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Grading of pituitary adenomas in acromegaly

Comparison of light microscopical, immunocytochemical, and clinical data*

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Summary. In a series of 284 adenomas from cases of acromegaly we studied major morphological variables using light microscopical techniques and compared them with immunocytochemical and clinical results.

Using our semiquantitative estimations many inter-relationships were observed. We established the density of secretory granules, nuclear pleomorphism and the rate of occurrence of multinuclear tumour cells, as essential features of tumour differentiation. Mitotic activity and invasive growth patterns did not reveal clear dependences.

Immunocytochemical analysis of 105 cases showed growth hormone (GH) in nearly all adenomas (98%), prolactin in 68%, and LH in 40%. The other hormones (ACTH, FSH, and TSH) were present at a much lower rate. Monohormonal GH-adenomas were found in only 29% of our cases.

Many different combinations of hormone content could be demonstrated without any relationship to morphological or clinical data. From the linear correlations and advanced method of semiquantitative evaluation, the granular density of the tumour cells is the most useful variable for subclassification and grading of pituitary adenomas in acromegaly.

Key words: Acromegaly – Immunocytochemistry – Pituitary adenoma – Tumour grading

Introduction

With few exceptions acromegaly is caused by adenomas of the pituitary anterior lobe (Melmed et al. 1983). Modern methods in pathological research have increased our knowledge about the structure and function of

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pituitary adenomas (Kinnman 1973; Landolt 1975; Horvath and Kovacs 1976; Saeger 1977; Halmi 1982) and although current concepts of classification (Saeger 1981; Melmed et al. 1983; Asa et al. 1984) reflect the use of these new techniques, the classical and so far accepted subdivision of adenomas is based on light microscopical findings (Williams et al. 1980).

The clinical significance of certain morphological variables seen by light microscopy such as granulation, mitotic activity, invasive growth, and particularly the immunocytochemical hormone content of the adenoma cells are often discussed (Young et al. 1965; Lewis and Van Noorden 1972; Robert 1973; Kinnman 1973; Horvath and Kovacs 1976; Saeger 1977, 1981; Klijn et al. 1980; Trouillas et al. 1980; Nieuwenhuijzen Kruseman et al. 1983) with inconsistent results probably due to small case numbers and lack of quantitative estimations.

In a large series of adenomas in acromegaly we analysed histological and immunocytochemical variables by semiquantitative methods using light microscopy, we established their relationships to clinical findings and discussed the importance of our results for classification and grading of pituitary adenomas in acromegaly.

Material and methods

From 1970 to 1983, 346 patients with acromegaly underwent transnasal microsurgery for a pituitary adenoma in the Department of Neurosurgery, University of Hamburg. The adenomectomies were done by two neurosurgeons (Prof. Dr. R. Kautzky, Dr. D.K. Lüdecke) using identical techniques.

Intraoperatively they measured the maximal tumour size by instillation of contrast medium into the tumour cavity after resection. 3 groups were established: small tumours up to 10 mm (size group (sg) 1), medium from 10 to 20 mm (sg 2), and large ones more than 20 mm (sg 3). They also estimated the incidence of suprasellar extension and invasive growth. Invasiveness was defined as infiltration into surrounding tissues like sella bone or dura was existent. Otherwise the tumour growth was classified as a non-invasive one.

The GH levels in plasma were measured before and shortly after the adenomectomies by a double antibody radioimmunoassay system (CEA-IRE-Sorin-Kit, Hormonlabor II. Med. Klinik; Prof. Dr. Voigt, Prof. Dr. Krieg, University of Hamburg). Postoperative basal GH levels less than 4.5 µg/l were considered to be evidence of complete tumour removal, whereas a higher value indicated surgical failure. We excluded all patients with preoperative radiation or surgery of the pituitary, due to the regressive alterations seen in those tumours (Anniko and Wersäll 1982). 284 patients with primary surgical intervention remained for this study.

