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Case report

Co-existence of two aneuploid stemlines in benign adenomas

A report of three cases with stemline heterogeneity*

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Summary. The co-existence of 2 or more aneuploid stemlines (DNA multiploidy) has been described in malignant human neoplasms and such cancers have often been found to be associated with a poor prognosis. Here 3 benign human adenomas with 2 co-existing aneuploid stemlines are described. Despite DNA stemline heterogeneity and large DNA indices up to 2.8 none of the adenomas recurred or gave rise to metastases after a simple excision during the follow-up of 8, 10 and 11 years. Two adenomas were hormonally active. Marked cellular atypia and frequent mitoses were seen in 1 of the adenomas but the other 2 tumours had little atypia. The present cases indicate that DNA stemline heterogeneity may occur in benign adenomas, and not even the presence of 2 aneuploid stemlines with greatly increased nuclear DNA content can be regarded as a conclusive sign of malignancy.

Key words: Flow cytometry – Adrenal neoplasms – Parathyroid neoplasms – Pancreatic neoplasms – DNA content – Multiploidy – Stemline heterogeneity

Introduction

Until recently, an abnormal nuclear DNA content (DNA aneuploidy) was considered to be a conclusive marker of malignancy (Barlogie et al. 1983) and to be the most common specific marker (Büchner et al. 1985). However, during the recent years a body of evidence has accumulated indicating that DNA aneuploidy as detected by flow cytometry may also occur in histologically benign neoplasms (Anniko et al. 1984; Ingh van den et al. 1985; Joensuu et al. 1986; Mattfeldt et al. 1987). An abnormal nuclear DNA content can therefore no longer be regarded as a conclusive sign of malignancy.

DNA multiploidy (the co-existence of 2 or more aneuploid stemlines within a tumour) may occur in human cancer, where it has been reported to be associated with poor prognosis (Tribukait 1984; Kallioniemi et al. 1988). The existence of DNA multiploidy in benign adenomas has not been established, and therefore 3 such adenomas seen by us are now described in detail. Two of these cases were encountered in a recent series of 164 endocrine adenomas analyzed for nuclear DNA content (Joensuu and Klemi 1988). In all present cases 2 co-existing aneuploid stemlines could be demonstrated indicating that not even DNA multiploidy is a conclusive sign of malignancy, and that human adenomas may be heterogeneous and simultaneously contain stemlines of cells with a different nuclear DNA content.

Material and methods

All available tissue material from the 3 tumours, was cut subserially for light microscopy and DNA flow cytometry after being fixed with neutral formalin and embedded in paraffin according to routine laboratory methods. The histological sections were reviewed independently by 2 pathologists (P.J.K. and K.A.A.).

DNA flow cytometry was done as described earlier (Hedley et al. 1983). In brief, 50 micrometer sections were cut, deparaffinized, digested with pepsin and stained with propidium iodide. A 5 micrometer control section was cut for light microscopy immediately adjacent to the 50 micrometer section used for DNA analysis to confirm that tumour tissue was analyzed. Nuclear DNA content was analyzed with a FACStar flow cytometer (Becton-Dickinson Immunocytometry Systems, Mountain View, CA). A 488 nm argon ion laser line run at 600 mW was used for fluorescence excitation. A 585 ± 42 nm band-pass filter was used in front of the red photomultiplier to block the laser light. For each histogram 20000 particles were analyzed. Eleven 50 micrometer sections from the adrenal ad-

