Virchows Arch [Pathol Anat] (1982) 396:167–186

Virchows Archiv A
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Sparsely Granulated Prolactin Cell Adenomas of the Pituitary Gland

Correlation of Ultrastructure with Plasma Hormone Level

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Summary. The possible relationship between the preoperative plasma prolactin levels of patients having a sparsely granulated prolactin cell adenoma of the pituitary gland and the morphology of the tumors was studied by means of quantitative electron microscopy. To this end, a number of ultrastructural variables were chosen which are generally regarded to be indicative of cellular activity and which could be determined in a quantitative or semiquantitative way. These variables were determined in 19 adenomas from 17 patients and plotted against the corresponding prolactin levels. It appeared that marked endocrine activity was associated with a small number of granules per cell, a high frequency of exocytosis, and a marked development of the rough endoplasmic reticulum. Granule size and development of Golgi apparatus and lysosomes were not at all, or only poorly correlated with the plasma hormone levels. Finally, the number of mitochondria per cell showed a totally unexpected inverse correlation with endocrine activity. Due to the close mutual correlation existing between several of the variables investigated, combining them in a multivariate analysis did not significantly improve the correlation with the hormone level.

Key words: Pituitary adenoma – Ultrastructural morphometry – Plasma prolactin level

The relationship between morphology and endocrine activity in pituitary adenomas has received much interest. Many investigators have attempted to assess the rate of secretory activity of these adenomas from their cellular ultrastructure (e.g. Cardell and Knighton 1966; Robert and Hardy 1975; Saeger et al. 1976; McComb et al. 1980); some have combined several ultrastructural variables in an "activity index" (Saeger 1973; Landolt 1975; Trouillas et al. 1980). Howev-

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0340-1227/82/0396/0167/$04.00

er, the correlation of endocrine activity and ultrastructure has been hampered by the very rough morphological criteria that have usually been applied: almost all were based on subjective estimations of the different cellular features, the results being expressed as 1–3 pluses per patient. Furthermore, plasma hormone levels were often not determined in every patient studied.

In the present study we tried to evaluate the significance of the various morphological features that have been considered indicative of cellular activity by other authors. To this end we selected from the pituitary adenomas surgically removed in our Department of Neurosurgery (head: Prof. A.J.M. van der Werf) 19 well documented cases of sparsely granulated prolactin cell adenomas. From each adenoma 15 cells were selected randomly and for each individual cell those morphological variables that could be evaluated in a quantitative or semi-quantitative way were determined. Subsequently we determined the correlation of the pre-operative plasma prolactin level and the average or median value of each variable in each adenoma. It appeared that some were good indicators of the endocrine activity of the tumors whereas others were of no value in this respect, or even gave a false impression.

Materials and Methods

Selection of Adenomas

In the literature four characteristics are generally accepted to be strong evidence for prolactin production by pituitary adenomas, and these critera were used for the selection of the adenomas to be included in the comparison of cellular ultrastructure and plasma prolactin level:

a) Hyperprolactinemia. A plasma prolactin level above normal does not in itself prove the existence of a prolactin secreting adenoma since the nontumorous part of the hypophysis can, in a number of conditions, secrete elevated amounts of prolactin (Kovacs et al. 1976; Schroffner 1976; Kovacs et al. 1977; Landolt 1978; Marrs et al. 1980). Excessive elevations of the plasma prolactin level, however, are considered to prove the presence of a prolactin producting adenoma (Schroffner 1976; Landolt 1978). In our experience (Assies et al. 1980), values above 200 ng/ml virtually exclude other causes of hyperprolactinemia. We measured the preoperative plasma prolactin levels of two or more blood samples in duplicate by radioimmune assay (Assies et al. 1974).

b) The Prolactin Response to an i.v. TRH Injection. In normal subjects thyrotropin releasing hormone (TRH) is a strong stimulus for prolactin secretion. Several groups of investigators have noted that this stimulating effect on prolactin secretion by pituitary adenomas is considerably decreased. A diminished TRH response can therefore be regarded as a strong indication of the presence of a prolactin cell adenoma (discussed in Assies et al. 1980; Marrs et al. 1980). We found that an increase of the plasma prolactin level 20 min after 200 μg TRH i.v. less than 3.5 times the basal level, virtually demonstrates the production of prolactin by a pituitary adenoma (Assies et al. 1980). The preoperative TRH response was determined in 13 of our 19 cases.

c) Specific Staining. Prolactin can be rendered visible in tissue sections either by immunohistology (Kovacs et al. 1975) or by staining with carmoisine (Brookes 1965) or erythrosine (Herlant 1960), although none of these technics seems to be fully reliable (Halmi and Duello 1976; Kovacs et al. 1976; Kovacs et al. 1977; Martinez et al. 1980). Dr. Johanna M. Berkvens (National Institute of Public Health, Bilthoven, The Netherlands) kindly performed and evaluated Brookes' carmoisine staining of those 12 adenomas of which sufficient material was available. During the work, she did not have any information regarding the endocrinological or ultrastructural features of the material.

d) Misplaced Exocytosis. Exocytosis of hormone granules, and especially exocytosis into narrow intercellular spaces rather than into perivascular spaces, is considered to be a highly characteristic feature of prolactin cell adenomas (Horvath and Kovacs 1974; Kovacs et al. 1975; Schroffner 1976; Horvath et al. 1977; Kovacs 1977; Kovacs et al. 1977; McComb and Kovacs 1978; Ryder et al. 1980). In every tumor studied, we made an extensive search for the occurrence of misplaced exocytosis (see below).

From the above criteria 19 sparsely granulated ("chromophobe") pituitary tumors of 17 patients were found to be prolactin cell adenomas. In the great majority of cases more than one of the four criteria was fulfilled.

Light Microscopy

Tissue was fixed in 4% neutral formalin, embedded in paraffin, and stained with haematoxylin and eosin. When possible, some material was also stained with Brooke's carmoisine.

Electron Microscopy

Small (1–2 mm^3) pieces of tissue were fixed with 2% cacodylate-buffered glutaraldehyde, postfixed with 1% OsO_4, block-stained with 0.25% uranyl magnesium acetate, and embedded in Epon. Intact tissue areas were selected in 2 or more blocks per adenoma on toluidine-blue stained semithin sections. Ultrathin sections corresponding to these areas were stained with uranyl magnesium acetate and Reynold's lead citrate. From each adenoma 15 cells were photographed. In accordance with McComb and Kovacs (1978) we selected cells that were sectioned roughly centrally, rather than performing morphometric analysis on completely random photographs. For the selection of the cells the following method was applied. At a low magnification in the electron microscope, at which neither cellular contours nor cytoplasmic details were discernible, well demarcated nuclei, distributed evenly over the section, were selected randomly. When, at a higher magnification, both the plasma membrane and the double nuclear membrane appeared to be sharply visible (and therefore cut roughly transversely), the cell was considered to be hit centrally, and photographed.