All adenoma samples were treated in the same way. After fixation in Bouin's solution the greater portion was embedded in paraffin and stained by several methods. For our quantitative estimations we used the slides stained by haematoxylin-eosin. Another part of tumour was embedded in Epon 812. From this material semithin sections were cut and stained with toluidine blue for analysis of the cytoplasmatic granules. We observed the morphological variables by a magnification of 400× (objective 40/0.65, ocular 10×/18) and examined the visual fields with more than 3/4 vital tumour cells and without severe regressive alterations like haemorrhage, fibrosis, or calcification. For diagnosis of mitoses we adopted the criteria published by Baak and Oort (1983).

In 105 cases since 1979, we took paraffin sections for immunocytochemical studies with the peroxidase-antiperoxidase-method (Sternberger 1979) using the Ortho HISTOSET (Ortho Diagnostic Systems). Dilution and time were as follows: normal goat serum (1:30) for 5 min; diluted primary antiserum overnight at 4° C; anti-rabbit IgG goat serum (1:10) for 30 min at room temperature; PAP-complex (1:30) for 30 min at room temperature, then incubation with 0.06% diaminobenzidine and 0.03% hydrogenperoxide in Tris-HCl-buffer for 10 min and counterstaining with haemalaun. The following primary antibodies were used: anti-GH

Table 1. Tumour size of adenomas in acromegaly and correlations to other clinical data ($n=284$)

Size group	Suprasellar extension %	Invasive growth %	Mean of basal GH levels log µg/l	Postop. GH level below 4.5 µg/l %	Average age years
1	4.1	16.3	1.41	94.4	46.2
2	17.3	30.9	1.55	77.1	44.1
3	63.4	65.6	1.75	62.4	40.1

(Kabi, 1:100); anti-Prolactin (Serono, 1:2000); anti-ACTH (Ferring, 1:200); anti-TSH (Kabi, 1:200); anti-LH (Kabi, 1:250), and anti-FSH (Panchem, 1:100). For our statistical evaluations we established a probability of error $P=0.05$ as significant level for all tests.

Results

Clinical findings

The adenomas in acromegaly showed great differences in size at the time of surgical removal. 93 of our 284 cases (32.75%) were microadenomas (sg 1), whereas 98 tumours (34.5%) measured between 10 and 20 mm (sg 2), and 93 (32.75%) were larger than 20 mm (sg 3). The incidence of suprasellar extension and the rate of invasive growth increased with the tumour size (Table 1).

From our patients 150 were females (52.8%) and 134 (47.2%) males. Sexual differences in tumour size, suprasellar extension, or invasive growth rate were not demonstrated.

The mean age of all cases was 43.5 years (standard deviation 12.4 y; median 43.5 y, range from 7 to 73 y). Mean age at tumour resection was younger ($\bar{x}=40.9$ y) for males than females ($\bar{x}=45.8$ y). A significant negative correlation was established between the age and tumour size (Table 1).

The preoperative basal GH levels in plasma ranged from 2.4 to 800 µg/l in our cases. Only one patient with symptoms of acromegaly showed a value below 4.5 µg/l. The distribution of GH levels was log-normal and for further analysis all data were transformed into their logarithms (base 10). The mean value was 1.57 log µg/l. The extent of basal GH increased with tumour size (Table 1).

The rate of surgical failure rose with increase of tumour size and preoperative basal GH levels.

Morphological findings

The density of cytoplasmic granules in tumour cells differed over a wide range. For evaluation we established four groups: ungranulated, sparsely, medium, and closely granulated cells. The two last groups were summared as densely granulated and then subdivided into 10%-steps of increasing

Table 2. Cytoplasmic granulation of the adenomas in acromegaly and its relation to other morphological, clinical, and immunocytochemical data

Rate of densely granul. cells	Number of cases	Rate of size group 3 (>20 mm)	Rate of pleo-morphism grade 1	Multi-nuclear cells per field	≥60% GH-positive cells	Ade-nomas with mitoses	Inva-sive growth	Postop. GH level below 4.5 µg/l
%	*n*	%	%	$\bar{x}$	%	%	%	%
≥80	25	20.0	8.3	2.63	90.9	42.8	32.0	76.0
70	40	27.5	15.4	3.32	64.7	30.8	32.5	77.5
60	33	24.2	15.3	3.64	80.0	50.0	36.4	75.5
50	22	22.7	23.8	3.76	75.0	42.9	36.4	81.8
40	17	35.3	18.8	2.90	63.6	50.0	62.5	70.6
30	27	14.8	22.2	3.00	75.0	75.0	25.9	85.2
20	22	50.0	42.9	4.40	50.0	62.5	40.9	77.3
10	60	41.7	31.5	4.79	38.9	27.8	40.0	76.7
0	12	58.3	36.4	4.25	0	50.0	45.5	66.7
correl. coeff. $r=$		−0.74*	−0.88**	−0.72*	0.84**	−0.24	−0.33	−0.27

