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Impulse cytophotometric DNA analysis in pituitary adenomas

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Abstract

Flow cytometric DNA analysis was carried out on 32 microsurgically removed pituitary adenomas. Additionally, the histograms of tumor cell nuclei of 7 patients were compared with those of the cultured cells from the same tumor samples.

The tumors were classified into 3 groups according to the proliferation index (PI) of the flow cytometric results: 1) tumors with DNA patterns of slow proliferation (PI under 10), to which the majority of the examined pituitary adenomas belonged; 2) pituitary adenomas with diploid karyograms and PI values from 10 to 15; 3) diploid or aneuploid karyograms with PI values above 15.
The third group were characterized histologically by increased chromatin content, nuclear polymorphism, mitoses, and extrapituitary infiltration of the tumor cells, and were, therefore, no longer benign. However, there was no direct relationship between the intensity of hormone secretory activity of the tumors and DNA ploidy. Cultured adenoma cells examined by flow cytometry remained stable in all cases but one.

Keywords: DNA analysis, flow cytophotometry, pituitary adenoma

1 Introduction

The classification of pituitary adenomas has been based on clinical findings in combination with conventional histological parameters. The introduction of electron microscopic and immunohistochemical methods has prompted more precise classification of the tumors, identifying the secretory hormone granules produced by tumor cells [9, 12, 16].
Flow cytophotometry (FCM) is a fluorescence method for serial examination to rapidly obtain information on DNA ploidy and the proliferative activity of tumor cells by measuring their nuclear DNA content during the cell cycle. Pathological proliferation and anaplasia of the tumor cells are indicated by increased impulses in the area of the S and G2+M phases, or by occurrence of poly- and/or aneuploid DNA patterns.

There are only a few reports on FCM investigations of pituitary adenomas [2–5, 13–15]. The correlations between DNA distribution in FCM, histological findings, and clinical parameters of pituitary adenomas examined on 32 patients will be reported here.

2 Material and methods

FCM analysis was performed on 32 cases of pituitary adenomas. The adenomas were microsurgically removed using the transsphenoidal approach. The mean age of the patients was 53.4 years, the youngest being 23 and the oldest 74. The sex ratio was 18 males to 14 females.

Patients were investigated with regard to endocrinological type of tumor, serum hormone levels, reactivity of thyroxin releasing hormone (TRH), glucose test, visual acuity and fields, X-ray and CT-scan of the sella turcica. In selected cases, carotid angiography was performed. Pituitary tissue obtained from two patients, one with metastatic breast cancer and one with prostate cancer (no metastases in the pituitary gland) served as controls. Histological and immediate FCM DNA analysis was applied to all specimens. In 7 cases, primary cell cultures were performed in addition.

For histological examinations, formalin-fixed paraffin sections were stained with hematoxylin-eosin (HE), trichrome-PAS [17], and reticulin (Gomori). Immunohistochemically, the hormone granules were identified using the peroxidase-antiperoxidase method [19] and a commercial antibody for human prolactin from DAKO, Denmark.

For tissue cultures, primary tumor cell monolayers were cultured in the medium TC 199 containing 15%