Ultrastructural Variables and Cellular Activity

A large number of morphological features have been regarded by others as indicators of the secretory activity of pituitary adenoma cells. Some of these seemed suitable for objective, quantitative or semiquantitative determination, and were therefore used in the present study.

a) Both the rough endoplasmic reticulum (RER) and the Golgi apparatus are directly involved in protein synthesis, and their development is therefore generally regarded as reflecting the state of activity of the cells (Cardell and Knighton 1966; Deaton and Dugger 1972; Saeger 1973; Lewis and Van Noorden 1974; Landolt 1975; Robert and Hardy 1975; Roy 1977; Robert 1979; Archer et al. 1980; Kameya et al. 1980; Martinez et al. 1980; Trouillas et al. 1980). We gave a preference to a semiquantitative estimation of the development of these organelles per cell over standard morphometric procedures that might be influenced by variations in the state of preservation of the adenomas studied (cf. Saeger et al. 1976). The development of both RER and Golgi apparatus of each cell photographed was therefore independently estimated, and expressed as 0–3 pluses by two of us (K.P.D. and N.J.) who were unaware of the clinical data of the patient concerned at the time of the estimation. The results obtained appeared to be identical in the great majority of the cells. The pluses awarded by the two investigators were added and the average value per cell was calculated for both RER and Golgi apparatus.

b) Mitochondria provide the energy necessary for protein synthesis, and their number per cell has therefore also been thought to be related to the rate of cellular activity (Cardell and Knighton 1966; Deaton and Dugger 1972; Saeger 1973; Archer et al. 1980). We counted the number of mitochondria per cell transsection with an electronic counting device (James 1975).

c) Lysosomes and related elements (dense bodies, multivesicular bodies, autophagic vacuoles, vacuolated bodies, and lipid inclusions) have been found to play a role in the regulation of hormone secretion by anterior pituitary cells by degrading excess intracellular hormone (Smith and Farquhar

1966). Large numbers of these elements are therefore thought to indicate a low degree of secretion (Saeger 1973, 1975a; Saeger et al. 1976; Trouillas et al. 1980). In view of the great diversity in shape and size of the lysosomal elements, we estimated the development of these organelles per cell according to the method applied for RER and Golgi apparatus. Again, there was an almost complete correspondence between the two independent investigators.

d) Until fairly recently, the very lack of granulation in "chromophobe" adenomas was regarded as an indication of endocrine inactivity. In recent years, however, it has been increasingly realized that a scarcity of granules in various types of pituitary cells might, on the contrary, be due to increased hormone secretion (Schelin 1962; Cardell and Knighton 1966; Deaton and Dugger 1972; Doniach 1972; Lewis and Van Noorden 1972; Saeger 1973; Kovacs et al. 1975; Landolt 1975; Robert und Hardy 1975; Saeger 1975A; Roy 1977; Landolt 1978; McComb and Kovacs 1978; Kameya et al. 1980; Martinez et al. 1980). However, an inverse relationship between granularity and endocrine activity of pituitary adenomas has by no means been established beyond doubt (Kovacs et al. 1975; Schroffner 1976; Kovacs 1977; Kovacs et al. 1977; Martinez et al. 1980; Trouillas et al. 1980; Kornfeld et al. 1981). We counted the number of hormone granules per cell transsection with an electronic counting device. No attempt was made to discriminate between mature and immature granules (Saeger 1973).

e) Prolactin granules are released by exocytosis (Ryder et al. 1980), and many hormone granules lying in invaginations of the cell surface are therefore regarded as a sign of secretory activity (Robert and Hardy 1975; Saeger 1975a; McComb and Kovacs 1978). For the quantification of exocytosis we selected 20 cells per adenoma as described above. The surface of each cell was then carefully screened for exocytosis at a high magnification in the electron microscope. When it could not be decided whether a granule lying in an intercellular space originated from the cell under investigation or from its neighbour, it was recorded as $^1/_2$.

f) Several authors have hypothesized that the size of hormone granules is influenced by the speed of hormone release from pituitary cells, and have accordingly suggested that the granule size might be related to the rate of cellular activity (Kovacs et al. 1975; Landolt 1975; Landolt and Rothenbühler 1977a; Landolt 1978; McComb and Kovacs 1978). However, the granule size is probably determined by other factors as well, including the rate of hormone synthesis and the rate of destruction of granules by lysosomes. A simple inverse relationship between granule size and plasma hormone level could therefore not be established (Landolt 1975; Kovacs et al. 1977; Landolt and Rothenbühler 1977a; Martinez et al. 1980; Kornfeld et al. 1981). On the basis of randomly taken micrographs (17.8000×), and using a Zeiss Particle Analyser, we measured at least 500 granules per tumor, which is reported to be the minimum number for a reliable determination of the granule size (Landolt 1975).

Statistical Analysis was performed with the aid of the BMDP package (Dixon and Brown 1979). The highest rates of correlation were obtained when some of the variables were used as the median rather than the arithmatic average value. For the same reason, some were expressed logarithmically.

Results

Adenomas Studied (Table 1). The 19 adenomas studied were derived from 17 patients, 9 male and 8 female, ranging in age from 28 to 67 years (av. 49.5 years). The two recurrences were operated 2 and 4 years, respectively, after the first operation. All patients had visual disturbances (diminished visual acuity and visual field defects) caused by compression of the optic chiasm and optic nerves by the tumor. In addition, most had endocrinological disorders such as loss of libido, impotence, amenorrhoea, and galactorrhoea. All operations were performed by the transcranial route.

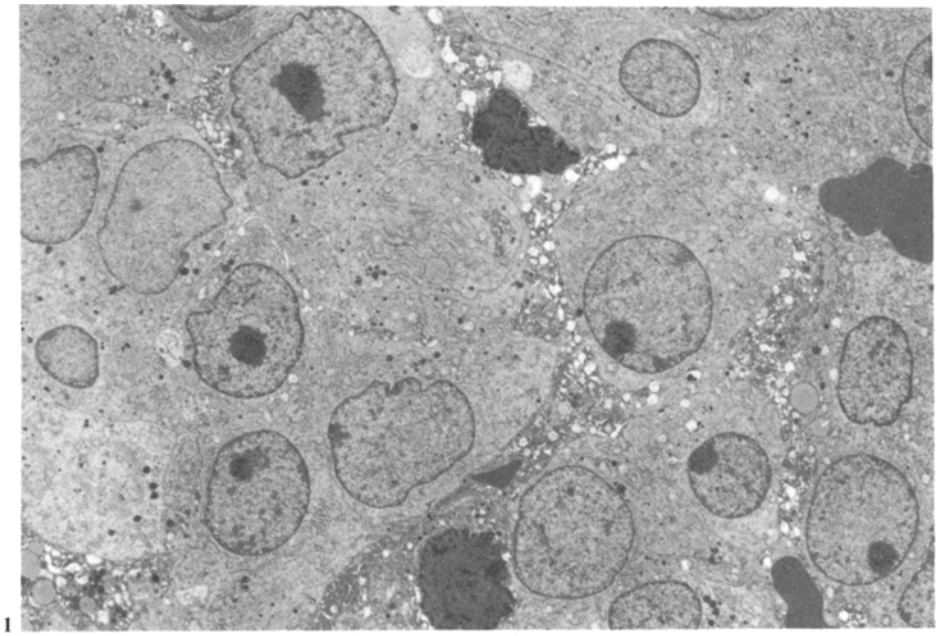
The preoperative plasma prolactin levels ranged from 10 tot 20.200 ng/ml, which means that the lowest levels recorded were at or even under the upper

Table 1. Adenomas selected

Adenoma No.	Sex	Age (years)	Main clinical symptoms (except visual disturbances)	Preoperative plasma prolactin level	response ratio in TRH test	Brookes' staining	Misplaced exocytosis
1	M	41	loss of libido; impotence	13	1.5		−
2	F	58	loss of libido; galactorrhoea	22	1.3		+
3	M	63	loss of libido; impotence	1,000		±	+
4	M	60	diminished libido; reduced potency	16			+
5[a]	F	28	secondary amenorrhea; galactorrhoea	380	1.1	+	+
6	M	66	−	10	0.9		−
7	M	61	loss of libido; impotence	13	1.8	+	+
8	F	66	galactorrhoea	5,100		+	+
9	F	39	secondary amenorrhea; galactorrhoea	11,200	1.3	+	+
10	M	37	loss of libido; impotence	7,650	2.9	+	+
11	F	56	galactorrhoea	21	1.6		+
12[b]	M	63	see Nr. 7	33		+	+
13	M	44	loss of libido; impotence	8,900	1.1		+
14	M	64	loss of libido; impotence; hypothyroidism	26		+	+
15	F	40	primary amenorrhoea; loss of libido; galactorrhoea	20,200	1.2	+	+
16	F	52	−	14	2.3	+	+
17[c]	M	67	see Nr. 3	125			+
18	F	41	secondary amenorrhoea; loss of libido; galactorrhoea	66	1.3	+	+
19	M	28	diminished libido; reduced potency	820	1.1	+	+

