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Immunohistochemical examination of the paraadenomatous "normal" pituitary

An evaluation of prolactin cell hyperplasia

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Summary. Prolactin cell hyperplasia has been described to occur in the paraadenomatous normal pituitary gland surrounding prolactinomas. However, compression of the glandular lobules and secretory cells alters profoundly the histological configuration of this tissue. No changes resembling those that occur during pregnancy are found. Immunohistochemical staining and counting of prolactin-(PRL-)secreting and growth hormone-(GH-)secreting cells in the normal, paraadenomatous pituitary gland obtained during extirpation of 24 prolactinomas and 5 adenomas causing acromegaly demonstrated that GH-secreting cells predominated in all biopsies obtained from acromegalic patients. PRL-secreting cells were more frequent than GH-secreting cells in 14 of 24 biopsies of the normal tissue surrounding prolactinomas. A particular predominance of PRL-secreting cells was found in patients with postoperative residual hyperprolactinemia. Direct comparison of adjacent sections demonstrates three cell types: One reacts with both antibodies and the other two react only with one or the other. We suggest that these groups are not stable but that cells belonging to one group can be transformed into cells belonging to the two other groups. Such a process, induced by extrahypophysary factors, may explain the shift of relative cell frequency observed in the normal pituitary gland surrounding prolactinomas.

Key words: Acromegaly – Hyperplasia – Immunochemistry – Pituitary gland – Prolactin

The etiology of pituitary adenomas remains unclear. They may arise as a result of changes in the normal pituitary gland caused by hypothalamic influences, peripheral endocrine derangements (e.g., long-standing hypothyroidism), or artificial hormone application (e.g., estrogen treatment of experimental animals); or they may develop in a setting of normal pituitary

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function unrelated to a previous endocrine disturbance (Landolt 1980; Reichlin 1980). We undertook this investigation on the premise that the study of biopsy specimens obtained from the paraadenomatous "normal" gland during operations on pituitary adenomas may provide an insight into this basic problem. A preexisting change in the distribution and number of the different secretory cell types in the gland may remain and coexist with the autonomously functioning adenoma to furnish information concerning the structure of the pituitary gland at the time of adenoma formation.

Few studies have been devoted to the structure of the paraadenomatous pituitary gland. Saeger (1977, 1981) demonstrated nodular adrenocorticotropin (ACTH) cell hyperplasia in tissues apart from ACTH cell adenomas in Cushing's disease and Nelson's syndrome. He found hyperplasia of prolactin-(PRL-)secreting cells in the biopsy material from 4 of 6 patients with differentiated prolactinomas, but observed no abnormalities in tissue surrounding growth hormone-(GH-)secreting adenomas. Kovacs and Horvath (1979) also noted increased numbers of PRL cells in the paraadenomatous tissue, and, like Saeger (1977, 1981), suggested that diffuse or multifocal prolactin cell hyperplasia may precede adenoma formation. However, both groups have rejected recently their own concepts (Asa et al. 1982; Saeger and Lüdecke 1983). Trouillas and collaborators (1976) found no PRL-cell hyperplasia in the vicinity of six prolactinomas; however, 2 of 5 biopsy samples obtained from normal gland adjacent to nonsecreting adenomas showed hyperactive PRL cells. Paraadenomatous PRL-cell hyperplasia was never observed in Robert's large series of prolactinomas (F. Robert, personal communication 1981).

The fundamental difference in the findings of these eminent scientists regarding the existence of paraadenomatous PRL cell hyperplasia is surprising. However, the discrepancy can be attributed to a number of factors.

– A major difficulty of these studies is that the diagnosis of hyperplasia was based on only qualitative criteria, which obviates a comparison of the findings. Quantitative criteria that would permit replication have been used only in two studies (Asa et al. 1982; Saeger and Lüdecke 1983).

– The different types of secretory cells are not distributed evenly throughout the gland. Certain cell types are located predominanty in the central (mucoid or basophilic) wedge, whereas others predominate in the lateral (acidophilic) wings (Baker 1974; Erdheim and Stumme 1909; Fowler and McKeel 1979; Halmi 1974; Kraus 1926; Phifer et al. 1970; Rasmussen 1929; Romeis 1940). Therefore, the relative number of individual cell types in a biopsy specimen depends heavily on the site from which the sample was taken – but it is difficult for the surgeon to define the exact site of biopsy because of distortions of the gland caused by the adenoma.