Table I. Summary of clinical cases with hormonal, histological and FCM results

No.	Age	Sex	Hormone-chemical analysis	Histological features	Flow cytometric results (relative peaks in %) HGR*	2C	4C	S	PI
1	27	f	cort., FSH, LTH & gonad. decreased	chromophobe	diploid	99.88	0.08	0.4	0.12
2	61	f	prolactinoma	chromophobe mixed with basophil cells	diploid	99.26	0.12	0.62	0.74
3	70	f	gonad. & thyr. decreased	chromophobe	diploid	98.52	0.85	0.63	1.48
4	44	m	prol. slightly increased; cort. decreased	chromophobe, recurrent after 10 years	diploid diploid diploid	98.41 98.7 96.06 99.22	1.4 0.85 2.03 0.58	0.19 0.45 1.91 0.2	1.59 1.3 (TC** 2 days) 3.9 (TC 7 days) 0.78 (TC 18 days)
5	58	f		chromophobe	diploid	98.2	1.1	0.7	1.8
6	74	f	inactive; Low T_3-syndrome	chromophobe & basophil	diploid	97.93	1.24	0.83	2.07
7	67	f	prolcatinoma	chromophobe	diploid	97.75 97.7	1.20 1.2	1.05 1.1	2.25 2.3
8	60	f	inactive	chromophobe; increased chromatin content, increased cell population	diploid	97.23 96.08	1.89 2.13	0.88 1.79	2.77 3.72
9	68	m	cort. decreased	chromophobe & eosinophil	diploid	97.75	3.15	1.1	4.25
10	61	m	inactive	chromophobe	diploid	95.68	3.57	0.75	4.32
11	47	m	prol. & FSH slightly increased	chromophobe, increased chromatin content	diploid	95.28	3.24	1.58	4.82
12	62	m	test., gonad., & cort. slightly decreased	chromophobe	diploid-hyperdiploid	95.08 88.46	2.63 6.76	2.29 4.78	4.92 11.54 (TC 21 days)
13	46	m	test. & cort. slightly decreased	chromophobe	diploid	95.41 95.64	3.29 3.14	2.3 1.22	5.59 4.36 (TC 8 days)
14	74	m	prolactinoma	chromophobe & eosinophil	diploid	94.36	4.79	0.85	5.64
15	58	f	TBG deficiency; low T_3-syndrome; cort. markedly decreased	chromophobe	diploid	93.30 93.1	5.14 5.85	1.56 1.05	6.70 6.70
16	60	m	prolactinoma	chromophobe	diploid	92.93 92.88 98.71	4.73 4.37 0.65	2.34 2.75 0.64	7.07 7.12 (TC 5 days) 1.29 (TC 22 days)
17	71	m	slight TBG induction	chromophobe, rare mitoses	diploid	94.75 98.50 93.51	5.38 1.25 4.66	1.87 0.25 1.83	7.25 1.50 (TC 5 days) 6.49 (TC 17 days)
18	53	f	prol. slightly increased	chromophobe, increased chromatin content, infiltration into surrounding tissue	diploid	92.70	4.6	2.7	7.30 (TC 10 days)
19	36	m	FSH markedly increased	chromophobe, increased chromatin content	diploid	92.34	5.14	2.52	7.66

No.	Age	Sex	Hormone-chemical analysis	Histological features	Flow cytometric results (relative peaks in %) HGR*	2C	4C	S	PI
20	45	m	marked TBG induction; cort. & prol. increased	infiltration; 2nd recurrence; post-irradiation	diploid	92.13	5.55	2.32	7.87
21	54	m	normal	chromophobe	diploid	92.10	5.25	2.65	7.9
22	53	m	GH increased; acromegaly	chromophobe & basophil; slight hyperchromasia	diploid	90.22	6.53	3.25	9.78
23	23	f	prolactinoma	chromophobe	diploid; moderate increase in 4C	89.45 89.7	8.49 8.19	2.06 3.11	10.55 10.30 (TC 36 days)
24	30	f	prolactinoma	chromophobe; increased chromatin content	diploid; moderate incrase in 4C	89.95	7.96	2.09	10.05
25	70	m	prolactin increased	chromophobe; moderate; polymorphism	diploid; moderate increase in 4C	89.90	8.22	2.68	10.90
26	62	m	prolactinoma	chromophobe; polymorphism sporadic mitoses	diploid; hyperdiploid; moderate increase in 4C	89.5	8.98	2.09	11.05
27	60	f	thyr. & prol. increased	chromophobe; moderate polymorphism; increased chromatin content	diploid; apparent increase in 4C	85.63	10.5	1.87	14.37
28	–	–	cort. & gonad. decreased; prol. increased	eosinophil, partly basophil; polymorphism	diploid; apparent increase in 4C	85.49	9.23	5.28	14.51
29	44	m	cort. & gonad. decreased; prol. increased	chromophobe; polymorphism; non-uniform chromatin content; pyknosis	diploid; apparent increase in 4C	84.61	10.18	5.21	15.39
30	52	f	prolactinoma	chromophobe; infiltration into surrounding tissue	diploid; apparent increase in 4C	84.23	11.18	4.59	15.77
31	48	m	test. decreased; FSH increased	chromophobe; polymorphism, mitoses recurrence	tendency toward polyploidy; apparent increase in 4C	81.38	11.25	3.62	18.62 (8C = 3.75)
32	50	m	low T_3 & T_4 syndrom; FSH, LTH & cort. decreased	chromophobe; polymorphism, mitoses; recurrence in 2 years	polyploid	27.83 65.84	64.56 21.74	3.29 12.42	72.17 (8C = 4.32) 34.16 (TC 16 days)

