



# IDENTIFICATION OF NUMERICAL CHROMOSOME ABERRATIONS IN ARCHIVAL TUMOURS BY *IN SITU* HYBRIDIZATION TO ROUTINE PARAFFIN SECTIONS: EVALUATION OF 23 PHAEOCHROMOCYTOMAS

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## SUMMARY

We have applied non-isotopic *in situ* hybridization (ISH) to interphase cell nuclei of 23 pheochromocytomas (18 primary and 5 metastatic tumours) within routine paraffin-embedded tissue sections. Each tumour was screened for numerical aberrations with a defined alphoid repetitive DNA probe set containing DNA probes specific for chromosomes 1, 7, 15, and Y. Normal adrenal medullas and other normal human cell types served as cytogenetic controls. Preservation of tissue morphology enabled targeted analysis of tumour cells. The presence of numerical chromosome changes in the tumour cells could easily be evaluated by comparing the ISH results of the DNA probes. Numerical abnormalities not previously reported in this neoplasm included overrepresentation of chromosomes 1 and 7, loss of chromosome 15, and both gain and loss of chromosome Y (*P* values <0.01). The percentage of aneuploid cell nuclei in a tumour correlated well with the percentage of cells in the 4C peak of flow cytometric DNA histograms from these neoplasms. We conclude that interphase ISH can be used for the identification of new and reported cytogenetic changes in tumour cell nuclei within archival tissue sections. This novel procedure also allows for retrospective analysis of previously not karyotyped material.

**KEY WORDS**—*In situ* hybridization, alpha-satellite DNA probes, paraffin sections, interphase cytogenetics, pheochromocytomas.

## INTRODUCTION

The currently held views in the development of cancer have led to intensive efforts to elucidate (tumour- and/or stage-specific) chromosome aberrations in malignant diseases. At present, knowledge in this area is growing rapidly, though relatively little information is available on most non-haematological neoplasms.<sup>1,2</sup> This is mainly due to difficulties in obtaining mitotic cells for karyotypic examination. Additional techniques

using approaches applicable to interphase cells would therefore be helpful.

In the last decade, non-isotopic *in situ* hybridization (ISH) with (peri)centromeric chromosome-specific DNA probes has emerged as a powerful and reliable instrument for the discrimination of numerical chromosome changes in interphase cell nuclei of haematological malignancies,<sup>3–5</sup> as well as solid tumours.<sup>6–11</sup> This technique has also been adapted for use on tissue sections, which allows the combination of cytogenetic and histological analyses.<sup>12–15</sup>

Pheochromocytomas are the most prevalent neoplasms of the adrenal medulla. A significant problem in this neoplasm is the unpredictability of

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its behaviour, apart from the severe hypertension which most patients suffer from.<sup>16</sup> These tumours should be regarded as potentially malignant, but in only 10 per cent do metastases eventually develop.<sup>17</sup> Importantly, reliable histopathological characteristics for the discrimination of pheochromocytomas that will and those that will not metastasize have not been described.<sup>18</sup>

DNA flow cytometry of pheochromocytomas has yielded conflicting data concerning the prognostic value of DNA aneuploidy,<sup>19–21</sup> although in a large recent study abnormal DNA ploidy patterns were found to be unfavourable.<sup>22</sup> Cytogenetic data on this tumour have been very limited. The only chromosomal abnormality found so far has been a +7 in one of three pheochromocytomas from patients with von Hippel–Lindau syndrome.<sup>23</sup> In sporadic tumours and as a part of MEN (multiple endocrine neoplasia) type 2, loss of heterozygosity has been detected on the tip of 1p.<sup>24–26</sup>

In this study we have applied *in situ* hybridization to paraffin-embedded tissue sections of 23 archival pheochromocytomas (18 primary and 5 metastatic tumours) to search for numerical chromosome aberrations. For this purpose, a targeted set of (peri)centromeric alphoid DNA probes specific for chromosomes 1, 7, 15, and Y was used. The tissue architecture on the sections was adequately preserved, permitting precise location and analysis of the tumour cells. With this method, a retrospective analysis of previously not karyotyped material became feasible.

## MATERIALS AND METHODS

### *Probe and probe labelling*

In this study (peri)centromeric and repetitive satellite DNA probes specific for chromosomes 1, 7, 15, and Y were applied. For *in situ* hybridization, complete plasmid DNA was labelled with biotin-14-dATP by nick translation (BRL, Gaithersburg, MD) according to the manufacturer's directions. Probe size ranged from 50 to 500 nucleotides. The DNA probes were stored at  $-20^{\circ}\text{C}$ .

### *Tissue preparation*

Twenty-six paraffin-embedded archival pheochromocytomas (1987–1991) were taken for this study: eleven specimens were from Nijmegen

University Hospital (1989–1991) and 15 came from the Erasmus University Hospital (1987–1991). The three archival normal adrenals (1987–1991) were obtained from nephrectomy specimens of patients with renal cell carcinoma. The 4  $\mu\text{m}$  paraffin sections were floated in warm ( $40^{\circ}\text{C}$ ) demineralized water and mounted on amino-alkylsilane (Serva, Heidelberg, Germany)-coated glass slides. Coating of the slides was performed according to the procedure of Rentrop *et al.*<sup>27</sup> Sections were air-dried for several hours at  $56^{\circ}\text{C}$  in a ventilating oven.