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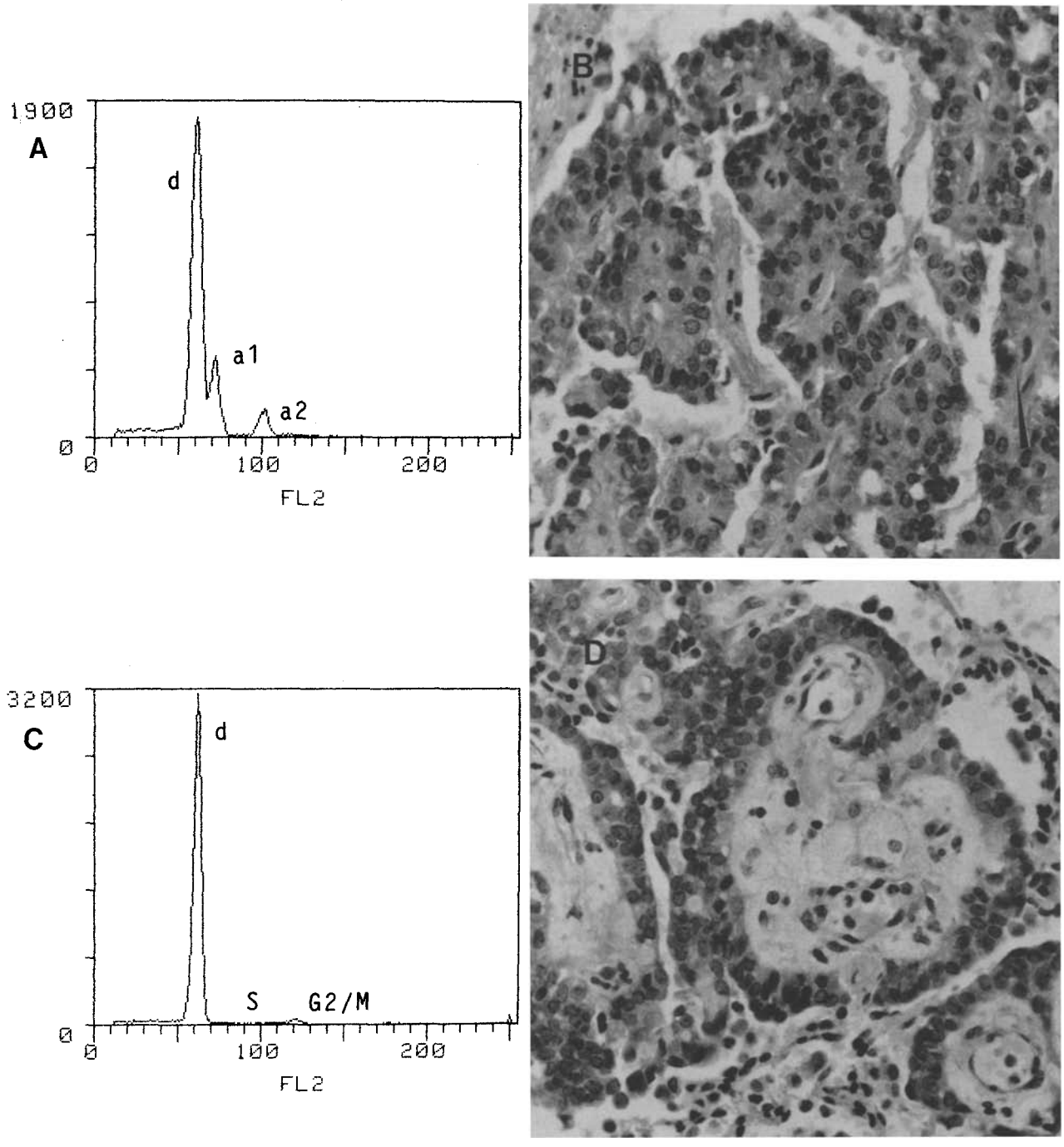

