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Ultrastructure, Immunohistochemistry and Hormone Release of Pituitary Adenomas in Relation to Prolactin Production*

Toru Kameya[1], Masaru Tsumuraya[1], Isamu Adachi[2], Kaoru Abe[2], Kiyoshi Ichikizaki[3], Shigeo Toya[3], and Reiko Demura[4]

[1] Pathology and [2] Endocrinology Division, National Cancer Center Research Institute, Tokyo, Japan

[3] Neurosurgery Division, Department of Surgery, School of Medicine, Keio University, Tokyo, Japan

[4] Department of Internal Medicine, Tokyo Women's Medical College, Tokyo, Japan

Summary. Fifteen cases of pituitary adenoma, 14 of which were associated with hyperprolactinemia, were studied by observation and granule morphometry of electron micrographs, immunohistochemistry and sequential observation of in vitro release with regard to hormone production, storage and secretion. Adenoma cells of 6 cases with marked elevation of plasma prolactin were sparsely granulated, showed characteristic ultrastrucures including the presence of small secretory granules, well developed Golgi and rough membranes, misplaced exocytosis, and positive or negative immunostaining for prolactin. These adenomas also showed vigorous release of the hormone into the circulation and/or culture medium. In vitro studies showed that negative immunostaining of adenoma cells did not preclude the production and secretion of the hormone. One densely granulated adenoma containing cells with numerous lactotroph type granules showed moderate release of prolactin into the circulation. In an acromegalic case associated with both high plasma growth hormone and prolactin, some cells were shown by immunohistochemistry to store both hormones. There were 4 adenomas which could not be shown to produce, store and secrete prolactin by any method available.

Key words: Pituitary adenoma – Prolactin – Ultrastructure – Immunohistochemistry – Morphometry.

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Abbreviations used in this paper: ACTH = adrenocorticotropic hormone, β-MSH = β-melanocyte stimulating hormone, hGH = human growth hormone, hPRL = human prolactin, LH = luteinizing hormone, FSH = follicle stimulating hormone, TSH = thyroid stimulating hormone, TRH = Thyrotropin-releasing hormone

Offprint requests to: Toru Kameya, M.D., Pathology Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo (104), Japan

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Introduction

A specific and highly sensitive radioimmunoassay of plasma prolactin (PRL) (Hwang, 1971) and a close radiological evaluation of the pituitary (Naidich, 1976) have led to frequent discoveries of prolactin-secreting tumors by clinical endocrinologists and radiologists. In a recent series of 69 documented pituitary tumors (Antunes, 1977), hypersecretion of PRL was found in 65% of all patients and in 79% of those seen prior to treatment. Nevertheless, in the consideration of an individual case, neither the PRL level nor the response to stimulation or suppression tests is helpful in defining the a etiology. Direct evidence of PRL secretion by the tumor should be obtained by several other methods including the observation of the presence of a significant number of PRL-containing cells in tumor tissue revealed by immunohistochemistry (Zimmerman et al., 1974, Kovacs et al., 1975, Saeger, 1975b, Corenblum et al., 1976, Kovacs et al., 1976, Osamura et al., 1978), in vitro synthesis of PRL (Hwang et al., 1971; Guyda et al., 1973) and exclusive in vitro release of the hormone by tumor itself (Demura et al., 1977). These observations are necessary, since interference with the production and/or release of prolactin-inhibiting factor (PIF) and other hypothalamic mediators, or with their transport to the adenohypophysis may result in increased PRL secretion by the non-neoplastic pituitary, regardless of the presence or absence of pituitary tumor.

The purpose of this paper is to characterize pituitary adenomas in cases which showed hyperprolactinemia, by electron microscopy, immunohistochemistry and radioimmunoassay of in vitro release of PRL.

Electron microscopists and immunohistochemists have recently classified PRL-producing adenomas (lactotroph adenomas) into densely granulated and sparsely granulated types (Saeger, 1975b; Horvath and Kovacs, 1976; Kovacs et al., 1977). We present evidence on the basis of morphometrical analysis of electron micrographs and immunohistochemical studies and time-sequence observations of in vitro release of PRL, in 6 adenomas. Supplemental evidence that the sparsely granulated type of adenoma often secretes a great amount of PRL and shows a defective storage of the hormone as secretory granules is also presented.

Materials and Methods

1. Materials (Table 1)

Fourteen cases of pituitary adenoma which had shown hyperprolactinemia with preoperative basal plasma prolactin (PRL) levels more than 30 ng/ml, and one case in which plasma PRL was not measured, were investigated. Five cases showed the Forbes-Albright syndrome, another 6 had no hormonal physical signs and the other 4 acromegaly.

In cases displaying Forbes-Albright syndrome, basal plasma PRL levels ranged from 60 to 19,000 ng/ml. A good hPRL response to TRH stimulation was observed in Cases 8 and 14. In Case 10 with the Forbes-Albright syndrome, a high biological PRL activity of 744 IU/g dry tissue weight had been measured in an extract of a surgically removed adenoma at another hospital in May, 1959 (Takatani et al., 1967) and autopsy was performed in our institution on May 29, 1975, recurrent tumor tissue being subjected to endocrinological study.