* $P<0.05$, ** $P<0.01$

granularity. From the 284 cases we could analyse granulation adequately in 258 (90.8%). Our examinations worked out a broad spectrum of granular density in the different tumours with the highest accumulations of cases in adenomas with 10% (60 cases) and 70% (40 cases) densely granulated acidophils. Using the most accepted subdivision of these adenomas, 120 tumours (46.5%) were densely granulated acidophil adenomas, 126 (48.8%) sparsely granulated, and 12 cases (4.7%) chromophobe ones. 2 adenomas were predominantly oncocytic (Saeger 1981). These cases were excluded from the further studies because of their uncertain biological significance.

The densely granulated adenomas were on average smaller than the sparsely granulated and chromophobe ones (Table 2). There was no demonstrable correlation of granular density to preoperative GH levels or the rate of postoperative GH normalisation (Table 2).

From our series 260 cases (91.5%) could be examined for the *nuclear pleomorphism*. For analysis we divided this morphological variable into two grades: – grade 0 (mono- or moderately pleomorphic nuclei): round to oval, mostly of the same size; – grade 1 (medium to distinctly pleomorphic nuclei): irregular forms and sizes, often considerably enlarged (Fig. 1 and 2).

The group showing pleomorphism grade 0 consisted of 199 (76.5%) of our cases, whereas grade 1 was seen in 61 adenomas (23.5%).

Comparing the different granulation densities, the chromophobe and sparsely granulated tumours revealed a higher rate of nuclear pleomorphism grade 1 than the densely granulated ones. We found a negative linear correlation between these variables in our series (Table 2).

Mitoses were rarely seen in adenomas causing acromegaly. With our restrictive criteria we found only 69 mitotic figures in 6,643 fields.

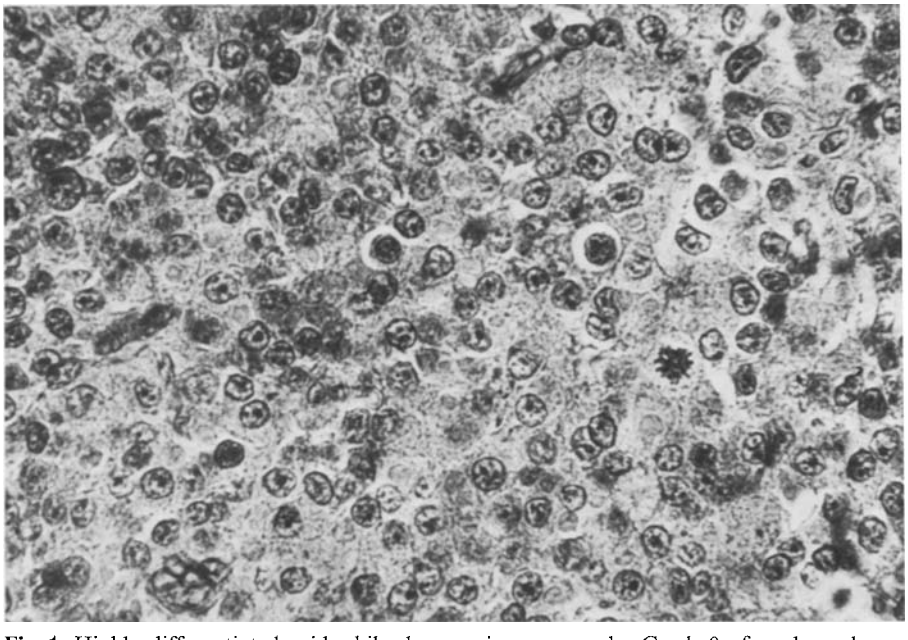

Fig. 1. Highly differentiated acidophil adenoma in acromegaly. Grade 0 of nuclear pleomorphism with round or elliptic nuclei, mostly of the same size; only a few multinucleated cells, one mitosis. Haematoxylin-eosin, 560 ×