Abbreviations: cort. = cortisol, FSH = follicular stimulating hormone, GH = growth hormone, gonad. = gonadotropin, LTH = luteinizing hormone, prol. = prolactin, TBG = thyroxin binding globulin, test. = testosterone, thyr. = thyrotropic hormone, *HGR = Histogram, **TC = tissue culture.

fetal calf serum for 1 to 4 weeks. At various intervals, the DNA histograms of cultured cells were compared with DNA karyograms of the corresponding fresh tissue specimen obtained at surgery. The preparation of primary cell cultures has been described previously [1, 18].

Like FEICHTER [10], we based our analyses of the DNA quantities and the corrections of the histograms were on the model of HAAG et al. [11]. This model allows an area integration, which, in turn, allows calculation of percentage values of a given phase (GO/G1, S, G2+M). The ascertainment of the phase parts further allows for the establishment of the proliferation index (PI):

$$PI = \frac{S + G2 + M}{G0/G1 + S + G2 + M} \cdot 100$$

The apparatus ICP-11 (Phywe, Göttingen) was used for impulse cytophotometry.

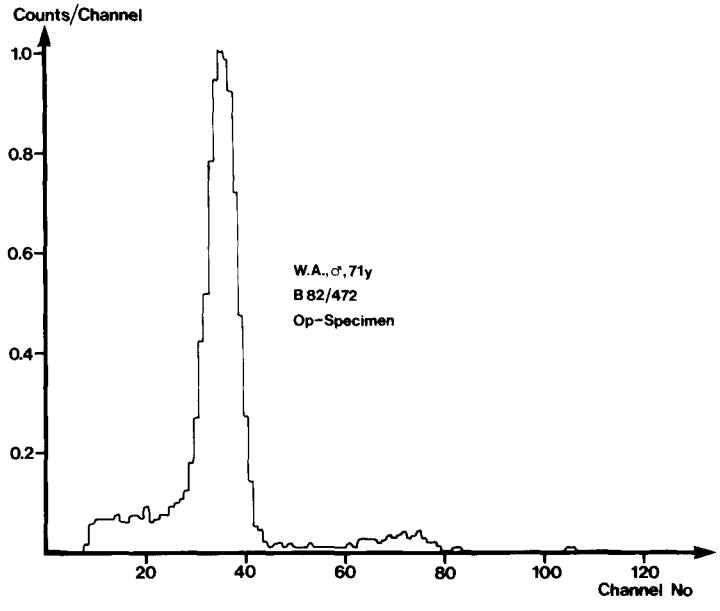

Figure 1a

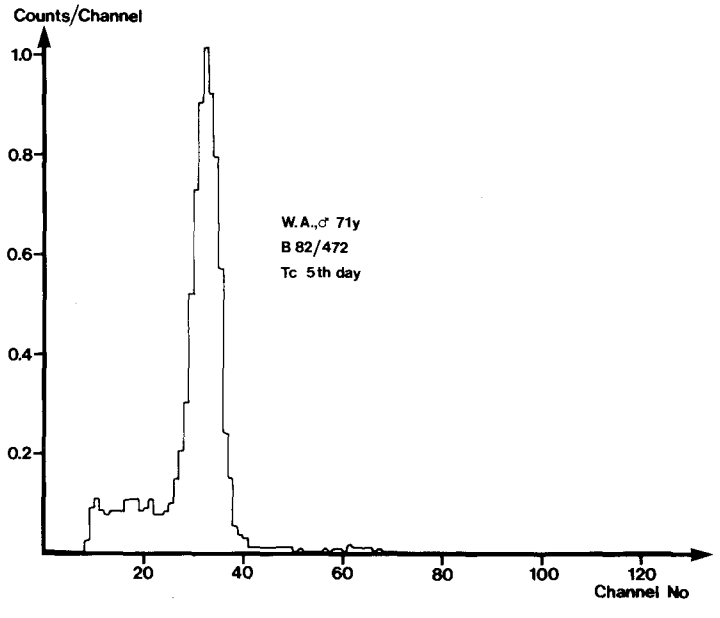

Figure 1b

