Silent Corticotropic Adenomas of the Human Pituitary Gland

A Histologic, Immunocytologic, and Ultrastructural Study

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Among 300 surgically removed pituitary adenomas, 17 tumors containing immunoreactive 1-39 adrenocorticotropin (ACTH) and/or 19-39 ACTH, β-lipotropin, and α-endorphin but unassociated with clinical signs of Cushing's disease have been detected. These neoplasms were divided into basophilic adenomas with strong periodic acid-Schiff (PAS) and lead-hematoxylin positivity and chromophobic tumors with moderate or no PAS and lead-hematoxylin positivity. The former were densely granulated tumors with a fine structure strikingly similar to that of functioning corticotropic cell adenomas. The latter were sparsely granulated with varying ultrastructural patterns. The marked morphologic diversity suggests that these adenomas, despite their similar immunocytologic characteristics, represent more than one entity. Clinically, the most common finding was a rapidly progressing visual defect. An unusually high incidence of infarction (5 cases) and recurrence (5 cases) was noted, underlining the importance of correct morphologic diagnosis and careful follow-up. (Am J Pathol 1980, 98:617-638)

SILENT ADENOMAS of the pituitary are tumors unassociated with clinical and biochemical evidence of overproduction of any known adenohypophyseal hormone. The incidence of such seemingly nonfunctioning neoplasms in our material of 300 pituitary adenomas is about 30%. The majority of these adenomas (22%) consists of incompletely differentiated cells with poorly developed cytoplasm and small, sparse secretory granules.[1,2,3] Due to the lack of morphologic markers indicative of cellular derivation, these tumors are classified as undifferentiated (precursor) cell adenomas or oncocytomas.[1,2] Silent tumors are very rare among growth hormone cell adenomas, and they did not occur among prolactinomas in our series. In an earlier report, however, we described a basophilic adenoma possessing the typical features of corticotropic cell adenomas and containing immunoreactive 1-24 adrenocorticotropin (ACTH) but unassociated with any signs of hypercorticism.[4] Subsequently,

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the routine immunocytochemical testing of pituitary tumors revealed further clinically nonfunctioning adenomas containing immunoreactive ACTH and the related peptides β-lipotropin and α-endorphin. This report deals with these neoplasms, placing special emphasis on some characteristics distinguishing these tumors from other silent pituitary adenomas.

Materials and Methods

Patient Material

Of 300 surgically removed pituitary adenomas investigated so far in this laboratory, 190 arose from growth hormone cells and/or prolactin cells; 39 derived from corticotropic cells; 1 adenoma was composed of thyrotropic cells; and 3 adenomas were composed of gonadotropic cells. The 67 tumors consisting of nononcocytic or oncocytic forms of incompletely differentiated precursor cells represented the largest single group of clinically silent adenomas. Seventeen apparently nonfunctioning tumors were, however, found among those that originated in corticotropic cells. These 17 adenomas were removed from 14 patients (9 male, 5 female) between 28 and 71 years of age (average age at the time of the last surgical intervention, 47 years).

Morphologic Techniques

Pieces of surgically removed pituitary adenomas were fixed immediately after removal. For light microscopy, tissues were fixed in 10% formaldehyde solution and embedded in paraffin. Sections 4–6 μ thick were stained with hematoxylin–phloxine saffron, periodic acid–Schiff (PAS), and lead–hematoxylin. For localizing adenohypophyseal hormones the immunoperoxidase technique was applied with the use of paraffin sections.[5–7] As primary antibodies, the following antiserums were tested: antihuman growth hormone (Imperial Cancer Research Foundation, London, England), antihuman prolactin (donated by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada), anti-1-24 ACTH (Organon, Oss, Holland, donated by Dr. S. Hane and Dr. P. H. Forsham, University of California Medical Center, San Francisco, Calif), antihuman 1-39 ACTH (Wellcome Reagents Ltd., Beckenham, England), antihuman 19-39 ACTH (Wellcome Reagents Ltd., Beckenham, England), anti β-lipotropin (donated by Dr. M. Chretien and Dr. M. Lis, Clinical Research Institute, Montreal, Quebec, Canada), anti-α-endorphin (donated by Dr. J. M. Polak, Postgraduate Medical School, London, England), antihuman glycoprotein hormone (thyroid-stimulating hormone [TSH], follicle-stimulating hormone [FSH], luteinizing hormone [LH]) α-subunit (donated by Dr. I. Kourides, Sloan-Kettering Institute, New York, NY), antihuman FSH (β-subunit), LH (β-subunit), and TSH (β-subunit) (donated by the National Pituitary Agency, University of Maryland, School of Medicine, National Institute of Arthritis, Metabolism and Digestive Diseases, Baltimore, Maryland).

For electron microscopy, pieces of adenoma tissue were fixed in 2.5% glutaraldehyde in Sorensen's buffer, postfixed in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol, and embedded in Epon 812. Semithin sections were stained with toluidine blue and examined with an optical microscope to select suitable specimens for fine structural study. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Results

Case Histories

A summary of the clinical findings and the results of the endocrine laboratory tests are shown in Tables 1 and 2.

Case 1

The case of this 42-year-old man has been reported in detail.[4] The patient had no symptoms suggestive of endocrine hyperfunction or hypofunction. Hormone studies revealed preserved pituitary function. At first he presented with severe headache, visual disturbances with total loss of vision in the right eye, and ophthalmoplegia. Roentgenographic examination showed marked sellar enlargement with clinoid erosion. Pneumoencephalography (PEG) showed a sellar mass with large suprasellar extension. Transphenoidal removal of the cystic hemorrhagic tumor resulted in dramatic improvement of vision and did not change the patient's eupituitary status. Two years later the patient's headaches recurred; and after another year he returned with headache, nausea, vomiting, visual disturbances, and palsy of the second, third, and sixth cranial nerves. A predominantly left-sided tumor, extending into the left parasellar area, was removed, and the patient's vision was significantly improved. The patient remained eupituitary.

Case 2

This 32-year-old woman had progressive visual disturbances for 3 years, leading to a left visual field defect. Sella studies indicated a predominantly suprasellar lesion. She had undetectable GH and low gonadotropin values. Thyroid indices, TSH level, and cortisol level were normal. The adenoma was successfully removed, and postoperative testing showed preserved pituitary function.