– Compression of the normal gland by the adenoma distorts the preexisting lobular structure. Lobules that were previously enlarged because of hyperplasia become compressed, and their size no longer can be used as a criterion of hyperplasia.

– The pressure of the adenoma exerted on the surrounding normal gland may destroy secreting cells. The different types of secreting cells may have a different resistance to pressure. This may alter the cellular composition of the glandular tissue.
– Compression of the hypothalamopituitary blood vessels may induce secondary cell changes because of the absence of stimulating and inhibiting hypothalamic factors.
– The histologic techniques used previously are not specific. Minor variations in the staining techniques may affect the apparent cell differentiation as seen under the light microscope. This is most important if a single cell secretes more than one hormone, for example PRL and GH (Halmi 1982; Stratmann et al. 1974). Precise data can be obtained only with immunohistologic techniques.

We will present the results of our quantitative study of PRL- and GH-cell numbers in the paraadenomatous normal pituitary gland of patients suffering from prolactinomas or acromegaly. These two conditions were chosen for study because of the similar intrapituitary distribution of the adenomas (Hardy 1973) and because PRL-cell hyperplasia has been described to occur in the paraadenomatous normal pituitary surrounding prolactinomas, whereas no analogous changes have been described for GH cells causing acromegaly (Saeger 1977, 1981). Cell differentiation was done with immunohistologic technique. The results were correlated with the outcome of adenoma extirpation in order to determine if the structure of the gland affects the endocrine result of surgery.

Material and methods

Biopsy specimens of the normal-looking pituitary tissue adjacent to secreting pituitary adenomas were obtained from 29 patients during selective transsphenoidal adenomectomy. Five of the patients had acromegaly and 24 patients harbored prolactinomas (15 microprolactinomas of 10 mm and less in diameter; and 9 macroprolactinomas of 11 mm or more in diameter). The serum PRL levels of the patients with prolactinomas ranged between 38 ng/ml and 5,200 ng/ml (normal 6–20 ng/ml). The specimens (diameter about 1 mm) were fixed within 30 s for 2 h in s-collidine buffered (pH 7.4) 2% osmium tetroxide, dehydrated in alcohol, and embedded in Epon (Landolt 1975). Pure osmium fixation was preferred to the more popular glutaraldehyde-osmium fixation technique because it offered better visibility of cell membranes due to a lower electron density of the cellular ground substance after omission of aldehyde prefixation.

Immunohistochemical analysis was done on random 0.5 μ thick plastic sections according to a slightly modified version of Sternberger's method (1979):
– Fix sections on glass slides covered with chromealumn gelatin (0.05 g chromealumn and 0.5 g gelatin dissolved in 100 ml distilled water at 40° C).
– Remove Epon in a 30% aqueous solution of hydrogen peroxide at room temperature during a period of 8 min.
– Wash the sections in distilled water and air-dry.
– Incubate in 3% normal goat serum in phosphate buffered saline (PBS) at pH 7.6 during 30 min at room temperature.
– Blot excess fluid.
– Incubate with the primary rabbit antibody diluted with PBS during 48 h at +4° C; follow

by incubation in the same fluid for 2 h at room temperature. The anti-hGH antibody is diluted 1:1,000, the anti-hPRL is diluted 1:120.[1]
– Rinse the sections in PBS three times for 2 min and blot.
– Incubate in goat-anti rabbit IgG link antiserum[2] diluted 1:10 in PBS for 30 min.
– Rinse the sections three times 2 min in PBS and blot.
– Incubate in 0.01 ml peroxidase-antiperoxidase complex[3] and 0.1 ml 10% goat serum diluted in 0.89 ml PBS for 30 min at room temperature.
– Rinse in PBS three times for 2 min.
– The peroxidase reaction was performed with a solution containing 0.05 g diaminobezidine[4] and 0.1 ml 10% hydrogenperoxide in 100 ml Tris buffer pH 7.6. The reaction time was 20 min at room temperature for the anti-hPRL staining and 8 min at room temperature for the anti-hGH staining. The sections were then washed in distilled water, dried, and embedded in Eukitt.

