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DNA-Flow Fluorescence – Cytometry of Ependymomas

Report on Ten Surgically Removed Tumours*

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Summary. The distribution of DNA is estimated from flow cytometric histograms in surgical specimens of ten ependymomas of different location and varying anaplasia. In three cerebral tumours grade I–II, including one ependymoma of the 4th ventricle, only limited elevation of the 4 C maxima was a prominent feature, corresponding to the microscopical frequency of typical mitoses. Four grade-III ependymomas showed aneuploid or polyploid histograms with stem lines. One frontal tumour was classified as "transitional" because of more numerous mitoses and abnormally elevated S and G2 + M phases, which increased in tissue culture. A correlation between the degree of anaplasia with the DNA pattern was difficult to pursue in two spinal ependymomas obviously lacking microscopical mitoses: Both – one a so-called tanycytic variant of grade I–II, and the other probably a metastasis from a cerebellar tumour – had a clear polyploid DNA histogram with a strikingly increased proliferation index, similar to the more malignant tumours of grade III. Also flow DNA measurements probably allow the decoding of heterogenous mixtures of tumour cells which are not always benign in ependymomas of lower grades of anaplasia microscopically.

Key words: DNA – Ependymomas – Flow fluorescence – Cytometry – Tissue culture

Introduction

Ependymomas, as is well known, orignate from cells that possess an obvious potential to develop characteristics of the primordial epithelial type, which normally lines the inner surface of the neural tube. According to their site, they are classified as supra- or infra-tentorial, spinal, or caudal tumours (Ringertz and Reymond 1949). Their frequency takes the sixth place of the gliomas (Henschen 1955; Zülch 1956, 1958; Rubinstein 1972; Zülch and Mennel 1974; Pia 1977; Russell and Rubinstein 1977). In their majority they are well-differentiated benign neoplasms characterized by an expanding growth. Merely 5% of the histologically proven cases show signs of striking anaplasia by use of a 3-grade scale of malignancy (Fokes and Earle 1969; Russell and Rubinstein 1977; Zülch 1979; Arendt 1974).

Hence, there are few reports which deal with the examination of the DNA content of these tumours as compared with most other human gliomas. Müller et al. (1977) cited that there had been no publications on this subject until 1977. They reported extensive DNA measurements with a microdensitometrical evaluation of Feulgen-stained cell nuclei (Böhm and Sandritter 1975) of ten ependymomas and two choroid plexus papillomas. Euploidy was not demonstrable though they examined six well differentiated low-grade tumours as contrasted with two anaplastic ependymomas, grade 3, exhibiting abnormal polyploidy. Subsequently, Lehmann and Krug (1980) used flow fluorescence cytophotometry (FCM) to examine 60 brain tumours, five of which were ependymomas in children and adults. They found DNA karyohistograms of a normal diploid type in three tumours grade I–II, whereas one grade III ependymoma showed polyploidy. In one further case, however, an unexpected abnormal tetraploid DNA pattern was detected in a histologically benign tumour.

The differences between these reports are difficult to explain because the methods were not the same.

Thus, there are some problems in understanding the relations between the DNA content of ependymomas and their prognosis because of the histological lesions which are demonstrable in each