Table 1. Data of clinical endocrinology in 15 cases of pituitary adenoma

Case No.	Age	Sex	Hormonal sign	Basal plasma hPRL (ng/ml) (2–20)[b]	Preoperative hPRL response to TRH
1	37	Female	F-A	19,000	no
2	24	Female	F-A	7,400	no
3	34	Male	Absent	1,200	no
4	29	Female	F-A	170	no
5	30	Female	Absent	600	no
6	45	Male	Absent	4,300	no
7	39	Male	Absent	500	no
8	45	Female	F-A	120	moderate
9	36	Male	Absent	n.m.	not done
10	39	Female	F-A	60	not done
11	45	Male	Absent	50	no
12[a]	37	Male	Acromegaly	350	no
13[a]	47	Male	Acromegaly	40	no
14[a]	31	Female	Acromegaly	31	good
15[a]	52	Male	Acromegaly	30	not done

hPRL = human prolactin, F-A = Forbes-Albright syndrome, n.m. = not measured

[a] Case of high plasma human growth hormone (hGH)

[b] Normal range

Out of 6 cases without hormonal signs, 5 were males and 1 female. Of these, 3 males and 1 female showed marked elevation of plasma PRL. In Case 12 in which in vivo and in vitro secretion behavior was reported previously (Demura, 1977), both hGH (190 ng/ml) and hPRL (350 ng/ml) were markedly elevated. In 3 other acromegalics, PRL levels were slightly elevated, and neither galactorrhea nor amenorrhea was detected.

2. *Methods*

a) Light Microscopy. Pieces of tumor tissue were fixed in 4% paraformaldehyde and Zamboni's fluids and embedded in paraffin. 4 μ sections were stained with hematoxylin-eosin. When needed, Herlant's erythrosin (Herlant, 1960) and Wilson-Ezrin's stainings (Wilson and Ezrin, 1954) were used.

b) Electron Microscopy. Tissues were fixed in phosphate buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated by graded alcohols and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed in a JEOL 100 U electron microscope. The size (diameter) measurement of more than 200 secretory granules of tumor cells of each case was performed on 5 to 6 randomly selected printed electron micrographs of 25,000 magnification. The number of secretory granules in cytoplasm in 5 to 6 randomly selected printed electron micrographs (12,000X, 17.5 cm × 24.5 cm), was counted. These measurements were made by a MOP/AMO3 semiautomatic quantitative picture analysing system (Kontron, Inc., Munich, West Germany). From these data, mean diameters ± standard deviations of secretory granules in nm, secretory granule number per unit area (in nm^2) of the cytoplasm, mean areas of secretory granules in nm^2 and mean percentages of areas occupied by secretory granules in cytoplasm in each tumor case were calculated.

c) Immunohistochemistry. In all 15 adenomas, indirect immunoperoxidase reactions (Nakane, 1967, Sternberger, 1970) for human PRL (hPRL) and human growth hormone (hGH) were performed on deparaffinized fixed tissue sections. Details of immunohistochemical techniques have been described previously (Kameya et al., 1974; Kameya et al., 1977). For control tests to absorb any

contaminating or cross-reacting antibodies to hGH, 1 ml of the anti-hPRL and anti-hGH sera diluted 1:20, and 1:500 were treated with 10 μg and 100 μg of purified hGH and the supernatant was used for immunohistochemistry, instead of unabsorbed sera. Absorption tests with purified hPRL could not be done, because sufficient amounts of the antigen were not available.

d) Primary Cell Culture. In Cases 2, 3, 5, 6, 9, 12 and 13, cells of minced adenoma tissue of approximately 2 mm^3, incompletely dispersed with 0.25% trypsin, were cultured in RPMI 1640 medium (Nissui Chemicals, Tokyo) containing 17% fetal calf serum at 37° C in a CO_2 incubator (5% CO_2 and 95% air). Complete medium changes were made twice a week and all media were separately frozen and stored at −20° C until hormone radioimmunoassay.

e) Radioimmunoassay (RIA) of Pituitary Hormones. Plasma and culture medium hGH and hPRL were measured by RIA using either Kitasato Biochemical Laboratory Kits, Sagamihara, Japan or Dainabot RIA Kits (Dainabot RI Research Institute, Tokyo). TSH, LH and FSH were measured by RIA kits which were supplied from the NIAMDD, U.S.A. and ACTH and β-MSH were radioimmunoassayed by the method reported previously by one of the authors (K.A.) (Abe et al., 1967).

Results

a) Light and Electron Microscopy (Table 2). Ultrastructural features of adenomas (Fig. 1), all of which were chromophobic and some faintly erythrosinophil, were very similar in high plasma PRL cases (Cases 1 to 6; 170 to 19,000

Table 2. Summary of electron microscopy, immunohistochemistry, and in vitro release of hPRL in 15 cases of pituitary adenoma

Case No.	EM findings rER	Golgi	Mitochondria	Misplaced exocytosis	Immunostaining for hPRL[b]	Maximal hPRL in culture medium (μg/ml)
1	+++	+++	+~++	+	positive	n.d.
2	+++	+++	+	+	positive	356
3	+++	+++	+~++	+	ambiguous	62
4	+++	+++	+~++	+	positive	n.d.
5	+++	+++	+~++	±	positive	45
6	+++	+++	++	++	positive	1,560
7	+	+	+	−	negative	n.d.
8	+	+	+++	−	negative	n.d.
9	+~++	++	+~++	−	ambiguous	2.3
10	+	+	+	−	positive	n.d.
11[a]	++	++	+~++	−	positive	n.d.
12[a]	++	+++	+	++	positive	1.5, 1.8 (hGH)
13[a]	++	+	+	−	negative	0.4, 1.2 (hGH)
14[a]	+~++	+	+	−	negative	n.d.
15[a]	+~++	+~++	+	−	negative	n.d.

hPRL = human prolactin

[a] Case of presence of cells positive for human growth hormone (hGH), rER = rough endoplasmic reticulum, − = absent, ± = rare, + = poorly developed or infrequent, ++ = moderately developed or occasional, +++ = well developed or frequent

[b] Immunoperoxidase method by use of anti-hPRL

[c] Maximal release of hPRL into 1 ml of culture medium in 3 to 4 days, n.d. = culture not done