Fig. 1. Examples of DNA histograms and histological sections of a pancreatic cystadenoma with 3 co-existent stemlines of cells with different nuclear DNA content (case 1). The histological control section of histogram (**A**) is shown in (**B**), and that of histogram (**C**) in (**D**). In DNA histograms, the number of analyzed particles is given on the vertical and DNA fluorescence (*FL2*) on the horizontal axis. Although both in sections (**B**) and (**D**) histology is essentially similar, the DNA content analysis from section (**B**) revealed 2 aneuploid stemlines of cells (*a1* and *a2*, modal channel numbers 73 and 103, DNA indices 1.18 and 1.66, respectively) in addition to the diploid peak (*d*, modal channel number 62), whereas only the diploid peak was seen in the analysis from section (**D**). *S* and *G2/M* are the corresponding phases of the cell cycle (in C 3.9% and 2.4%, respectively). Van Gieson × 500

enoma, and 2 from the parathyroid and pancreatic adenoma were analyzed for DNA content. All samples were analyzed twice using the double-run technique to exclude the possibility of artifactual peaks generated during flow cytometry (Joensuu and Klemi 1988).

In histogram analysis the peak with the lowest DNA content was taken as the diploid peak, and the DNA index (DI) was calculated by dividing the modal channel number of an aneuploid peak by the modal channel number of the diploid peak.

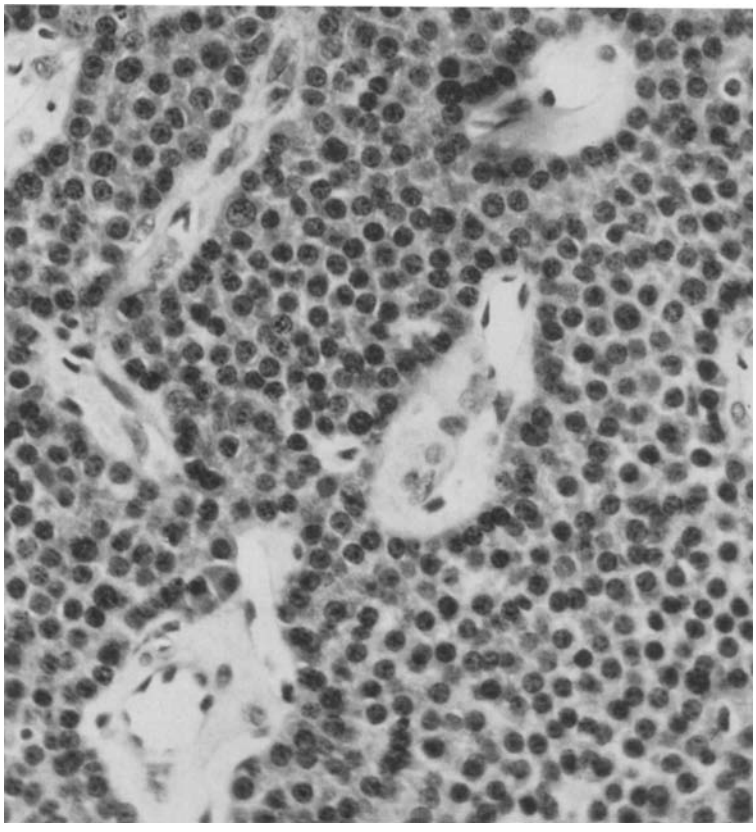

Fig. 2. A histological section of a parathyroid adenoma (case 2). The tumour consists of small, roundish cells in a fibrous stroma. No atypia or mitotic figures are seen. van Gieson × 500

Case reports

Patient 1. A 26 year-old previously healthy woman had laparotomy because of a tumour in her right epigastrium in January 1975. A 10 cm diameter, partly cystic tumour was totally removed from the head of the pancreas by a simple excision. Histologically the tumour is a papillary cystadenoma with few mitoses and no signs of invasion (Fig. 1). No tumour recurrence or metastases have been discovered during the follow-up of 10 years.

The DNA histogram had 2 aneuploid peaks with DIs 1.18 and 1.67 in addition to the diploid peak. In an analysis from another part of the same tumour, only 1 diploid stemline was found despite the fact that the histological control section contained similar adenoma tissue (Fig. 1).

