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Case Report

Pituitary Adenoma Producing Thyrotropin and Prolactin

An Immunocytochemical and Electron Microscopic Study*

Theresa M. Duello and N.S. Halmi**
Department of Anatomy, University of Iowa

Summary. A pituitary adenoma with suprasellar extension that had caused hyperthyroidism due to secretion of excess thyrotropin (TSH), as well as mild hyperprolactinemia, was studied with differential staining, immunocytochemistry and electron microscopy. Most cells of the tumor stained lightly with aldehyde thionin, which demonstrates the granules of normal thyrotrops, and immunocytochemically with antiserum to the hormone-specific β chain of TSH. A minority of the cells was immunoreactive for prolactin. Electron microscopy revealed light cells interspersed with highly pleomorphic dark cells. Both were sometimes multinucleated, and contained variable numbers of small secretion granules, multiple Golgi complexes, and abundant endoplasmic reticulum.

Key words: Pituitary gland – Adenoma – Thyrotropin – Prolactin.

Introduction

The rare thyrotropin (TSH)-producing adenomas of the human hypophysis are of 2 varieties: those that arise in response to primary hypothyroidism of long standing, and those that cause hyperthyroidism by secretion of excess TSH (Linquette et al., 1971). Several cases with concurrence of thyrotoxicosis and pituitary adenoma published in the early literature may have belonged in the second of these categories, but conclusive evidence of TSH hypersecretion by pituitary tumors awaited the advent of sensitive bio- and radioimmunoassays for TSH in serum. Table 1 summarizes the 11 previous cases in which frank elevation of serum TSH (or at least TSH levels inappropriately high in view

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For offprints contact: N.S. Halmi, M.D., 1-450 BSB, University of Iowa, Iowa City, Iowa 52242, USA

Table 1. Cases of pituitary adenoma with elevated or inappropriate serum TSH levels and hyperthyroidism

Case No., reference, age (yr), sex	Serum TSH		Tumor histology	Additional information
	Bio-assay mU/ml	Radio-immuno-assay μU(ng)/ml		
1. Jailer and Holub (1960), 51, F	1.8[a]	N[b]	N	TSH assay by the method of D'Angelo et al. (1951)
2. Lamberg et al. (1969), 51, F	2	N	largely chromophobic	TSH assay by McKenzie (1958) method. Acromegaly. Multiple endocrine adenomatosis type I
3. Linquette et al. (1969), 33, M	0.21[c]	N	TSH cells (tetrachrome)	TSH assay by McKenzie (1958) method. Acromegaloid features. Serum growth hormone: 12 ng/ml[d]
4. Hamilton et al. (1970), 50, M	N	17[e]	chromophobic	–
5. Faglia et al. (1972), 25, M	1.8	avg. 24	N	TSH bioassay by McKenzie (1958) method
6. Faglia et al. (1972), 47, M	0.6	avg. 8.4[c]	N	TSH bioassay by McKenzie (1958) method
Reschini et al. (1976)	N	7–12	N	–
7. Hrubesch et al. (1972), 40, F	N	32–42	N	–
8. Mornex et al. (1972), 21, F	N	42	pleomorphic; cells aldehyde thionin+	Tumor TSH assay by McKenzie (1958) method. Electron microscopic study of tumor
9. O'Donnell et al. (1973), 28, M	N	28	fibrotic (chromophobic?)	Tumor TSH by radio-immunoassay
10. Horn et al. (1976), 22, F	N	23	acidophilic-chromophobic; +immuno-fluorescence for TSH	Galactorrhea. Serum PRL > 150 ng/ml[f]. Electron microscopic study of tumor
11. Baylis (1976), 23, M	N	4.5–16.2	chromophobic	Serum PRL 73 ng/ml

[a] Upper limit of normal: 0.6 mU/ml
[b] N = not done or not examined
[c] Inappropriately high in view of elevated blood thyroid hormone levels
[d] Upper limit of normal: 5 ng/ml
[e] Upper limit of normal given as 5–10 μU(=ng)/ml
[f] Normal range: 5–25 ng/ml (women); 5–20 ng/ml (men)

of serum thyroid hormone in the hyperthyroid range) occurred along with clinically or pathologically diagnosed pituitary tumors.