[a] patient was operated in 20th week of pregnancy
[b] same patient as adenoma Nr. 7
[c] same patient as adenoma Nr. 3

normal level [15 and 20 ng/ml in males and females, respectively (Assies et al. 1974)]. Eight of the 19 preoperative prolactin levels were higher than the maximum value (200 ng/ml) that we found for nontumorous pituitaries. All response ratios in the TRH test that were recorded were below the minimum value (3.5) that we found in normal individuals and in individuals with other types of pituitary adenomas. With one exception which yielded an equivocal result, the adenomas to which the Brookes stain was applied stained positively, albeit frequently patchy and with highly variable intensity. A TSH producing pituitary



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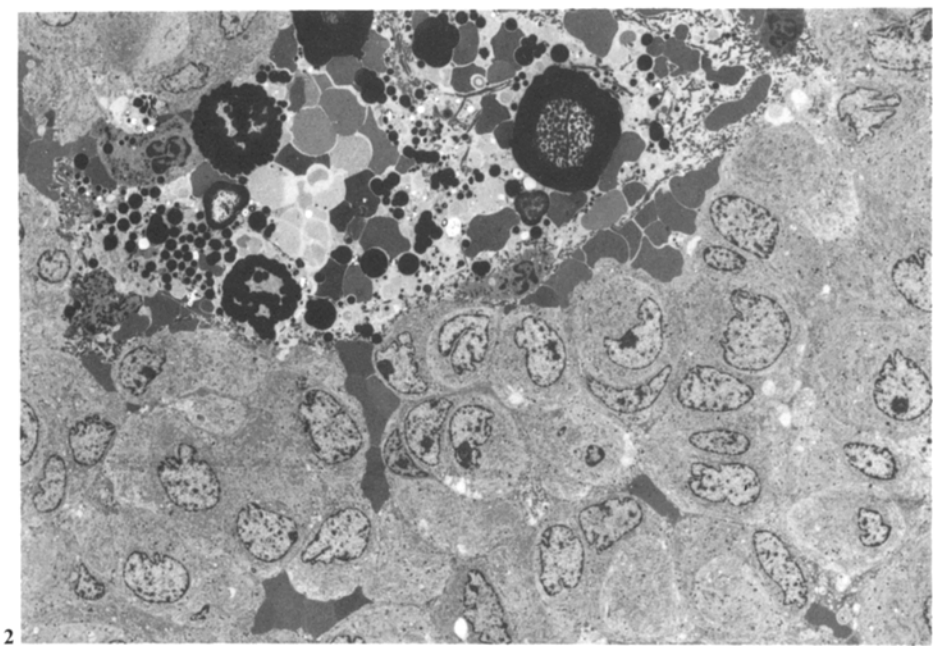

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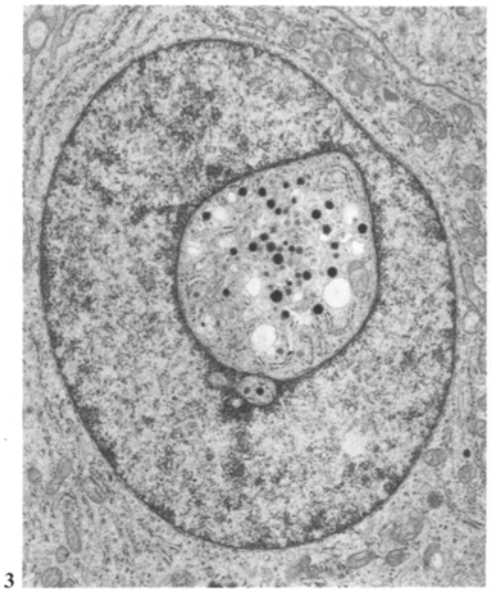
Fig. 1. Survey of a prolactin cell adenoma, mainly consisting of large, pale cells. The nuclei have a prominent nucleolus and little heterochromatin. Note the presence of several free erythrocytes and a few irregular, degenerating cells. × 2,000

Fig. 2. Haemorrhagic area with extensive calcium deposits interspersed with fibrin strands and free erythrocytes. Note the loose coherence of the cells. × 1,100



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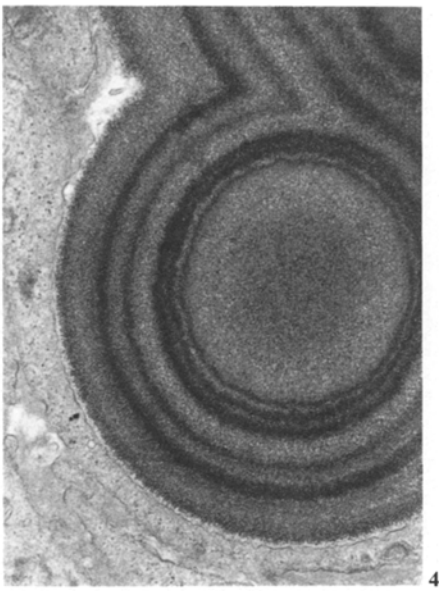

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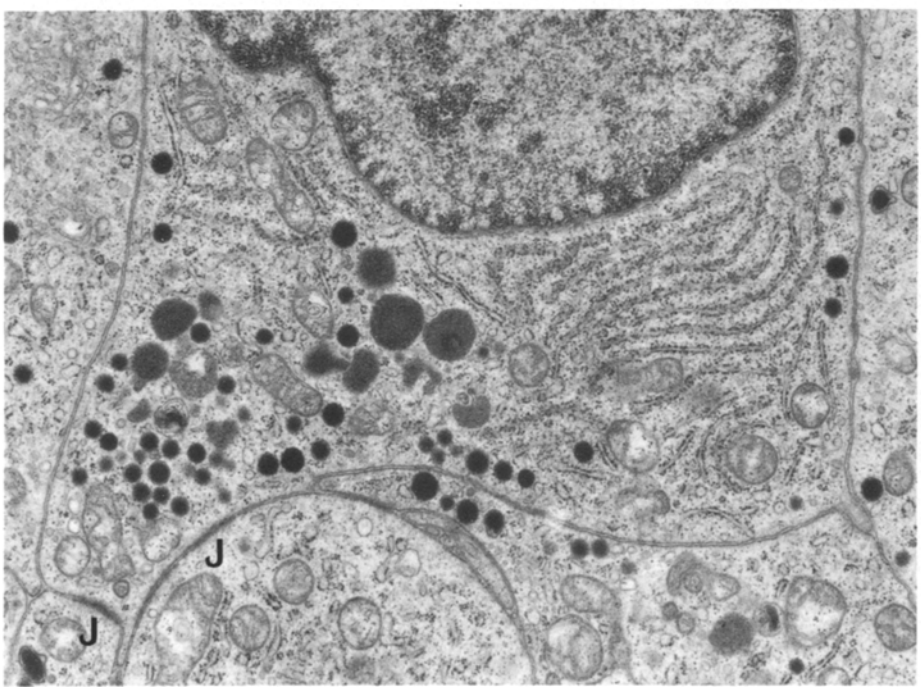