### *Pretreatment*

The sections were deparaffinized at room temperature in xylene  $3 \times 10$  min, in ethanol  $4 \times 2$  min, and immersed in 0.3 per cent  $\text{H}_2\text{O}_2$  in methanol for 20 min to block endogenous peroxidase activity. The slides were then rinsed twice in phosphate-buffered saline (PBS)

Subsequently, the sections were incubated in  $2 \times \text{SSC}$  ( $1 \times \text{SSC} = 0.15 \text{ mol/l NaCl} + 0.015 \text{ mol/l sodium citrate}$ ) at  $70^{\circ}\text{C}$  for 30 min. This incubation allows shorter pepsin digestion times and renders increased preservation of morphology. A proteolytic treatment with pepsin (Sigma, St Louis, MO; 4 mg/ml in 0.2 M HCl) was applied at  $37^{\circ}\text{C}$  (see below). The slides were then rinsed twice in distilled water and immersed in  $2 \times \text{SSC}$  until denaturation.

The pepsin digestion time had been carefully evaluated for each tumour in a separate experiment by a time series (5, 10, 15, and 20 min). The optimal pepsin time was judged by the following two criteria: ISH signal intensity and tissue morphology. In seven tumours, no adequate ISH signal was obtained within the pepsin time series. Four of these seven tumours showed an acceptable result with a longer pepsin digestion time, resulting in 23 ISH-positive tumours (89 per cent of all cases). The remaining three negative tumours were all 'Nijmegen' specimens from early 1989, possibly representing differences in fixation protocols in the two hospitals.

### *In situ hybridization*

Production of single-stranded DNA for ISH was accomplished by heat denaturation. Slides were immersed for 2 min at  $70^{\circ}\text{C}$  in 70 per cent formamide.  $2 \times \text{SSC}$  at pH 7. Formamide (Merck, Darmstadt, Germany) was deionized by treatment

with a mixed-bed ion-exchange resin (Dowex AG501-X8; Bio-Rad Labs, Richmond, CA). Then the slides were dehydrated in an ethanol series [70 per cent (cold), 83 per cent, 96 percent] and air-dried at room temperature.

The chromosome-specific repetitive DNA probes were denatured for 5 min at 70°C in the hybridization mixture, which contained 1–2 µg/ml probe DNA, 500 µg/ml sonicated herring sperm DNA (Sigma), 0.1 per cent Tween-20, 10 per cent dextran sulphate, and 2 × SSC in 60 per cent formamide at pH 7. After denaturation, the mixture was put immediately under a glass coverslip covering the section.

ISH was performed overnight at 37°C in a humidified chamber. Then the coverslips were removed by immersing the slides briefly in 2 × SSC. Next the slides were washed three times for 3 min each in 60 per cent formamide in 2 × SSC, pH 7, at 42°C and subsequently two times for 5 min each in 2 × SSC, pH 7 at 42°C. The slides were then immersed in PBS+0.05 per cent Tween-p20 (PBST).

#### *Histochemistry*

For immunoperoxidase staining, the slides, in PBST, were incubated with mouse-*a*-biotin [Dakopatts, Glostrup, Denmark; 1/100 in PBS+5 per cent non-fat dry milk (PBSM)] for 30 min at 37°C. They were then rinsed twice in PBST. (All subsequent washes in PBST were for 10 min at room temperature). An incubation with biotin-labelled horse-*a*-mouse (Vector, Burlingame, CA; 1/200 in PBSM) followed for 30 min at 37°C, and the slides were washed in PBST. In the meantime, the ABC complex (Vectastain Elite ABC Kit: 20 µl. avidin+20 µl biotin-labelled peroxidase in 1 ml of PBSM) was prepared and complex formation was allowed for 30 min at room temperature. Next the ABC complex was incubated with the sections for 30 min at 37°C and non-bound complex was removed by rinsing in PBST. The probe-related signal was developed with DAB–H<sub>2</sub>O<sub>2</sub> (0.5 g/l–0.05 per cent, respectively, in PBS with 0.01 M imidazole), at neutral pH, for 5–15 min at room temperature. Slides were then rinsed in distilled water for 15 min, the signal was amplified with CuSO<sub>4</sub> (0.5 per cent in 0.9 per cent NaCl) for 5 min at room temperature, and the slides were again rinsed in distilled water. Finally, the sections were counterstained with haematoxylin (10–20 min), rinsed in tap water, dehydrated in ethanol,

immersed briefly in xylene, and mounted in Permount (Fisher Scientific, Fair Lawn, NJ).

#### *Evaluation of ISH results*

The DNA probe set was analysed for each phaeochromocytoma (plus controls) on consecutive 4 µm sections in a previously defined tumour area of ± 5 × 5 mm. This approach reduced artefacts due to differences in section thickness or tumour heterogeneity, respectively. For each of the probes, 100 'intact' (=spherical) and non-overlapping 4 µm nuclear slices were counted by two independent investigators and the number of solid DAB spots per nuclear fragment was scored (0, 1, 2, 3, 4 and >4 spots per nuclear slice). The individual DNA probe spot distributions were then compared and totalled. In our protocol no discrepancies emerged using these criteria.<sup>15</sup> The numerical chromosomal aberrations were evaluated statistically by means of the Kolmogorov–Smirnov test.<sup>15,28</sup> An aberrant probe spot distribution was considered to represent a numerical aberration if a *P* value greater than 0.01 (maximum difference >12 per cent) was calculated.