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Table 1. Ependymomas

No.	Sex	Age	Localisation	Grade	DNA histogram	Op. Tumor specimen/Tissue cult.							Survival time
						G0/G1 (%)	S (%)	G2+M (%)	S (%)	G2+M (%)	PI-a (%)	PI-b (%)[a]	
1	f	30 yr	IInd ventricle	I–II	Diploid	92.4	5.32	2.22	–	–	7.54	–	3 d
2	f	2 yr	IVth ventricle	I–II	Diploid	93.9	5.9	0.2	–	–	6.1	–	1.9 yr
3	f	45 yr	Frontal	I–II	Diploid	95.7	2.6	1.7	–	–	4.3	–	2.6 yr (dead)
4	m	42 yr	Frontal	II–III	Diploid; elevated prolif. phase partic. in TC	87	7.4	5.6	15.3	13.6	13	28.9	1.8 yr (alive)
5	m	18 yr	IVth ventricle	III	Diploid-hyper-tetraploid	24.13	2.72	73.15	–	–	75.87	–	2 d
6	m	21 yr	Frontal	III	Diploid; hyperdiploid; octaploid (polyploid)	22.25	7.8	69.95	–	–	77.75	–	1.9 yr
7	f	42 yr	Occipital	III	Diploid; hyperdiploid; octaploid (polyploid)	22.5	26.58	50.92	11	61	77.5	75	1.5 yr
8	m	14 yr	Occipital (Tu-reced.) post-radiat.	III	Tetra-octaploid (polyploid)	3.46	6.44	81.82	24.55	69.12	96.54	93.67	2.6 yr
9	f	33 yr	Sacral-metast.	I–II	Tetra-octaploid	18.1	4.86	77.13	–	–	81.99	–	1.2 yr
10	m	32 yr	Cervical	I–II	Diploid-tetra- and octaploid (polyploid)	20.1	6.66	63.25	–	–	69.91	–	2 wk (dead)

[a] PI-a = Proliferation index from fresh tumor tissue
PI-b = Corresponding values from tissue culture

individual. Hence, we wish to contribute our FCM observations on ten ependymomas surgically removed from variable sites; some of the tumours we could compare subsequently with the DNA distribution in their primary tissue cultures.

Material and Methods

The tumour samples were obtained surgically from ten patients of the neurosurgical department during a 2-year period. In each case, the microscopical diagnosis and FCM examination was performed immediately after the craniotomy (Ahyai et al. 1983). Cell cultures were also prepared, for later cell nuclei isolation, within 1.5 h. According to the method described by Dittrich and Göhde (1969) the staining for DNA was performed with a solution of ethidium bromide, pH 7.5, after RNAse pre-treatment. Primary tumour cell monolayers were cultured from 1 to a maximum of 4 weeks by using medium TC 199 with fetal calf serum. At different intervals the DNA karyograms of the tissue culture cells could be compared with the DNA histogram of the corresponding fresh tissue specimen taken at craniotomy. The cultivated cells were fixed in 96% ethanol in falcon-flasks. Trypsination was avoided. Instead, a 30-min pepsination followed by RNAse treatment was applied regularly. The DNA content of cell nuclei was measured in an ICP-11 (PHYWE, Göttingen) or an ICP-22 (ORTHO-Instruments), respectively. Normal mouth epithel cells, harvested daily from the same individual, served as a standard reference for the determination of diploid or heteroploid DNA equivalents. Analysis of the relative DNA quantities within the histogram curves based on the model of Baisch et al. (1975) which permits a practical adequate assessment of the separate sections in the histograms. Hence, a (semiquantitative) synopsis of the most interesting relative portions of the cell cycle phases concerning at least 8,000–10,000 cell nuclei per measurement was available.

Results

Our observations are summarized in Table 1. The FCM data of eight cerebral and two spinal ependymomas, classified in a 3-grade scale of histological malignancy, are detailed. In cases 1–3 two cerebral and one infra-tentorial ependymomas grade I–II had a normal diploid DNA pattern with G0/G1 peaks around 92%–95%. The G2 + M maxima in the cerebral tumours were up to 2.22%, whereas the G2 + M portion of the infra-tentorial ependymoma was only 0.2%, a relatively low value due probably to the infrequency of mitoses.

The S-phase values did not extend to an extraordinary height, but reached the upper limits of normality in two cases. Unfortunately, for technical reasons the control of the three tumours in tissue culture was not possible. Hence, we introduced in Table 1 the so-called proliferation index (PI), as proposed by Feichter et al. (1983). It corresponds with the coefficient between the rate of $G2 + M + S \times 100$ divided by the total sum of $G0/G1 + S + G2 - M$.