Case 3

This 56-year-old woman had no previous symptoms suggestive of endocrine dysfunction. She was undergoing anticoagulant therapy for calf vein thrombosis when she experienced severe headaches and complete loss of vision in both eyes with ophthalmoplegia. She appeared eupituitary. No preoperative endocrine testing was undertaken in this emergency situation. The transphenoidal removal of a large necrotic hemorrhagic tumor, extending into the anterior portion of the third ventricle, left the patient with thyroid and adrenal deficiency, requiring replacement therapy.

Table 1—Clinical Features and Morphologic Characteristics of Silent Corticotropic Adenomas

Case, sex, age	Endocrine history	Leading clinical symptoms	Radiologic findings of the sellar region	Light-microscopic findings					Ultrastructure	Remarks
				PAS	Lead-hematoxylin	ACTH Δ	β-LPH	α-END		
1,* M, 43 yrs	Recurrent (basophilic) adenoma with no endocrine history	First adm: headache, sudden loss of vision, ptosis of right eyelid. Second adm: headache, diplopia, nausea, vomiting, loss of vision in left eye, palsy of 2nd, 3rd, 6th cranial nerves.	First adm: x-ray: markedly enlarged sella with erosion of clinoids and deepening of floor PEG: suprasellar extension. Second adm: x-ray: enlarged pituitary fossa, CT scan, PEG: predominantly left-sided tumor extending into left parasellar area.	3+	3+	(1-24): + (19-39): 2+	2+	3+	Densely granulated corticotropic adenoma containing Type I microfilaments. Markedly enhanced lysosomal activity. Necrosis in 2nd biopsy.	Focal hemorrhages in the first biopsy; widespread necrosis in the recurrent tumor.
2, F 32 yrs	None	Visual disturbances (3 yrs), visual field defect.	Skull x-ray: minor enlargement of pituitary fossa. PEG: large suprasellar extension.	2+	+	(1-24): +	+	NT	Partly densely, partly sparsely granulated adenoma with Type I microfilaments. Incomplete development of Golgi complex in sparsely granulated cells.	
3, F 56 yrs	None	Severe headache, acute loss of vision, ophthalmoplegia.	Skull x-ray: sellar enlargement with erosion of the dorsum. PEG: marked suprasellar extension.	+	NT	(1-24): +	+	+	Remnants of a densely granulated tumor with large lysosomal bodies. Necrotic changes.	Surgical specimen was mostly necrotic.

4, M, 42 years	None	Headaches (5 yrs); abnormal EEG (2 yrs); blackout spells; history of depression.	Enlarged pituitary fossa	Small surgical specimen was not suitable for histochemical study					Densely granulated corticotropic adenoma with typical features.	Good tissue preservation in EM specimen.
5, F, 46 yrs	Cushingoid appearance (more than 25 yrs); secondary amenorrhea (12 yrs); sellar enlargement, "empty sella" syndrome (3 yrs PTA)	Left 6th nerve palsy, head ache, and fever (5 months PTA); headache, bilateral neck soreness, occipital pain, sudden reduction of vision (1 wk PTA).	Skull x-ray: slightly enlarged sella. PEG: erosion of the left anterior clinoid, filling of sella by soft tissue mass and substantial suprasellar extension elevating the supraoptic recess of the 3rd ventricle.	3+	3+	(1-39): 2+	2+	2+	Densely granulated adenoma with signs of advanced necrosis.	Evidence of hemorrhage in histologic specimen.
6, M 42 yrs	Decreased libido (10 mos)	Progressive visual blurring, retroorbital pain.	Skull x-ray: enlarged sella. PEG: sellar mass with suprasellar extension.	+	NT	(1-24): +	+	2+	Sparsely granulated adenoma with teardrop granules.	
7, M, 54 yrs	Hypopituitarism	Severe weight loss, lack of energy, general malaise.	Skull x-ray: greatly enlarged sella. PEG: sellar mass with moderate suprasellar growth and extension into the sphenoid sinus.	–	–	(1-39): +	+	+	Same	
8, F, 40 yrs	Post pill amenorrhea (5 yrs); transient ↑ of 17-ketosteroids (3 yrs PTA); sellar enlargement (3 yr)	Bitemporal hemianopsia, symptoms suggesting schizophrenia.	Skull x-ray: grossly enlarged sella. PEG: large suprasellar extension.	+(weak)	+(weak)	(1-39): – (19-39): +	–	+	Same	

Table 1—Continued

Case sex, age	Endocrine history	Leading clinical symptoms	Radiologic findings of the sellar region	Light-microscopic findings					Ultrastructure	Remarks
				PAS	Lead-hematoxylin	ACTH Δ	β-LPH	α-END		
9, M, 44 yrs	Recurrent pituitary ("chromophobe") adenoma causing hypopituitarism	Left temporal hemianopia.	Tomography: sellar mass with extension into sphenoid sinus and nasopharynx. PEG: moderate suprasellar extension and posterior growth into interpedunclear fossa.	2+	2+	(19-39): 3+	+	+	Same	Only the recurrent adenoma was studied.
10, M, 71 yrs	None	Blurring of vision (6 wks); bitemporal visual field defect.	Skull x-ray; diffusely expanded sella with thin floor. PEG: slight suprasellar extension.	+	2+	(19-39): 2+	+	+	Same	
11, M, 60 yrs	None	Progressive visual disturbances, bitemporal hemianopia.	PEG: sellar mass with large suprasellar growth displacing the 3rd ventricle.	2+	3+	(1-39): +	–	2+	Same	
12, F, 28 yrs	Recurrent pituitary adenoma with no endocrine symptoms	Rapidly developing bitemporal hemianopia leading to blindness.	First adm: expanded sella with soft tissue mass extending into the left sphenoid sinus. Second adm: CT scan: recurring discrete mass in the left anterior portion of the sella with small suprasellar extension.	+	–	(19-39): +	+	+	Well-differentiated adenoma with small spherical secretory granules. In the recurrent tumor nuclear pleomorphism and accumulation of SER were also evident.	Hemorrhage and necrosis in the first surgical specimen.