The following control experiments for specificity of the immunological reactions were done: (1) Saturation of the hGH-antibody with either 25 µg hGH[5] per 1 ml of 1:1,000 diluted anti-hGH or with 2 µg hPRL[6] per 1 ml of 1:1,000 diluted anti-hGH. (2) Saturation of the hPRL-antibody with either 2 µg hPRL per 1 ml of 1:120 diluted anti-hPRL or with 10 µg hGH per 1 ml of 1:120 diluted anti-hPRL. (3) Replacement of the antibody by normal rabbit serum diluted with PBS 1:120 and 1:1,000.

The slides were examined with a phase contrast microscope. Random photographs were made of 600–1,000 pituitary cells from each patient. The final magnification was ×1,800. All secretory cells and the population reacting with anti-hPRL or anti-hGH were counted. The proportions of the hGH and hPRL cells were calculated for each case.

A direct comparison of the cells reacting with anti-hGH and anti-hPRL was made in randomly chosen cases. One partner of pairs of adjacent tissue sections was fixed downside-up on the glass slide in order to expose the frontside and the backside of the same section-surface in two slides. One section was stained with anti-hGH the other with anti-hPRL. The negatives of one of the two series were enlarged upside-down in order to obtain superimposable pictures.

The percentages of the PRL cells and GH cells found in the individual biopsy specimens obtaind from patients with PRL-secreting adenomas and with acromegaly were compared with the Wilcoxon rank test. The prevalence of PRL cells or GH cells in the normal pituitary of the different groups of adenomas (acromegaly, prolactinomas with postoperative normal PRL, prolactinomas with postoperative elevated PRL) was tested with a modification of the 2 × 2 contingency table for small samples designed by Ott and Free (1969).

Results

The control experiments demonstrated that the immunohistochemical reactions were specific. There was a positive reaction after incubation of the tissue sections with the antibody and with the antibody reacted with the noncorresponding antigen (e.g., anti-hGH plus hPRL), whereas no reaction product was seen in sections incubated with the antibody saturated with the corresponding antigen (e.g., anti-hGH plus hGH) nor after replacement of the antibody by normal rabbit serum (Figs. 1, 2). The percentages of

1 The hGH antiserum was purchased from Wellcome Diagnostics, Dartford, England. The antiserum to hPRL was kindly provided through the National Pituitary Agency, Baltimore, MD by Dr. A.F. Parlow, Torrance, CA, USA.
2 Polysciences, Inc., Warrington, PA, USA.
3 Sternberger-Meyer, Inc., Immunohistochemicals, Jarrettsville, MD, USA.
4 Polysciences, Inc., Warrington, PA, USA.
5 Christiaens Bioproducts, Bruxelles, Belgium generously donated by Dr. M. Celio, Dept. of Anatomy, University of Zurich.
6 Calbiochem-Behring Corp., San Diego, CA, USA generously donated by Dr. M. Celio, Dept. of Anatomy, University of Zurich.