According to Feichter et al. (1983) the PI is a parameter of the quantity of cells which are on the way to divide, including those in the stage of mitosis. The three mentioned cases showed some degree of malignancy varying between grades I–II. The highest PI is that of the supra-tentorial ependymoma of case 1 which showed some hypercellularity and polymorphism of cell nuclei demonstrating a variable chromatin network and scattered mitoses. The latter could not be found in case 2, but were also seen in some areas of case 3 (Fig. 1) which presented the lowest PI.

A histologically transitional type of a supratentorial ependymoma grade II–III is represented in case 4 (Fig. 2). This tumour exhibited a diploid DNA histogram with abnormally increased S and G2 + M phase values; the latter corresponds probably to the more frequent incidence of mitoses. The same peaks are manifested in the tissue culture controls, extending the PI 3–4-fold which contrasts with the above mentioned first cases of three ependymomas grade I–II.

Our cases 5–8 can be summarized in a further group; they present with drastic decreases of their G0/G1 proportions in favour of the considerably enhanced G2 + M peaks and the abnormally elevated S phase values. This was also found in the primary tumour cell cultures (cases 7, 8). Figure 3 presents the polyploid and aneuploid DNA histograms from the tumours of cases 6, 7 and 8 corresponding to three supra-tentorial ependymomas grade III. Microscopically, there were striking cell nuclear polymorphism, mitoses, giant cell formation, necroses, and vascular proliferations. One relapsing tumour (case 8) presents the lowest G0/G1 peak, with elevated PI in the whole series and a very high S phase value in tissue culture. This is probably the result of the foregoing therapeutic X-ray irradiation. The PI of the remaining three ependymomas grade III (cases 5–7) accounted for more than 70%. In the surgical samples it surpasses 77%.

The tumour cells of the tissue cultures reached their maximum PI of 93.67% in case 8. This tumour showed also a clear parallelism in vitro with the DNA distribution in the freshly removed surgical specimen. The striking increase of the S phase values in tissue culture cells contrasts with the S phase of the surgical sample, independently of the rather stable G2 + M proportions. Thus, an accumulation of pre-mitotic nuclei or a relative decrease of G2 + M phase cells may have its reason probably in some kind of synchronizations. Cases 9 and 10 of Table 1 were patients of approximately the same age. Both presented intra-spinal ependymomas of a similar differentiation. There were abnormal peaks in 4 C, altough no mitoses could be detected microscopically. However, case 10 being a tanycyctic variant of