Patient 2. A 55 year-old previously healthy woman had sudden pain in the left flank, and ureteral calculi were suspected. fS-Ca was clearly elevated 3.7–3.9-3.8 mmol/l (ref. range, from 2.3 to 2.6 mmol/l), fS-Pi low 0.8-0.5-0.5 mmol/l (ref. range, from 0.7 to 1.3 mmol/l), P-Cl was 110 mmol/l (ref. range, from 100 to 108 mmol/l), and S-parathormone 2.4 microgr/l (ref. range, from 0.22 to 0.50 microgr/l) suggesting hyperparathyroidism. A 3 cm diameter encapsulated tumour was excised from the area of the thyrothymic ligament below the right thyroid lobe at neck surgery in May 1980. S-Ca returned to normal after surgery, and no tumour recurrence or metastases were seen during regular follow-up visits until May 1985. According to a postal inquiry, the patient was alive 8 years after surgery in 1988.

A well encapsulated neoplasm is seen in paraffin sections, with little cell atypia, no mitoses or capsular invasion. The histology is compatible with a parathyroid adenoma (Fig. 2). In flow cytometric analysis two aneuploid DNA stemlines with DIs about 1.4 (1.35 and 1.46 in repeated analyses) and 2.0 (1.96 and 2.00) were seen in addition to the diploid peak (Fig. 3).

Patient 3. A child born in September 1975 developed skin pustules, greasy hair, enlarged penis, acne, and increased pubic hair after his first birthday. S-testosterone 7.5-6.4–9.5 nmol/l and diurnal U-dehydroepiandrosterone 20.6-19.6–26.6 micromol/l were repeatedly elevated, but P-cortisol morning and evening levels were normal. A tumour above the right kidney was seen on intravenous urography and angiography. A well encapsulated suprarenal tumour, 7 cm in diameter and about 200 gr in weight, was excised at laparotomy in May 1977. The tumour was not attached to the neighboring organs, and no ascites or metastases were seen. The histological diagnosis was adrenal adenoma, and no further therapy was given. All signs of virilization disappeared after surgery, and the laboratory tests returned to normal. No tumour recurrence was noted in regular follow-up visits during 8 years following surgery, and according to a postal inquiry, the patient was alive 11 years after surgery in 1988.

Severe cellular atypia and frequent mitoses including some atypic mitoses were seen on microscopy (Fig. 4). However, no capsular or angioinvasion could be demonstrated in any of the sections investigated.

In DNA analysis 2 stemlines with an abnormal DNA content were seen, in addition to the diploid peak (Fig. 5). The DIs of the aneuploid peaks were about 1.5 (range, from 1.44 to 1.54 in different analyses) and 2.8 (range, from 2.70 to 2.95). A diploid DNA histogram was obtained from a histologically