3 Results

Histological examinations revealed 26 chromophobe and 6 mixed cell adenomas. The results are summarized in Table I in which the tumors are arranged according to their proliferation activity as expressed by PI. The percentages of cells, determined by impulse cytophotometry and corrected following HAAG's method, are represented in the various phases of the cell cycle. The PI did not exceed the limit of 5 in the control pituitary tissues.

Pituitary adenomas were classified in 3 groups, according to their PI and DNA patterns. The first group (PI from 0.12 to 9.78) was comprised of 22 adenomas which had an almost unimodal diploid cellular composion (Figure 1a). The S-phase varied from a minimum of 0.4% to a maximum of 3.25%. The G2+M(4C) quantities ranged from 0.8% to 6.53%. The tumors of this group, which consisted histologically of 17 chromophobe and 5 mixed cell adenomas, exhibited no anaplastic features except rare mitoses (Case 17) and slight infiltrative growth

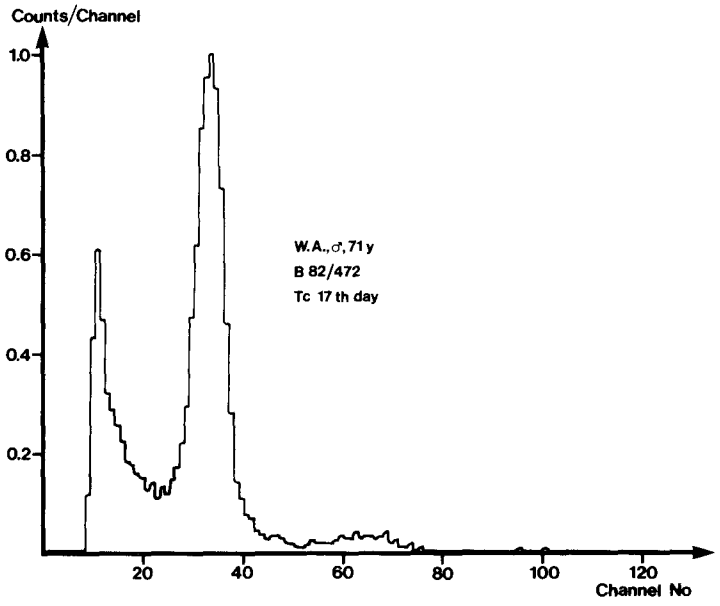

Figure 1c. Chromophobe adenoma showing unimodal diploid DNA histogram in the biopsy specimen (a) and in tissue cultures of day 5 and 17 (b, c).