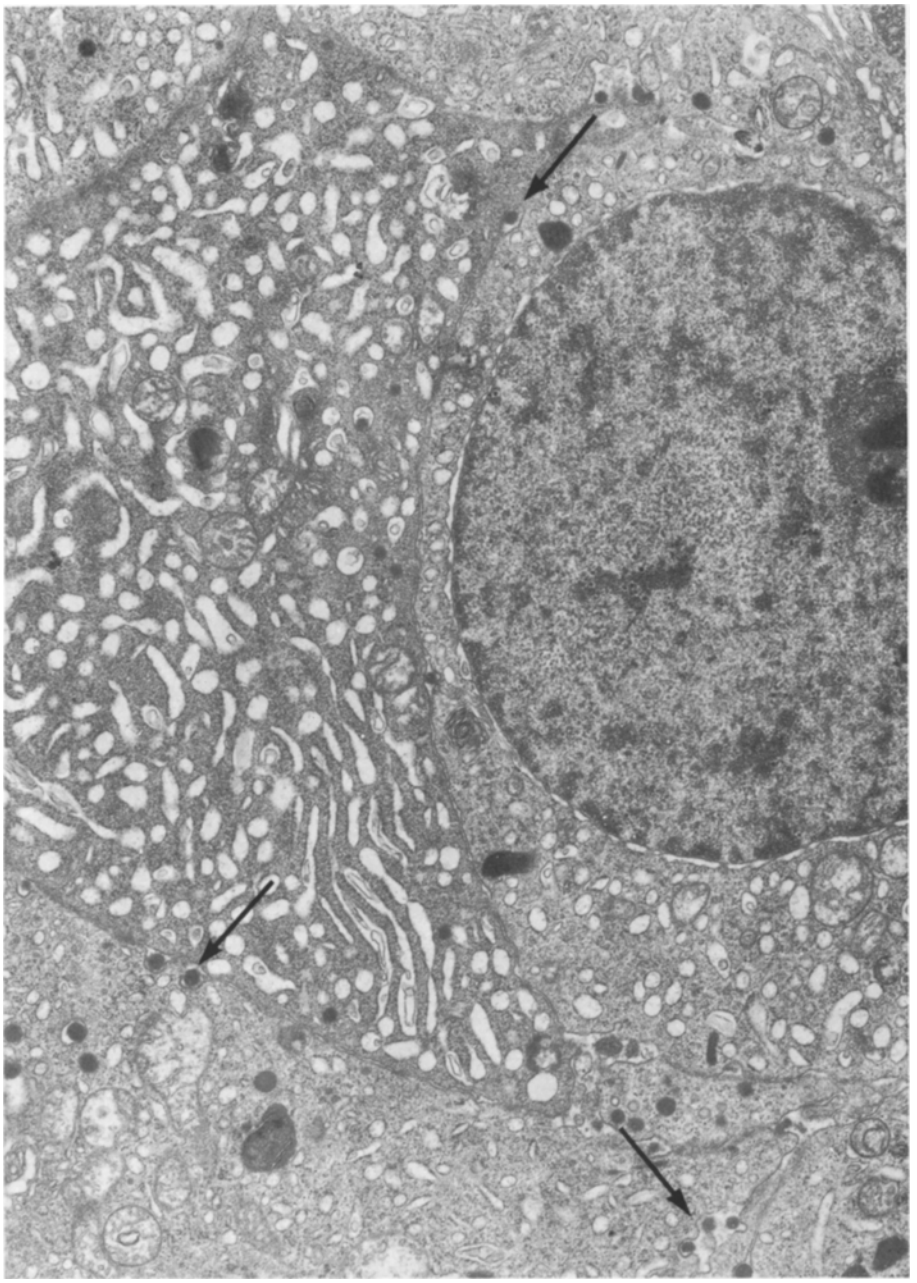


Fig. 1. Electron micrograph of sparsely granulated adenoma cells (Case 1). Well-developed endoplasmic membranes and small secretory granules with occasional polymorphic ones. Arrows indicate "misplaced" exocytosis of secretory granules. × 12,500

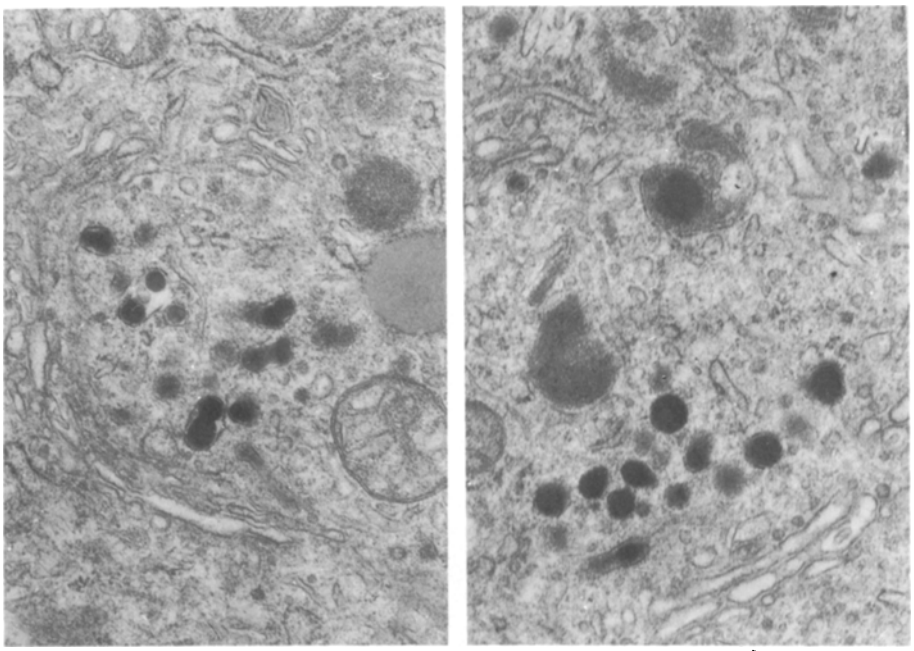


Fig. 2. Electron micrograph of a sparsely granulated adenoma cell (Case 6). A well-developed Golgi complex with two or three fusing granules in membrane-bound compartments. ×25,000