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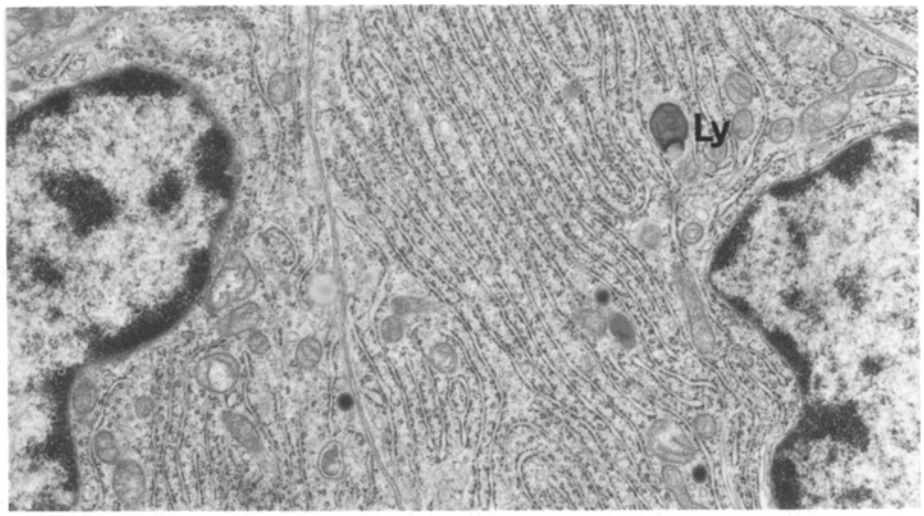
Fig. 3. Intranuclear cytoplasmic inclusions in which hormone granules have accumulated. ×8,200

Fig. 4. Detail of a calcium deposit, showing its concentric structure and an intimate association with the surrounding cell. ×18,000

Fig. 5. Adenoma cells with smooth outlines and poorly developed junctions (*J*). The cytoplasm contains a well developed RER, consisting of parallel membranes, a considerable number of hormone granules (102 in the complete cell transsection), and many lysosomal elements of various shapes and sizes. ×15,000



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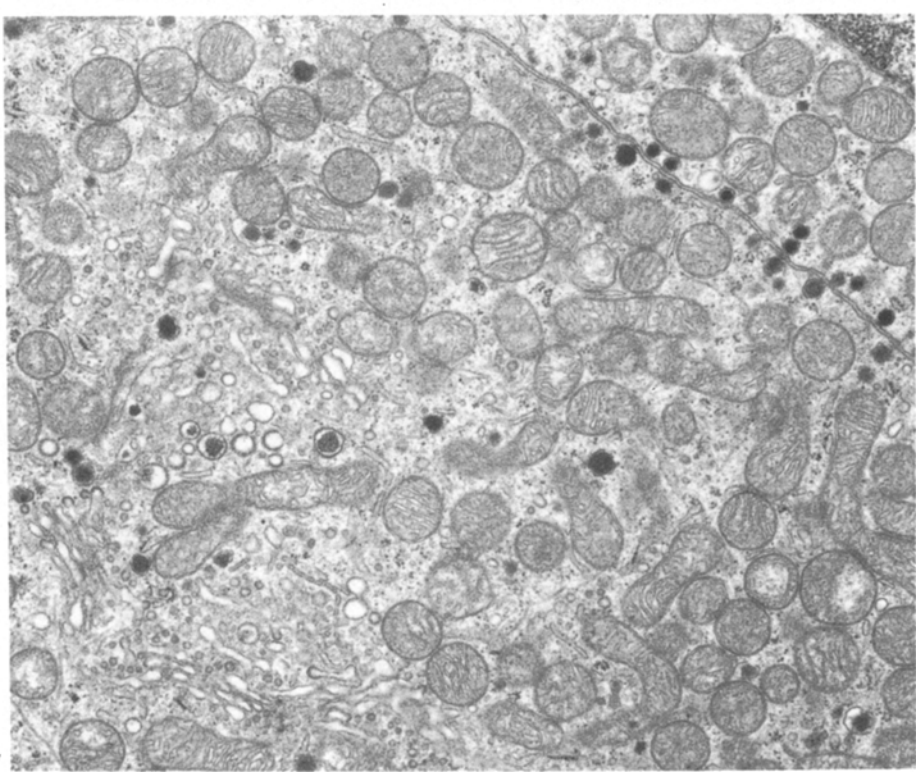

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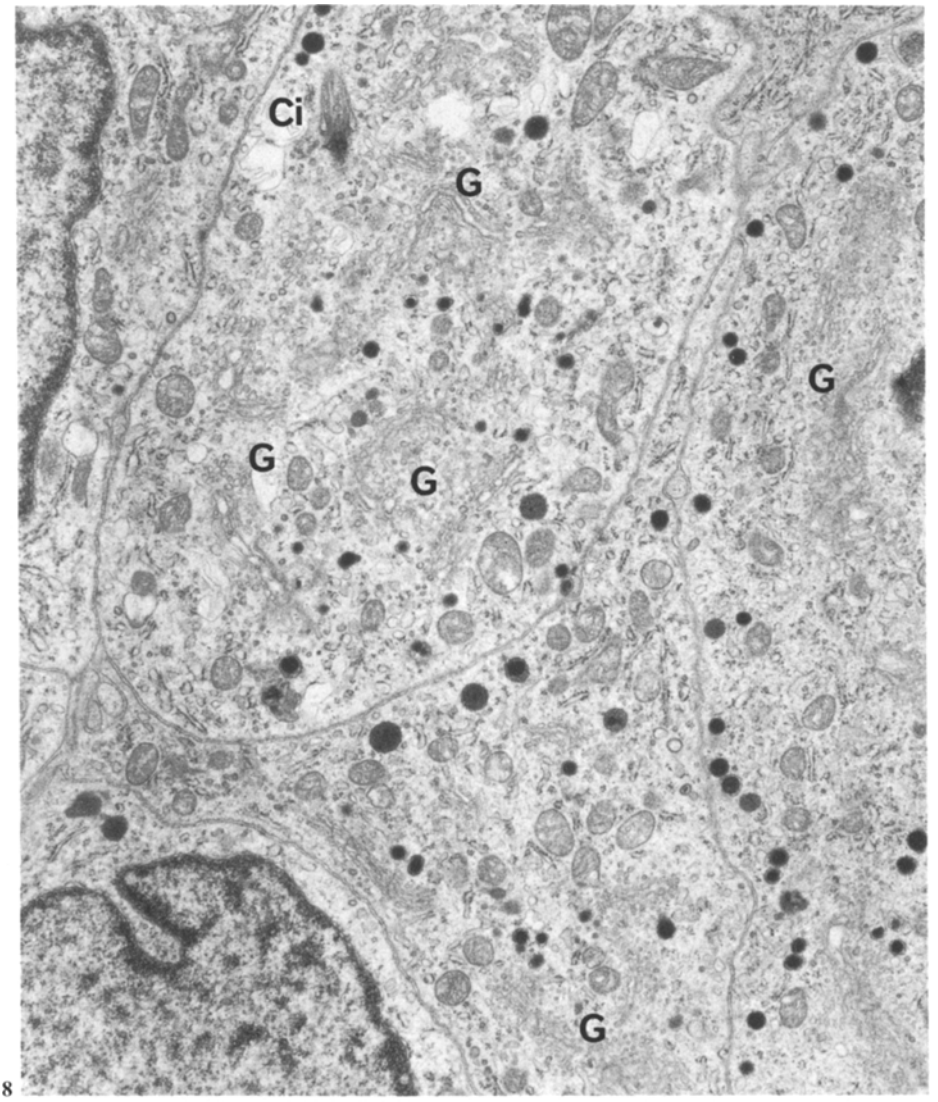
Fig. 6. Adenoma cells with extensively developed RER, consisting of stacks of parallel membranes. *Ly*, lysosomal element ×15,000