#### *DNA cytometry*

Flow cytometric DNA histograms of the paraffin-embedded tissue blocks were obtained by standard dewaxing and isolation procedures. A Facscan (Becton-Dickinson, Sunnyvale, CA) was used for measurement and analysis of propidium iodide (Sigma) stained specimens. Leukocytes were applied for calibration purposes. In the analysis, a peak with a DNA index of 1.8–2.2 was assigned 4C.

## RESULTS

A DNA probe set was selected for the screening of chromosomes 1, 7, 15, and Y. In 23 phaeochromocytomas (18 primary and 5 metastatic tumours) taken from 21 patients (11 male and 10 female; mean age 41.0 years; Table I), high-quality *in situ* hybridization (ISH) was obtained that was suitable for subsequent interphase analysis. DNA probes for chromosomes 1, 7, and Y were chosen since numerical aberrations of these chromosomes are frequently observed in solid tumours. Furthermore, we have previously seen monosomy 15 (and loss of the Y chromosome) in predominantly

Table I—Twenty-three pheochromocytomas: tumour/patient features

Tumour No.	Sex/age years	Tumour site	Occurrence*
1	M/56	Adrenal/primary	Unknown
2	M/59	Adrenal/primary	Sporadic
3	M/25	Adrenal/primary	NF
4	M/43	Adrenal/primary	Sporadic
5	F/45	Adrenal/primary	Sporadic
6	M/9	Adrenal/primary	Unknown
7	F/35	Thorax/metastasis	Sporadic
8	F/36	Kidney/metastasis	Sporadic
9	F/53	Adrenal/primary	Sporadic
10	F/44	Adrenal/primary	Sporadic
11	M/63	Adrenal/primary	Sporadic
12	F/50	Bladder/primary	Sporadic
13	M/41	Adrenal/primary	Sporadic
14	M/40	Adrenal/primary	Sporadic
15	M/28	Adrenal/primary	Sporadic
16	M/65	Adrenal/primary	Sporadic
17	F/47	Adrenal/metastasis†	MEN2
18	F/60	Adrenal/primary	Sporadic
19	M/25	Lymph node/metastasis‡	Sporadic
20	M/25	Adrenal/primary	Sporadic
21	F/26	Adrenal/primary	Sporadic
22	F/30	Adrenal/primary	NF
23	F/37	Thorax/metastasis§	Sporadic

\*Sporadic or related to hereditary diseases: multiple endocrine neoplasia type 2 (MEN2), von Hippel-Lindau syndrome (VHL), von Recklinghausen's neurofibromatosis (NF). In the case of metastasis or recurrence, the original tumour is referred to.

†Recurrence of the tumour.

‡Metastasis of tumour 20.

§Recurrence of tumour 7.

high-stage and grade bladder tumours.<sup>29</sup> For that reason the chromosome 15 DNA probe was included. In order to evaluate the ISH results of the tumours, control experiments were performed on normal adrenal medullas (three cases, mean age 60.0 years). Normal stromal cells in the specimens, if present, were utilized as internal (diploid) controls.

In nine control tissues, ISH showed low percentages of hyperdiploid cell nuclei (<5 per cent). In this respect, a difference was observed between leukocyte (four cases: mean value 0.5 per cent) and adrenal cortex controls (four cases: mean value 2.7 per cent). Leukocyte nuclei showed a diploid distribution (Figs 1A, 1B, and 2A), while ISH with the DNA probe set to three normal adrenal medullas revealed the existence of hyperdiploid cell nuclei for all probes (range 8–20 per cent; see also Figs 1C and 2B). Diploid ISH patterns without

hyperdiploid nuclei were also seen in normal kidney, prostate, and gastric specimens (not shown). Therefore, the presence of 5–25 per cent hyperdiploid cells in the ISH distribution pattern of a pheochromocytoma was regarded as normal ('euploid'). Using this definition, a euploid ISH pattern was observed in 15 pheochromocytomas and an aneuploid distribution in 8 (Table II; Figs 2C and 2D).