Fig. 3. Electron micrograph of a sparsely granulated adenoma cell (Case 6). A Golgi complex with lysosome-like granules in the process of digestion of a secretory granule. ×25,000

ng/ml) with or without the Forbes-Albright syndrome. Golgi complexes and rough and smooth vesicular membranes were extensively developed (Fig. 1). Small secretory granules were sparsely and randomly dispersed in the cytoplasm, occasionally concentrated in concave zones of Golgi complexes (Figs. 2 and 3). Secretory granules measuring 150–250 nm in diameter predominated (Fig. 14), but two or three fusing granules of the same size encased in a single membrane-bound compartment were also seen. Some of these appeared to be in the process of incorporation into large round granules and heterogeneously or homogeneously dense large pleomorphic granules (300–500 nm). The latter granules have been described as lactotroph granules in animal and human pituitaries, and small number were present in most cases. However, in Case 6 a considerable number were observed whereas in Case 5 they were extremely rare. The large heterogeneous pleomorphic granules often containing smaller dense granules were difficult to distingush from secondary lysosomal granules (Fig. 3). The "misplaced" exocytosis or extrusion of granules in the intercellular spaces described by some authors (Horvath and Kovàcs, 1974; Robert and Hardy, 1975) was constantly found with variable frequencies, most frequently in Case 6. It occurred at sites between apposing cell membranes, remote from blood vessels and basement membranes, where normal exocytosis was found (Figs. 1 and 4).

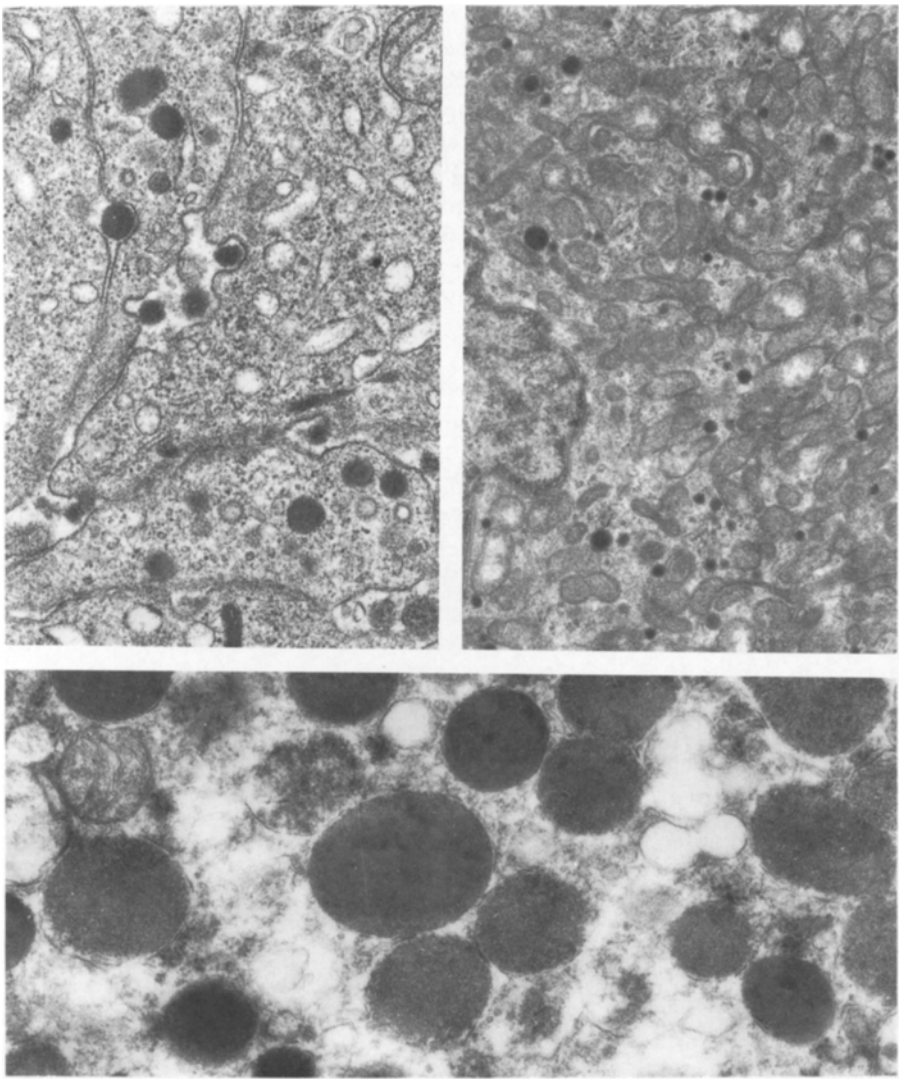


Fig. 4. Electron micrograph of sparsely granulated adenoma cells (Case 1). Several features of "misplaced" exocytosis of secretory granules. × 25,000

Fig. 5. Electron micrograph of an oxyphilic adenoma cell (Case 8). Numerous mitochondria and scattered small secretory granules. × 12,500

Fig. 6. Electron micrograph of a densely granulated adenoma cell (Case 10). Numerous very large pleomorphic granules were packed in the cytoplasm. × 25,000

The mean percentages of area occupied by secretory granules ranged from 0.43% to 1.7% in Cases 1 to 9. The values were very similar among these cases and might represent the lower capacity of storage of the hormone produced by adenoma cells in the form of secretory granules (Fig. 15). In Case 7, the cytoplasm was only sparsely granulated and large granules were absent. In

Case 8, the cytoplasm was packed with numerous mitochondria and contained only a small number of secretory granules, representing the oxyphilic adenoma (Figs. 5 and 14) described by some authors (Kovacs and Horvath, 1973; Landolt and Oswald, 1973; Saeger, 1975a). In Case 10, tumor cells were mostly filled with large, round or oval secretory granules (Figs. 6, 14 and 15) which were acidophilic, erythrosinophilic and simulated the typical non-neoplastic large lactotroph granules described in many experimental animals and in man, and in a few cases of adenoma (Peake et al., 1969). The adenoma of Case 11, which had a lower plasma PRL value (50 ng/ml) and no hormonal symptoms, consisted of cells with moderately or highly dense populations of small secretory granules. Typical lactotroph granules were absent (Figs. 14 and 15).