Fig. 7. Part of a cell with a large horseshoe-shaped Golgi apparatus in which developing granules are present. The cytoplasm further contains many mitochondria (392 in the complete cell transsection) and many granules (253 in the complete cell transsection). Note the preferential localization of mature granules along the cell borders. ×19,000



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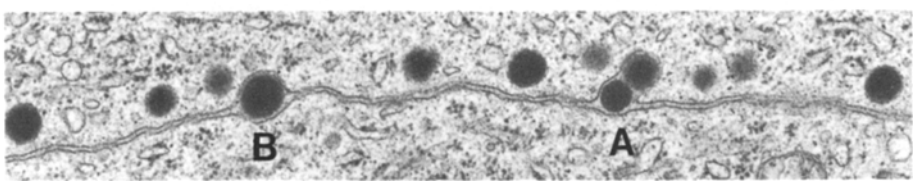

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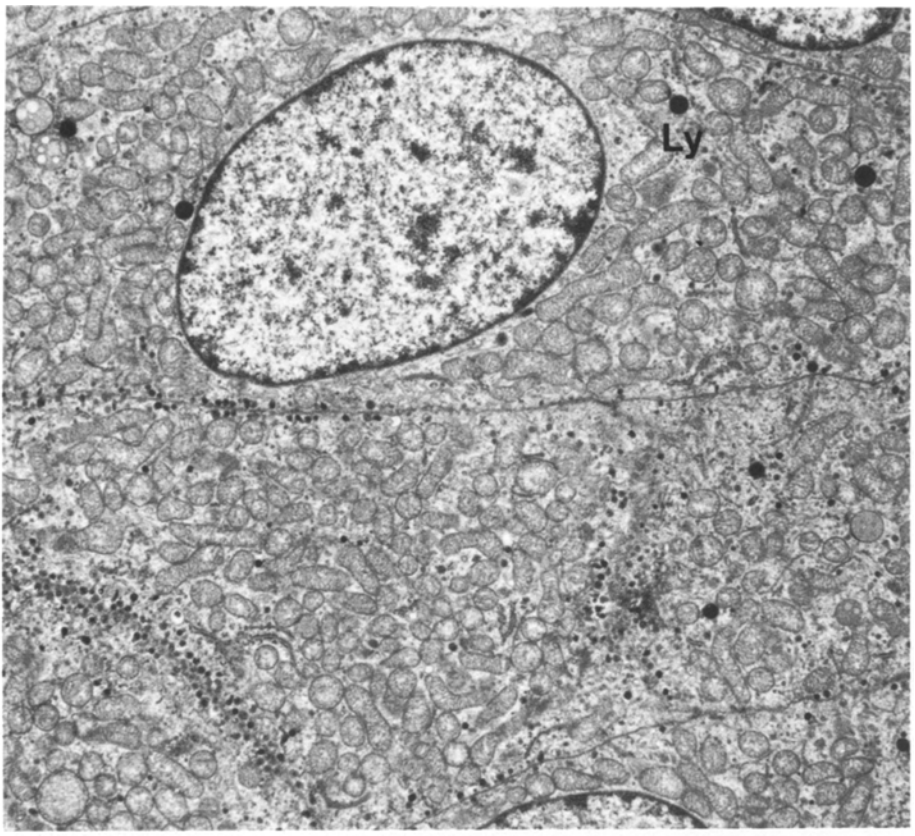
Fig. 8. Adenoma cells in which considerable portions of the cytoplasm are occupied by Golgi apparatus (some of them marked *G*). In contrast, the RER is only poorly developed. *Ci*, solitary cilium. ×12,000

Fig. 9. Hormone granules near the plasma membrane. The granules at *A* are apparently in the process of exocytosis, the granule at *B* is lying freely in the intercellular space. ×29,500



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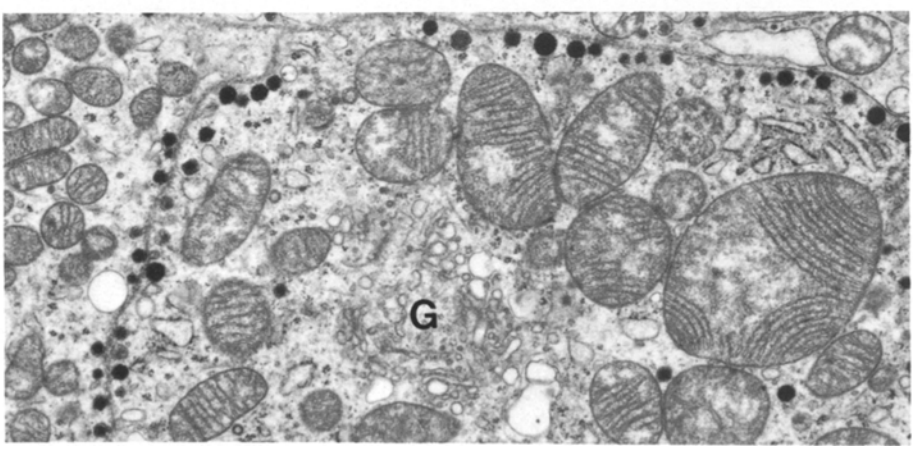

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Fig. 10. Adenoma cells filled with mitochondria, and also containing many hormone granules. The larger dense bodies (one of them marked *Ly*) represent lysosomes. × 7,000

Fig. 11. Mitochondria of giant size, and one of them with irregular morphology, in a mitochondrium-rich adenoma. Compare the size of the aberrant mitochondria with that of the mitochondria in the neighbouring cell at left. Note the well developed Golgi apparatus (*G*) and the localization of the hormone granules near the cell surface. × 14,000

adenoma that was included as a control was fully negative. Examples of misplaced exocytosis were found in 17 of the 19 adenomas.

Morphology. The morphology of most adenomas corresponded closely to the extensive descriptions of sparsely granulated prolactin cell adenomas in the literature (Horvath and Kovacs 1974; Kovacs et al. 1975; Robert and Hardy 1975; Saeger 1975a; Kovacs 1977; Kovacs et al. 1977; McComb and Kovacs 1978; Kameya et al. 1980). They generally consisted of irregular cell clumps and cords, frequently containing a central small blood vessel, and separated by thin sheets of connective tissue. Most cells were large, rounded and pale, frequently alternating with some irregular, degenerating cells (Fig. 1). There was, however, a gradual transistion to adenomas with extensive haemorrhagic areas. In such areas many cells were almost solitary, and mixed with variable amounts of stagnant blood. Calcium deposits that were present in some adenomas (Landolt and Rothenbühler 1977b) were located predominantly in the haemorrhagic areas (Figs. 2 and 4).

The cells had a large, ovoid, centrally placed nucleus with a prominent nucleolus and little heterochromatin (Figs. 1 and 2). Intranuclear cytoplasmic inclusions were not uncommon (Fig. 3). The cell outlines were smooth with occasional inconspicuous junctions (Fig. 5).

The RER was usually well developed, and often formed conspicuous stacks of parallel leaflets (Fig. 6); occasionally, "Nebenkern" formations were found. In other tumors, the RER was less abundantly developed, and sometimes it consisted mainly of small ribosome-bearing vesicles. One or more horseshoe-shaped Golgi apparatus were found in most cells (Fig. 7), sometimes occupying a considerable portion of the cytoplasm (Fig. 8). Usually the Golgi apparatus contained immature secretion granules, characterized by a wide electron-lucent halo (Fig. 7). Mature granules, round or slightly pleomorphic, and measuring about 150–250 nm, were often concentrated along the cell borders (Figs. 5, 7, 10 and 11). Granules in the process of exocytosis or lying freely in the intercellular space were numerous in many adenomas (Fig. 9). The cells generally contained a considerable number of mitochondria; in some adenomas, especially in the oldest patients, they filled most of the cytoplasm (Figs. 7 and 10). In the cells with the largest numbers of mitochondria, they sometimes showed irregular cristae and giant sizes (Fig. 11). Lysosomes and related elements (Smith and Farquhar 1966) were found in most cells (Figs. 5, 6 and 10). Solitary cilia of the "9+0"type (Dingemans 1969) were a regular finding (Fig. 8).