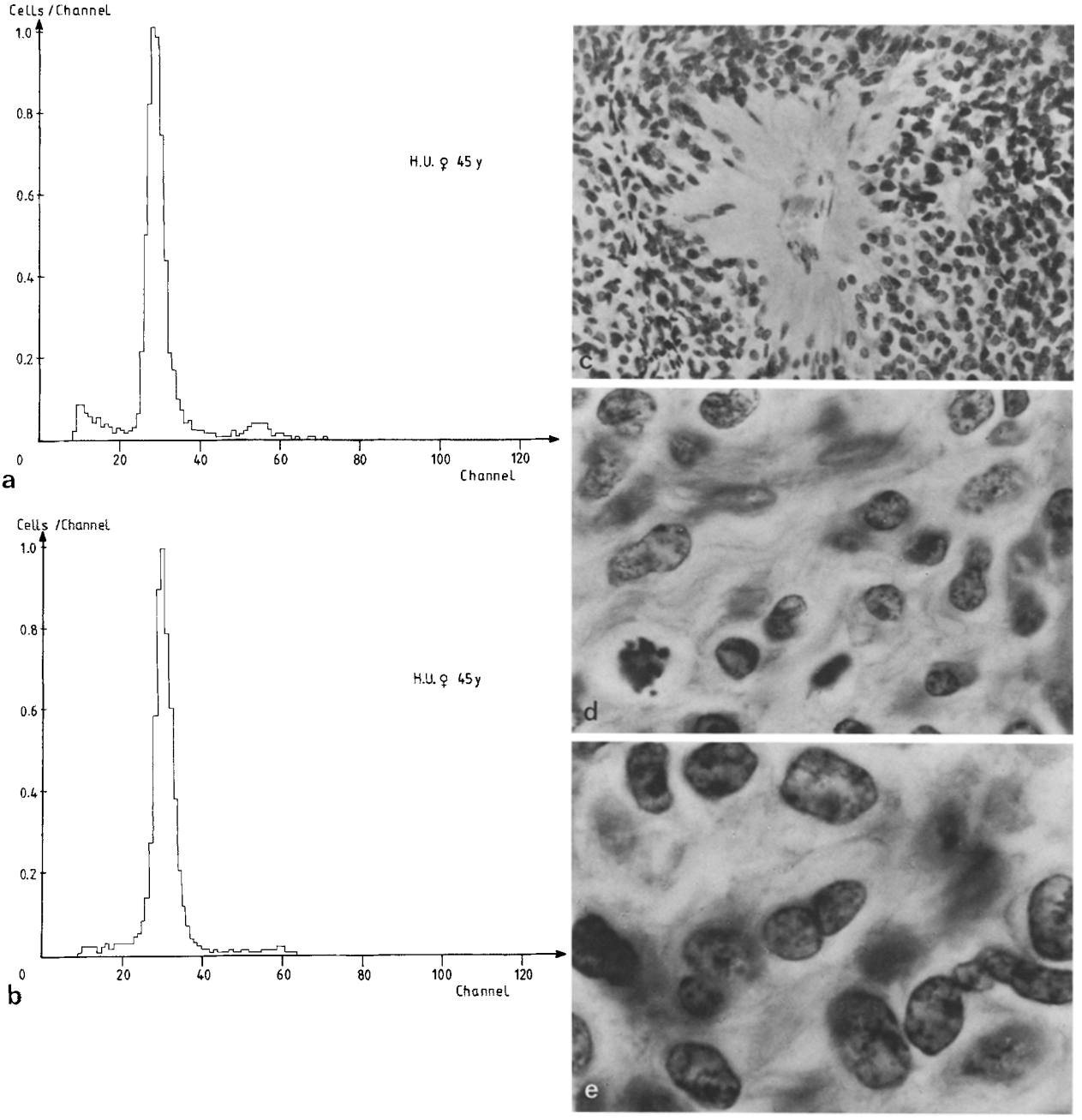


Fig. 1a–e. *Case 3:* woman, aged 45 years. Supra-tentorial "cellular" ependymoma grade I–II. Diploid DNA pattern. The small 4 C-peak may vary to some extent in the tissue specimen when two samples of the same tumor (**a** and **b**) are compared. The incidence of mitoses is in agreement with a grade I–II ependymoma of cerebral (frontal lobe) site. Microscopically clear variation of nuclear configuration and chromatin network (**c–e**)

ependymoma (Friede and Pollak 1978) showed a clear polyploid histogram with increased G2 + M maxima and a PI of 69.95% max. These peaks differed considerably in case 9, which presented a fairly well circumscribed tumour which had spread to the lumbar region, after a cerebellar ependymoma had been removed 2 years before (Fig. 4a), followed by therapeutic X-ray irradiation. The short survival period of only 2 weeks in case 10 was caused by post-operative complications. A remarkable feature of both spinal tumours is the occurrence of an abnormal octaploid peak, indicating aneuploid stem lines, although no evidence of anaplastic changes or cytological de-differentiation – apart from a variation in nuclear size and of chromatin networks – was detected (Fig. 4b).

