G_1 cells (2 n), the smaller peak the tetraploid G_2 cells (4 n). The cells between are in S phase. Dotted line: Magnification x 4. Proportion of G_1 cells 95.6%, S phase cells 2.8% and G_2 cells 1.6%. Number of cells measured 39.000. Small tumour. Patient 1

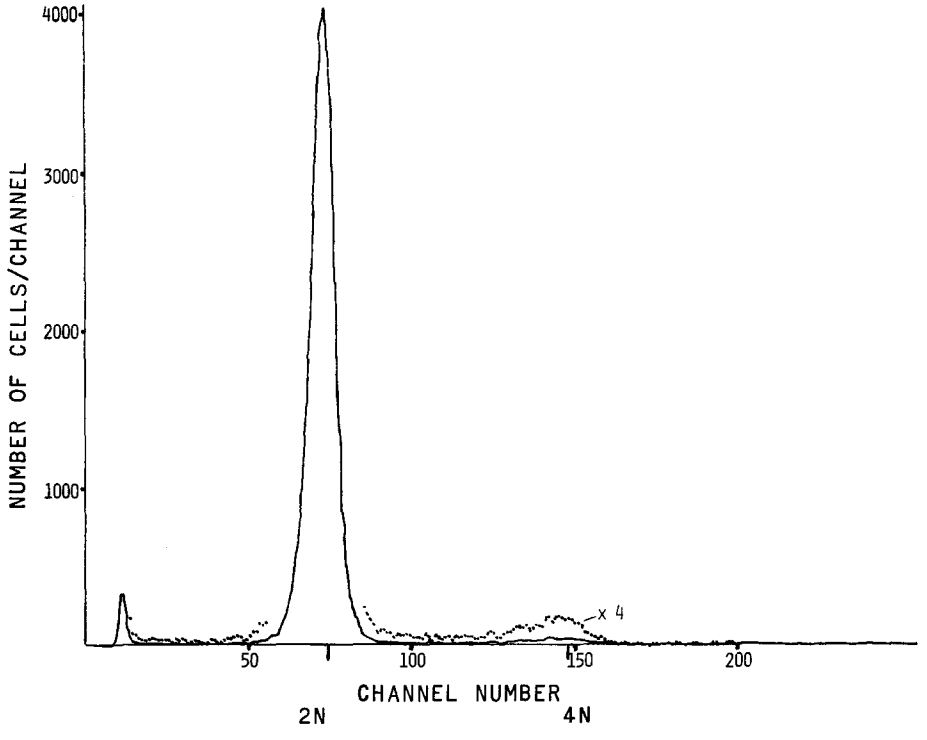

Fig. 5. DNA histogram. In the position of the G_2 peak (tetraploid level) the G_1 portion of the aneuploid population is easily seen, representing 14.4% of all cells. Number of cells measured: 38.000. Large tumour. Patient 1

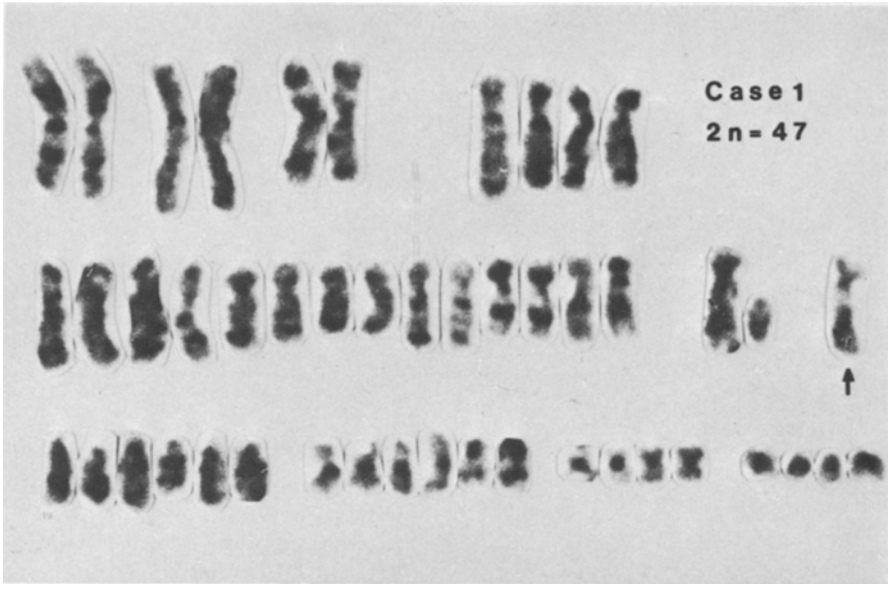

Fig. 6. Deviant cell showing marker chromosome (arrow). Patient 1

Table 1. Cytogenetic assessment of papillary carcinomas of the bladder by chromosome and DNA analysis