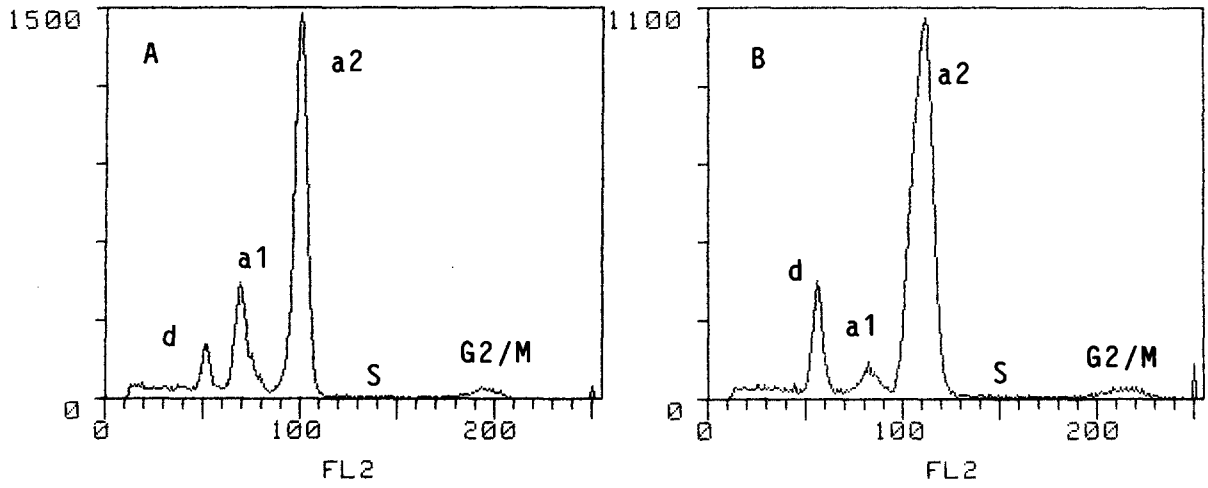

Fig. 3. DNA histograms obtained from a parathyroid adenoma (case 2). The lettering and axes are as in Fig. 1. (**A**) 2 aneuploid stemlines *a1* and *a2* (modal channel numbers 69 and 100 with DNA indices 1.35 and 1.96, respectively) are seen in addition to the diploid peak (modal channel number 51). *S%* of *a2* is 3.7 and *G2/M%* 4.7. (**B**) In another analysis of the same adenoma 2 aneuploid peaks (modal channel numbers 82 and 112, DIs 1.46 and 2.0) were once more demonstrated. *S%* of *a2* is 3.7 and *G2/M%* 5.0