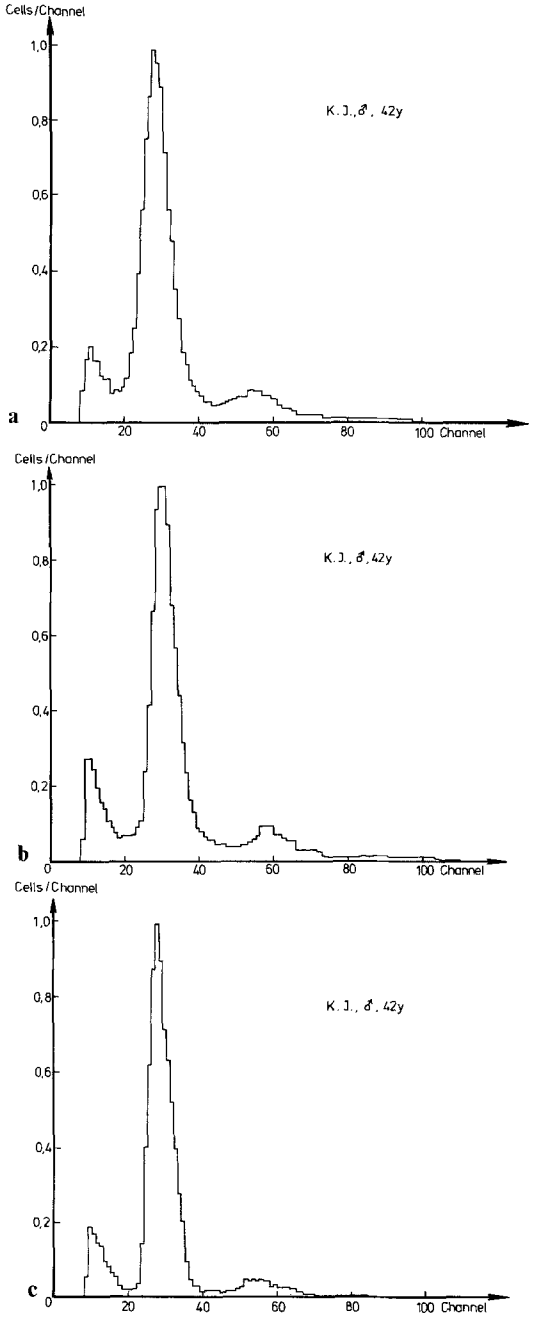


Fig. 2a–c. *Case 4:* male, aged 42 years. Supra-tentorial "cellular" ependymoma, "transitional type" grade II–III. The diploid bimodal pattern of DNA distribution is stable during a 3-week tissue culture with slow decrease of 4C and S phase peaks due to reduced quantity of tumour cells. **a** Surgically removed tumour tissue. **b** Tissue culture day 10. **c** Tissue culture day 20