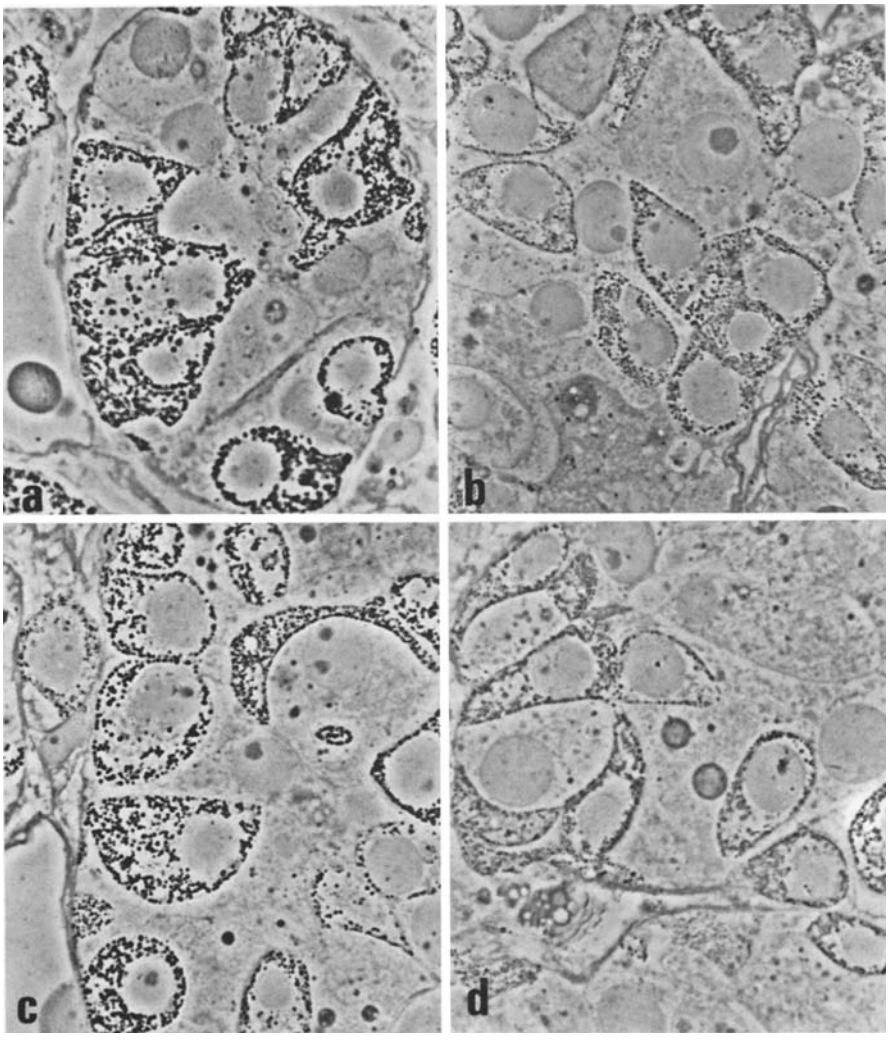

Fig. 1 a–d. Normal paraadenomatous pituitary tissue stained with anti-hPRL. Deposition of reaction product on PRL cells (**a**). No reaction product is seen after saturation of the antibody with hPRL (**b**) and after omission of the anti-hPRL antibody (**d**). The addition of hGH to the anti-hPRL antibody does not alter the positive reaction (**c**). The fine granules visible in the unstained cells (**b**, **d**) respresent the phase contrast picture of the osmiophilic granules that are seen also in unreacted control sections. Phase contrast micrograph, × 1,040

cells reacting with anti-hGH or anti-hPRL revealed extensive variations between the two groups of patients (Table 1); this was not surprising because no attempt had been made to obtain the biopsy specimens from a predefined area (e.g., acidophilic wing or mucoid wedge) of the pituitary gland. The percentages of GH cells and PRL cells were higher in the biopsy specimens obtained from patients with PRL-secreting adenomas than in the samples obtained from the patients who had acromegaly, but comparison of the