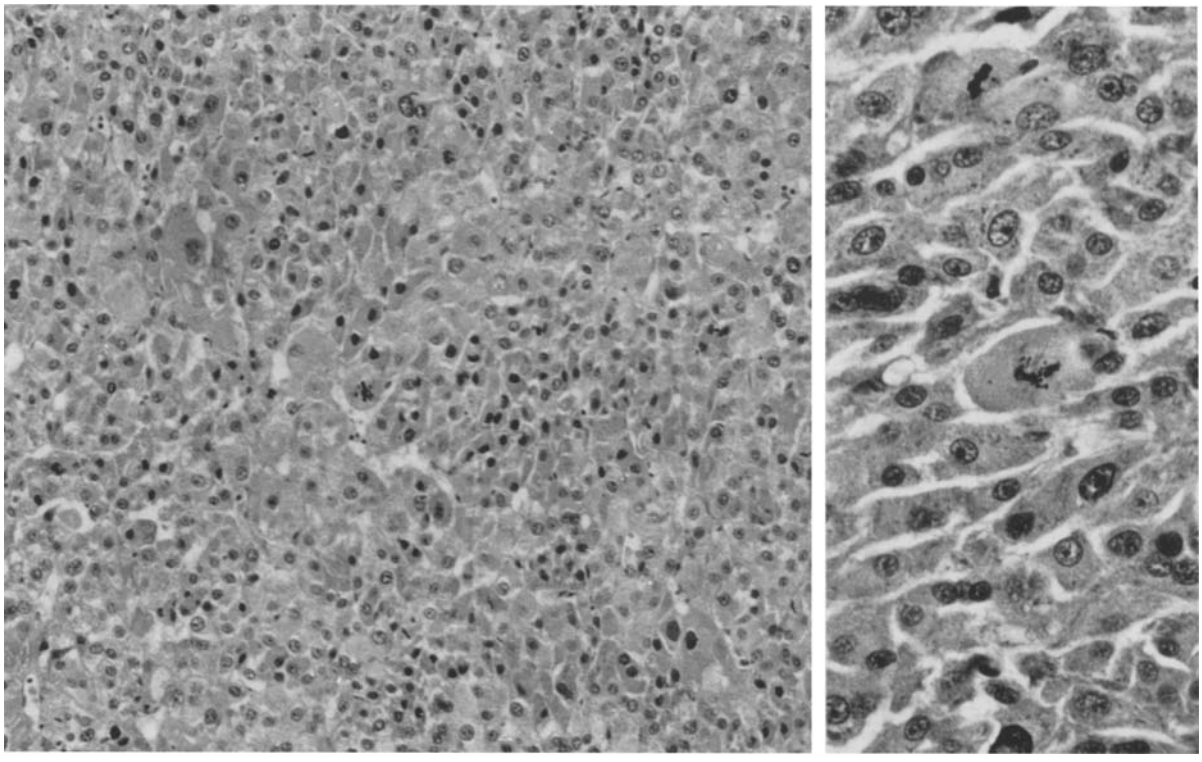

Fig. 4. A histological section of an adrenal adenoma (case 3). The tumour consists of pleomorphic cells with numerous mitotic figures. van Gieson (× 200 and × 500)

benign abdominal lymph node removed together with the adrenal tumour (Fig. 5D). In multiple DNA analyses of the neoplasm, either the 1.5 or the 2.8 stemline was present together with diploid cells, and in some sections all 3 peaks were present simultaneously (Fig. 5).

Discussion

DNA stemline heterogeneity in different parts of the same tumour has been found in several types of human cancer (Ljungberg et al. 1985; Hidde-