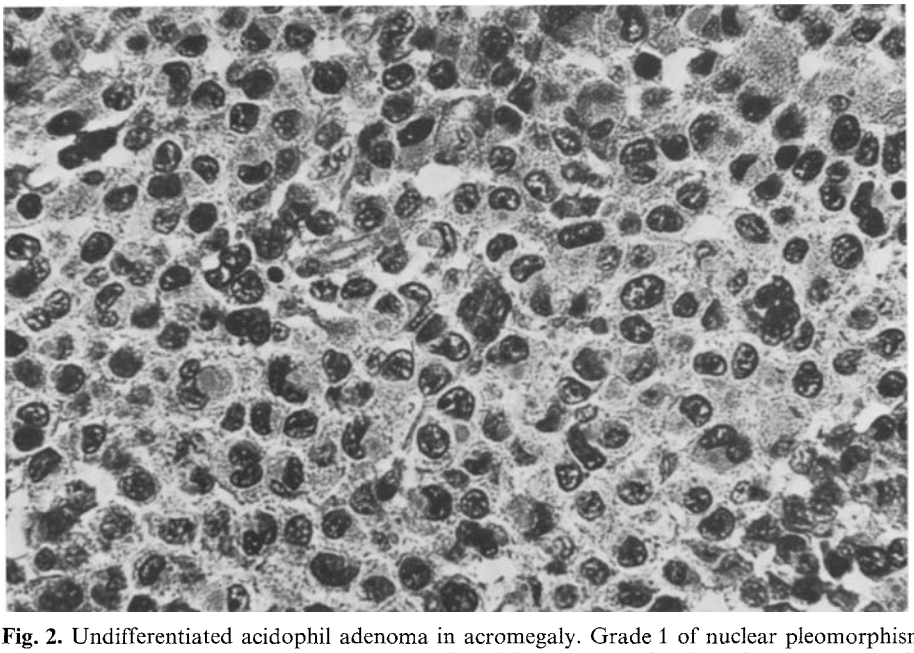

Fig. 2. Undifferentiated acidophil adenoma in acromegaly. Grade 1 of nuclear pleomorphism with many irregular and sometimes enlarged nuclei; many multinucleated cells, no mitosis. Haematoxylin-eosin, 560 ×

Table 3. Frequency and rate of hormone content in pituitary adenomas in acromegaly ($n=105$)

Hormone	Negative cases		Relative part of hormone-positive cases									
			+		++		+++		++++		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
GH	2	1.9	2	1.9	5	4.8	32	30.5	64	61.0	103	98.1
Prl	34	32.4	26	24.8	29	27.6	12	11.4	4	3.8	71	67.6
LH	63	60.0	34	32.4	6	5.7	2	1.9	0		42	40.0
ACTH	94	89.5	7	6.7	1	0.9	1	0.9	2	1.9	11	10.5
FSH	95	90.5	10	9.5	0		0		0		10	9.5
TSH	99	94.3	6	5.7	0		0		0		6	5.7

\+ only few hormone-positive cells demonstrable
\+ + between 10% and 30% of tumour cells hormone-positive
\+ + + between 30% and 60% of tumour cells hormone-positive
\+ + + + more than 60% of tumour cells hormone-positive

Table 4. Different combinations of hormone content of the adenomas in acromegaly revealed by peroxidase-antiperoxidase immunostaining ($n=105$)

Number of hormones	Hormone combinations	Number of cases	
n		*n*	%
1	GH	30	28.6
	Prl	2	1.9
2	GH + Prl	27	25.7
	GH + LH	2	1.9
3	GH + Prl + LH	24	22.9
	GH + Prl + ACTH	6	5.7
	GH + LH + TSH	2	1.9
	GH + LH + FSH	1	1.0
4	GH + Prl + LH + FSH	5	4.8
	GH + Prl + LH + ACTH	1	1.0
5	GH + Prl + LH + ACTH + TSH	2	1.9
	GH + Prl + LH + ACTH + FSH	1	1.0
	GH + Prl + LH + FSH + TSH	1	1.0
6	GH + Prl + LH + ACTH + FSH + TSH	1	1.0

The greatest number was demonstrated in a chromophobic adenoma with pleomorphism grade 1 which showed 4 mitoses in 31 visual fields. For statistical evaluations we used only the adenomas with 40 random selected fields, but because of the mostly small biopsies this quantity could be counted in only 85 cases. 53 of them (62.4%) did not contain any mitotic figures, 23 (27.1%) revealed one, 6 (7.1%) two, and the highest frequency of three mitoses was seen in 3 cases (3.4%).