Table 1 shows that there was remarkably high incidence of documented or suspected hypersecretion of hormones other than TSH among these cases: excess growth hormone (GH) casued acromegaly in one (case 2), and mildly elevated serum GH levels were present, along with acromegaloid features, in another (case 3); one adenoma apparently produced large amounts of prolactin (PRL) and thereby caused galactorrhea (case 10), and a fourth tumor (case 11) was associated with an asymptomatic elevation of serum PRL.

In 4 of the cases in Table 1, there was no morphologic study of the adenoma. In 4 others, only conventional histologic techniques were applied to it. Only in 2 (cases 3 and 8) were modern histologic staining methods used. Immunofluorescence staining for TSH was carried out in case 10. Two adenomas (case 8 and 10) were studied electron microscopically. In no instance was the whole gamut of modern morphologic methods applied to a tumor.

Our case is that of a woman whose pituitary adenoma had caused hyperthyroidism by secretion of excess TSH, along with mild hyperprolactinemia. The surgically removed tumor was studied with advanced differential staining methods, immunoperoxidase techniques and electron microscopy.

Case

A 52-year-old woman had mildly elevated plasma protein-bound iodine levels and, for 4 years, was treated by her physician with propylthiouracil. After examination at the University of Iowa Hospitals, she was given a therapeutic dose (6.1 mCi) of ^{131}I. She remained euthyroid to mildly hyperthyroid, and gradually developed headaches and visual field defects. When serum TSH levels determined by radioimmunoassay on several occasions proved elevated (to as much as 230 μU/ml; upper limit of normal 10 μU/ml), the existence of a pituitary adenoma causing hyperthyroidism by secretion of excess TSH was diagnosed (Dr. J.D. Brown), and surgical removal of the tumor was recommended. Preoperatively, blood was sampled every 20 min for 8 h, and serum TSH levels were $59 \pm$(SD) 15 μU/ml; serum PRL (38 ± 15 ng/ml) was also above the normal range (5–25 ng/ml). A solid, friable pituitary tumor with suprasellar extension, about 3 cm in maximal diameter, was then removed transphenoidally (Dr. J.C. Van Gilder). Twelve days after the operation, serum TSH had dropped to 12.4 ± 1.9 μU/ml, and serum PRL to 21.2 ± 14 ng/ml.

Differential Staining

Pieces of the tumor were fixed in Bouin's fluid, embedded in paraffin and cut at 5 μm. In addition to the battery of stains previously recommended for the study of the hypophysis (McCormick and Halmi, 1971), tissue was also stained with the Brookes method (1968), the carmoisine component of which has an affinity for the secretory granules in some PRL cell (lactotrop) tumors (Kovacs et al., 1975; Halmi and Duello, 1976). The adenoma showed considerable pleomorphism. Most cells were fairly large and spindle-shaped, but others were round or ovoid and often contained several nuclei. Accumulations of extracellular fluid occurred among the tumor cells (Fig. 1). Many neoplastic cells contained fine granules that stained lightly with aldehyde thionin, a dye which well demonstrates thyrotrops (TSH cells) in the normal hypophysis (Ezrin and Murray, 1963). None of the cells stained with carmoisine.

Immunocytochemical Staining

Thyrotrops and lactotrops were demonstrated in 5-μm sections of the Bouin's fluid-fixed tumor with the immunoglobulin-enzyme bridge method of Mason et al. (1969), as modified by Sternberger et al. (1970). The procedure included the following steps: 1) initial incubation of sections with