Even at superficial examination, it was evident that cells with an "active" morphology (i.e. with well developed RER and Golgi apparatus, and with many mitochondria) and "inactive" cells (i.e. with many lysosomal elements and stored hormone granules) could not be clearly distinguished. In fact, morphological features were frequently found in unexpected combinations (Figs. 5, 7, 8 and 10).

Correlation of Plasma Prolactin Level and Ultrastructural Variables. The results of the quantitative morphological analysis are given in Table 2. The values determined are plotted graphically against the plasma prolactin levels in Figs. 12–

Table 2. Ultrastructurally observed variables of cellular activity

Adenoma No.	Granules per cell[a]	Exocytosis per 20 cells	Mitochondria per cell[a]	Development of RER	Granule size[a] (nm)	Development of Golgi apparatus	Development of lysosomal elements
1	44	0	19	2.3	150	2.3	2.2
2	46	1.0	177	1.8	119	1.7	0.4
3	34	2.5	48	3.3	166	2.8	1.5
4	73	2.0	50	2.3	181	1.8	1.9
5	39	8.5	49	3.9	243	2.2	2.0
6	96	0	95	2.0	134	1.7	2.1
7	109	1.5	141	1.9	150	0.9	2.5
8	10	6.5	16	3.9	212	2.1	2.1
9	9	9.0	35	3.3	181	2.5	1.9
10	13	10.0	26	2.9	212	2.9	1.3
11	92	2.0	151	1.9	166	2.8	2.5
12	111	1.0	137	2.0	150	2.6	2.7
13	15	5.0	33	4.1	174	3.6	3.1
14	103	1.0	133	2.6	166	2.7	2.6
15	24	4.5	27	3.3	212	3.7	2.4
16	126	1.0	90	2.6	134	3.9	2.8
17	18	2.0	47	5.1	251	5.3	3.9
18	46	3.0	42	2.7	197	2.5	2.3
19	32	1.5	67	3.3	181	3.5	3.1

[a] Median value

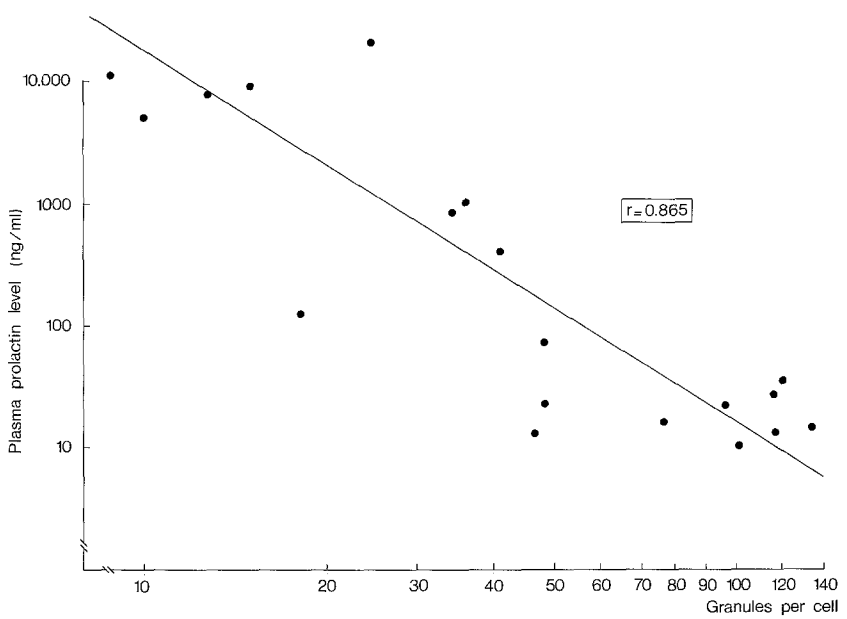

Fig. 12. Correlation of the median number of granules per cell transsection with the plasma prolactin level

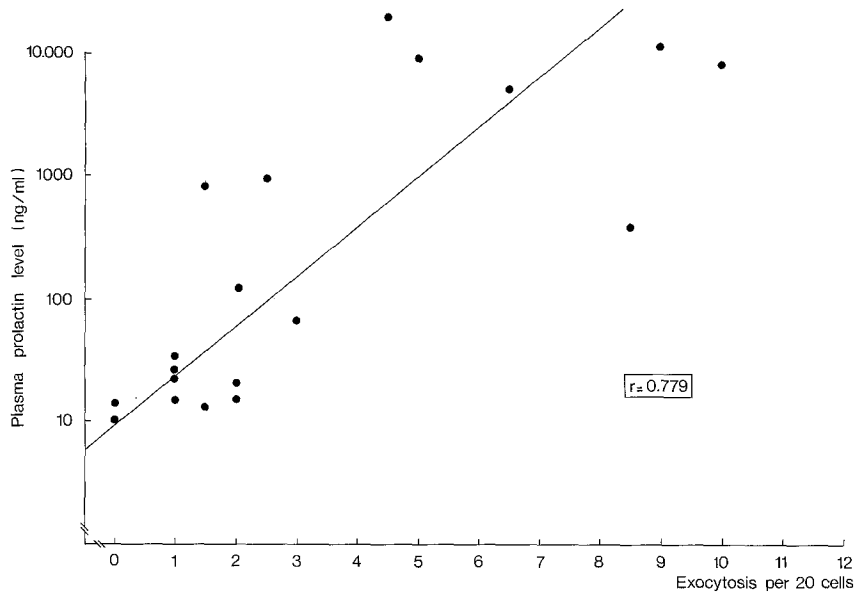

Fig. 13. Correlation of the frequency of exocytosis per 20 cell transsections with the plasma prolactin level

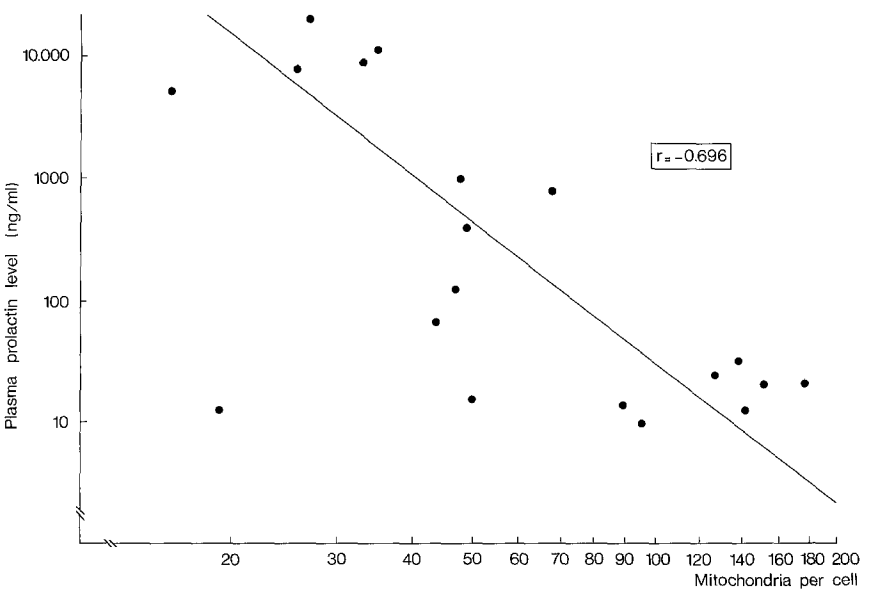

Fig. 14. Correlation of the median number of mitochondria per cell transsection with the plasma prolactin level