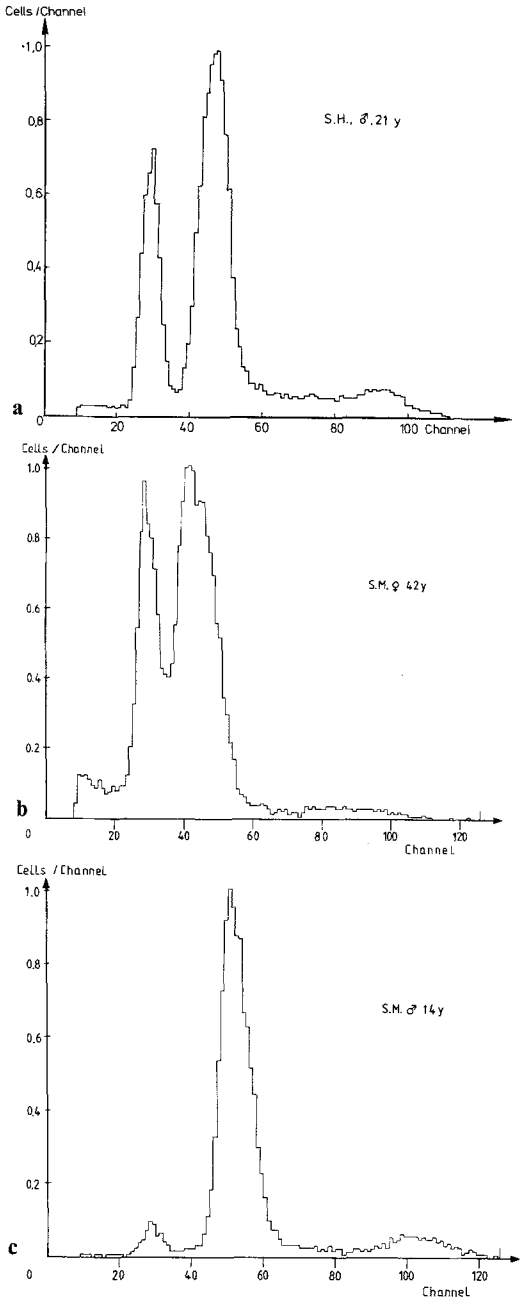


Fig. 3a–c. DNA distribution in 3 supra-tentorial ependymomas grade III. **a** *Case 6:* polyploid and aneuploid DNA pattern. **b** *Case 7:* aneuploidy with a marked hypotetraploid peak. **c** *Case 8:* aneuploidy with very decreased 2 C peak, but increased 4C. The values of tissue culture cells were similar for each case. Case 8 was a relapsing ependymoma after X-ray irradiation, probably the reason for diploid cell reduction

Discussion

Ependymomas exhibit considerable histocytological variation, as has been described for tumours of different site. Those located near the foramen Monroi, in the cerebello-pontine region, the spinal cord dorsal column or the myxo-papillary types of the filum terminale are examples described in the literature (Zülch and Kleinsasser 1957). With regard to patterns of de-differentiation, the occurrence of anaplasia grade III is chiefly manifested in the supra-tentorial