13, M, 44 yrs	Recurrent "chromophobe" adenoma with no endocrine history other than hypopituitarism	Longstanding blindness in right eye, decreasing peripheral vision in left eye, bilateral optic atrophy.	Skull x-ray, tomography: large sellar mass eroding the dorsum and the sellar floor. Angiography: huge pituitary tumor extending superiorly and laterally.	–	–	(1-39): +	+	+	Incompletely differentiated adenoma with numerous low-density secretory granules measuring up to 600 nm.	Both tumors showed identical features.
14, M, 51 yrs	Panhypopituitarism (3 yrs)	Fatigue, loss of libido, cold intolerance.	Skull x-ray: markedly enlarged sella with uneven floor and erosion of the dorsum. PEG, angiography: pituitary mass with parasellar extension laterally but no suprasellar extension.	2+	+	(1-39): 2+	+	+	Adenoma with immature features. Cells contain small (less than 150-nm) secretory granules and aggregates of SER.	

* Case is published in detail.[4]

PTA = prior to admission; N = normal; ↑ = increased; ↓ = decreased; PEG = pneumoencephalography; NT = not tested; Δ = Immunoperoxidase technique, using anti (1-24), (1-39), or (19-39) ACTH, β-lipotropin, α-endorphin.

Case 4

This 42-year-old man had bitemporal headaches for 5 years. Six months before hospitalization he began to experience blackout spells. He had an enlarged sella turcica. Endocrine tests were normal. A pituitary adenoma was removed by the transphenoidal route.

Case 5

This 46-year-old woman had a Cushingoid appearance (truncal obesity, florid cheeks, buffalo hump) for many years without the biochemical manifestations of Cushing's disease. She became amenorrheic at the age of 34. At 39, a slightly enlarged sella was noted, accompanied by bitemporal visual field defects. Three years later, a PEG showed a largely "empty sella." Hormonal studies disclosed no signs of hypercorticism. Two years later, she presented with severe headaches, bilateral neck soreness, and occipital pain. She experienced a rather sudden reduction of visual acuity with increased bitemporal visual field loss. PEG documented further erosion of the sella by a large, soft tissue mass. The patient's Cushingoid features appeared to be more marked than previously. Because of sudden visual field changes, she was placed on Dexamethasone prior to surgery, and no laboratory investigation was carried out. At transphenoidal operation, a deficient sellar floor and a bulging soft tissue mass were found. The tumor, compartmentalized by dense stroma and containing dark hemorrhagic fluid, was only partially removed because of numerous adhesions to neighboring structures. Surgery resulted in dramatic improvement in the patient's visual acuity and field defect. She remained Cushingoid postoperatively. Steroid determinations showed normal values, while plasma ACTH levels appeared to be elevated on two occasions.

Case 6

This 42-year-old man gave a 10-month history of decreased libido and progressive visual blurring. Retroorbital pain had been present for about 3 months. Endocrine studies indicated no hormonal hypersecretion. PEG revealed a sellar mass with suprasellar extension, which was removed by the transphenoidal approach.

Case 7

This 54-year-old man was admitted to the hospital for the investigation of severe weight reduction, loss of muscle bulk and strength, general malaise, and pallor. A routine skull x-ray showed a greatly enlarged sella. On further clinical examination, he appeared to be hypopituitary, with preserved thyroid function. He had no visual field defects. Sella studies

showed a large tumor with suprasellar and infrasellar extension. Following transphenoidal removal of the neoplasm, the patient felt well for 2 years, when he was readmitted because of back pain. A metastatic survey demonstrated multiple osteolytic foci, which proved to be the metastases of an anaplastic carcinoma whose primary site was unknown. He died a few weeks later. No autopsy was performed.

Case 8

The endocrine history of this 40-year-old woman went back 5 years, when her menses ceased after 18 weeks' use of an oral contraceptive. Three years later, she was found to have an enlarged pituitary fossa. Her pituitary function was assessed as normal. However, she had elevated 17-ketosteroid levels, suppressed by short-term prednisone therapy. At this time, she experienced profound psychological changes and a marked surge of libido. Three years later, a partial bitemporal hemianopia was noted with further sellar enlargement. PEG demonstrated a sellar mass with a large domed suprasellar extension. Endocrine studies indicated no abnormality, with the exception of a slightly elevated serum prolactin level. Transphenoidal removal of a large pituitary tumor resulted in major improvement in her visual fields. Normal menses resumed 5 months postoperatively.

Case 9

This 44-year-old male patient had a transfrontal craniotomy for removal of a large pituitary tumor 15 years previously and had been on full replacement therapy with cortisone, thyroxine, and testosterone. He presented with left temporal hemianopia. Investigation of the sella revealed a large pituitary lesion with suprasellar and parasellar extension. By the transphenoidal approach, a large friable tumor was removed, followed by improvement of visual fields and visual acuity.

Case 10

This 71-year-old man had a long history of ventricular arrhythmia and mild essential hypertension. No endocrine or neurologic symptoms were apparent when he presented with a 6-week history of visual disturbances. Skull x-ray and PEG showed a large sellar lesion. Endocrine studies revealed no impairment of pituitary function. Shortly after the transphenoidal removal of a pituitary adenoma, the patient's blood pressure rose sharply, followed by coma and death. Autopsy revealed subdural hemorrhage and cerebral edema.

