SING primarily immunocytochemical analysis, the results from several studies have provided conclusive evidence that pituitary neoplasms may be bihormonal or plurihormonal: producing two or more hormones.\textsuperscript{6,12,14,23,24}

Based on careful ultrastructural and immunoelectron microscopic investigation, it has become apparent that these adenomas can be divided into three separate classes: monomorphous, bimorphous, and pluri-morphous tumors.\textsuperscript{7,10,12,14,23,24} Monomorphous adenomas consist of one distinct cell type and two or more hormones are synthesized in the same cell. Bimorphous and pluri-morphous adenomas are composed of two or more cell types and each hormone is produced in different cell populations. In some adenomas one cell type can predominate; in others various areas contain groups of similar cells; and in others a gradual transformation seems to exist between the cell types. Despite these overlaps, the separation of tumors into monomorphous, bimorphous, and pluri-morphous classes for diagnostic purposes has value and is useful as a practical classification. The most frequent hormone combinations are growth hormone (GH) and prolactin (PRL), or GH, PRL, and the \( \alpha \) subunit of the glycoprotein hormones and/or \( \beta \)-thyrotropin (TSH) or follicle stimulating hormone (FSH), luteinizing hormone (LH) and/or the \( \alpha \) subunit.\textsuperscript{5,18,23,24} Other hormone combinations within one tumor are extremely rare.

We recently investigated a pituitary adenoma that had been removed from a 62-year-old man. The tumor contained both GH and adrenocorticotropic hormone (ACTH) and apparently consisted of two different cell types. Because this hormone combination is extremely rare, we studied the tumor by using several morphological techniques including immunocytochemical analysis, transmission electron microscopy, immunoelectron microscopy, and in situ hybridization. In this paper we report the clinical and morphological findings of this case.
Case Report

History. This 62-year-old man had long-standing diabetes mellitus that was controlled with diet and an oral course of hypoglycemic agents. In 1995 the diagnosis of acromegaly was made on the basis of characteristic physical features.

Examination. The patient had a large prominent forehead, jaw, and tongue and was visibly acromegalic. He had no complaints, was in a good physical state, and had no visual disturbances or breathing difficulties. His blood pressure was 150/92 mm Hg and his blood glucose level was 8.2 nmol/L. No cardiovascular abnormalities were noted and his chest x-ray film was normal.

Clinically, the patient had euthyroidism, and his blood triiodothyronine and thyroxin were within normal ranges. His blood hormone levels were as follows: GH 13.7 \( \mu \)g/L (normal, 5–275 \( \mu \)g/L); insulin-like growth factor (IGF)-I 973 \( \mu \)g/L (normal 48–275 \( \mu \)g/L); PRL 10 \( \mu \)g/L (normal 0–18 \( \mu \)g/L); TSH 0.2 mU/L (normal 0.4–5.5 mU/L); FSH 11.3 IU/L (normal 2–11 IU/L); LH 15.6 IU/L (normal 3–18 IU/L); and cortisol 361 nmol/L (normal 193–690 nmol/L).

Magnetic resonance (MR) imaging demonstrated a large pituitary mass on the right side of the gland, which shifted the hypophyseal stalk to the left of the midline (Fig. 1).

Operation. In 1996, 1 year after the diagnosis of acromegaly had been made, the patient underwent pituitary surgery via the transsphenoidal approach. The tumor was subtotally resected.

Postoperative Course. Recovery was uneventful and the patient had no complaints. There was no diabetes insipidus, and he was given 5 mg prednisone per day. His blood GH and IGF-I levels decreased after surgery but remained above normal ranges. Because it was believed that the patient had residual disease, he received radiotherapy and was advised to take bromocriptine tablets.

Pathological Investigation of the Tumor

Investigative Methods

For light microscopy tissue was fixed in 10% buffered formalin immediately after surgical removal, dehydrated in baths of graded ethanol, and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin and periodic acid–Schiff (PAS). For immunocytochemical analysis, the streptavidin-biotin-peroxidase complex method was applied by using antibodies raised against GH, PRL, ACTH, \( \beta \)-endorphin (\( \beta \)-END), \( \beta \)-TSH, \( \beta \)-FSH, \( \beta \)-LH, and the \( \alpha \) subunit of the glycoprotein hormones. We used MIB-1 as a cell proliferation marker. Additional slides were immunostained for p53 oncoprotein by using a mouse monoclonal antibody that detects both wild and mutant forms. For double immunostaining, we used two different chromogens (diaminobenzidine and nickel chloride). Details of the methods, sources of supplies, dilutions of antibodies, and control procedures have been described in previous papers.