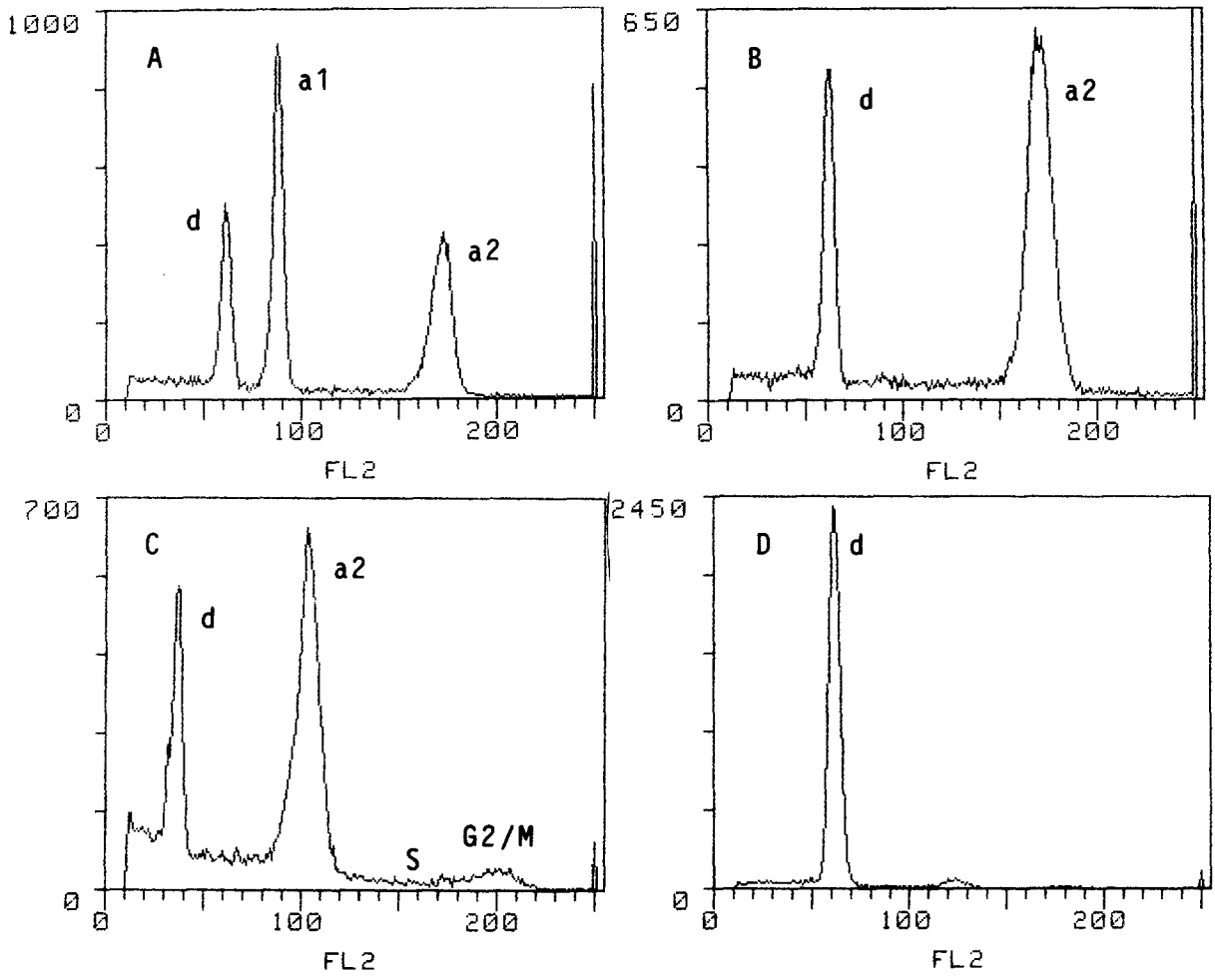

Fig. 5. Examples of DNA histograms produced from an adrenal adenoma (case 3). (**A**) 2 aneuploid stemlines *a1* (modal channel number 88) and *a2* (channel 173) with DIs 1.44 and 2.84 are seen in addition to the diploid peak (*d*, channel 61). (**B**) Only *a2* (modal channel number 169, DI 2.73) can be seen in addition to the diploid peak (channel 62). (**C**) By changing photomultiplier settings the diploid peak was adjusted on channel 37, and the corresponding *S* and *G2* phases of *a2* (channel 103, DI calculated as 2.78) could be seen. *S* phase fraction of *a2* is 11.6% and *G2/M%* 7.7, but cell debris is also present making the calculations uncertain. (**D**) A diploid histogram obtained from a histologically benign abdominal lymph node excised simultaneously with the adenoma

mann et al. 1986; Hiddemann et al. 1987). DNA multiploidy has also been described in some conditions generally regarded as premalignant, since Stenzinger et al. (1984) have described the occurrence of 2 aneuploid stemlines in addition to a diploid stemline in 2 large congenital melanocytic nevi, and van den Ingh et al. (1985) found 2 aneuploid stemlines with DIs 1.17 and 1.84 in a colorectal tubulovillous adenoma with severe atypia. To our knowledge DNA multiploidy has not been previously described in benign adenomas.

By some criteria (van Slooten et al. 1985), which may be debatable, the large adrenal neoplasm of patient 3 with numerous mitoses and severe atypia might also be classified as adrenal carcinoma or as a premalignant condition. Because no sign of invasive growth was seen either histologically or at surgery, and since no tumour recurrence or metastases developed during the follow-up, the original diagnosis of adenoma was not changed to carcinoma. The tumour was excised at the age of 19 months, and assuming a constant growth rate, it should have recurred sooner than 28 months after surgery (9 months' gestation period added to 19 months). Therefore, a 96-month regular follow-up time is likely to be sufficient, and the child has apparently been cured by the simple excision of the well encapsulated tumour. However, there is no reason why the other 2 cases now described should be considered as premalignant, since they were both histologically and clinically typical benign adenomas.

A diploid histogram was obtained in addition to a histogram with aneuploid stemlines from the adenoma tissue of patient 1 (Fig. 1), indicating that some adenoma cells had diploid DNA content. Thus, in this case at least 3 co-existing stemlines with DIs 1.00, 1.18 and 1.66 were present in the same benign adenoma. The diploid cells seen in histograms prepared from the other 2 adenomas may represent either diploid adenoma cells or stromal cells and blood leukocytes, or both.

In conclusion, the present cases indicate that all human neoplasms with multiploid DNA content do not fulfil the present histological or clinical criteria for malignancy. DNA multiploid adenomas may be cured by conservative surgery. DNA stemline heterogeneity may occur in human adenomas, and DNA multiploid adenomas with large DNA indices may be hormonally active.

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