Table 2—Biochemical Findings in Cases of Silent Corticotrophic Adenomas

Case, sex, age	Laboratory tests
1, M 43 yrs	First adm: tests showed no signs of endocrine dysfunction. For details see Kovacs et al.[4] 18 months prior to second adm: T_3, T_4: N; cortisol: normal diurnal variation Cortisol: 2000: 4.2 μg% after 1 mg dexamethasone 0800: 0.4 μg% Second adm: no preoperative tests done
2, F, 32 yrs	Preoperative tests: PI cortisol: 8.5, 9.8 μg% (AM) 17-ketosteroids: 8.9 μg/24 hrs (N) 17-OH-steroids: 9.2 μg/24 hrs (N) GH: undetectable TSH: 6.6 μU/ml FSH: 6.0 mIU/ml Thyroid indices: normal Postoperative tests: Cortisol: 10.5 μg% GH: 2 ng/ml→17.9 ng/ml } with normal response to hypoglycemia TSH: 10.7 μU/ml
3, F, 56 yrs	Preoperative testing was not done Postoperative tests: AM cortisol: 2.6 μg% (↓) T_3: 29.1% T_4: < 1 μg% (↓)
4, M, 42 yrs	Preoperative tests: PI cortisol: 16.0 μg% (AM), 8.0 μg% (PM) 17-ketosteroids: 6.6 μg/24 hrs (N) 17-ketogenic steroids: 9.7 μg/24 hrs (N) Thyroid indices: normal
5, F, 46 yrs	3-year preoperative tests: Cortisol: 18.0 μg% (N) (No change during hypoglycemia) GH: 1.0 ng/ml → 4.5 ng/ml LH: 1.2 mIU, FSH: 6.9 mIU } no response to LHRH 17-ketosteroids: 6.0–11.0 μg/24 hrs (N) 17-OH-steroids: 7.2–10.4 μg/24 hrs (N) Postoperative tests: ACTH: 150 pg/ml (3 wks) ACTH: 220 pg/ml (5 wks) ACTH: 50–75 pg/ml (3 mos) (N) 17-ketosteroids: 4.3–6.7 μg/24 hrs (N) Urinary free cortisol: 40–69 μg/24 hrs (N)
6, M, 42 yrs	Preoperative provocative tests: Cortisol: 8.6 μg% → 16.6 μg% GH: 2 ng/ml → 11.5 ng/ml TSH: 1.8 mIU/ml → 19.0 mIU/ml FSH: 1.7 mIU/ml → 3.9 mIU/ml Prl: 7.6 ng/ml → 20.5 ng/ml Thyroid indices: within normal range Postoperative tests: PI cortisol: 12.5 μg%; no change during hypoglycemia
7, M, 54 yrs	Preoperative values: Cortisol: 2.0 μg% → 4.0 μg% GH: 3.0 ng/ml → 6.0 ng/ml TSH: 6.0 μU/ml; no response to TRH LH: 0.9 mIU/ml, FSH: 0.4 mIU/ml } no response to LHRH Prl: 7.0 ng/ml; no response to TRH PI cortisol: 0800: 4.5 μg%, 2000: 0.3 μg% 17-ketosteroids: 1.5 μg/24 hrs (↓) Thyroid indices: within normal range Postoperative tests: Cortisol: 5.1 μg% → 14.3 μg% 17-ketosteroids: 10.1 μg/24 hrs (N) Urinary free cortisol: 50 μg/24 hrs (N)

Table 2—Continued

Case, sex, age	Laboratory tests
8, F, 40 yrs	Preoperative values: Cortisol: 10.8 μg% → 16.7 μg% GH: 3.7 ng/ml → 30.0 ng/ml TSH: 4.5 μU/ml LH: 5.6 mIU/ml; no response to LHRH FSH: 16.1 mIU/ml → 58.7 mIU/ml Prl: 30 ng/ml → 60.0 ng/ml (sl. ↑) Pl cortisol: 0800: 12.8 μg%, 2000: 5.3 μg%
9, M, 44 yrs	Preoperative tests: GH: 3.0 ng/ml; no response to hypoglycemia Prl: 6.6 ng/ml → 10.0 ng/ml The patient is hypopituitary and on full replacement therapy
10, M, 71 yrs	Preoperative tests: Pl cortisol: 0800: 8.0 μg% (N) 2000: 5.3 μg% (N) 17-ketosteroids: 7.2–8.6 μg/24 hrs (N) 17-ketogenic steroids: 8.8–12.1 μg/24 hrs (N) GH: 1.7 ng/ml TSH: 3.5 μU/ml LH: 21.5 mIU/ml Prl: 4.9 ng/ml Testosterone: 843 ng/dl (N) Thyroid indices: normal
11, M, 60 yrs	Preoperative provocative tests: Cortisol: 5.0 μg% → 14.5 μg% GH: 1.0 ng/ml; no change during hypoglycemia TSH: 2.5 μU/ml → 7.0 μU/ml FSH: 2.0 mIU/ml, LH: 0.3 mIU/ml } no response to LHRH Prl: 28.0 ng/ml (sl. ↑); no response to TRH Thyroid indices: low normal values
12, F, 28 yrs	Tests prior to first operation: LH: 0.3 mIU/ml FSH: 0.1 mIU/ml Prl: 70.2 ng/ml (↑) Postoperative tests: Prl: 8.7 ng/ml → 29.2 ng/ml GH: 6.6 ng/ml On cortisone maintenance
13, M, 44 yrs	Preoperative tests: (On replacement with prednisone and L-thyroxin) Prl: high normal with good response to TRH LH, FSH: normal baseline level responding to LHRH
14, M, 51 yrs	Preoperative tests: Cortisol, GH: low baseline level with no response to hypoglycemia TSH: undetectable LH: 8.0 mIU/ml, FSH: 10.0 mIU/ml } blunted response to LHRH Testosterone: 37.0 ng/dl (N: 325-1500 ng/dl) Pl cortisol: 0800: 1.3 μg%/low) Postoperative (3 mos) Pl ACTH: 180.0 pg/ml (↑) while on full replacement therapy

In the stimulation tests 200 μg TRH iv (TSH, Prl), 100 μg LHRH iv (FSH, LH), and hypoglycemia (GH, cortisol) were used.

→ denotes value post stimulation.

N = normal; ↑ = increased; ↓ = decreased; sl. = slightly; GH = growth hormone; Prl = prolactin; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyrotropic hormone; TRH = thyrotropin-releasing hormone; LHRH = luteinizing hormone-releasing hormone.

Case 11

This 60-year-old man gave a 1-year history of progressive visual disturbances. He had a bitemporal visual field defect. PEG demonstrated a sellar mass with large suprasellar growth displacing the third ventricle. Endocrine studies revealed normal adrenal and thyroid function. The baseline blood prolactin level was slightly elevated. A large pituitary adenoma was incompletely removed.

Case 12

This 28-year-old woman experienced an alarmingly rapid development of bitemporal hemianopia that led to legal blindness over a period of 5 weeks. Tomography demonstrated a sellar lesion with infrasellar extension. As surgery was required urgently, provocative endocrine testing was not carried out. A blood sample taken during anesthesia showed elevated blood prolactin concentrations and undetectable levels of FSH and LH. The transphenoidal removal of the soft, friable, partially hemorrhagic tumor tissue resulted in prompt optic decompression with full recovery of visual acuity and normal visual fields. Postoperatively, her blood prolactin level was normal. Blood levels of FSH and LH were low, with sluggish or flat response to LHRH. However, she became pregnant 5 months later. Shortly the patient again noted a reduction of vision in the left eye, which further deteriorated in the following weeks. In another transphenoidal operation, the recurrent adenoma was removed with careful preservation of the nontumorous gland. She received postoperative radiation during the second trimester of pregnancy. Later, she delivered a healthy, full-term male infant.