The ISH results of the 23 pheochromocytomas are summarized in Table II. Six tumours carrying a numerical aberration were observed. The aberrant DNA probe distributions were evaluated statistically by the Kolmogorov-Smirnov test ( $P < 0.01$  values; see also Fig. 3). In a metastatic pheochromocytoma (tumour 8), loss of chromosome 15 was seen (Fig. 3C). Lower numbers of #15 ISH spots per nucleus can be seen than, for example, of #7 (Figs 4A and 4B). Fibroblasts present in this

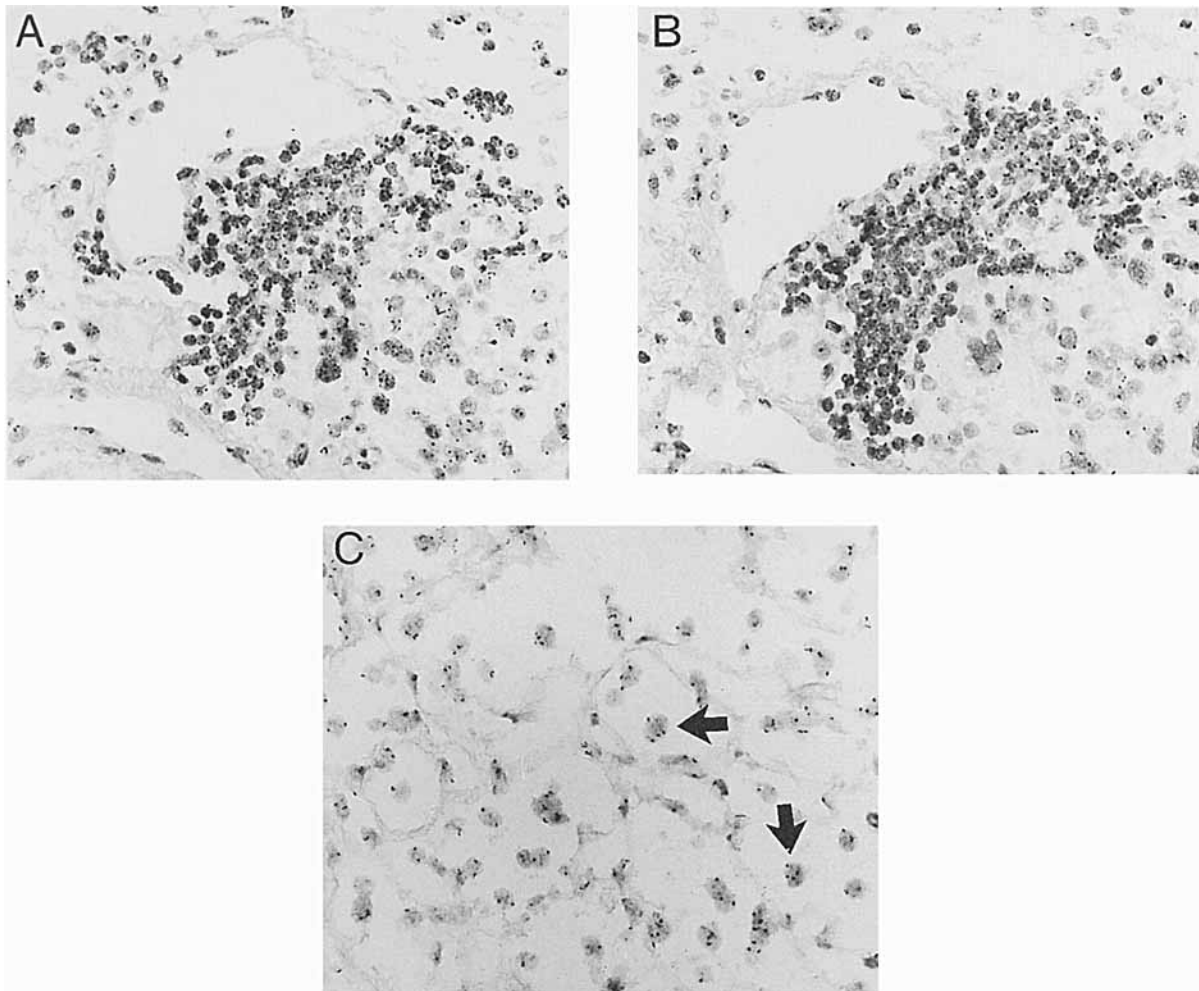


Fig. 1—(A) *In situ* hybridization (ISH) with the chromosome 1-specific DNA probe to a leukocytic infiltrate within 4  $\mu$ m sections of tumour 2. The ISH-related spots were visualized with immunoperoxidase/DAB (black); haematoxylin was used as a counterstain (grey). No hyperdiploid leukocytes can be distinguished. (B) Same as A, chromosome Y DNA probe. Note the haploid hybridization pattern when compared with the chromosome 1 DNA probe. (C) ISH with the chromosome 7-specific probe to a normal adrenal. Hyperdiploid nuclei can be discriminated in the normal adrenal medulla (arrows)

specimen revealed a diploid ISH distribution. Tumour 15 showed an increase of ISH spots for chromosome 7 when compared with the other probes (Fig. 3D). On average, these nuclei contained more spots for the #7 probe than, for example, for #1 (Figs 4C and 4D). Loss of the Y chromosome was seen in tumours 3 (Fig. 4B) and 14. This Y-loss is restricted to the tumour cells (Fig. 4E). Overrepresentation of chromosomes 1 and Y was observed in phaeochromocytomas 13 and 2 (Fig. 3A). In the latter patient, the normal haploid Y pattern was seen in leukocytes present in the same specimen (Figs 1A and 1B). Polymor-

phism of chromosome 15 repetitive DNA was found in tumour 16 by comparing the probe histograms of the tumour cells with the cortical (and leukocyte) control cells present in this specimen ( Figs 3E and 3F). A high number of nuclei with only one ISH spot were observed for the #15 probe (Fig. 4F).