For electron microscopy small fragments of tissue were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in baths of graded ethanol, processed through propylene oxide, and embedded in an Epon-Araldite mixture. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with the aid of a transmission electron microscope. For immunoelectron microscopy, the double-immunogold labeling method was applied by using two sizes of gold particles. Details of the technique have been described in previous publications.

In situ hybridization with \( ^{35} \)S autoradiography was used to demonstrate messenger (m)RNAs for GH and proopiomelanocortin (POMC), the prohormone of ACTH. Details of this method have been described previously.

Control procedures included predigestion with ribonuclease A.

Morphological Findings

Light microscopic examination of the tumor revealed a pituitary adenoma composed of middle-sized cells exhibiting no significant cellular or nuclear pleomorphism (Fig. 2). The cytoplasmic staining was variable; the majority of adenoma cells appeared to be chromophobe and PAS negative. The minority of cells showed varying degrees of cytoplasmic basophilia and PAS positivity. In a few foci, faint images of extracellular asteroid masses were seen.
By using immunocytochemical analysis we found that the majority of cells displayed variable positivity for GH, whereas the minority of adenoma cells were strongly immunoreactive for ACTH and β-END (Fig. 3). These latter cells were unevenly scattered and represented approximately 8 to 15% of all adenoma cells. Occasional cells were immunopositive for PRL; these cells were interpreted as being interspersed, trapped nontumorous adenohypophyseal cells. Immunostainings were negative for β-TSH, β-FSH, β-LH, and the α subunit of the glycoprotein hormones. The cell proliferation index, which was determined by using MIB-1 antibody, was low, less than 1%. No immunoreactivity was evident for p53 oncoprotein. Double immunostainings at the light microscopic level showed that GH and ACTH were present in two different cell types.

Transmission electron microscopy revealed a well-differentiated pituitary adenoma composed of two distinct cell types (Fig. 4). The majority of cells were predominantly large, elongated, or angular with long cytoplasmic processes. The fairly uniform ovoid nuclei contained moderately developed nucleoli and small amounts of finely stippled heterochromatin. The abundant cytoplasm harbored moderately or well-developed rough-surfaced endoplasmic reticulum membranes and a prominent Golgi apparatus. Some adenoma cells were sparsely granulated, whereas others were densely granulated. Secretory granules were spherical or ovoid and measured up to 600 nm. Many adenoma cells contained elongated or geometrically shaped secretory granules, suggesting crystallization within their substance, a phenomenon seen in densely granulated somatotrophic adenomas. The mitochondria displayed regular features in most cells; however, in a few adenoma cells the mitochondrial content was increased, indicating oncocytopoietic transformation.

The second cell population was scattered singly or formed small groups among the somatotrophs. These latter cells were rather small, compact, spherical, or polyhedral, and possessed unremarkable nuclei, inconsiderable rough-surfaced endoplasmic reticulum membranes and Golgi apparatuses, and numerous secretory granules measuring 200 to 400 nm. The granules were dented, drop-shaped, heart-shaped, or irregular and displayed the characteristic ultrastructure of corticotroph granules. It is noteworthy that type I intermediate filaments were not apparent in these cells. The extracellular asteroid masses represented foci of endocrine amyloid produced by the somatotrophs. Although transition between the two cell types was not prominent, several somatotrophs harbored a few dented or heart-shaped secretory granules characteristic of corticotrophs.

Immunoelectron microscopy demonstrated that there was GH in the secretory granules of the somatotrophs and ACTH in the secretory granules of the corticotrophs. In several somatotrophs, however, ACTH could also be localized in the GH-containing secretory granules (Fig. 5). Thus the two hormones were present in the same cell, even in the same secretory granule.

In situ hybridization revealed strong expression of GH mRNA in the majority of tumor cells (Fig. 6 left) and a weak signal for POMC in the minority of tumor cells (Fig. 6 right). In situ hybridization was negative for the mRNA of the α subunit of the glycoprotein hormones. These findings can be interpreted to mean that GH and ACTH were not only stored, as shown by immunocytochemical analysis, but also produced by the adenoma cells.