Case 13

This 31-year-old man developed total bitemporal hemianopia with macular involvement. At that time, he refused surgery and was treated with irradiation. Subsequently, his vision returned in the left eye, but he remained blind in the right eye. He was on replacement therapy with l-thyroxin and prednisone. At the age of 42 he again noticed decreasing peripheral vision in the left eye. This time, radiologic studies indicated a huge pituitary mass with suprasellar and parasellar extension. At transfrontal surgery, a large tumor was removed, with decompression of the optic nerves. A recurrence 15 months later necessitated a second operation. The patient is on replacement therapy.

Case 14

This 51-year-old man had a 3-year history of fatigue, loss of libido, cold intolerance, and weight loss. At the time of hospitalization he was on replacement therapy with l-thyroxin and prednisone. On physical examination he appeared hypopituitary, with fine wrinkled skin, loss of facial and body hair, and atrophy of external genitalia. He had developed gynecomastia 1 year previously but had no galactorrhea. Laboratory tests indicated hypopituitarism. Skull x-ray and carotid angiography documented a large sellar mass with parasellar growth. Visual fields were normal. The tumor was removed by transphenoidal surgery. Three months postoperatively, while the patient was on full replacement therapy, plasma ACTH determinations showed elevated levels.

Light-Microscopic Findings

A summary of the morphologic findings is shown in Table 1. The adenomas were basophilic, with strong PAS and lead–hematoxylin positivity in Cases 1, 2, and 5, and exhibited a sinusoidal pattern frequently seen in functioning corticotropic adenomas. The histologic structure was lost due to pituitary apoplexy in the second biopsy of Case 1 and in Case 3. In Case 4, the small surgical specimen was insufficient for histologic and histochemical evaluation. The tumors in Cases 6–14 were chromophobic, with varying positivity for PAS and lead–hematoxylin, and exhibited a diffuse growth pattern. The immunoperoxidase technique revealed varying amounts of immunoreactive ACTH, β-lipotropin, and α-endorphin in the adenomas tested. Only weak positivity was obtained when using antiserum raised against 1-24 ACTH. The staining was more intense with anti-1-39 and even more so with anti-19-39 ACTH. A moderate or weak reaction was seen with anti-β-LPH in most cases. An inverse relationship appeared to exist in some tumors between the intensity of immunostaining for ACTH and that for α-endorphin. In these cases, high ACTH content was associated with moderate or weak staining for α-endorphin, and vice versa. When comparing consecutive sections stained for 1-39, 19-39 ACTH, or α-endorphin, a different distribution of reacting cells was often found. Immunostaining for GH, Prl, β-FSH, β-LH, and β-TSH and for glycoprotein hormone α-subunit was negative in all cases.

Electron-Microscopic Findings

In nonadenomatous human pituitaries, the most common corticotropic cell, showing positive immunostaining for ACTH,[8] is an oval or angular cell possessing spherical and irregular secretory granules of varying elec-

tron density, measuring 250–450 nm. This cell type usually contains Type I microfilaments [1,2] and undergoes Crooke's hyalinization in cases of hypercorticism. The electron-microscopic application of the immunoperoxidase technique,[8] using anti-19-39 ACTH and anti-α-endorphin as primary antibodies, revealed numerous positive cells exhibiting different features.[9] Some cells contained secretory granules with a diameter of only 200–250 nm; others contained granules in the range of 250–450 nm. Cells with granules measuring up to 700 nm and over were fairly common. In addition to granule size and electron opacity, striking variations were seen in the morphologic characteristics of granules. Some cells possessed spherical granules; others possessed spherical and irregular or teardrop-shaped granules. The overwhelming majority of pituitary adenomas associated with Cushing's disease or Nelson's syndrome are composed of densely granulated corticotropic cells of the most common type.

The silent adenomas in Cases 1–5 consisted solely or mostly of densely granulated cells having the fine structural features of corticotropic cells occurring in functioning corticotropic cell adenomas. The oval or angular cells possessed a moderately or well-developed, often dilated rough endoplasmic reticulum (RER), numerous free ribosomes, prominent Golgi complex, and spherical as well as irregular secretory granules with varying electron density and measuring 250–700 nm (majority: 300–400 nm). Type I microfilaments occurred in Cases 1, 2, and 4. In the recurrent tumor of Case 1, as well as in Cases 3 and 5, except for the morphologic characteristics of secretory granules, most of the cytoplasmic details had been lost due to advanced necrotic changes. As an added feature, markedly increased lysosomal activity (autophagy and crinophagy) was noted in Cases 1 and 3. In Case 2, a considerable number of sparsely granulated cells were seen with the absence or incomplete development of the Golgi apparatus.

The fine structure of pituitary adenomas in Cases 7–11 showed similar characteristics. The closely apposed, middle-sized cells were oval or angular, with spherical or somewhat irregular nuclei moderately rich in chromatin. The nucleoli were inconspicuous. In the abundant cytoplasm, moderately to well developed RER was evident in the form of parallel stacks or randomly distributed slim cisternae. Slight dilation of RER profiles occurred. In several cells, smooth-walled tubular ER dispersed in the cytoplasm was also noted. The prominence of the Golgi complex varied from case to case and from cell to cell. It usually occupied a large area and consisted of 4–6 mostly flat sacculi and a varying number of vesicles. Forming granules were infrequently seen. The rod-shaped or oval, moder-

ately dense mitochondria were present in fair number and showed no obvious abnormality. No oncocytic change was noted in any of the tumors. The secretory granules varied in shape and electron density and measured 150–450 nm, most of them being about 250 nm. Numerous granules exhibited the characteristic teardrop shape. Accumulation of secretory granules along the plasmalemma was common. Lysosomes were not numerous. A few fine Type I microfilaments were noted in only 1 tumor. Long intercellular junctions of the adherent type were present in all adenomas.