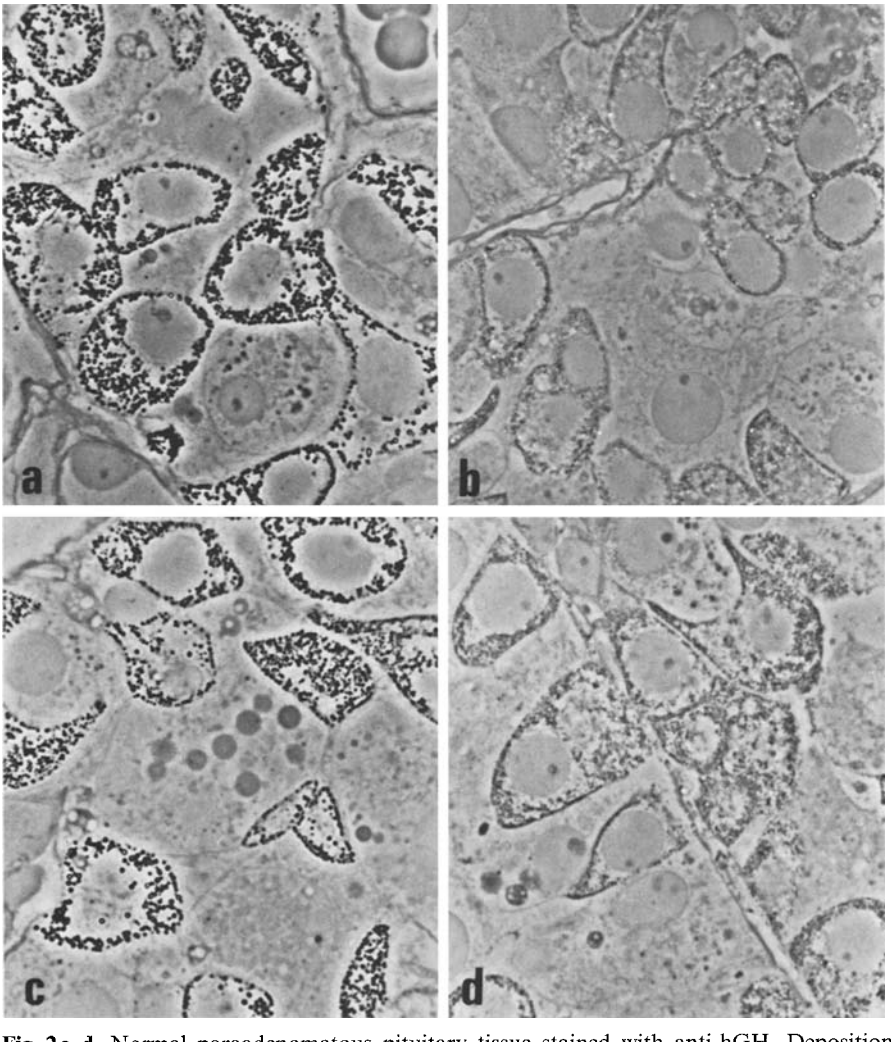

Fig. 2a–d. Normal paraadenomatous pituitary tissue stained with anti-hGH. Deposition of reaction product on GH cells (**a**). No reaction product is seen after saturation of the antibody with hGH (**b**) and after omission of the anti-hGH antibody (**d**). The addition of hPRL to the anti-hGH antibody does not change the positive reaction (**c**). The fine granules visible in the unstained cells (**b, d**) represent the phase contrast picture of the osmiophilic granuleas that are seen also in unreacted control sections. Phase contrast micrograph, × 1,040

individual percentages did not show significant differences (Wilcoxon rank-test). No significant differences were found in the percentage of PRL cells between the two subgroups of prolactinomas ("normal PRL after adenomectomy" and "elevated PRL after adenomectomy") with respect to microprolactinomas (diameter 10 mm or less) (Table 2).

The frequency of each cell type must be related to either a standard or among cell types in order to eliminate the influence of the intrapituitary

Table 1. Percentage of growth hormone- and prolactin-secreting cells in normal paraadenomatous pituitary gland

Adenoma type	Number of cases	Growth hormone cells		Prolactin cells	
		Percent of all cells in specimen	Range (%)	Percent of all cells in specimen	Range (%)
Acromegaly	5	29.4	9–48	25.0	7–37
Prolactinoma	24	41.8	15–73	41.0	14–73

Table 2. Percentage of prolactin cells in the normal pituitary adjacent to surgically treated macroprolactinomas and microprolactinomas

Prolactinoma group	Number of cases	Prolactin cells	
		Percent of all cells in specimen (average)	Range (%)
Postoperative PRL *within* normal limits:			
Total	9	37.6%	18–52
Microprolactinomas[a]	8	37.0%	18–52
Postoperative PRL *above* normal limits:			
Total	15	43.0%	14–73
Microprolactinomas	8	48.5%	21–73

[a] Diameter $\leqq 10$ mm

Table 3. Predominating cell type in normal gland adjacent to pituitary adenomas

Adenoma type	Predominating cell type (number of cases)	
	PRL-cell	GH-cell
Acromegaly	0	5
Prolactinomas		
Postoperative PRL *within* normal limits	3	6
Postoperative PRL *above* normal limits	11	4

origin of the individual specimen. In Table 3 the biopsy specimens are grouped according to the predominating cell type and the underlying disease. PRL cells were more frequent than GH cells in the normal pituitary gland surrounding prolactinomas and were less frequent than GH cells in the tissue surrounding adenomas causing acromegaly. PRL cells were also more