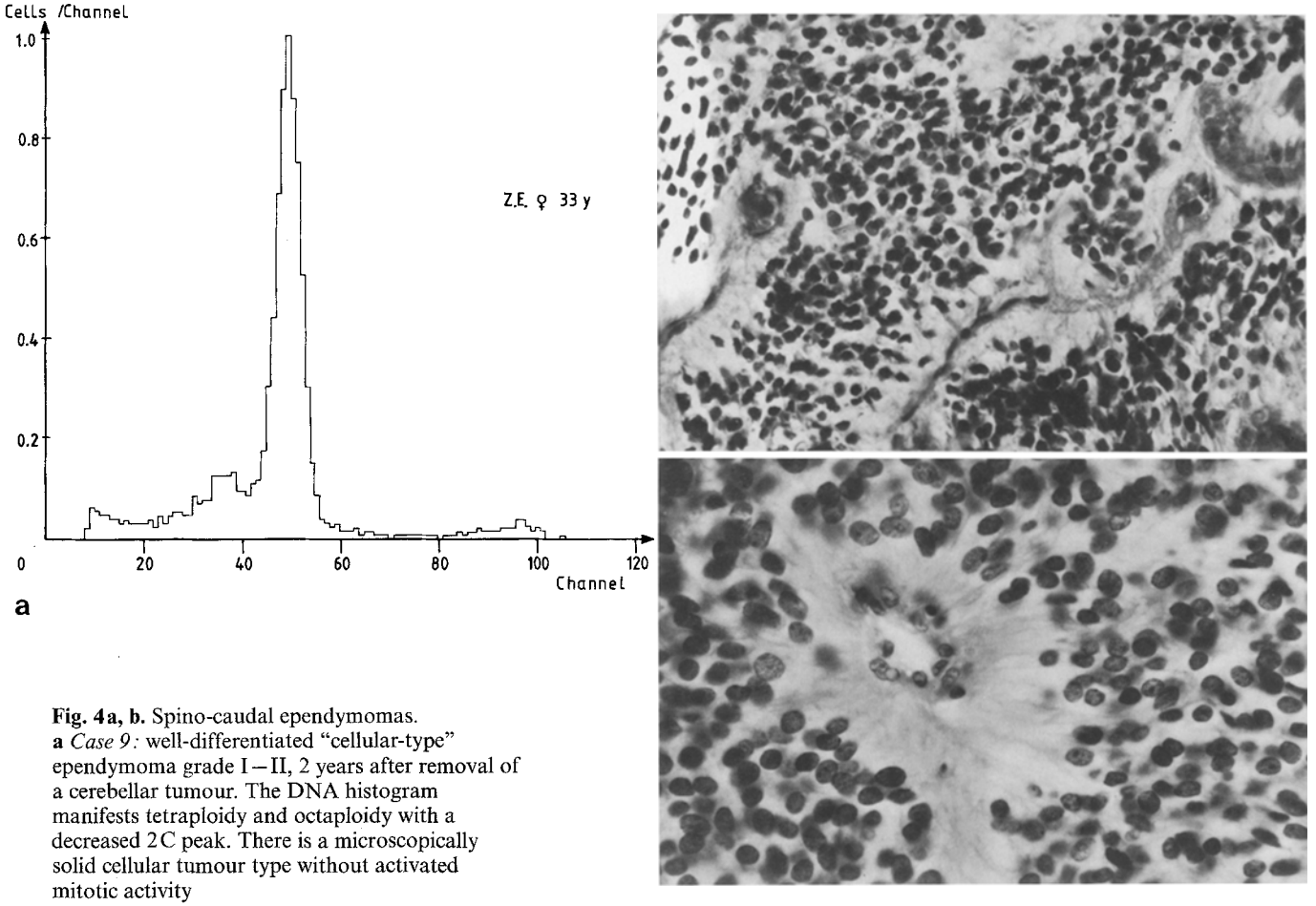
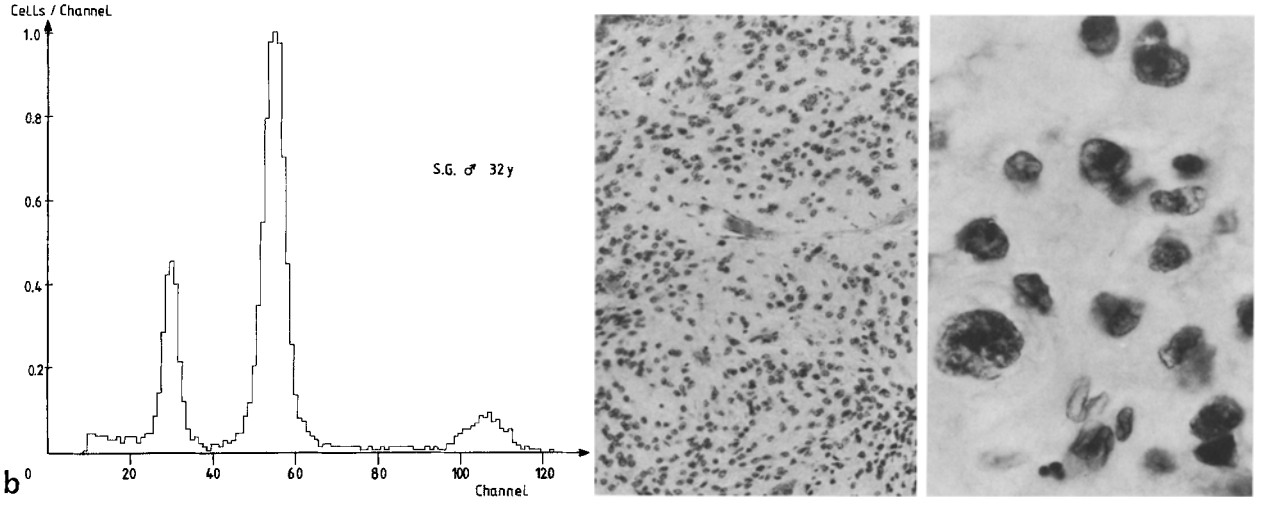


Fig. 4a, b. Spino-caudal ependymomas.
a *Case 9:* well-differentiated "cellular-type" ependymoma grade I–II, 2 years after removal of a cerebellar tumour. The DNA histogram manifests tetraploidy and octaploidy with a decreased 2C peak. There is a microscopically solid cellular tumour type without activated mitotic activity



b *Case 10:* intramedullary (cervical) ependymoma of a tanycytic variant grade I–II presenting a few enlarged or hyperchromatic nuclei in some areas (HE). The DNA pattern is polyploid showing stem lines in 4C and 8C

ependymomas (Arendt 1974), whereas changes up to grade II can be met in both supra- and infra-tentorial tumours. As a concomitant feature, originally stressed by Zülch (1958), the incidence of microscopical mitoses can be established preferably in the ependymomas of cerebral location. This statement seems to offer a good opportunity to match the patterns of DNA content detected by FCM in tumours of different location and to compare the relation to their histological conditions.