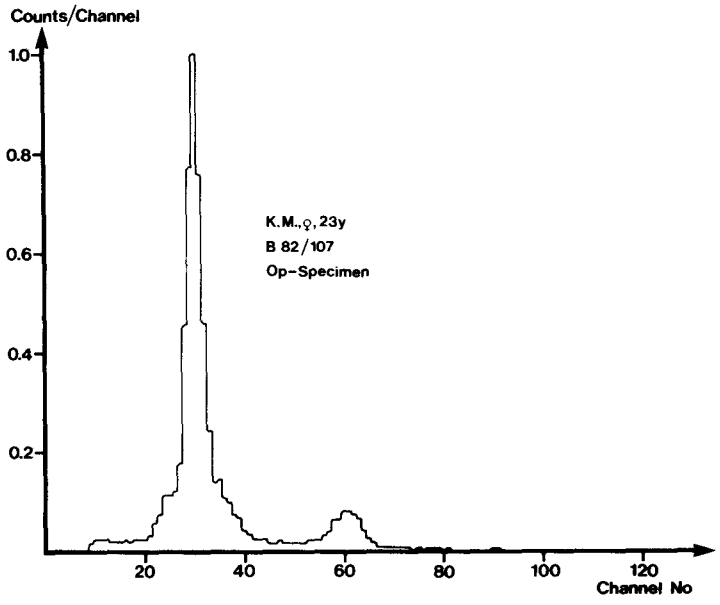

Figure 2a

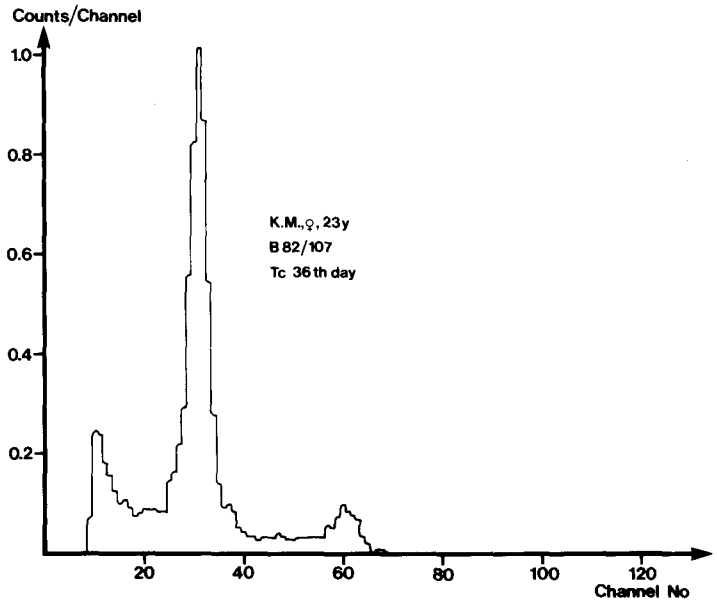

Figure 2b. Prolactinoma (Case 23) with moderate increase of 4C peak (a). The karyogram of the tissue culture (36 days) shows approximately the same DNA distribution except for a minimal increase in S-phase (b).

into the surrounding dura (Cases 18 and 20). Endocrinologically, there were 4 prolactinomas whose karyograms, however, showed no changes. Of the 5 tissue cultures of adenomas from this group (Figure 1b, c), only that of Case 12 showed some change (a moderate increase of S and 4C areas in the later phase of the cell culture).

The second group (PI from 10 up to 15; cases 23–28) included 5 chromophobe adenomas (three of these were prolactinomas) and one a mixed cell adenoma, showing moderate increase of 4C peaks (7.96–10.5%) (Figure 2a). The S-phase proportion remained under 3% in the first 5 adenomas in this group; it amounted to 5.28% only in the adenoma from Case 28 and reached up to 3.11% in the tissue culture from Case 23 (Figure 2b).

The third group (PI above 15) included 4 chromophobe adenomas (one of these, Case 30, was