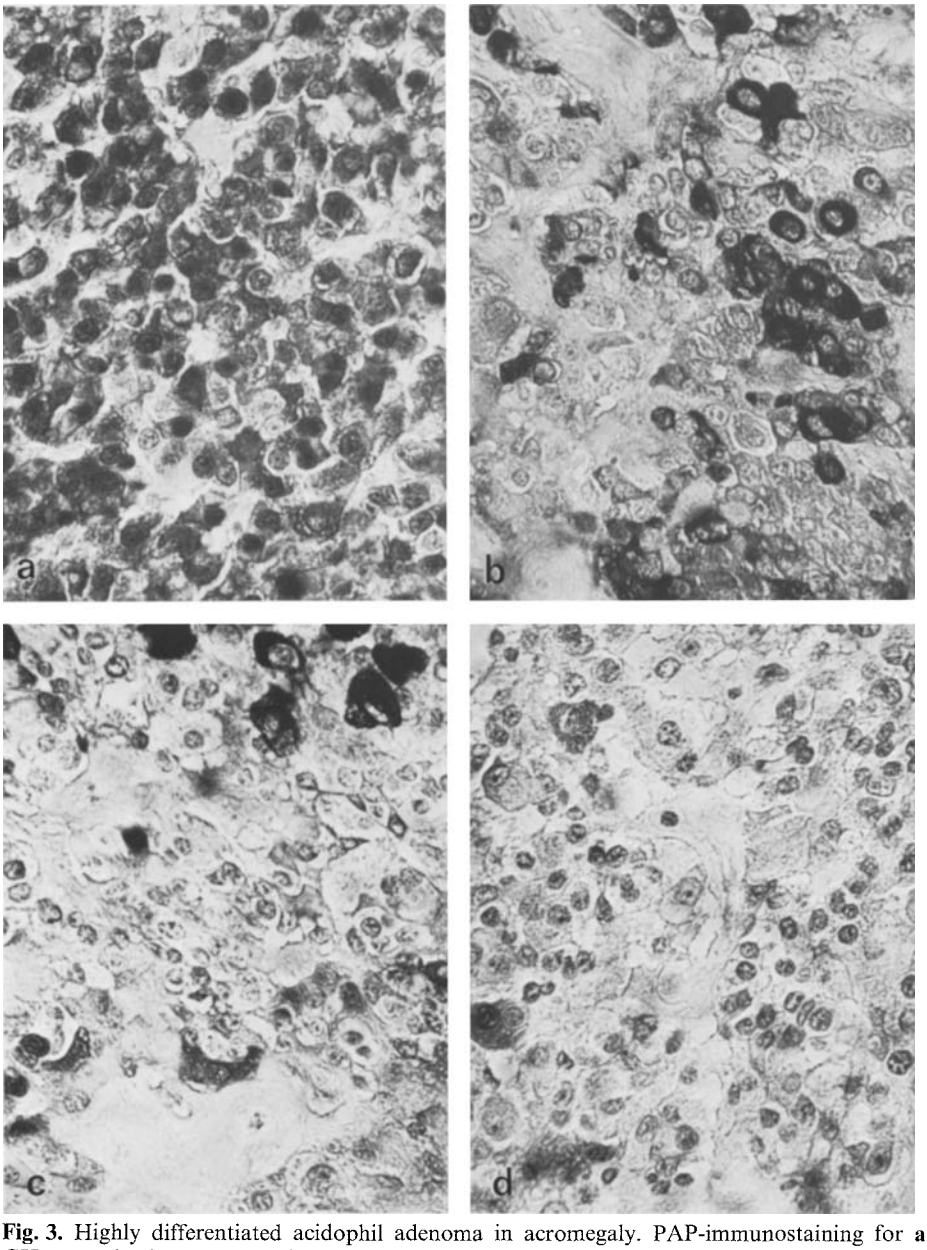

Fig. 3. Highly differentiated acidophil adenoma in acromegaly. PAP-immunostaining for **a** GH, **b** prolactin, **c** LH, and **d** FSH. Most of the tumour cells are GH-positive. LH and prolactin are positive in a considerably smaller amount, FSH is visible only in very few cells. 560 ×