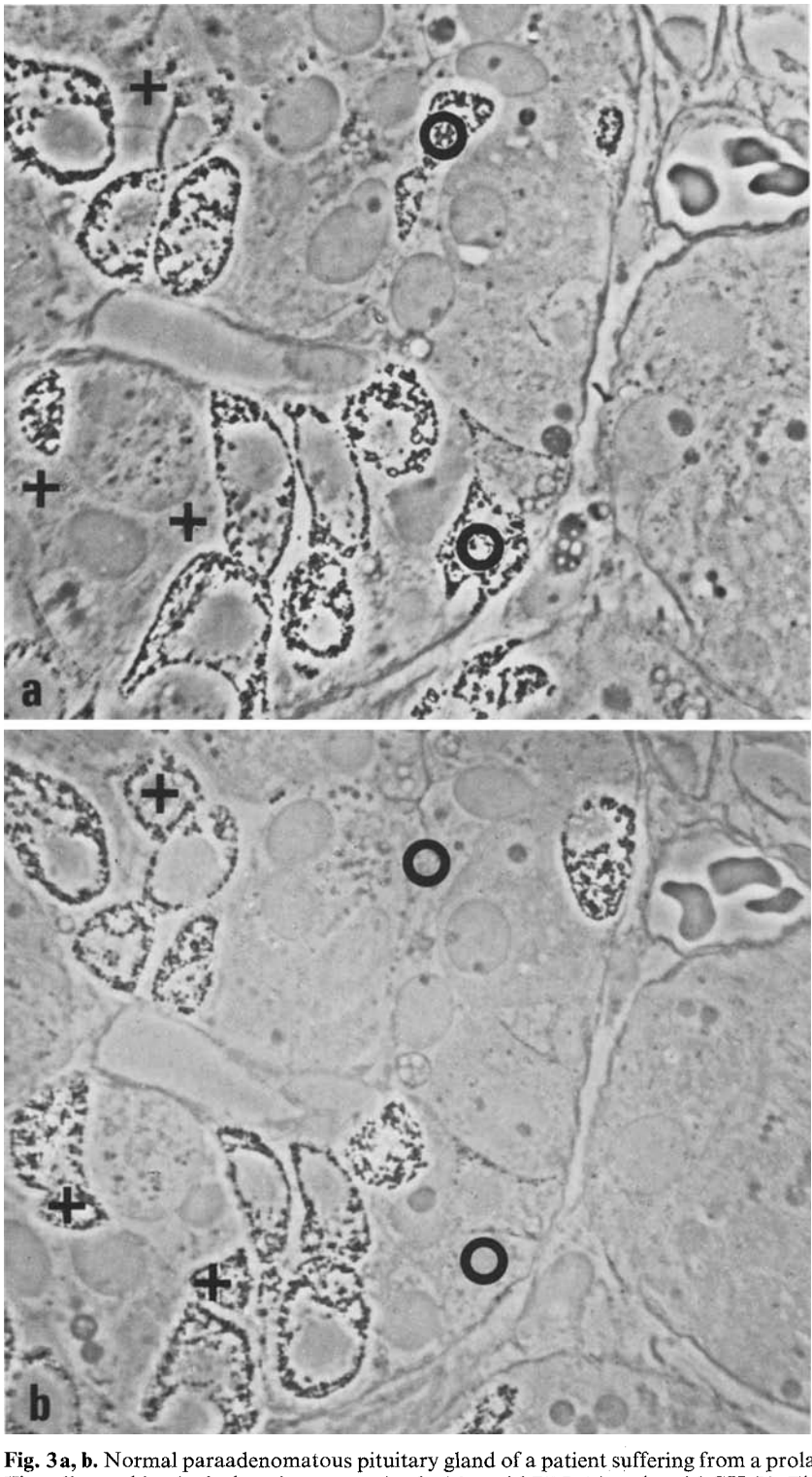

Fig. 3a, b. Normal paraadenomatous pituitary gland of a patient suffering from a prolactinoma. The adjacent histological sections are stained with anti-hPRL (**a**) and anti-hGH (**b**). The majority of the stained cells react with both antibodies. A minority is stained only with anti-hPRL (○) or anti-hGH (+). Phase contrast micrograph, ×1,160