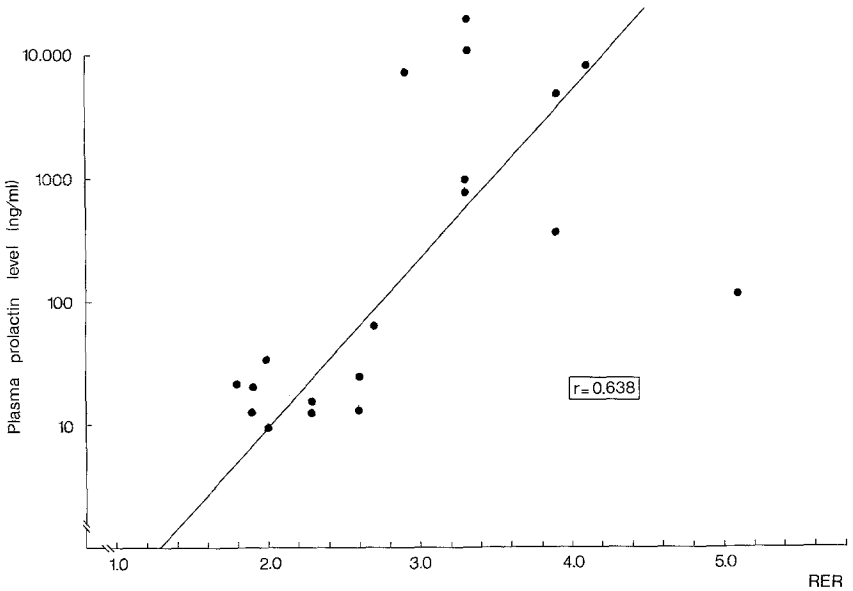

Fig. 15. Correlation of the development of the RER with the plasma prolactin level

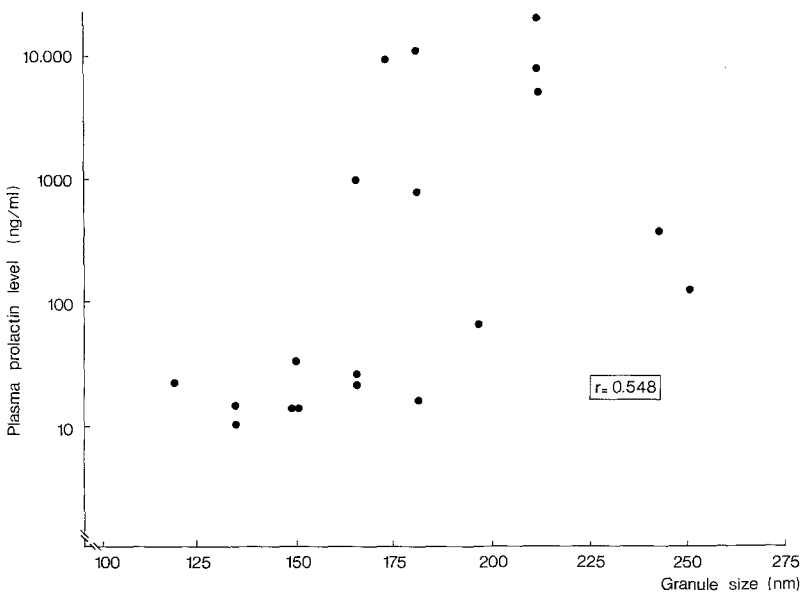

Fig. 16. Correlation of the granule size with the plasma prolactin level

18. There was a highly significant correlation of the plasma prolactin level and the number of granules per cell (Fig. 12). As expected, the correlation was negative, meaning that actively secreting adenomas had few hormone granules retained in the cells. Also the frequency of exocytosis was closely correlated with the plasma prolactin level (Fig. 13): actively secreting adenomas usually

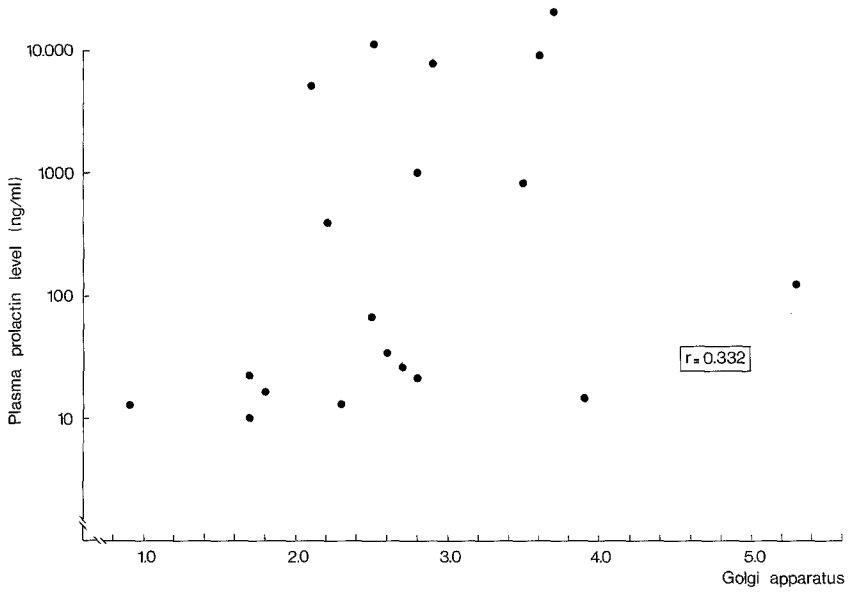

Fig. 17. Correlation of the development of the Golgi apparatus with the plasma prolactin level

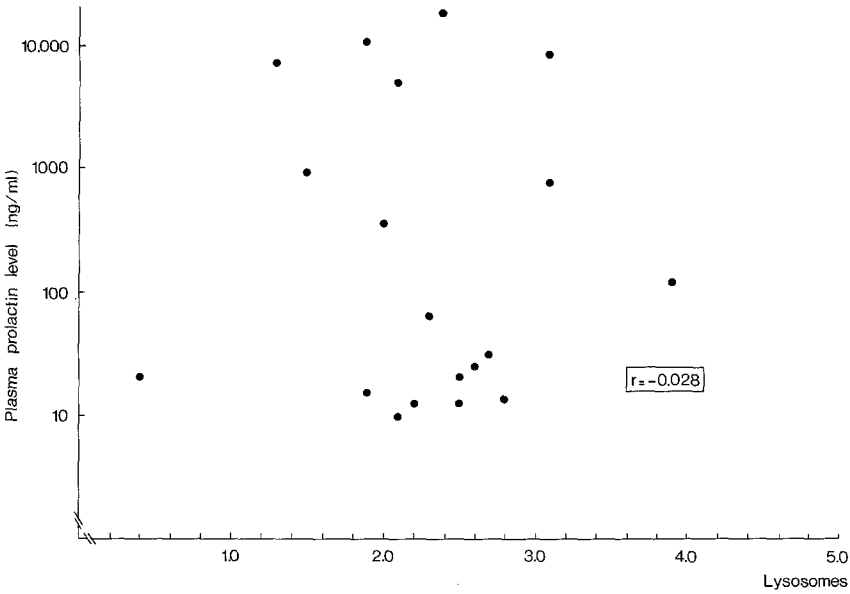

Fig. 18. Correlation of the development of the lysosomal elements with the plasma prolactin level

showed many granules in the process of exocytosis. The number of mitochondria and the development of the RER were somewhat less closely correlated with the plasma prolactin level (Figs. 14 and 15). Unexpectedly, the number of mitochondria per cell was inversely correlated with the plasma prolactin level, i.e. actively secreting adenomas were usually relatively poor in mitochondria. The