The adenomas of pleomorphism grade 0 contained mitoses in 22 of 65 cases (33.8%), whereas in tumours of grade 1, 10 of 20 adenomas (50%) had mitotic figures. A significant difference in these rates could not be obtained ($X^2 = 1.68$). Comparing the frequency of cell divisions with the granulation density we found no clear dependences (Table 2).

Table 5. Comparison of pure GH-adenomas, GH-prolactin-containing adenomas, and mutihormonal adenomas (with more than three different hormones) in acromegaly

Type of adenoma	Tumour size >20 mm		Mean of basal GH levels log µg/l	Adenomas with mitosis	
	n	%		*n*	%
Pure GH-adenomas	11/30	36.7	1.50	3/7	42.8
GH-Prl-adenomas	8/27	29.6	1.59	4/8	50.0
Multihorm. adenomas	4/11	36.4	1.59	2/5	33.3

The *multinuclear tumour cells* were easy to identify, problems of distinction only occured if borders between tumour cells were unclear. We counted all polynuclear cells per field and calculated the average rate for each adenoma. To avoid greater random variations we only took cases with at least 10 examined fields for analysis.

The rate of occurrence of these cells depended clearly on the grade of nuclear pleomorphism and from the density of granulation. In adenomas of pleomorphism grade 0 an average of 3.1 multinuclear cells was seen, while cases in grade 1 revealed a mean value of 6.3 per field. The average of multinuclear cells increased with the loss of granules (Table 2).

No correlation was seen between mitotic rate and number of polynuclear cells. In adenomas without mitoses a visual field contained a mean value of 3.8 and in tumours with cell divisions 3.7 of these cells ($X^2 = 0.01$).

Because the biopsies of adenomas were often small and without surrounding tissue the histological features of *invasive growth* could only be seen in 9 of our cases. However, a macroscopical infiltration of surrounding structures was found by the neurosurgeons in about 1/3 of adenomas. Because of these difficulties in histological diagnosis we took the intraoperative estimations as a standard for further analysis of invasiveness. From our series, 106 tumours (37.3%) showed invasive growth, 176 (62.0%) were non-invasive, whereas two could not be characterized sufficiently. A clear correlation of invasiveness with tumour size was demonstrable (Table 1). The infiltration rate increased distinctly with the maximal diameter of the adenomas. Within the different size groups invasive growth influenced the frequency of complete removal in a variable way. While in size groups 1 and 3 only slight variations were found, medium sized adenomas showed marked variation demonstrating the relevance of invasion to clinical outcome. The non-invasive tumours were completely removed in 55 of 66 cases (84.8%), the invasive ones in only 17 (58.5%) of 29 cases ($X^2 = 7.7$).

Comparing the density of granulation with invasiveness we found no clear dependence (Table 2). The slightly higher incidence of infiltration in adenomas with low differentiation was not significant ($r = -0.33$).

Adenomas with pleomorphism grade 1		Multinuclear cells per field	Invasive growth		Rate of postop. normalisation of GH levels	
n	%	$\bar{x}$	*n*	%	*n*	%
12/28	42.9	4.56	15/30	50.0	21/29	72.4
8/26	30.3	5.15	11/25	44.0	19/27	70.4
4/11	36.4	4.45	6/11	54.5	8/11	72.7

The adenomas of pleomorphism grade 0 were invasive in 68 of 198 cases (34.3%), the tumours of grade 1 in 27 of 60 (45.0%). Statistical analysis revealed no significant difference ($X^2 = 2.24$). Mitoses were found in 16 of 43 non-invasive (37.2%) and in 16 of 42 invasive adenomas (38.1%). These results suggest that the invasiveness of adenomas in acromegaly is not influenced by the histological features of tumour differentiation.