The percentages of hyperdiploid nuclei (more than 2 ISH spots) correlated well with the cytometric DNA histograms, especially when ISH criteria for tumour ploidy were used (Table II and Discussion). No relationship between tumour site and ISH ploidy or aberrations was observed. Further,

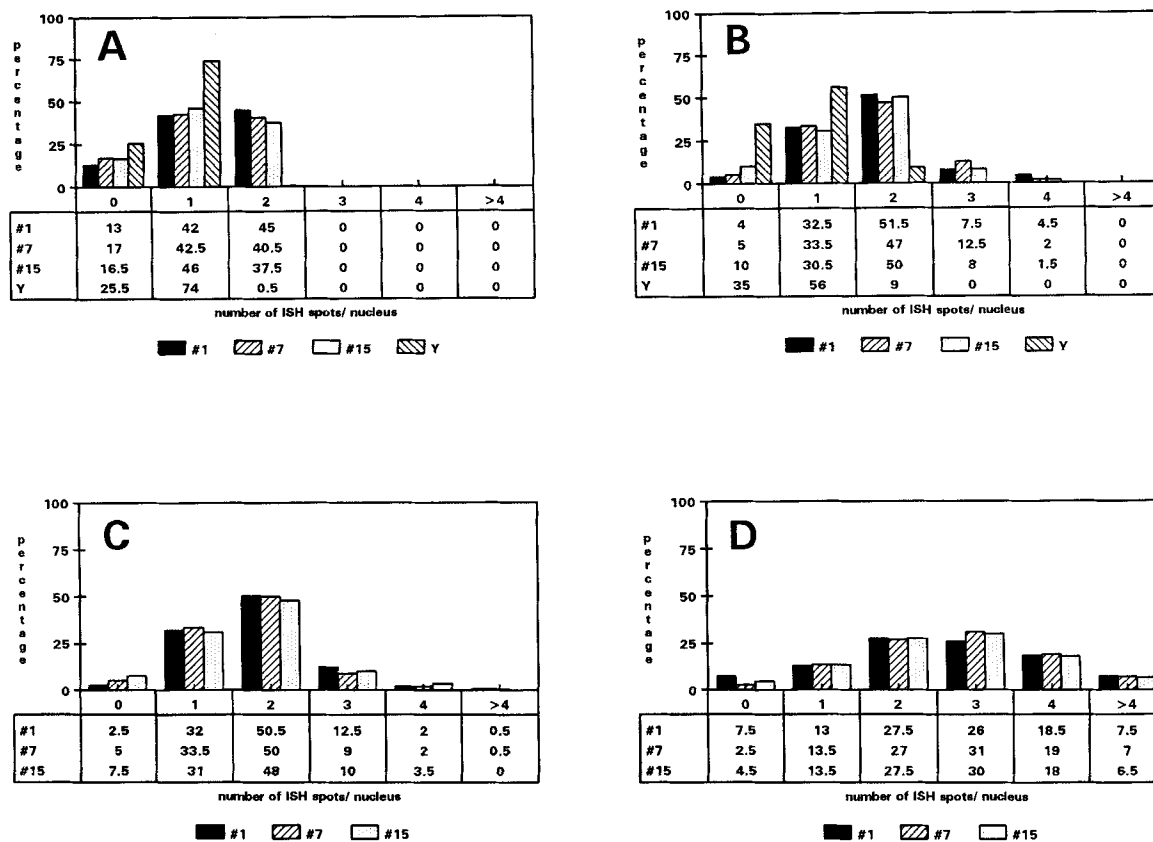


Fig. 2—DNA probe frequency distributions (with tables) of the number of hybridization spots per nucleus for control and pheochromocytoma specimens after ISH with the probe sets to  $4\ \mu\text{m}$  tissue sections. For all of the probes, 100 spherical and non-overlapping nuclei were counted by two independent investigators. The results were added and plotted as a percentage per probe. (A) Leukocytes (in tumour 2) showing the diploid ISH profile. (B) Normal adrenal medulla (Fig. 1C), demonstrating the tissue-specific euploid pattern. (C) Patient 22, showing a distribution pattern which is comparable to those in normal adrenal medullas, without numerical chromosome changes. (D) Patient 5. An aneuploid pattern with many hyperdiploid cells is seen

no correlation was found between the occurrence of the disease and the ISH results. This could, however, be due to the low number of hereditary diseases in this study.

## DISCUSSION

Non-isotopic *in situ* hybridization with chromosome-specific DNA probes to paraffin-embedded archival tissue sections allowed us to screen 23 pheochromocytomas for the presence of numerical changes of selected chromosomes. Despite the sectioning artefact, which yields sliced nuclei, specific chromosome aberrations were detected and evaluated statistically. The tissue architecture was sufficiently retained, enabling

histological examination and precise location of tumour cells. Thus, non-tumour cells, such as fibroblasts, blood cells, and other normal tissue components, could be excluded from analysis. This is an advantage over techniques using isolated cells or tissue extracts. Moreover, the normal stromal cells can be used for control purposes with respect to hybridization efficiency and patient genetics. This is exemplified in tumour 16, containing a polymorphism for alpha-satellite 15 DNA. This polymorphism would otherwise have been interpreted as a loss of chromosome 15. Finally, ISH to archival material renders the possibility of targeted retrospective cytogenetic analysis, e.g., on cells that were not, or could not be, inspected by conventional cytogenetic procedures.