Case 12 had acromegaly and marked elevation of plasma hGH and hPRL. The adenoma was acidophilic, slightly eythrosinophilic and moderately or sparsely granulated (Fig. 15). The size of granules in this case varied from cell to cell and within individual cells. Some had large spherical GH-type granules, some had mixed large GH-type and pleomorphic lactotroph type granules, others had mostly smaller granules, mixed with scattered large spherical ones (Figs. 7, 8 and 14). Cystoplasmic processes were extensively developed. Organelles of rough membranes and Golgi complexes were moderately or well developed and misplaced exocytosis and intracytoplasmic filamentous aggregates (Schochet et al., 1972) for fibrous bodies (Kovacs et al., 1977) were encountered occasionally (Table 2).

The last 3 cases (Cases 13 to 15) were acidophilic and orangeophilic adenomas. Ultrastructural features appeared identical to those of other reported cases which showed acromegaly and were called by Kovacs et al. (Kovacs et al., 1977) densely granulated GH cell adenoma. Cells with the features described in Cases 1 to 6 were infrequently encountered in Case 13 and never encountered in Cases 14 and 15 (Figs. 14 and 15).

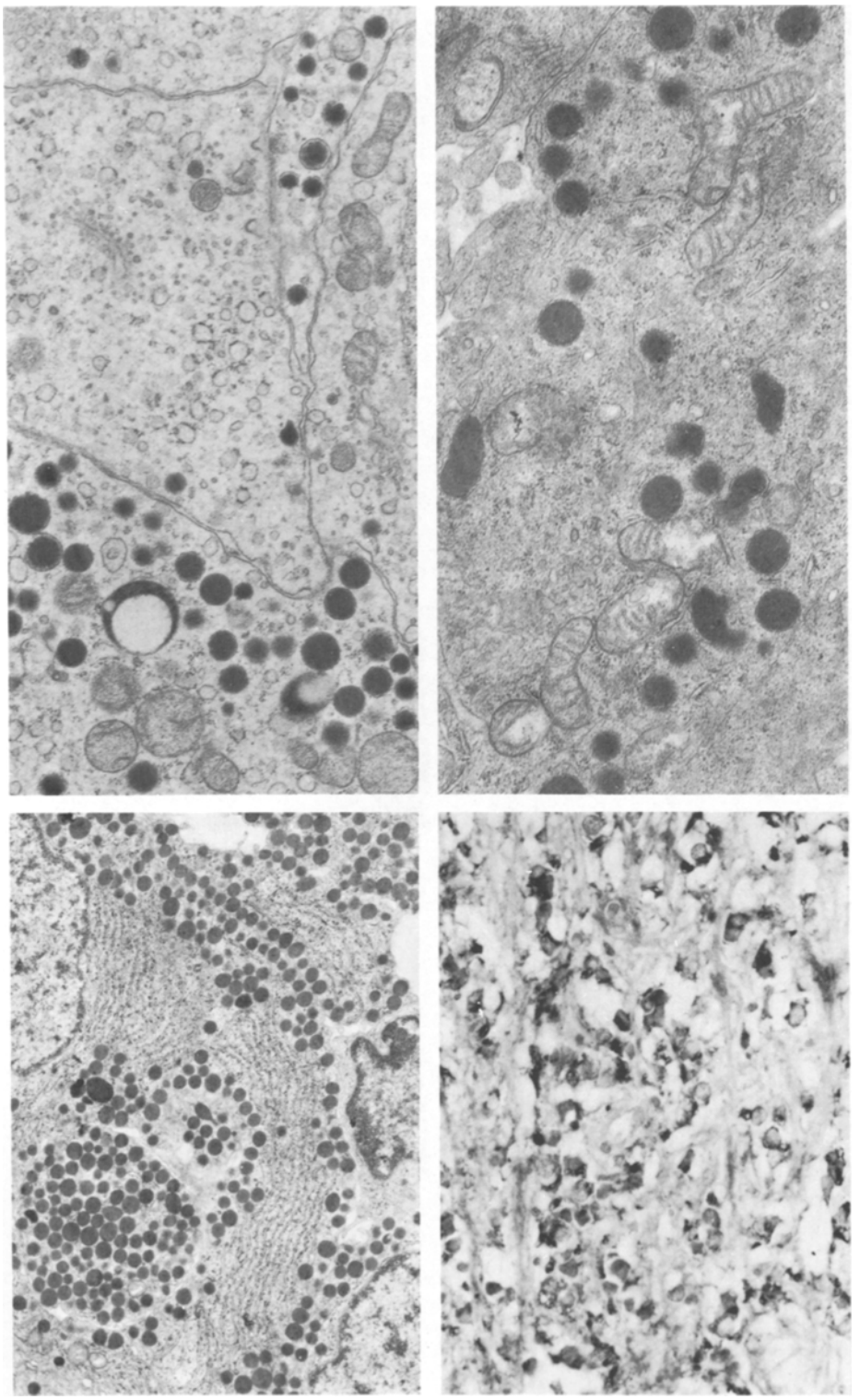
b) Immunohistochemistry. Immunostaining of adenoma cells for hPRL was positive in Cases 1, 2, 4, 5 (Fig. 10), 6, 10, 11 (Fig. 12) and 12 (Fig. 13), negative in Cases 7, 8, 13, 14 and 15, and ambiguous in Cases 3 and 9 (Table 2). Positive cells were usually distributed at random, staining was patchy, often weak and in solitary or cluster forms. Reaction deposits were often localized on one side of the nucleus (Fig. 10). Very faint diaminobenzidine deposits did not permit judgment of whether Cases 3 and 9 contained positive cells or not.