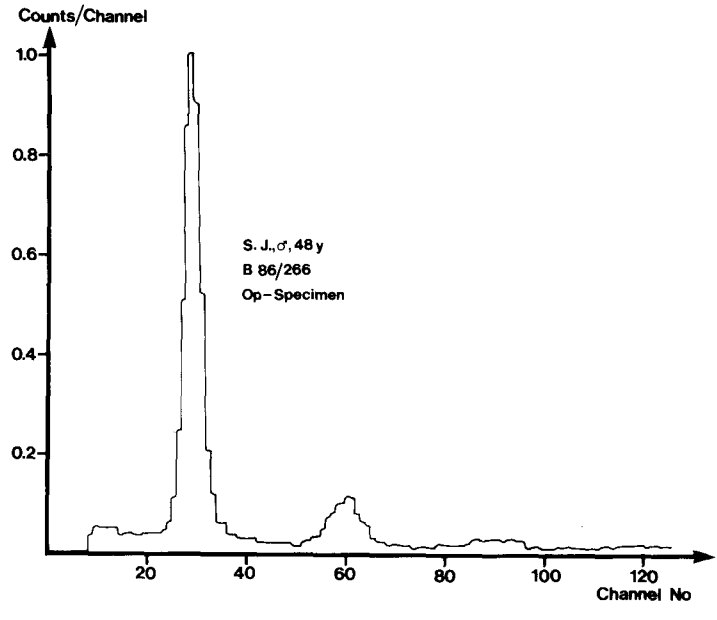

Figure 3a

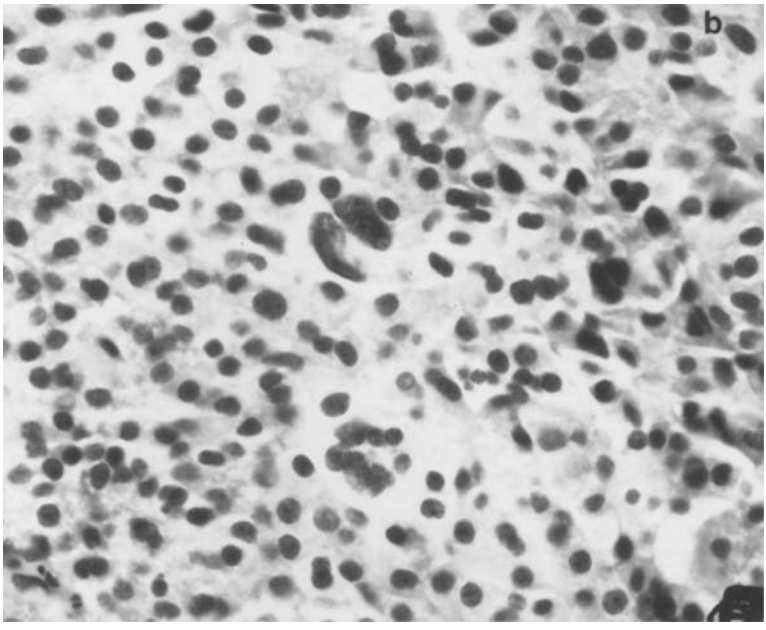

Figure 3b. A recurrent chromophobe adenoma (Case 31) which shows the increased 4C area and tendency towards polyploidy (a). Histologically, there is prominent polymorphism and increased chromatin contents of tumor cell nuclei (b); H&E, x 400.

a prolactinoma 2 of these exhibited diploid (Cases 29 and 30) and 2 aneuploid DNA karyograms (Cases 31 and 32). Case 31 was a recurrent adenoma with a tendency towards polyploidy (Figure 3). The 4C area with tetraploid cell clones was greatly increased in Case 32 (64.56%) (Figure 4a). The increase of tetraploid cells (21.74%) was demonstrable in the tissue culture after 2 weeks (Figure 4b). No significant increase of S-phase could be observed. Histologically, increased chromatin contents, nuclear polymorphism, infiltrative proliferation of the tumor cells into the surrounding tissue, and apparent mitoses were present (Figure 4c).

There was no direct correlation between DNA ploidy and secretion of prolactin, growth hormone, or FSH. Of the 8 prolactinomas, 1 (Case 26) showed a hyperdiploid nuclear DNA pattern and another (Case 30) an increasing 4C (11.18%) and PI (15.77).