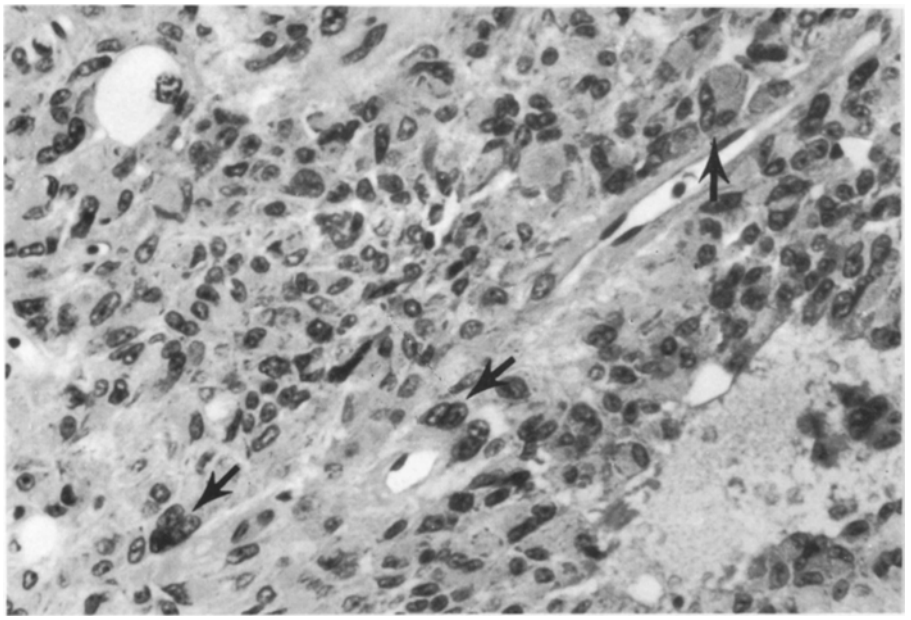

Fig. 1. Typical field of adenoma. Tumor cells spindle-shaped or round to ovoid. Arrows point to multinucleated cells. HE × 400

an antiserum to the β chain of bovine TSH[1] (1:100) at 4° C for 3 days or an antiserum to rat PRL[2] (1:100) at 4° C for 4 days; 2) incubation with sheep anti-rabbit gamma globulin[3] (1:50 for TSH, 1:1000 for PRL) for 10 min; 3) incubation with PAP[4] (horseradish peroxidase-anti-horseradish peroxidase complex) (1:50) for 10 min; and 4) reaction of the peroxidase with a solution of 3,3′ diaminobenzidine hydrochloride (0.3 mg/ml) and H_2O_2 (0.05%).

Specificity controls entailed the substitution of normal rabbit serum for the hormone-specific antiserum, prior incubation of the antiserum to the β chain of bovine TSH with bovine TSH[2] or rat TSH[2] (50 µg/ml) for 48 h at 4° C, and prior absorption of the antiserum to rat PRL with either rat PRL[2] or rat GH[2] (50 µg/ml) for 48 h at 4° C.

Immunostained thyrotrops constituted the majority of cells. They exhibited considerable pleomorphism, marked granularity of the cytoplasm, and occasional multinucleation (Fig. 2a). Prior incubation of the antiserum with bovine TSH abolished staining in most cells and greatly decreased it in even the most intensely stained ones (Fig. 2b). Absorption with rat TSH failed to affect staining. The intensely immunostained lactotrops were far fewer in number, irregularly ovoid to elongated, and occasionally multinucleated (Fig. 2c). Prior addition of rat PRL to the PRL antiserum abolished staining (Fig. 2d) whereas absorption with rat GH did not. No staining was observed if normal serum was substituted for the immune serum.