Fig. 7. Electron micrograph of adenoma cells with both hGH and hPRL production (Case 12). Sparsely and moderately granulated cells with small and large spherical secretory granules. ×12,500

Fig. 8. Electron micrograph of adenoma cells with both hGH and hPRL production (Case 12). Mixed shperical and pleomorphic large granules in one cell. ×12,500

Fig. 9. Electron micrograph of densely granulated cell adenoma cells (Case 13). Adenoma cells contained predominantly GH-type granules. ×7,500

Fig. 10. Adenoma of Case 5 immunostained by anti-hPRL. Immunoperoxidase method. Nuclei lightly stained with methyl green. ×136



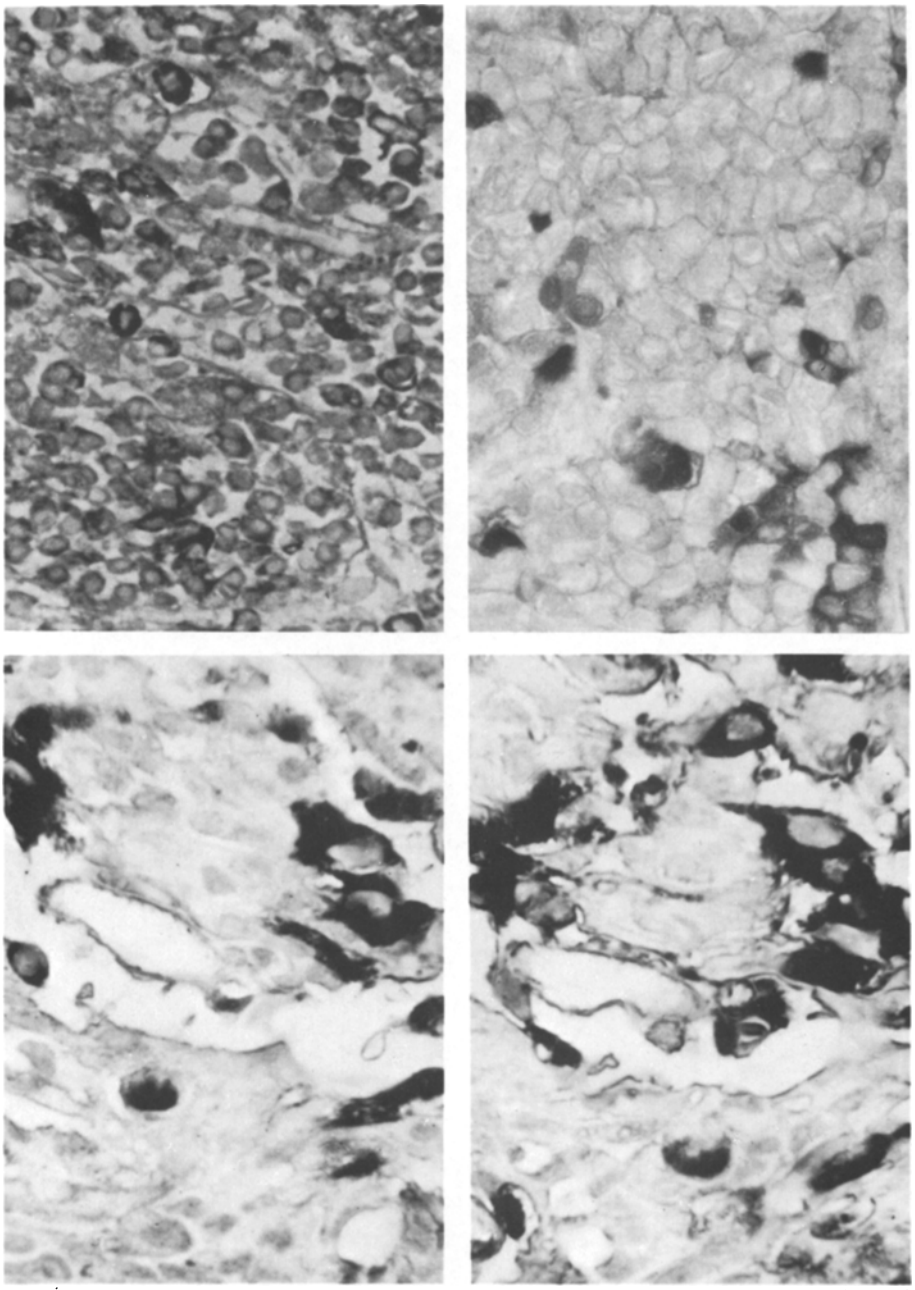


Fig. 11. Adenoma of Case 10 immunostained by anti-hPRL. Immunoperoxidase method. Nuclei lightly stained with methylgreen. ×200

Fig. 12. Adenoma of Case 11 immunostained by anti-hPRL. Immunoperoxidase method. Nuclei unstained. ×200

Fig. 13a and b. Adenoma of Case 12 with hGH and hPRL production. **a** and **b** show the same area of adjacent sections. **a** Immunostained by anti-hGH, and **b** Immunostained by anti-PRL. Immunoperoxidase method. Nuclei lightly stained with methylgreen. ×1,400