Immunoperoxidae staining demonstrated the presence of all six hormones examined in different frequencies and combinations. GH was demonstrable in all but two of the 105 stained cases (98.1%), prolactin in 71 (67.6%), and LH in 42 adenomas (40.0%). The other hormones were found at a much lower rate: ACTH in 10.5%, FSH in 9.5%, and TSH in 5.7% of our series. Table 3 shows the semiquantitative distribution of the hormone-positive in relation to other tumour cells.

When comparing the number of GH-positive cells with the density of granulation we found a positive linear correlation between these variables (Table 2). The chromophobe or sparsely granulated tumours had a smaller number of GH-positive cells in immunostaining than the densely granulated acidophil adenomas. In contrast to this result dependence of the rate of GH-positive cells to preoperative basal GH levels was not found. Our studies revealed many different combinations of hormone content in the adenomas (Table 4). Monohormonal GH-adenomas were seen in 30 cases (28.6%), in 44 tumours (41.9%) we found more than two positive hormones (Fig. 3) and in one case all six were demonstrable.

For further analysis we subdivided our series into the groups proposed by Scanarini and Mingrino (1979): (1) pure GH-adenomas (monohormonal) 30 cases, (2) mixed GH-Prolactin-adenomas (bihormonal) 27 cases, and (3) adenomas with more than three hormones (multihormonal tumours) 11 cases. For these groups the light microscopical and clinical features were compared (Table 5). We did not find any significant difference for the factors analysed indicating that the spectrum of hormone content is not dependent on tumour differentiation in our series.

Discussion

In the classification of adenomas in acromegaly the cytoplasmic granulation was often used as the predominant criterion (Young et al. 1965; Lewis and Van Noorden 1972; Robert 1973; Horvath and Kovacs 1976; Melmed et al. 1983). Most of the authors classified the granular density in acidophil tumours with Orange-G staining by light microscopy, even though this method allows for only approximate estimations. So far, the adenomas in acromegaly were subdivided into 3 groups: (1) densely granulated acidophil adenomas (with 50% or more copiously granulated cells), (2) sparsely granulated acidophil adenomas (with less than 50%), and (3) chromophobe adenomas (without any dense granulation).

Because of limited reliability several publications have reported very different ratios for these subclasses. Therefore we preferred the examination of Epon-embedded semithin sections, allowing semiquantitative analysis of the granules. In our opinion this more detailed evaluation is necessary for an advanced light microscopical classification (Saeger et al. 1976; Saeger 1981) and easy identification of chromophobe tumours and oncocytomas.

Our studies showed a broad continuous spectrum of granulation density, but no natural gap was visible. Although a subdivision of these tumours has to be artificial, it gives evidence of prognostic value due to its clinical correlations. For the diagnosis of a highly differentiated GH-cell adenoma we require a ratio of 60% or more densely granulated acidophils in our own material.

The nuclear pleomorphism of adenomas in acromegaly has usually been considered to be a feature of tumour differentiation (Young et al. 1965; Horvath and Kovacs 1976; Saeger 1977; Melmed et al. 1983), although some authors have taken a pluriform appearance of the nuclei as a sign of an increased secretory activity (Müller 1969). Relationship to granulation density (Melmed et al. 1983) indicated a stronger possibility for the first view, but this correlation could not always be demonstrated (Lewis and Van Noorden 1972; Robert 1973).

Our results revealed a linear increase of pleomorphism accompanied by loss of granulation, supporting a relationship to tumour differentiation, but the subjective criteria used in the definition of pleomorphism grades restricts this factor for tumour grading.

The frequent occurence of multinuclear cells in adenomas of acromegaly has been reported for a long time. Müller (1969) related an increase of these cells to higher secretory activity, whereas others (Saeger 1977, 1981; Melmed et al. 1983) found a relationship with tumour differentiation. We can support the latter result from the correlations found to nuclear pleomorphism and density of granulation in this study.