Electron Microscopy

Portions of the tumor were cut into 1-mm^3 pieces and fixed in 1% glutaraldehyde in 0.1 M Sorensen's buffer (pH 7.4).[5] Pieces were post-fixed for 1 h in 1% OsO_4 in 0.1 M Sorensen's buffer. All pieces

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1 Kindly supplied by Dr. J.G. Pierce

2 Generously provided by Dr. A.F. Parlow or A.E. Wilhelmi for the NIAMDD Hormone Distribution Program

3 Purchased from Antibodies, Inc., Davis California

4 Gift of Dr. L. Sternberger

5 This concentration of glutaraldehyde was used because electron microscopic immunocytochemical study of the tumor is also planned, and higher concentrations may adversely affect antigenicity of hormones

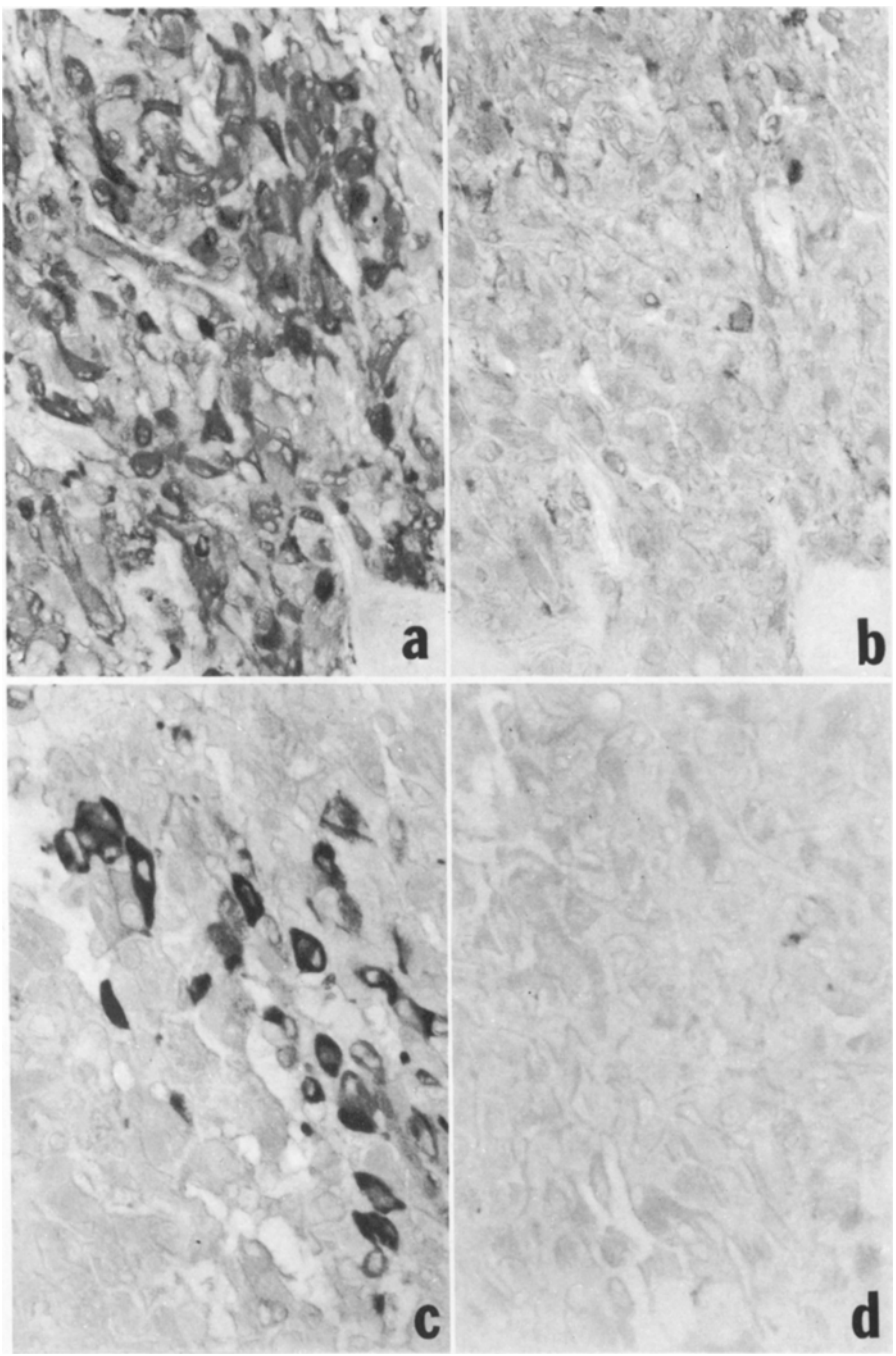

Fig. 2. a Adenoma immunostained with antiserum to bovine TSH-β. **b** Adjacent section. Virtually complete abolition of staining by prior absorption of antiserum with bovine TSH. **c** Immunostained PRL cells. Note difference in shape and distribution compared with a. **d** Adjacent section. Staining abolished by prior incubation of anti-PRL with rat PRL. $\times 300$