Patient	Age	Sex	Grade	Histologic invasion	Mean number of chromosomes	Marker chromosomes	By cytoflow predicted number of chromosomes
1.	73	M	IIa	Lamina propr.	46	(+)	46, 92
2.	72	M	IIa	None	46	(+)	-
3.	67	M	IIa	None	46		46
4.	39	F	IIa	None			46
5.	57	M	IIb	Lamina propr.	43	(+)	46
6.	59	M	IIb	None	44	+	-
7.	71	M	IIb	None	47		46
8.	56	M	III	T. muscularis	43	+	43 (?), 78
9.	74	M	III	T. muscularis	46-47, 92	+	-
10.	65	M	III	T. muscularis	54	+	-
11.	61	M	III	T. muscularis	62	+	78
12.	66	M	III	T. muscularis	65	+	69
13.	59	F	III	T. muscularis	68	+	74, 100
14.	61	F	III	T. muscularis	72	+	85
15.	64	F	III	T. muscularis	80	+	99
16.	68	M	III	T. muscularis	80	+	89

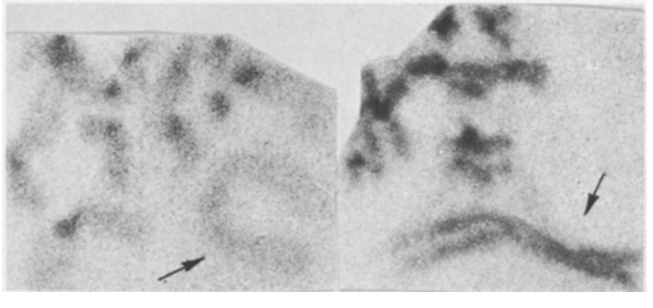

Fig. 7. Portion of two C-banded metaphases. Arrows indicate the larger marker chromosome without C-bands. Patient 15