**Discussion**

Blevins, et al., reported the case of a 40-year-old woman with acromegaly and Cushing’s disease. In the woman’s pituitary two separate synchronous adenomas, one producing GH and the other ACTH, were found. In a 76-year-old woman, two strongly demarcated distinct...
pituitary adenomas, one composed of somatotrophs and the other of corticotrophs, were noted by Apel and colleagues. In that case, only GH overproduction was documented with no evidence of ACTH excess. Blevins, et al., mentioned two additional patients who presented with GH and ACTH hyperactivity, probably due to pituitary tumors. However, in these two cases GH and ACTH production could not be conclusively demonstrated because of limitations in the available morphological methods.

A careful and extensive literature search revealed only one previously published case of a single pituitary adenoma that produced GH and ACTH simultaneously. This patient, described by Arita and coauthors, was a 29-year-old woman with acromegaly, obesity, amenorrhea, hirsutism, excessive pigmentation, acne, and diabetes mellitus. Blood levels of GH, ACTH, and cortisol were elevated, and computerized tomography scanning demonstrated a pituitary tumor destroying the sellar floor. Transsphenoidal subtotal adenomectomy was performed in that case and resulted in reductions in blood GH, ACTH, and cortisol concentrations. Histological analysis revealed that the tumor was an acidophilic pituitary adenoma. Immunocytochemical analysis showed diffuse GH and sporadic ACTH immunoreactivity. No cells were found to contain both hormones. Electron microscopy revealed that the tumor consisted of two separate cell types, somatotrophs and corticotrophs. Arita and coworkers claimed that their report was the first in which a pituitary adenoma had been confirmed endocrinologically and morphologically to produce GH and ACTH. Our case was that of an older man and ACTH hypersecretion was not evident clinically or biochemically; this indicated that somatotrophs with GH hypersecretion were associated with adenomatous corticotrophs representing silent subtype I cells. Arita and coworkers studied their tumor by using histological and immunocytochemical methods and transmission electron microscopy; our case was also investigated by using in situ hybridization.

Nontumorous human adenohypophyseal cells may produce two hormones, such as GH and PRL or FSH and LH. Two or more hormones can also be generated by one single adenoma cell. To our knowledge, cells synthesizing GH and ACTH have not described in nontumorous or in adenomatous adenohypophyseal cells. In the case described by Arita and coworkers and in our case, the tumors were mixed adenomas, that is, they were composed of two separate cell types, one producing GH and the other ACTH.

The cytogenesis of pituitary adenomas that consist of two different cell populations is not known and remains to be elucidated. Most, if not all, pituitary adenomas are monoclonal; they are assumed to arise in a single cell. It is not clear as to how the concept of monoclonality can be reconciled with adenomas comprising two different cell populations. It may well be that some pituitary adenomas are not monoclonal and that the insult that causes the neoplastic transformation affects two different cell types. Alternatively, it is conceivable that some pituitary tumors originate in an uncommitted stem cell, which, because of unknown factors, can differentiate into two separate cell types. Multidirectional differentiation could explain the development of plurihormonal tumors. It is also possible that one cell type can transdifferentiate as a result of subsequent mutations during tumor progression to another cell type; this has been shown in the pituitaries of rats made hypothyroid by chemical thyroidectomy. Subsequent mutations may lead to lineage infidelity and the formation of another cell type. In our case immunoelectron microscopy conclusively documented the presence of ACTH in the secretory granules of several somatotrophs, which was associated with changes in secretory granule morphology. This suggests that the tumor was originally a somatotropic adenoma that began to produce

Fig. 5. Immunoelectron micrograph depicting GH immunoreactivity (10-nm gold particles) in the secretory granules of some somatotrophs and ACTH immunoreactivity (20-nm gold particles) in the secretory granules of corticotrophs. The ACTH labeling is also evident in several secretory granules of somatotrophs (arrowheads). Original magnification × 19,800.

Fig. 6. Photomicrographs. Original magnification × 400. Left: Silver grains representing GH mRNA are evident in many adenoma cells. In situ hybridization for GH mRNA. Right: A weak signal is seen for POMC mRNA in scattered adenoma cells. In situ hybridization for POMC mRNA.
Pituitary adenoma producing GH and ACTH

ACTH as a result of mutations that occurred during tumor progression. At present one can only conclude that the cytogenesis of mixed pituitary adenomas is obscure and that more work is required to obtain a deeper insight into the development and progression of pituitary adenomas that are composed of two distinct cell populations and are capable of synthesizing two separate hormones that differ in chemical composition, immunoreactivity, and biological action.

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References


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Address reprint requests to: Kalman Kovacs, M.D., Ph.D., Division of Pathology, St. Michael’s Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8, Canada.