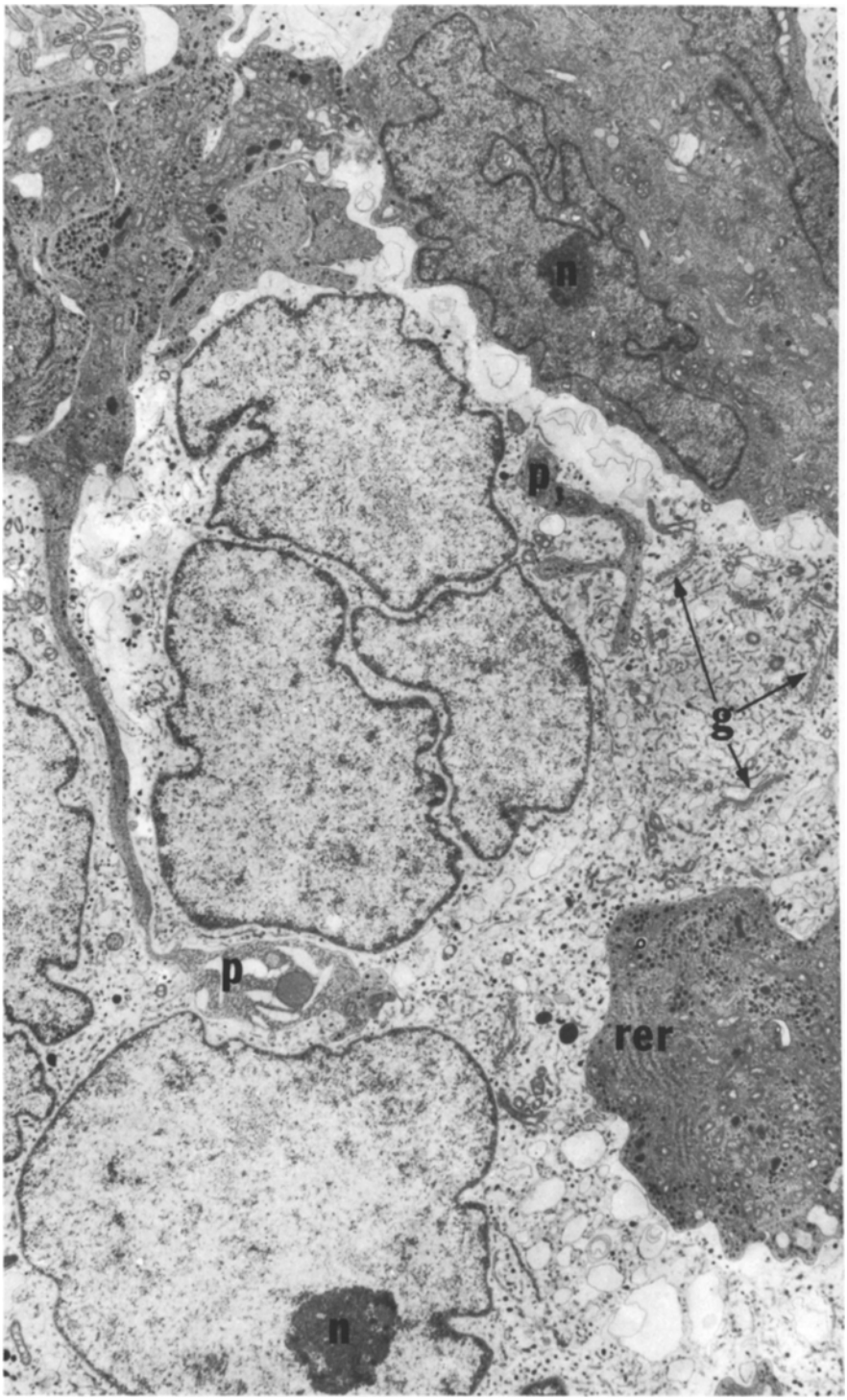

Fig. 3. Electron micrograph showing dark cell processes (*p*) branching through light cell syncytium. Note large nucleoli (*n*), several Golgi complexes (*g*), rough endoplasmic reticulum (*rer*), and the absence of light cell plasma membranes. ×6900