The adenomas in Cases 12 and 13 showed a different and unique fine structure. The tumor in Case 12 consisted of closely apposed, irregular cells with spherical or elongated nuclei and very prominent nucleoli. The large cytoplasm was well differentiated with well-developed RER, prominent Golgi apparatus, a large number of mitochondria showing regular features, and numerous, predominantly spherical secretory granules showing slightly varying electron density and measuring only 100–200 nm. Several cells possessed some tubular smooth endoplasmic reticulum (SER) and lysosomes were common. In the recurrent tumor of Case 12, marked nuclear pleomorphism and further accumulation of SER were observed.

The adenoma in Case 13 consisted of rather small polyhedral cells with irregular nuclei. The cytoplasm contained a few stacks of poorly developed RER, inconspicuous Golgi complexes, and a fair number of rod-shaped, moderately dense mitochondria, frequently showing focal cavitations. The secretory granules were numerous in the majority of cells, measuring 100–600 nm. The abundance of secretory granules, however, was not very obvious, because most of them, especially the larger ones, exhibited an unusually low electron opacity. The morphologic characteristics of the secretory granules, with light, uneven flaky content and the irregular, frequently discontinuous limiting membranes, showed a striking similarity to those of the delta cells of the islets of Langerhans—never seen before in a pituitary adenoma.

The adenoma in Case 14 showed rather immature features, with moderately developed cytoplasmic organelles, very small (less than 150 nm), mostly spherical secretory granules, and moderate oncocytic change. Many cells contained aggregates of SER—a characteristic apparently shared by some silent corticotropic adenomas and the incompletely differentiated elements of the acidophilic cell line.

Discussion

Two unexpected facts have emerged from the investigation of silent corticotropic adenomas: their relatively high incidence (they account for

43% of all corticotropic cell tumors) and their morphologic diversity. The possibility that these adenomas—despite similar immunocytochemical characteristics—represent more than one entity must be considered.

The strongly basophilic adenomas of the group exhibit fine structural features and immunocytologic properties similar to those of active corticotropic adenomas.[1,2,10] The cause of "silence" in these adenomas may be complex. In three basophilic tumors, markedly enhanced lysosomal activity was noted, which, due to intracellular disposal of secretory material, may be responsible for the lack of secretion.[4] One tumor contained numerous cells possessing an incompletely developed Golgi complex with a collapsed appearance harboring no secretory granules. Such an abnormality may lead to defective packaging and release of biologically inactive products. It is also conceivable that the substance(s) synthesized by the adenoma cells and recognized by specific antibodies contains the amino acid sequence specific for ACTH but is biologically inactive ("big" ACTH).[11,12] It must be kept in mind, however, that the basophils in the pars intermedia and in the posterior lobe ("basophil invasion") have immunocytochemical and ultrastructural features strikingly similar to those of some nontumorous corticotropic cells and of cells of ACTH-secreting tumors associated with Cushing's disease or Nelson's syndrome (unpublished observations). Pars intermedia cells contain immunoreactive ACTH, β-lipotropin, and α-endorphin; hence they are closely related to corticotropic cells but do not actively secrete ACTH; not even the most extensive "basophil invasion" leads to Cushing's disease.[13] There is also a growing body of evidence that unlike anterior lobe corticotropic cells, the intermediate lobe corticotropic cells of the rat are unresponsive to the hypothalamic corticotropin-releasing factor (CRF).[14] Therefore, adenomas arising in the basophils of the intermediate or posterior lobe are likely to be clinically silent. Rasmussen and Nelson [15] reported two cases of basophilic adenomas of intermediate lobe origin found incidentally at autopsy. In one case (of a 77-year-old man) no clinical signs possibly linked to pituitary neoplasm were present. The other patient (a 55-year-old woman) had a Cushingoid appearance (hirsutism, florid face, truncal obesity, purple striae) for many years. The adrenals, however, were normal in both cases, and no Crooke's hyalinization was noted in the nontumorous part of the pituitaries. We have also seen a similar tumor at the autopsy of a 68-year-old woman who had hypothyroidism and gouty arthritis and died of bronchopneumonia. She had no symptoms of hypercorticism; and the weight and gross and histologic appearance of the adrenals were normal. The pituitary adenoma, basophilic and exhibiting strong PAS positivity, was not only sharply demarcated but also separated by the narrow pituitary cleft

from the pars distalis. The large tumor occupied a part of the posterior lobe and invaded the distal part of pituitary stalk. There is a striking resemblance between Rasmussen and Nelson's [15] second case and our Case 5: a 46-year-old woman who had a Cushingoid appearance for several years without any biochemical evidence of hypercorticism. Unfortunately, the exact localization of pituitary adenomas and their anatomic relation to the surrounding tissue in surgical material are largely lost, and the assumption of a pars intermedia origin of these tumors is purely conjectural.

Eleven tumors (including 2 biopsies from Cases 12 and 13) exhibiting moderate or no PAS positivity showed three different patterns by electron microscopy: 6 adenomas had teardrop-shaped granules, the tumor in Case 12 possessed very small spherical secretory granules, and the tumor in Case 13 contained secretory granules similar to those of delta cells of Langerhans islets. One adenoma (Case 14) was composed of incompletely differentiated cells.

In order to explain these variations, the following possibilities can be entertained: 1) The adenomas consist of the densely and sparsely granulated variants of the same cell type. This assumption would permit considerable differences in the fine structural appearance of cells (as in the cases of densely and sparsely granulated growth hormone cell adenomas).[1,2] The marked morphologic differences in granules and the lack of ACTH oversecretion, however, would remain unexplained. 2) The sparsely granulated cells represent the committed, incompletely differentiated precursors of corticotropic cells that are not yet capable of secreting bioactive hormones. This hypothesis would be acceptable in a few cases of adenomas with immature features. However, most silent sparsely granulated corticotropic adenomas appear to be well differentiated, with prominent RER, SER, and Golgi complex. 3) The cells giving rise to silent corticotropic adenomas are peptide-producing cells that belong to the same cell line as corticotropic cells but have a different yet undisclosed secretory function. This assumption would sufficiently explain the lack of bioactive ACTH secretion and also the elevated plasma ACTH, as measured by RIA, found in the postoperative period in the two patients tested so far. One of them (Case 5) was eucorticoid; the other (Case 14) was panhypopituitary and on replacement therapy well before pituitary surgery. It is also relevant to mention that the marked morphologic variations found in corticotropic adenomas were seen within the cell population containing immunoreactive ACTH and α-endorphin in the nontumorous human pituitary as well. Among these cells, one type [8] is identified so far as corticotropic. It was also learned recently that corticotropic cells con-

tained not only ACTH, β-lipotropin, and endorphins but also calcitonin.[16,17] These findings reinforce the assumption that the corticotropic cells represent a heterogeneous cell population consisting of different subtypes with hitherto unknown specific functions.