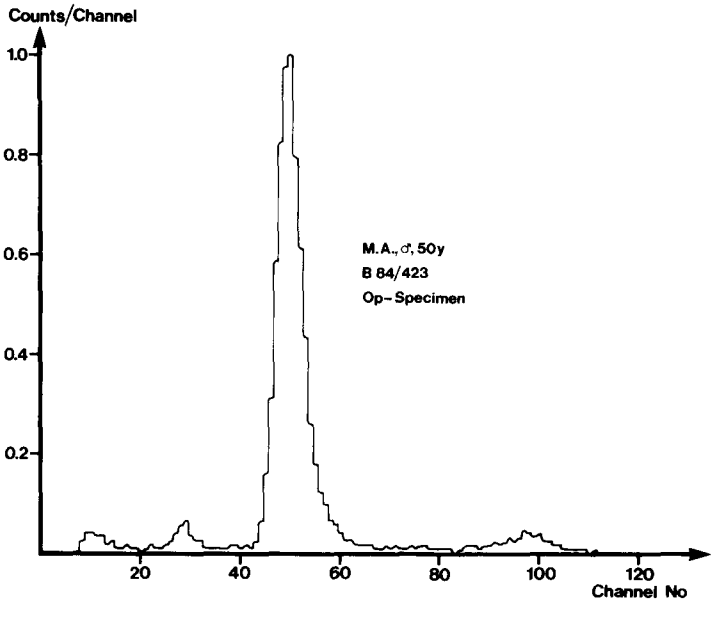

Figure 4a

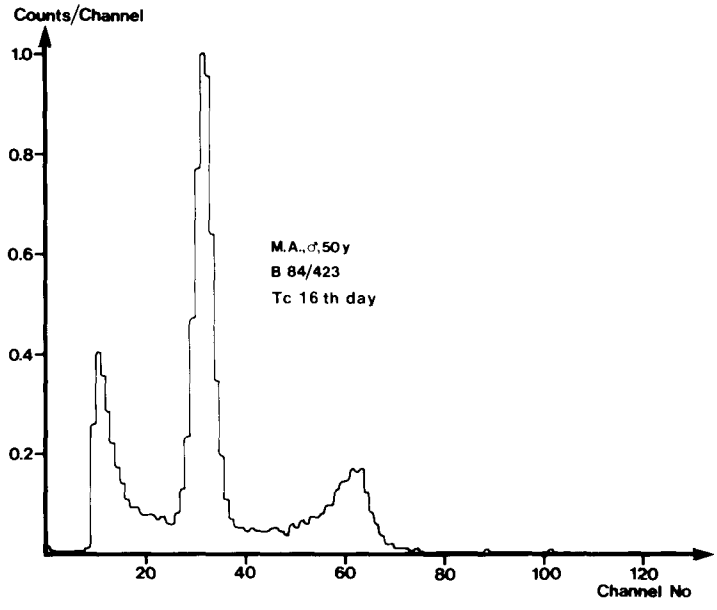

Figure 4b

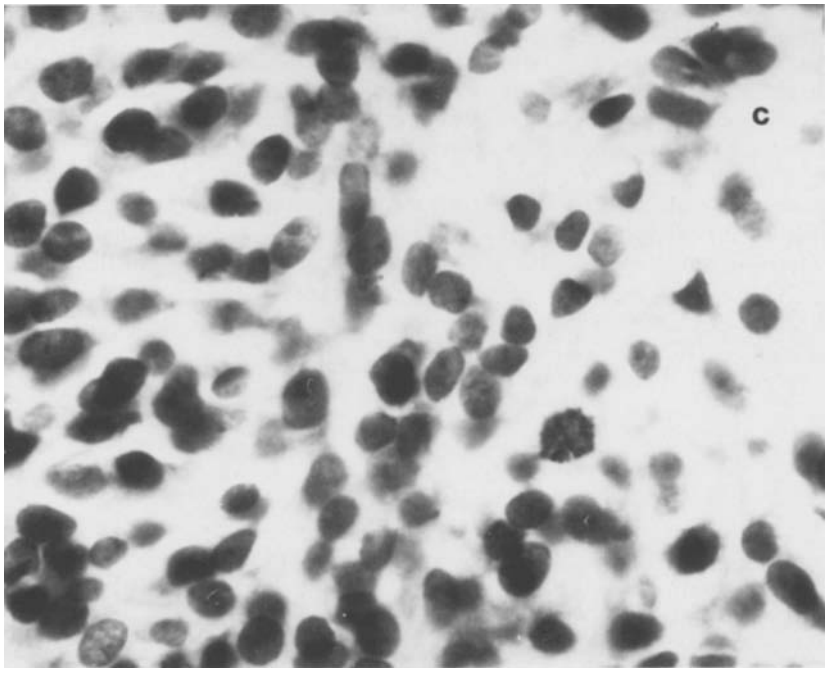

Figure 4c. The karyogram of Case 32, a recurrent chromophobe adenoma two years after the first surgery. This shows aneuploid DNA-patterns with an extremely increased number of tetraploid cells in 4C phase (64.56%) and stemlines in the octaploid area (a). In tissue culture 16 days), an apparent increase of 4C-phase (21.74%) can still be observed (b). Microscopically, mitoses and slight nuclear polymorphism can be seen (c); H&E; x 480.

4 Discussion

The proliferative activity of the tumor cells was apparently increased in the second and third group of our series. Anniko et al. [4] reported a high percentage of aneuploid karyograms (41%) in their DNA investigation of 29 pituitary adenomas. In our series this could be observed only in 3 out of 32 cases (approximately 10%), and in two of these there was a tendency towards polyploidy (Cases 31 and 32). Anniko et al. [3] indicated in another study of 24 pituitary tumors that there is no correlation between the degree of ploidy and the histological or ultrastructural features. In still another investigation,

ANNIKO et al. [5] reported that the serum hormone levels of growth hormone and prolactin in tumors causing acromegaly or prolactinomas could not be correlated to the DNA ploidy. However, they did find that pituitary adenomas with an aneuploid nuclear DNA content occur mainly in cases of acromegaly with concomitant secretion of prolactin and growth hormone. LÜDECKE et al. [15] found 20% aneuploid DNA patterns which occurred especially often in prolactin secreting tumors (36%) and even to up 7% in hormonally inactive pituitary adenomas. Admittedly, the material we examined consisted mainly of chromophobe adenomas in contrast to that of the cited authors. In our series of 8 prolactinomas, one adenoma (Case 30) showed an increase in 4C (11.18%) and PI (15.77) and another one (Case 26) a diploid-hyperdiploid DNA distribution.