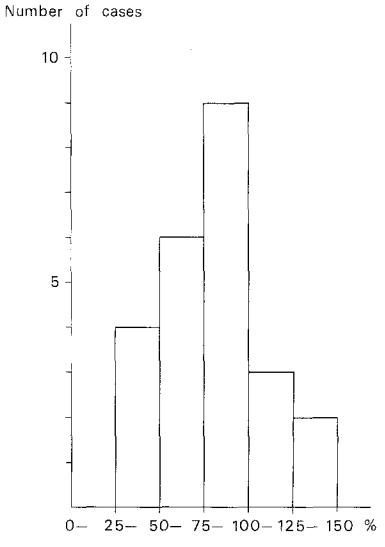

Fig. 4. Sum of percentages of PRL and GH cells calculated from results obtained from 24 biopsies of normal pituitary tissue adjacent to prolactinomas. Note that this sum is above 100% in one-fifth of the cases

frequent in the paraadenomatous pituitary adjacent to prolactinomas not cured by adenomectomy than in the gland of patients who were cured by surgical intervention. The difference were significant ($P<0.01$) according to the modified 2×2 contingency table.

A further analysis of our data demonstrated that the sum of the percentages of PRL cells and GH cells in the paraadenomatous pituitary ranged between 29.7% and 146.7% (Fig. 3). About one-fifth of the samples (5 of 24) had values above 100%, demonstrating that, at least in this group of specimens, a number of cells reacted with both antibodies. This could be shown also by a direct comparison of superimposable photographs of adjacent tissue sections (Fig. 4). Three cell types were observed. Most cells reacted with both antibodies. Two cell types found in small numbers reacted only with one antibody: either with anti-hPRL or with anti-hGH.

Discussion

Erdheim and Stumme (1909) examined in detail the enlargement of the pituitary gland that occurs during pregnancy. They observed enlargement of the cell nuclei and of the cytoplasma of the main cells (also called pregnancy-cells), which are equivalent to PRL cells, beginning during the fourth month of pregnancy and reaching a maximum between the seventh month and the time of delivery. This caused enlargement of the glandular lobules, the diameter of which increased from 50–70 μm in women who had never been pregnant to 250 μm during the first pregnancy and to 350 μm during subsequent pregnancies. Rare mitoses were observed in the pregnancy cells. Circumscribed collections of pregnancy cells were described as "adenomatous hyperplasia." These findings were confirmed by Romeis (1940) who

distinguished two cell types that contained different granules: the alpha granules (corresponding to GH granules) and the eta granules (PRL granules). The effect of pregnancy on the number of PRL cells as compared to the number of GH cells was determined by Goluboff and Ezrin (1969). Using a modification of Brooke's stain they differentiated the cell types in a random sampling that was proportional to the cell number in the whole gland. They described somatotrophs, prolactin cells, and mixed cells. The prolactin cells and mixed cells were only 1.6% the number of somatotrophs in premenopausal women, but in women in the third trimester of pregnancy their number increased to an average of 242% or 2.4 times as many PRL cells and mixed cells as somatotrophs. The PRL cells were larger during pregnancy than were the GH cells. Halmi and collaborators (1975) observed giant PRL cells in pregnant women.

We conclude from these observations that changes in the prolactin cell population seen during pregnancy are threefold: (1) There is an increase in the volume of the PRL cells (hypertrophy). (2) There is also an increase in the number of PRL cells relative to GH cells because of mitotic division (hyperplasia). (3) These two processes result in enlargement of the glandular lobules and of the whole gland.

The paraadenomatous normal pituitary of patients bearing prolactinomas, however, shows enlargement of neither the glandular lobules nor the prolactin cells. To the contrary, the lobules are compressed; they are small and form a pseudocapsule around the adenoma (Hardy 1969, 1975). This is even the case if the adenoma is situated in a pituitary gland that shows pregnancy-induced PRL-cell hyperplasia (see Erdheim and Stumme 1909, Fig. 16). In addition, the acidophilic cells surrounding a prolactinoma in a case with prolactin cell hyperplasia in the paraadenomatous normal pituitary are no larger than other secretory cells (see Saeger 1977, Fig. 1b). Therefore, hypertrophy of PRL-secreting cells present in the pituitary of pregnant women does not occur in the paraadenomatous pituitary.