The most important practical problem arising from the present investigation was the high incidence of apoplexy, infarction, and recurrence of silent corticotropic tumors; the reason for that is not yet known. No fine structural alterations different from those occurring in other adenoma types [18] were noted in the vasculature of the surviving areas adjacent to the necrosis. It is conceivable that proliferating tumor tissue outgrows its blood supply, causing hypoxic injury. Alternatively, the cause of infarction in some cases may be related to the anatomic site of tumors. If an adenoma originates in the posterior portion of the median wedge (where there is a concentration of corticotropic cells) or in the intermediate lobe and grows upward into the posterior lobe, it may lead to the compression of the portal vessels. Such neoplasms may cut off their own blood supply, thereby "committing suicide." The similarly high incidence of recurrences seems to reflect the proliferative tendency and relatively rapid growth of some of these tumors.

The need for correct morphologic diagnosis and careful follow-up in cases of silent corticotropic adenomas cannot be overemphasized. As preliminary results indicate, the immunoreactive ACTH level of the plasma may be elevated in some cases, and it could be used as a marker either in the preoperative endocrine study or in the postoperative period to detect residual tumor and recurrence. Present investigations strongly indicate the usefulness of immunocytochemistry and electron miroscopy in the identification of pituitary adenomas. The application of these techniques cannot any longer be regarded as an academic exercise, and they should be fully exploited whenever possible to assure accurate diagnosis of hypophyseal lesions.

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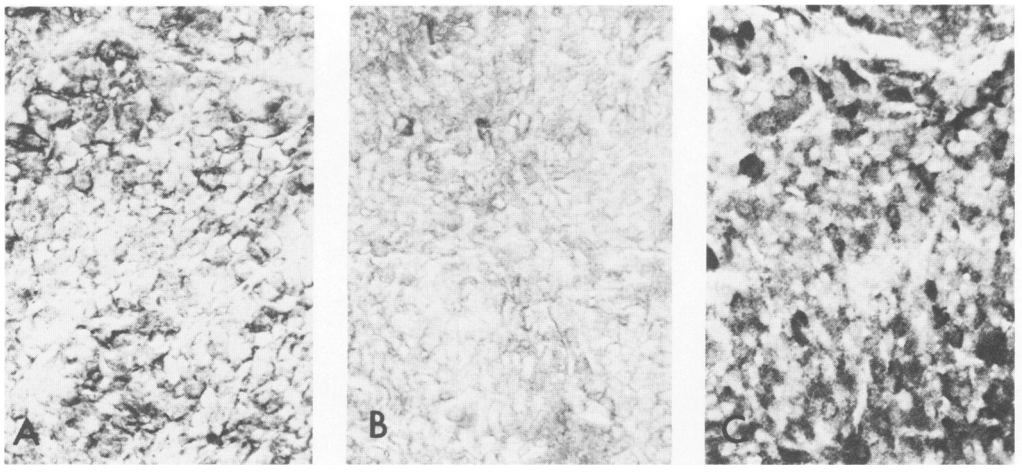
[*Illustrations follow*]



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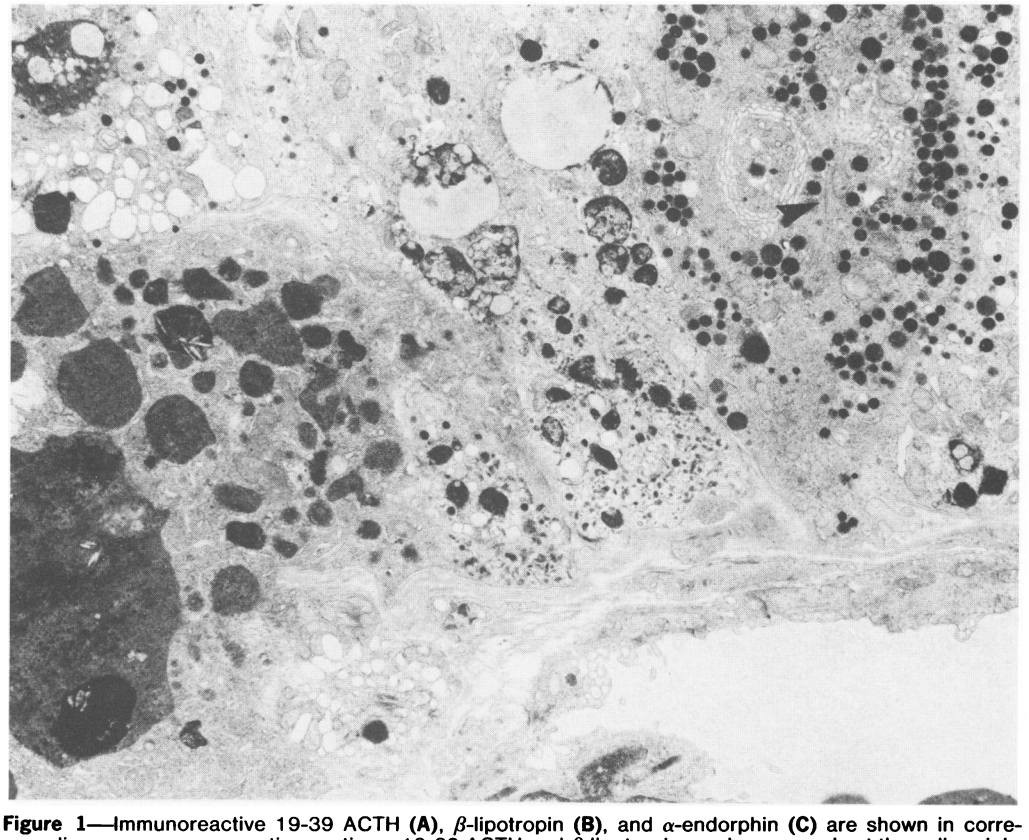
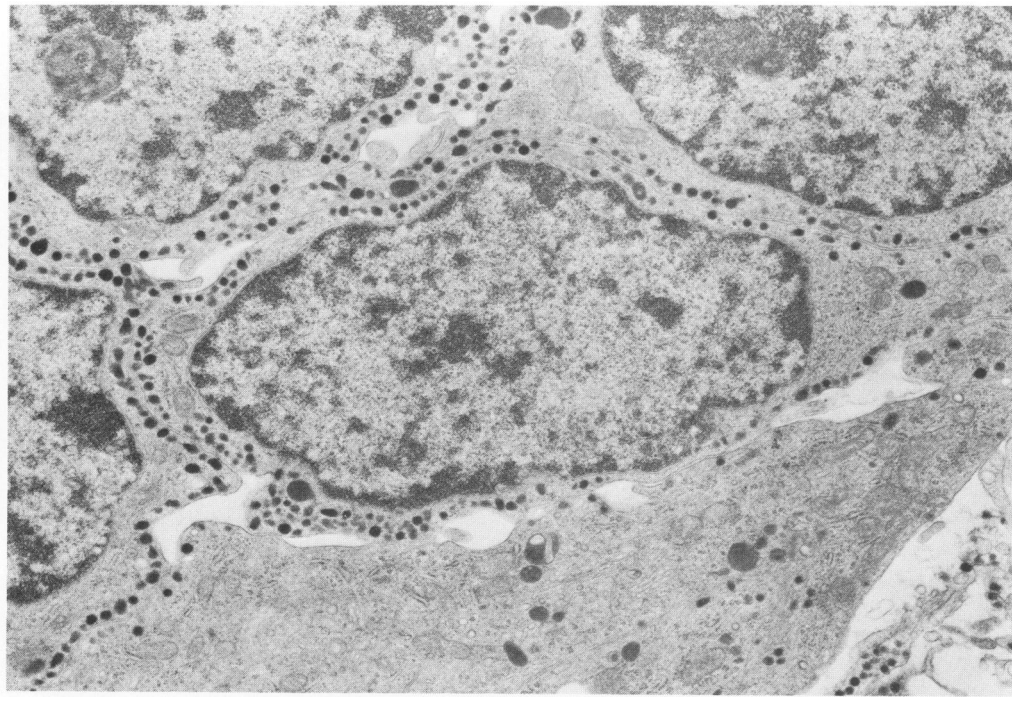