Indeed, the cerebral tumours of cases 1 and 3 show G2 + M peaks, 8–11-fold higher than the infratentorial IVth ventricle tumour of case 2 which is probably due to a lack of mitoses in the latter. This view is supported by the ependymoma grade II–III in case 4, which exhibits an abnormal increase of S phase and G2 + M peaks in the presence of numerous mitoses. Generally the series of ependymomas, reported here, proves by no means homogenous, neither concerning their location nor with regard to their various degrees of differentiation and the relation between cytohistology to DNA patterns. Although we present a rather small collection, we can conclude from our data that polyploidy and aneuploidy with various manifestation of stem lines, are seen typically in the four ependymomas grade III, where these stem lines are stable during primary tissue cultivation. One of these ependymomas occurred in a young man (case 5) and expanded in his IVth ventricle. This hypercellular, widely vascularized tumour showed some necrotic areas, but no striking cell polymorphism and relatively few mitoses, whereas the nuclear chromatin networks varied markedly in size and density. An unquestionable 4 C-peak elevation is followed by a largely increased peak in channel 69. Hence, the DNA histogram supplies more information on the presence of dubious cell clones manifested in the hypertetraploid stem line than the histological tissue preparations. A striking feature is the extraordinary decrease of the G0/G1 peak in the surgically removed relapsing occipital ependymoma of case 8, whereas the S phase in the tissue culture control increased up to 24.55%! This may be due to a partial synchronization as a possible result of antecedant therapeutic X-ray irradiation. A considerable increase of S phase and G2 + M during tissue culture has been demonstrated also in case 4, which had no irradiation or cytostatic therapy, probably due to an unfolding of proliferative activity. A quite obvious discrepancy is demonstrable for our two spinal tumours. In both (cases 9 and 10), polyploid DNA karyograms could be well established. The G2 + M peaks and the PI show strikingly increased values comparable to the group of ependymomas of grade III, although no similar signs of anaplasia were demonstrable. Under such circumstances it seems also reasonable to suppose that the FCM measurement of DNA distribution is superior in the documentation of heterogeneous, possibly not benign tumour cells which are very difficult to categorize on the basis of their microscopical characteristics.

In general, the detection of varying patterns in the DNA content of ependymomas reported first by Müller et al. (1977) can be confirmed from the results of our FCM studies. Reviewing, however, the data of the most similar cases, the conclusion may be drawn that there are probably two subgroups in our series: One comprises the tumours of histological grade I–II which show a diploid DNA distribution obviously common to the majority of benign or low-grade blastomas; the other refers to malignant grade III tumors showing clear hetero- and polyploid DNA patterns. The question why the latter are nonmandatory in the "transient" ependymomas grade II–III, and why, on the other side, polyploid and stem lines can be a surprising feature in spinal ependymomas of low-grade I–II, must remain open. Lehmann and Krug (1980) reported a well-differentiated supra-tentorial ependymoma with clear polyploidy. Furthermore, they were able to show evidence of a small hypertetraploid cell population dispersed in an otherwise normal karyogram of a young patient who survived a triplicate craniotomy in a very good fashion. Those observations can support the results under discussion and our conclusion that a correlation between the prognosis of ependymomas and their location in the CNS must not be predetermined. The demonstration of heterogenous tumour cell clones, however, within the karyograms points probably to an inherent potency for development of malignancy also in low-grade ependymomas. Hence, the DNA analysis by means of FCM can furnish useful supplement for the assessment of tumour prognosis. Its correlation with the ependymomas of various clinical course, however, needs further elucidation.

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