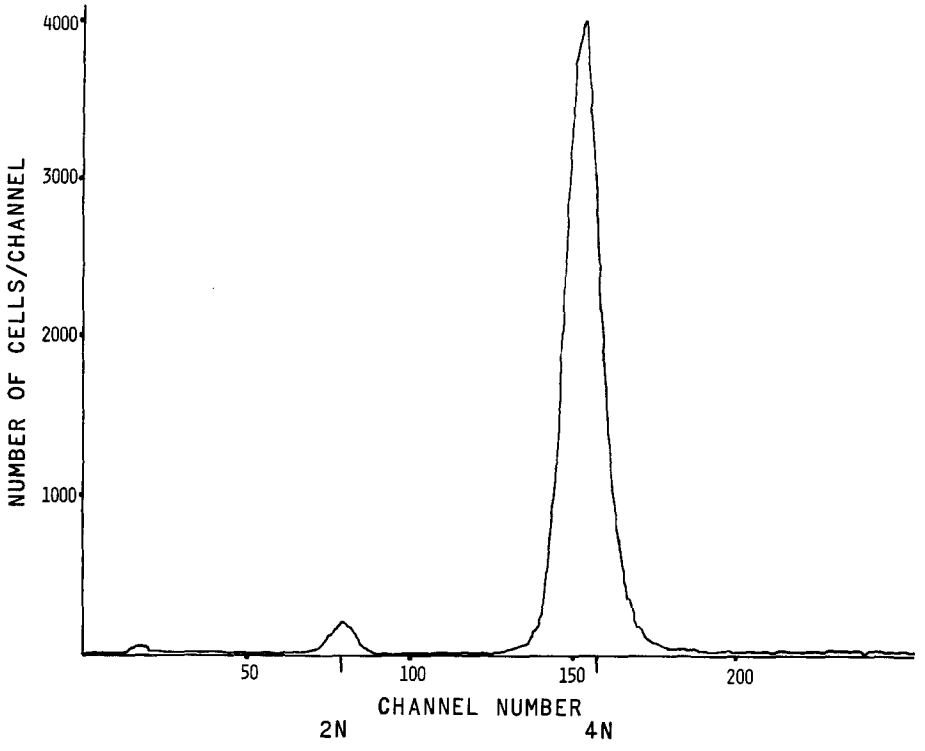

Fig. 8. DNA histogram showing an aneuploid cell population. The small peak to the left corresponds to normal diploid cells and the large peak to G_1 cells of an aneuploid cell line. Patient 16

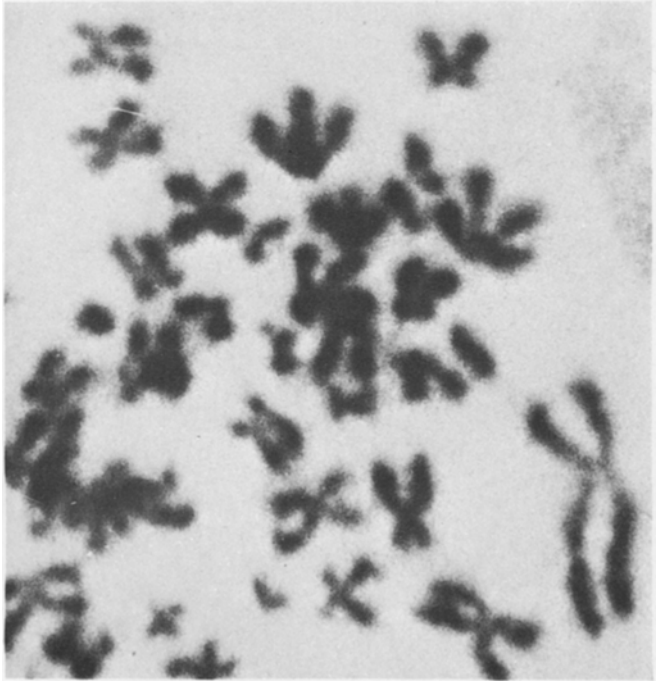

Fig. 9. Clearly identifiable marker chromosome in a poorly spread metaphase from a papillary carcinoma grade III. Patient 15

Falor and Ward (3) state that marker chromosomes are a characteristic of clinically more aggressive bladder tumours. Several of these markers were large - probably derived from group A chromosomes. However, Sandberg (9) studied 137 noninvasive and invasive papillary tumours and found markers in both groups. In noninvasive carcinomas (which may correspond to our grade IIa and IIb tumours) marker chromosomes were present in 64%, while in invasive tumours one or more marker chromosomes were present in 100%.

The present study seems to confirm the above results. In our study all tumours of malignancy grade III had marker chromosomes in the majority of the cells analysed. In tumours of lower malignancy (grade I and II) marker chromosomes were also observed but only in occasional deviant metaphases. In the majority of cases the marker chromosomes seemed to be derived from group A chromosomes, perhaps indicating some specificity in epithelial bladder tumours.

The more malignant tumours (grade III) constantly showed high ploidy, predominantly in the hypertriploid - hypotetraploid region (15). Thus a predominance of polyploid cells and the presence of deviant chromosomes are common findings in the more aggressive bladder tumours.

The flow cytometry DNA analyses generally were in good agreement with the modal numbers obtained by karyotyping with the exception of 2 cases with peridiploid deviations. At present it is not possible to demonstrate such neardiploid deviations by flow cytometry alone. Further development of flow cytometry systems is likely to increase their resolution capacity. On the other hand, the large number of cells which can be analysed by cytometry permits identification of tumour stem lines with high ploidy levels which may not be identifiable by karyotyping when only

a limited number of metaphases are available. In addition, the degree of proliferation of tumour cell populations (15), another important tumour property, can be assessed by flow cytometry.

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Ingrid Granberg-Öhman M.D.
Department of Morphology
Huddinge University Hospital
S-141 86 Huddinge
Sweden