In contrast, the number of mitoses did not reveal any clear dependence to differentiation. As described before (Lewis and Van Noorden 1972; Landolt 1975) they were rarely seen in adenomas. Young et al. (1965) found mitotic figures in 44% of their cases and noticed a correlation with granular density and tumour size. In our cases only 35% of adenomas with 40 exam-

ined fields contained at least one mitosis without any relation to other morphological or clinical data. In our opinion this variable is not useful for grading adenomas in acromegaly. As indicated by several case reports (literature see Saeger 1981) a considerably higher rate of mitotic figures may be an important feature in pituitary carcinomas.

The significance of invasive growth in pituitary adenomas has been discussed for a long time. Infiltration of surrounding tissues could not be correlated sufficiently with other morphological variables (Martins et al. 1965), and metastases appeared very rarely in pituitary tumours (Saeger 1981; Melmed et al. 1983). Corresponding with the most widely accepted definition we used the term carcinoma if a discontinuous growth was evident, without mestastases the tumours were considered to be invasive adenomas. In our 284 cases we did not find any sign of a pituitary carcinoma.

In spite of the importance of the different growth patterns for tumour removal no characteristic differences in morphology could be found in former studies (Robert 1973; Landolt 1975). Some authors assumed an increase of invasiveness in the adenomas with poorer differentiation (Horvath and Kovacs 1976; Melmed et al. 1983). Our findings demonstrate a clear correlation of invasive growth rate with the tumour size. Within the size groups a comparison of non-invasive and invasive adenomas did not reveal differences in any variable. We therefore suppose that invasiveness is not mainly influenced by the grade of tumour differentiation, but much more by the size of adenomas and other unknown factors.

Immunocytochemical studies have recently demonstrated many different combinations of hormone content in adenomas causing acromegaly (Scanarini and Mingrino 1979; Heitz 1979; Fukaya et al. 1980; Saeger and Lüdecke 1983; Kanie et al. 1983; Melmed et al. 1983). In accordance to these results we established several combinations with very unequal frequencies (Table 4).

The rate of prolactin-positive adenomas in acromegaly varies between 35% (Nieuwenhuijzen Kruseman et al. 1983) and 68% (Fukaya et al. 1980) in the literature, the largest series (Melmed et al. 1983) contained 37% of all cases. Our analysis revealed 67.6% prolactin-positive adenomas, of which 1/4 had 30% or more positive tumour cells. Fukaya et al. (1980) demonstrated 57% LH-positive cases in their study of 23 adenomas in acromegaly. Our findings of 40% positive tumours confirmed a high incidence of LH in these tumours. Pure GH-adenomas were found in 59% of cases reviewed by Melmed et al. (1983), while in our series only 28.6% of adenomas contained only GH in their granules. This very low rate of monohormonal GH-adenomas contrasts remarkably with other tumour-groups in our material evaluated with the same technique (prolactinomas with 68% isolated prolactin content, adenomas in Cushing's disease with 85% isolated ACTH content; see Saeger and Lüdecke 1983). This difference is not clearly understandable and needs further clarification.

In this study we were not able to prove the current concepts of tumour classification in acromegaly of Kovacs and colleagues (Horvath and Kovacs 1976; Corenblum et al. 1976; Horvath et al. 1977, 1981, 1983; Melmed et al. 1983; Asa et al. 1984) due to the lack of electron microscopical exami-

nations. The complexity and range of hormone combinations found in our series make it difficult to subdivide the adenomas into clearly distinguishable groups (see Halmi 1982).

Moreover, we did not find any correlations of clinical and morphological variables. An exclusive subclassification based on immunocytochemical results (Scanarini and Mingrino 1979; Martinez and Barthe 1982) seems to be without great clinical relevance.

Conclusions

Light microscopical examination of pituitary adenomas in acromegaly is useful in estimating the grade of tumour differentiation. The density of cytoplasmic granules reveals many clear correlations with morphological, clinical, hormonal, and in part immunocytochemical data. Together with nuclear pleomorphism and the rate of occurrence of multinuclear cells it is the most relevant indicator of tumour differentiation. Due to its importance for classification and the inadequate demonstration in Orange-G staining we recommend semithin sections stained with toluidine blue as a semiquantitative method of assessment.

Mitotic activity, invasive growth patterns and various hormone combinations reveal no clear dependences on grade of differentiation in the adenomas. In our opinion these variables are not very useful for classification.

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