Table II—Cytogenetics and ploidy patterns of 23 phaeochromocytomas by ISH to 4  $\mu$ m sections with a panel of chromosome-specific repetitive DNA probes (1, 7, 15, and Y) and DNA flow cytometry

Tumour No.	Cytometry*	Aneuploidy†	Aberrations‡
1	Tetraploid	29	
2	Tetraploid	15	+Y
3	Diploid	22	-Y
4	Tetraploid	26	
5	Tetraploid	53	
6	Diploid	22	
7	Diploid	14	
8	Tetraploid§	47	- 15
9	Tetraploid	15	
10	Tetraploid	36	
11	Aneuploid	21	
12	Diploid	6	
13	Tetraploid	47	+1
14	Tetraploid	13	-Y
15	Diploid	17	+7
16	Tetraploid	23	(15)
17	Tetraploid	6	
18	Tetraploid	19	
19	Aneuploid	39	
20	Aneuploid	40	
21	Diploid	18	
22	Tetraploid	13	
23	Tetraploid	9	

\*Ploidy of the tumour, according to Nativ *et al.*:<sup>22</sup> diploid= $\leq$ 15 per cent cells in the 4C peak; tetraploid= $>$ 15 per cent cells in the 4C peak; aneuploid denotes the presence of a third peak other than 2 or 4C.

†Percentage of cells with more than two hybridization spots.

‡Kolmogorov-Smirnov test:  $p < 0.01$ .

§Image cytometry on another tissue block of the same tumour.

||Tumour and control cells of this patient showed strong polymorphism for alpha-satellite 15 DNA.

The ISH hyperdiploid nuclei (three or four spots) in the normal adrenal medullas are likely to be tetraploid cells (and not G<sub>2</sub>M). An ISH-G<sub>2</sub>M cell shows an increase in spot size, but not doubling of spots, after hybridization with (peri)centromeric DNA probes.<sup>8,30</sup> Further, if doubling should occur, these spots should appear as a pair, not located far apart (e.g., Fig. 1C). Tetraploid populations in DNA histograms of normal adrenal glands were also observed by other investigators.<sup>19,20</sup> In a recent study on phaeochromocytomas, an upper limit as high as 15 per cent was regarded as normal for the percentage of nuclei found in the 4C peak of normal adrenals.<sup>22</sup> We

therefore conclude that the hyperdiploid cells seen by us in normal adrenal medulla reflect a tetraploid DNA content. This replication phenomenon is also found in other normal tissues, e.g., the liver.

A fair correlation was found between the percentage of ISH hyperdiploid cells and the amount of cells in the 4C peak of the flow cytometric DNA histograms of the phaeochromocytomas (Table II). If we use the ISH criteria for ploidy (diploid, euploid and aneuploid= $<$ 5 per cent, 5-25 per cent, and  $>$ 25 per cent hyperdiploid cells) on both DNA cytometric and ISH data, a good correlation is found in ploidy definition. Then a disagreement on ploidy pattern appears in only 4/23 cases. It can thus be speculated that phaeochromocytomas contain low percentages of (diploid) G<sub>2</sub>M cells, since the latter are not discriminated by ISH.<sup>8,30</sup> We did not see a correlation between ploidy and tumour progression (primary, metastatic). Such a correlation appeared inconsistent in several DNA cytometric studies.<sup>19-21</sup> The existence of tetraploid populations in normal adrenal medulla might be related to this controversy.

An overrepresentation of chromosome 7 was found in a primary tumour (Table II). This might have prognostic significance. It has been suggested that bladder cancers with +7 show more aggressive biological behaviour.<sup>31</sup> In support of this was the correlation found between chromosome 7 copy number, determined by ISH, and bladder tumour grade, stage, and proliferation index.<sup>11</sup> An underrepresentation of chromosome 15 was detected in a metastatic phaeochromocytoma. It could indicate a late cytogenetic event during tumour progression. Previously, loss of chromosome 15 was observed by us in high-stage and grade bladder tumours.<sup>29</sup> Loss of chromosome 15 was also found in non-seminoma germ cell tumours, when compared with seminomas.<sup>32</sup> These authors advocated a model in which seminomas were regarded as preceding the more pathogenic non-seminomas. Y-chromosome aberrations, mostly losses, can be seen in many solid tumours. In our study we could demonstrate that this event is restricted to the tumour cells.