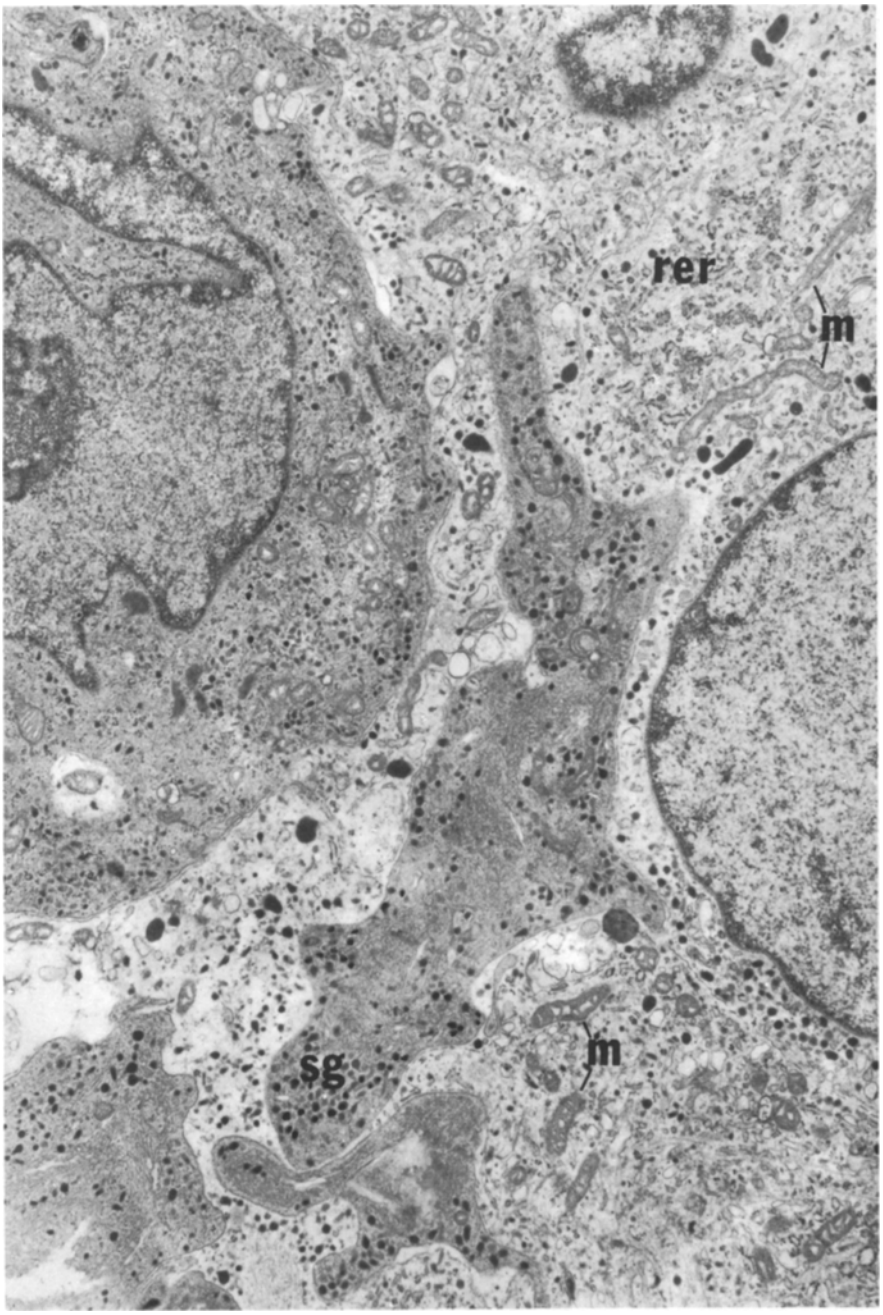

Fig. 4. Many, randomly distributed secretion granules (*sg*) in light and dark cells. Elongated mitochondria (*m*), rough endoplasmic reticulum (*rer*). × 11,000

were dehydrated in a graded ethanol, series followed by propylene oxide, and embedded in Epon 812. Thick (1-μm) sections were cut with an LKB MT2 microtome and stained with toluidine blue. Silver sections were cut from selected areas, stained with uranyl acetate and lead citrate, and viewed in a Philips 300 electron microscope.

Two types of cells, evident in thick sections, could be distinguished on the basis of the staining intensity of their nuclear and cytoplasmic matrix: dark and light cells (Figs. 3 and 4). Transitional forms were not seen. The 2 types differed in the degree of cellular and nuclear pleomorphism, the dark cells as a rule exhibiting greater irregularities in shape. The plasma membrane of dark cells was easily demonstrated, whereas that of the light cells was often difficult or impossible to discern. The light cells thus appeared to form a syncytium within which the dark cells branched (Fig. 3). Multinucleated forms of both light and dark cells were seen, all nuclei possessing large, prominent nucleoli. Both types of cells showed similar signs of secretory activity (Fig. 4). Rough endoplasmic reticulum was abundant, occurring in stacks of 4 to 7 cisternae, often in long parallel arrays. Smooth endoplasmic reticulum was evident, though not as abundant, as were free ribosomes. Cells often contained as many as 7 Golgi complexes, each with 4 or more cisternae, exhibiting varying degrees of dilatation. Long worm-like mitochondria with atypical cristae were numerous in both types of cells, as were myelin bodies, autophagic vacuoles, secretory granules, and other structures of an unknown nature. The secretion granules showed an apparently random distribution, and varied considerably in numbers from cell to cell. The distribution of granule sizes in light cells was unimodal, with a mode of 150 nm and a maximum of 225 nm (500 granules). Granule