Histologically, there were rare mitoses and nuclear hyperchromasia in Case 26 and infiltrating growth into the surrounding dura in Case 30. Also in 3 out of 7 patients with slight hyperprolactinemia (Cases 27–29) and elevated proliferation activities, apparent nuclear polymorphism of the tumor cells was ascertained. Therefore, the question remains whether a direct correlation exists between the proliferation activity or ploidy in the DNA distribution and hormonal secretory activities. An aneuploid DNA distribution pattern could be observed in only one case (Case 26) of a prolactinoma but never in mixed cell adenomas. In contrast, we found variations in DNA patterns, for example, in Cases 31 and 32, with poly- and aneuploidy, corresponding to the histological anaplastic features (Figure 4c). LILIKWARGAWIDJAJA [13] studied 23 endocrinologically active and 22 inactive pituitary adenomas and found no significant difference between the proliferative activity of the tumor cells and hormone secreting adenomas. According to this author, the G2+M fractions more frequently exhibited striking variations than the S-phases. Typical dissimilarities in the DNA distribution were poly- and/or aneuploidy with the so-called stem lines. These usually do not occur in the benign cell populations but are found in more than 90% of all malignant neoplasms and precancerous states [6–8]. As mentioned above, we observed only one recurrent pituitary adenoma with a tendency towards polyploidy.

In conclusion, we distinguished 3 different groups of pituitary adenomas according to the increasing PI values: 1) diploid histograms with PI values up to 10; 2) diploid karyograms with PI from 10 to 15; 3) diploid or aneuploid tumors with PI above 15. The latter group in our classification may reflect a more prominent polymorphism and increased frequency of mitoses. These tumors seem to be no longer benign and possibly incline to a relapse, as seen in Case 31. The FCM analysis may thus contribute to offer more relevant information on the biological behavior of such tumors.

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