2

Figure 1—Immunoreactive 19-39 ACTH **(A)**, β-lipotropin **(B)**, and α-endorphin **(C)** are shown in corresponding areas on consecutive sections. 19-39 ACTH and β-lipotropin can be seen only at the cell periphery, in contrast to the strong positivity for α-endorphin over the entire cytoplasm. Basophilic silent corticotropic adenoma, first biopsy, Case 1. Immunoperoxidase technique. (×250) (With a photographic reduction of 11%) **Figure 2**—Fine structural appearance of the basophilic adenoma of Case 1 is seen. Note the abundance of large lysosomal bodies. One cell retained its original features, including Type I microfilaments (*arrowhead*). (×7100) (With a photographic reduction of 11%)

3



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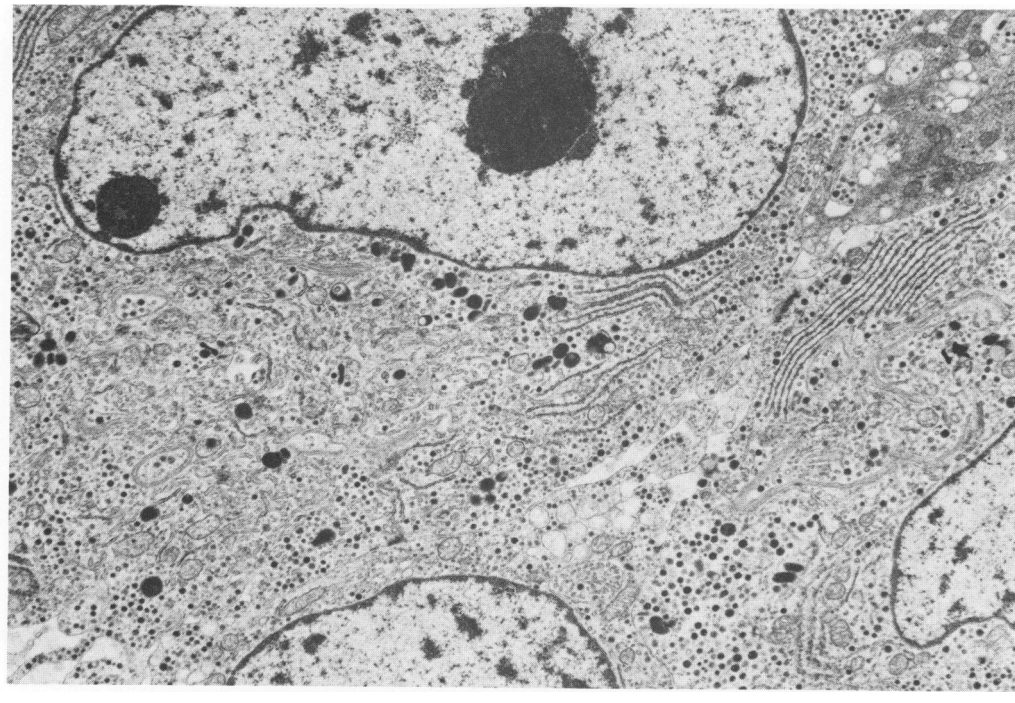

Figure 3—Well-differentiated adenoma with numerous teardrop-shaped secretory granules measuring 200–250 nm along the plasma membranes. Chromophobic silent corticotropic adenoma, Case 9. (×11,200) (With a photographic reduction of 8%) **Figure 4**—The adenoma in Case 12 had well-developed cytoplasm containing prominent RER and SER, large Golgi complexes, and small (less than 200 nm in diameter) spherical secretory granules. Several small lysosomal bodies are also seen. Chromophobic silent corticotropic adenoma, first biopsy. (×7000) (With a photographic reduction of 8%)