size distribution in dark cells was also unimodal, but more variable, with modes for individual cells ranging from 75 to 150 nm (maximum: 240 nm; 555 granules measured in 4 cells). Microtubules and microfilaments could be seen in close association with the plasma membranes, or the pericapillary spaces in the absence of an intact plasma membrane.

Discussion

The staining properties of thyrotropic hypophysial adenomas that cause hyperthyroidism have not been adequately explored in most cases. The classification as "chromophobic" must be viewed with reservations if only conventional techniques are used (cf. McCormick and Halmi, 1971). In the 2 cases where sophisticated histologic methods were applied to such tumors (Herlant's tetrachrome, aldehyde thionin) (Linquette et al., 1969; Mornex et al., 1972), the staining of the cells resembled that of normal thyrotrops, as it did in our case.

The adenoma examined by Mornex et al. (1972) was so pleomorphic at the light microscopic level that even the diagnosis of chordoma was considered. Classification of the tumor as a thyrotropic adenoma was not final until bioassay revealed that it contained substantial concentrations of TSH. Our adenoma (Fig. 1) also showed considerable, although less pronounced, pleomorphism, contrasting sharply with the monotonous picture presented by the majority of pituitary adenomas.

In one case where immunofluorescence staining was applied to a thyrotropic adenoma (Horn et al., 1976), cells containing TSH were demonstrated. They were not illustrated, nor can the specificity of the staining be judged from the paper. The lactotrops in the same tumor were identified only by histologic and electron microscopic criteria. In our case, immunoperoxidase staining showed 2 dissimilar populations of cells, reacting with antiserum to the β chain of TSH (Fig. 2a) and with anti-PRL (Fig. 2c), respectively. The specificity of both staining reactions was documented by abolition or marked attenuation of staining after incubation of the immune sera with the appropriate antigens (Fig. 2b and d).