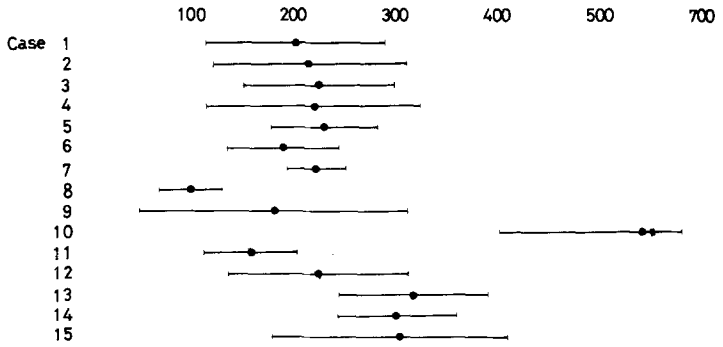


Fig. 14. Mean diameters ± S.D. (standard deviations) nm of secretory granules of randomly selected adenoma cells in 15 cases. Each diameter of more than 200 secretory granules was measured by a semiautomatic quantitative picture analysing apparatus MOP/AMO3 (Kontron) and the mean diameter ± S.D. of each case was calculated

In Case 10, most cells of the autopsy adenoma material reacted to anti-hPRL with variable intensities and did not react to anti-hGH (Fig. 11). In case 11, cells randomly reacted to anti-hPRL (Fig. 12) and anti-hGH in different cells.

In Case 12, immunostaining with anti-hGH revealed numerous positive cells while a few scattered cells positively stained with hPRL. However, in many areas, clusters of cells stained positively both for hGH and hPRL on two sets of adjacent sections. Numerous cells were negative for both hGH and hPRL (Figs. 13a and 13b).

Numerous cells of Cases 13, 14 and 15 were positive for hGH. Adenomas of Cases 1 to 9 revealed no reaction to anti-hGH.

Immunostaining by anti-hPRL (1:20 and 1:500) treated with hGH (10 and 100 μg) did not change the intensity of the positive immunoreaction to non-treated anti-hPRL, and immunostaining by anti-hGH (1:500) treated with hGH (100 μg) was completely negative in Cases 11, 12, 13, 14 and 15. No definite reaction deposit of diaminobenzidine was observed in the cytoplasm of adenoma cells when several batches of non-immunized serum were applied instead of specific antiserum. In Case 6, very few cells were stained by anti-FSH serum.

c) hPRL Release of Primary Culture of Adenomas. Vigorous release of hPRL into culture medium in contrast to other pituitary hormones (except in Cases 12 and 13) was confirmed at the initial stage of culture and occasionally enhanced or maintained for some periods at high levels, followed by gradual decrease from levels of 10^2 to 10^6 ng/ml of medium at 3 or 4 days. Continued detection was possible for up to 100 days in Cases 2, 3, 5, 6, 9 (Table 2 and Fig. 16), 12 and 13 (Table 2 and Demura, 1977). The first three cases were especially prominent in terms of hPRL release. The in vitro results were in accordance with marked release of the hormone into circulation in vivo (Table 2). One month after culture, adenoma cells of Case 3 were confirmed to have the same features as the original tumor cells by electron microscopy, including the amount of organelles, size of secretory granules and number of secretory granules per

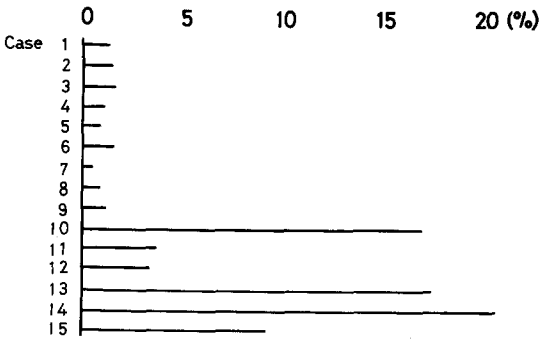


Fig. 15. Mean percentages of area occupied by secretory granules in whole cytoplasmic area of randomly selected adenoma cells in 15 cases. These were calculated from the mean granule diameters obtained in Fig. 14 and granule numbers per unit area of cytoplasm, which were also measured by the same apparatus mentioned in Fig. 14

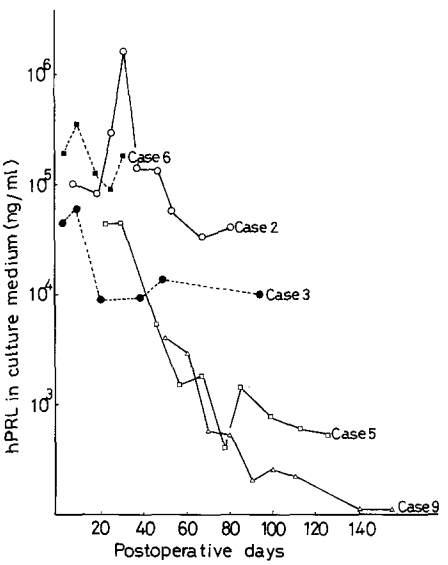


Fig. 16. hPRL release (ng/ml) from adenoma cells (Cases 2, 3, 5, 6 and 9) into 1 ml culture medium (PRMI 1640 medium containing 17% fetal calf serum) in 3 to 4 days in primary cultures. Initial cell counts per plate were not made

unit area. In Cases 12 and 13, a significant release of both hPRL and hGH was observed (Table 2). Because of incomplete dissociation of cells, which prevented counting cells per culture plate, and because of the unknown rate of cell viability, comparison of hormone release rates per cell could not be made among these 7 cases.

LH and FSH were constantly detected at levels of 10 ng/ml in all 7 cases cultured but levels were much lower than those of prolactin. Other pituitary hormones were below levels of detectability; below levels of 10 ng/ml for hGH, except in Cases 12 and 13, and below 200 pg/ml ACTH and β-MSH. In Case 8, five pituitary hormones were detected in considerable amounts (hGH; 55,000 ng, PRL: 98,500 ng, ACTH: 25,300 pg, β-MSH: 3,200 pg, TSH: 208 μU, each per millilitter of medium), which might have been due to contamination with non-neoplastic pituitary tissue, because the presence of both non-neoplastic and tumor tissue fragments in the histologically examined specimens was confirmed in this case. These data were therefore excluded from evaluation of the hormone release by tumor cells.