We can speculate on the nature of the numerical changes found by calculating the total number of ISH spots in all 200 cells counted for a DNA probe with an aberrant distribution (Fig. 3) relative to the mean value of the other autosomal probes in the same tumour. For tumour 15, this probe value (#7/mean of #1+#15) would be 1.42. This suggests that the chromosome 7 overrepresentation in

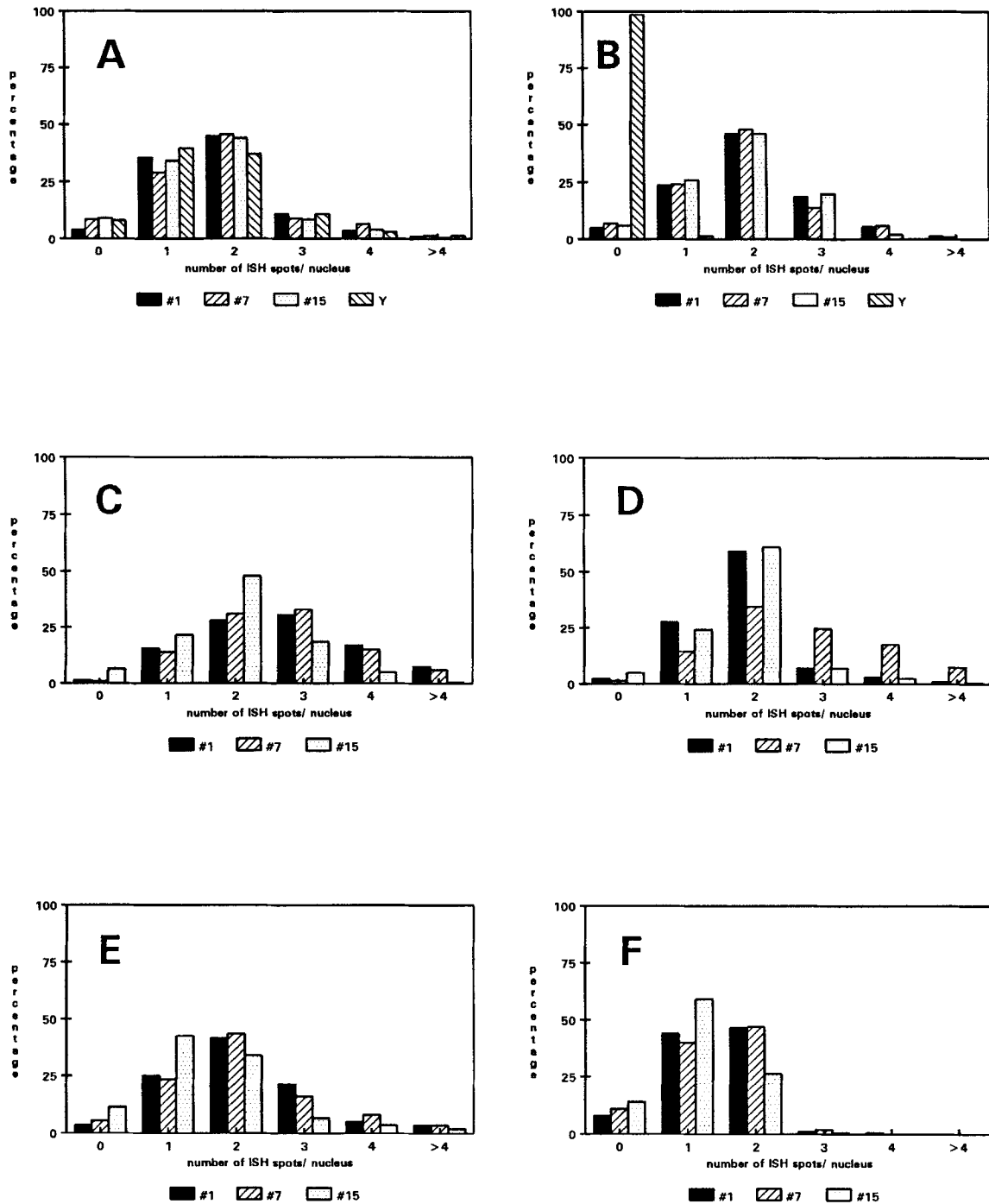


Fig. 3—DNA probe frequency distributions (without tables) of the number of hybridization spots per nucleus for abnormal pheochromocytoma specimens after ISH with the probe sets to 4  $\mu$ m tissue sections. (A) Tumour 2, containing an extra copy of the Y chromosome. The Y profile follows the autosomal distribution. (B) Loss of chromosome Y in tumour 3. (C) Specimen 8: loss of chromosome 15, demonstrated by a shift to the left (as compared with the other DNA probes) of the #15 distribution. (D) Overrepresentation of #7 in tumour 15. The 7 profile is shifted to the right. (E) A shift to the left of the #15 profile in tumour 16. (F) Same specimen, adrenal cortex control. The same pattern is seen, indicating polymorphism of chromosome 15 alpha-satellite DNA