Table 3. Correlation coefficients and regression coefficients for the logarithm of the plasma prolactin level as dependent varible in the regression equation

	Correlation coefficient (r)	Standard error of estimate	Intercept	Regression coefficient	Probability of regression line (p)
No. of granules per cell	−0.8653	0.6171	6.7098	−2.7552	<0.001
Frequency of exocytosis	0.7787	0.7725	1.3123	0.2995	<0.001
No. of mitochondria per cell	−0.6964	0.8836	7.1990	−2.7401	<0.001
Development of RER	0.6380	0.9481	−0.1652	0.8449	0.003
Granule size	0.5477	1.0302	−0.4646	0.2813	0.015
Development of Golgi apparatus	0.3324	1.1611	1.2053	0.4003	0.164
Development of lysosomes	−0.0285	1.2306	2.3930	−0.0454	0.908

Table 4. Correlation matrix for the morphometric features

	No. of granules per cell	Frequency of exocytosis	No. of mitochondria per cell	Development of RER	Granule size	Development of Golgi apparatus
No. of granules per cell	1					
Frequency of exocytosis	−0.7370	1				
No. of mitochondria per cell	0.7946	−0.5828	1			
Development of RER	−0.7158	0.4779	−0.6220	1		
Granule size	−0.5945	0.6215	−0.6038	0.7873	1	
Development of Golgi apparatus	−0.3139	0.0417	−0.2695	0.6605	0.4361	1
Development of lysosomes	0.0673	−0.2398	−0.0842	0.4444	0.2956	0.6253

size of the hormone granules was only weakly correlated with the plasma prolactin level (Fig. 16). Finally, no significant correlations were found for the development of the Golgi apparatus (Fig. 17) and for the frequency of lysosomal elements (Fig. 18).

Statistical Analysis. In order to analyse the dependence of the hormone level on the activity of the cells as described by the morphological data, (multiple) regression was carried out. In Table 3 the result of single regression analysis is given. Table 4 gives the correlation between the morphological features. In Table 5 the result of multiple regression analysis is given. As can be deduced

from the results of the multiple regression, 82% (multiple $R^2=0.8250$) of the variance of the hormone level can be explained. The standardized regression coefficients in Table 5 show that the number of granules per cell and the frequency of exocytosis are responsible for a high proportion of the regression. The standard error of estimate indicates that, on the average, the predicted hormone level may deviate from the actual levels by 0.64 units on the logarithmic scale.

Table 4 shows that several morphological features are correlated with each other. It is also clear from Table 5 that most of the variables do not add significantly to the regression. Because of this insignificant part the multiple regression gives too high a value for the explained variance. It was therefore considered worthwhile to analyse the regression in a stepwise manner. In this case the logarithm of the number of granules per cell appeared to be selected as first of the features entered in the analysis, with 74% of the variance explained after the first step. Further steps did not significantly add to the regression, so that the results are of course identical to those of the univariate regression (Table 3, with the number of granules per cell as the entry).

Discussion

The data presented here provide an insight into the ultrastructural features associated with secretional activity of sparsely granulated prolactin cell adenomas. Some of the variables investigated showed a correlation with the plasma hormone level that might be anticipated on the basis of the literature, whereas others did not. The close but inverse relation between granule content and secretory activity fully answered our expectations, and the same was true for the high frequency of exocytosis and the marked development of the RER that were found in actively secreting adenomas. That the correlation was somewhat less for the RER than it was for the granule content and the exocytosis may be due to the uneven, polar distribution of the RER in most cells: frequently, the development of the RER cannot be deduced from a particular cross-section of a cell. An uneven distribution, together with a very great variability in shape and size, may explain the total lack of correlation found in the case of the lysosomal elements; an alternative explanation is that only a proportion of the elements in this heterogenous group are directly involved in hormone degradation. An uneven cellular distribution of the Golgi apparatus does not seem likely to explain the lack of correlation that we found for this organelle. In particular, the frequent presence of well developed Golgi apparatus even in the most inactive adenomas constitutes a striking finding that is difficult to explain. It demonstrates that one has to be extremely cautious in deducing the rate of cellular activity from morphology alone. Also our finding that the size of the hormone granules is no more than weakly correlated with the rate of secretion is difficult to explain. Apparently, granule size is an intrinsic property of the adenoma cells, and only partially dependent on the speed of granule formation and release (Landolt and Hosbach 1974).

The last morphometric variable investigated, the number of mitochondria per cell, exhibited a totally unexpected inverse correlation with the plasma

hormone level. The adenomas containing few mitochondria per cell formed the most active group and those with moderately increased numbers of mitochondria still caused a marked elevation of the plasma prolactin level, whereas the adenomas containing 90 or more mitochondria per cell were all very inactive. The adenomas in the latter group resembled pituitary oncocytomas as described by others (Kovacs and Horvath 1973; Landolt and Oswald 1973; Kovacs et al. 1974; Landolt 1975; Saeger 1975b; Horvath et al. 1977; Gjerris et al. 1978; Kalyanaraman et al. 1980; Martinez et al. 1980). According to most of these descriptions, the RER is scanty, some mitochondria have an aberrant morphology, and the Golgi apparatus may be surprisingly well developed, and this corresponds with our observations. A striking finding was the presence of many secretory granules in the oncocytic cells: the numbers of mitochondria and of granules per cell even constituted the morphometric features with the highest mutual correlation (Table 4). Several investigators have mentioned the existence of intermediate or transitional stages of oncocytic transformation (Kovacs and Horvath 1973; Kovacs et al. 1974; Landolt 1975). This probably corresponds to our finding of a fully gradual transition from only few mitochondria per cell transsection to values of 150 and more. We think our observations demonstrate that the oncocytic adenomas in our material constitute one endpoint of the range of variability of sparsely granulated prolactin cell adenomas rather than forming a separate group. The nature of the apparent metabolic inefficiency of the mitochondria in oncocytic adenomas is as yet obscure.

An intriguing question is why we found a better correlation of morphology and plasma hormone level than most other authors. As outlined in the introduction, this can be partially attributed to the quantitative methods used by us as opposed to the more subjective grading systems applied by several others. However, this explanation does not hold for the difference between our results and those of McComb et al. (1980), who used methods comparable to ours but were nevertheless unable to find any correlation of subcellular morphology and plasma prolactin level. We think the main reason for this difference is that the plasma prolactin levels in our study cover a much greater range – 10–20,200 ng/ml as opposed to 42–10,000 ng/ml in the study of McComb et al. Examination of our Figs. 12–18 demonstrates that little correlation would have been apparent if our data would have been limited to prolactin levels between 42 and 10,000 ng/ml. An additional factor may have been our method of quantitation in which several morphometric variables were estimated in a semiquantitative way, thus avoiding many of the errors that may be caused by the different degree of swelling of the various organelles that is caused by suboptimal tissue preservation.

In conclusion, our observations demonstrate a considerable degree of correlation between a number of morphometric features of sparsely granulated prolactin cell adenomas and the preoperative plasma prolactin levels. Due to the close mutual correlation that existed between several of the variables studied, combining them in a multivariate analysis did not significantly improve the correlation with the hormone level. It should be pointed out that complete correlation cannot be expected since our study was directed solely to the morphology of the cells, not taking into account the number of viable cells per adenoma, which is very hard to determine (McComb et al. 1980).

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Accepted March 18, 1982