Discussion

Our results showed that pituitary adenomas accompanied with hyperprolactinemia can be classified into miscellaneous categories on the basis of light microscopic, ultrastructural, immunohistochemical and hormone release studies.

Recent findings revealed that while some hPRL-producing tumors of the human pituitary belong to the acidophilic group, many belong to the chromophobes. The cytoplasm of cells of the latter group almost always contain some types of secretory granules (Mirouze et al., 1969; Racadot et al., 1971; Le Beau and Foncin, 1972; Guinet et al., 1973; Saeger, 1975a; Saeger, 1975b; Horvath and Kovacs, 1976; Kovacs et al., 1977). The ultrastructural features of Cases 1 to 6 were extremely similar and coincided closely with those of previously reported cases. In every case, in addition to markedly well-developed rough-surfaced membranes and Golgi complexes, typical lactotroph type secretory granules were infrequent. Smaller granules measuring 100 to 300 nm were sparsely distributed in the cytoplasm, often revealing "misplaced" or abnormal exocytosis as described by Horvath and Kovacs (Horvath and Kovacs, 1974). Our morphometrical analysis of secretory granule size and granule population (granule number per unit area of cytoplasm) clearly showed that the first 6 cases, all associated with marked hyperprolactinemia, and Case 9, were sparsely granulated and possessed predominantly small secretory granules. These could be regarded as immature, and did not grow or differentiate to larger pleomorphic granules. In contrast, in Case 10, which was associated with moderate hyperprolactinemia, the adenoma was densely granulated and possessed large lactotroph type granules (Saeger, 1975a, Kovacs, 1977). The mean and the standard deviation of the diameter of secretory granules in each case may represent an intrinsic property of tumor cells and the area of the cytoplasm occupied by secretory granules may represent the storage capacity of the hormone in tumor cells, provided that a greater portion of the hormone in cells is stored in secretory granules. Immunohistochemical prolactin-containing cells were observed in 5 of the 7 cases (Cases 1 to 6 and 9) but the reaction was often weak and patchy. Overwhelming release of PRL in culture medium in five of these 7 cases indicated that these adenomas were capable of viorously secreting hPRL. All these findings strongly suggest that the sparsely granulated adenoma associated with hyperprolactinemia possesses a high capacity for production and secretion of the hormone, but defective storage capacity. The densely granulated adenoma (Peake et al., 1969; Racadot et al., 1971; Le Beau and Foncin, 1971; Guinet et al., 1973), as seen in Case 10, associated with moderate hyperprolactinemia, may possess moderate capacity for production and secretion of the hormone and a high storage capacity.

Our immunohistochemical results for hPRL in this group were approximately in accordance with those of other authors (Zimmerman et al., 1974; Kovacs et al., 1975; Saeger, 1975b; Kovacs et al., 1976; Osamura et al., 1978). Weak, ambiguous or negative results in definitly high PRL producing adenomas (i.e., Cases 2, 3, 6 and 9) were also mentioned by some of those authors (Kovacs et al., 1975; Saeger, 1975b). They suggested that PRL was not stored in this group in sufficient amounts to render it identifiable by immunostaining. There-

fore, occasional negative findings with immunostaining may not totally rule out PRL secretion by tumor.

In 7 cases subjected to tissue culture, we detected the release of small amounts of pituitary hormones other than hPRL into culture medium. Whether these hormones were produced by adenomas or contaminated by non-tumorous elements is yet to be clarified. However, the fact that a small number of FSH-containing cells were detected by immunostaining in Case 6 suggests simultaneous production of hPRL and FSH by the tumor itself. In this connection, Teraoka (1972) reported that LH was detected in culture media in 10 out of 13 cases of pituitary adenomas, which consisted of two acromegalic and 11 "non-functioning" cases. He did not demonstrate whether the release of LH was caused by genuine production and secretion by tumor cells or by contamination of non-tumorous LH cells.

A number of studies have shown that both hPRL and hGH were often overproduced by eosinophilic and chromophobic adenomas (Guyda et al., 1973; Zimmerman et al., 1974; Corenblum et al., 1976; Demura et al., 1977). In Case 12 with acromegaly and marked elevation of plasma hGH and hPRL, the features of the adenoma were different from those of usual types of densely and sparsely granulated GH cell adenoma and those of mixed GH and PRL cell adenoma described by several authors (Schlein, 1962; Guyda et al., 1973; Saeger, 1975a; Saeger, 1975b; Corenblum, 1976; Kovacs et al., 1977).

We tried to clarify whether or not immunoreactive hGH and hPRL were present in the same cells in this case. We used adjacent paired sections, immunostained by anti-hGH and anti-hPRL respectively, and identified the same cells in the paired sections (see Ueda et al., 1973; Phifer et al., 1974). Our analysis revealed that both hormones were detected in the same cells, at least in some areas of the adenoma. Previous authors (Zimmerman et al., 1974; Kovacs, 1977) have also suggested that some pituitary adenoma cells might contain both GH and PRL but they did not demonstrate definite evidence. Ueda et al. (Ueda et al., 1973) showed that some cells of pituitary transplant of a spontaneous mammotropic pituitary tumor (MtT, strain W83) of the rat, stained with both anti-PRL and anti-GH. The results of our ultrastructural, immunostaining and in vitro hormone release studies suggested that the classification of this case as acidophilic stem cell adenoma may be justified, in the sense that the committed acidophilic precursor cell would be the source of this category, although the functional and morphological entity was differently defined by its denominators (Horvath et al., 1977).

In Cases 7, 8, 14 and 15, no evidence of PRL production was obtained by examination of the tumors. In some of them, in which a good hPRL response was observed by TRH stimulation, hyperprolactinemia might be related to hypothalamo-hypophyseal disturbance rather than to production of the hormone by the tumors themselves (Kleinberg et al., 1977).

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