Electron microscopy in the case of Horn et al. (1976) showed that the cells interpreted as thyrotrops were elongated and had granules 90–200 nm in maximum diameter. The cells believed to be lactotrops had larger granules. No illustrations were provided. No electron micrographs accompanied the paper by Mornex et al. (1972) either. [In a separate article, Curé et al. (1972) depicted tubular inclusions in stromal cells of the same tumor.] From the description, it is nevertheless clear that the tumor whose ultrastructure Mornex et al. studied had several features in common with ours: cells with bizarre processes, multiple nuclei, and small secretory granules (which, however, were apparently less numerous and less widely distributed than in our case, being most heavily concentrated in the cell processes). The multiple Golgi complexes, extensive endoplasmic reticulum, and many secretion granules in the cells of the present tumor suggest vigorous secretory activity. Appreciable numbers of secretory granules demonstrable with the electron microscope may be the basis of the staining with aldehyde thionin at the light microscopic level found both by Mornex et al. (1972) and by us. Two other TSH-producing tumors recently examined that were aldehyde thionin negative showed only few secretion granules under the electron microscope. Incidentally, they were also unreactive with the same antibody to bovine TSH-β that was used in this study (Samaan et al., 1977).

In our case, immunocytochemical staining indicated that TSH and PRL were apparently produced in different sets of cells. There is no reason to identify these with the electron microscopically demonstrated dark and light cells, respectively. These ultrastructural designations are purely descriptive and have no known functional correlates. Cells with dark cytoplasm have also been described in cases of apparently non-functional chromophobic adenomas (Kuromatsu, 1968; Schechter, 1973). It is possible that the same tumor cells can produce both TSH and PRL. Tissue from a TSH- and PRL-secreting adenoma grown in culture elaborated both hormones and responded to factors known to release TSH, PRL or both from non-tumorous hypophyses (Marcovitz et al., 1977). In that study an organ culture, not a monoclonal explant of the tumor, was investigated, so TSH and PRL could have been produced by different cell types.

Why there should be an association of TSH- and GH-overproduction in some adenomas (Table 1, cases 2 and 3) is not clear. The concurrent hypersecretion of TSH and PRL is easier to understand. Any hypophysial tumor large enough to impinge on the hypothalamus can interfere with the tonic inhibition of pituitary PRL secretion normally exerted by the hypothalamus. This may account for the hyperprolactinemia in the case of Baylis (1976), and has not been rigorously ruled out for the case of Horn et al. (1976) either, in the absence of immunocytochemical evidence for PRL in the tumor. It is more likely, however, that the tumor described by them did, like that in our case, produce both TSH and PRL. Since hypothalamic TSH-releasing hormone (TRH) also causes PRL secretion from the pituitary (Bowers et al., 1971; Jacobs et al., 1971), it is possible (Horn et al., 1976) that the primary disturbance underlying mixed thyro-lactotropic hypophysial adenomas is increased production of TRH.

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Note Added in Proof:

After submission of the manuscript, 2 more cases of TSH-producing pituitary adenomas with accompanying hyperthyroidism were described [Kourides, I.A., Ridgway, E.C., Weintraub, B.D., Bigos, S.T., Gershengorn, M.C., Maloof, F.: Thyrotropin-induced hyperthyroidism: use of alpha and beta subunit levels to identify patients with pituitary tumors. J. Clin. Endocr. **45**, 534–643 (1977); Tolis, G., Bird, C., Bertrand, G., McKenzie, J.M., Ezrin, C.: Pituitary hyperthyroidism. Case report and review of the literature. Amer. J. Med., in press]. In the first of these cases, there was concomitant acromegaly, and the tumor contained acidophilic as well as mucoid cells; in the second, the adenoma was classified as chromophobic on the basis of staining with hematoxylin and eosin, and TSH was localized in its cells by an immunoperoxidase method.