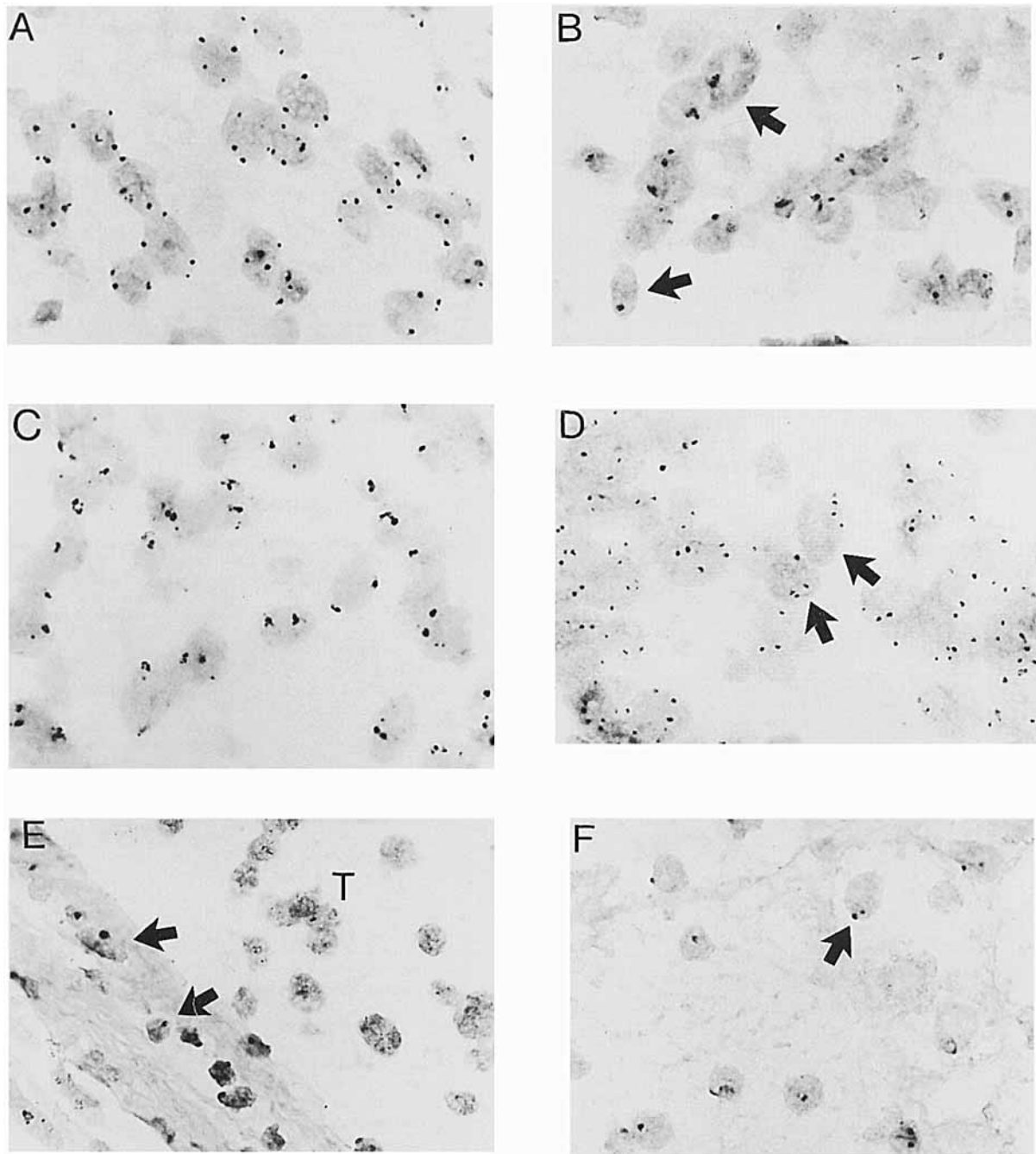


Fig. 4—ISH with the chromosome 7-specific DNA probe to the (metastatic) tumour 8. An aneuploid pattern with many hyperdiploid nuclei is seen. (B) Loss of chromosome 15 in the same tumour. Two nuclei with only one spot are arrowed. (C) #1-ISH to specimen 15. Many nuclei carry two spots. (D) ISH to the same tumour with the #7 DNA probe. Note that the mean number of spots per nucleus is higher than in C. Many nuclei containing three or four spots can be seen (two are arrowed), indicating a gain of chromosome 7. (E) ISH with the Y-specific probe to the pheochromocytoma of tumour 3. Chromosome Y is absent in tumour cell nuclei (T), but present in stromal cells (arrows). (F) Adrenal cortex control, confirmed by absence of chromogranin A staining, in specimen 16. Many nuclei contain only one spot, whereas some display a large and a small signal denoting chromosome 15 polymorphism (arrow)

this neoplasm was caused by a trisomy. Y values in normal adrenals and control cells range from 0.4 to 0.6, being roughly in accordance with Y haploidy. The chromosome 1 over- and chromosome 15 underrepresentation in pheochromocytomas 13 and 8 have values of 1.19 and 0.75, respectively. These numbers are probably biased by cytogenetic heterogeneity in these very aneuploid tumours (Table II).

In this investigation, we focused on the detection of numerical chromosome changes. Aberrations of chromosomes 1, 7, 15, and Y were disclosed in a tumour that is marked by an almost complete absence of karyotypic information.<sup>23</sup> However, many (early) genetic events in oncogenesis and progression are of a structural kind, e.g., loss of the tip of 1p in pheochromocytomas.<sup>26</sup> Preliminary ISH experiments on archival pheochromocytomas demonstrated the feasibility of detecting unique regional DNA probes (e.g., for the relevant 1p36 band) in the paraffin-embedded sections (Tersteeg and van Dekken, unpublished observations).

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