The second change of PRL-secreting cells seen during pregnancy is the increase of their number in relation to GH-secreting cells as described by Goluboff and Ezrin (1969). The relative number of PRL cells in comparison to GH cells does not depend much from the intrapituitary location of the examined biopsy since GH cells and the more frequent, small PRL cells (mammotrophs I) show a similar distribution within the pituitary gland (Baker and Yu 1977). Our results show that PRL cells predominate over GH cells in the paraadenomatous pituitary gland in the majority of prolactinomas (Table 3), whereas this was never the case in patients with acromegaly. This difference cannot be attributed to the effect of pressure on the pituitary stalk or on the surrounding normal gland because the pressure was probably similar in both groups of adenomas.

Paraadenomatous PRL-cell hyperplasia, has to be expected in the group of microadenomas with persisting hyperprolactinemia after adenomectomy. It is not expected to be present in patients with normal postoperative PRL because it would produce persistence of hyperprolactinemia. However, not every case in which hyperprolactinemia persists after extirpation of a micro-

prolactinoma is necessarily a case of PRL-cell hyperplasia – incomplete extirpation of the adenoma may explain residual disease. Nonetheless, there is a significant difference between the number of cases in which PRL cells predominate in the group of patients with postoperative PRL above normal limits in comparison to those with normal postoperative PRL. This indicates that, at least in some cases, residual hyperprolactinemia may indeed be caused by an increase of the proportion of PRL cells as compared to GH cells in the paraadenomatous pituitary.

Saeger and Lüdecke (1983) recently have rejected the previous concept of Saeger (1977) concerning the PRL-cell hyperplasia occurring in the paraadenomatous pituitary adjacent to prolactinomas. The authors calculated the percentage of PRL-cells in immunostained small biopsy specimens obtained during surgical extirpation of prolactinomas. The cell number was compared with an average cell number obtained from immunostained sections of "normal" pituitaries removed for cancer treatment. This method, however, disregards the pronounced regional variation of PRL-cell distribution as shown in Table 1. The authors did not mention if the cancer patients had undergone previous endocrine treatment or not.

Cell counts on midhorizontal, immunostained sections through pituitary glands obtained from autopsy files did not demonstrate increased numbers of PRL cells in pituitaries containing "prolactinomas" in comparison to normal pituitaries obtained from men and nulliparous women (Asa et al. 1982). However, the authors did not mention wether these adenomas had caused elevated PRL-levels in the patients serum, as in our group of patients, or if the prolactinomas belonged to the group of incidental, adenoma-like changes in the glandular architecture described by Costello (1936) that do not cause hyperprolactinemia and have a different biological nature than prolactinomas (Heitz 1979; Landolt 1980; Burrow et al. 1981). The material studied therefore is probably not comparable with our biopsy specimens.

In their experiments performed in estrogen-treated male rats, Stratmann and collaborators (1974) demonstrated that the mitotic division of PRL cells alone cannot explain the increase in the number of PRL cells in the adenohypophysis, but that "uncommitted mammosomatotroph" cells, which under normal conditions bear the features of GH cells, can be transformed into PRL cells under the influence of estradiol. This finding defines the mechanism by which a change in the relative frequency of PRL cells and GH cells can occur without a corresponding increase in the number of cells present in a single pituitary gland lobule. In our material, the "uncommited mammosomatotroph" cells may be represented by the population of cells reacting with both the anti-hPRL and anti-hGH antibodies. Proof of the presence of such cells is evident in our finding that the sum of PRL cells and GH cells exceeds 100% in about one-fifth of the biopsy specimens examined and by the results of our superposition of photographs of adjacent tissue sections stained for the two antibodies.

We suggest that such a shift of GH cells into the group of PRL-GH-cells and of PRL-GH-cells into the group of cells producing only PRL may occur in the paraadenomatous pituitary of some prolactinomas. This "pro-

lactin cell-shift" may be the expression of a functional and structural change preceding the formation of a prolactinoma. Its persistence and slow increase after selective adenomectomy may also explain the high incidence of recurrent hyperprolactinemia without evidence of new tumor formation observed within 6 years after surgical PRL normalization in patients with microprolactinomas (Serri et al. 1983).

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