Pituitary Adenomas

An Immunohistochemical Study of Hormone Production and Chromogranin Localization

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Tumors from 42 surgically resected pituitaries and from 13 autopsy cases were studied immunohistochemically with polyclonal antisera to 7 anterior pituitary hormones and with a newly developed monoclonal antibody directed against human chromogranin for evaluation of the distribution of chromogranin in normal and neoplastic pituitaries. In addition, a prospective study was done for assessment of the prevalence, morphology, and endocrine cell types of pituitary tumors in 100 autopsy subjects. When these 55 pituitary adenomas were examined with monoclonal antibody (LK2H10) directed against human chromogranin, selective staining of normal adenohypophyseal cell types and pituitary tumors was observed. Most null-cell adenomas (12/14) were positive for chromogranin, whereas all prolactin (PRL)-producing adenomas (19/19) were negative. Growth hormone (GH) adenomas were focally positive (9/9). All oncocytomas (2/2), 1 thyrotropin (TSH) adenoma, and a follicle-stimulating hormone/luteinizing hormone adenoma were positive for chromogranin. One or more adenomas were present in 14% of the autopsy cases. The tumors occurred most frequently in patients in the fifth through the seventh decades of life. Immunohistochemical staining of 13 adenomas revealed 1 TSH, 1 ACTH, and 4 PRL-producing tumors, whereas 7 other tumors, which were null-cell or undifferentiated adenomas, failed to stain for any of the seven principle pituitary hormones. These results indicate that antibody LK2H10 to human chromogranin is useful in the immunohistochemical characterization of pituitary adenomas. Incidental pituitary microadenomas from autopsy-derived pituitaries most commonly produce PRL, or they belong to the null-cell or undifferentiated tumor group. (Am J Pathol 1984, 116:464–472)

SEVERAL STUDIES have shown that incidental pituitary adenomas are relatively frequent autopsy findings when the glands are thoroughly examined microscopically.[1-4] Until recently, however, it has been difficult to classify such adenomas, since conventional histologic methods do not allow reliable identification of adenohypophyseal cell types.[1] It is now possible to classify both normal and neoplastic pituitary cells with respect to specific hormonal production by immunohistochemical methods.[5,6]

Most studies of incidental pituitary adenomas have been retrospective.[2,4,7-9] In one prospective study, prolactin (PRL) was the only hormone identified by immunohistochemistry, and the majority of the adenomas lacked this hormone.[3] In another prospective study of incidental pituitary adenomas in patients over 80 years of age, 9 of 17 adenomas were prolactinomas, whereas the other tumors failed to stain with antibodies against corticotropin (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), or thyrotropin (TSH).[2]

Null-cell or undifferentiated pituitary adenomas are pituitary tumors which are derived from patients lacking clinical or biochemical evidence of increased

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hormone secretion and are negative for pituitary hormone production by immunohistochemistry.[10] These tumors can be diagnosed by electron microscopy or by exclusion after complete clinical and immunohistochemical studies. Neuron-specific enolase, which is a general marker of neuroendocrine cells and tumors,[11] has recently been found in some null-cell adenomas.[12]

We recently described a monoclonal antibody to chromogranin (LK2H10) which can be used to detect this molecule in normal anterior pituitary gland tissues and in pituitary tumors.[13] Because null-cell adenomas contain endocrine granules and chromogranin is associated with endocrine secretory granules, antibody LK2H10 was examined for its use as a probe to identify null-cell pituitary adenomas.

The purpose of this study was to analyze the distribution of chromogranin in pituitary adenomas. In addition, pituitary adenomas discovered in a prospective autopsy series were analyzed for hormone production.

Materials and Methods

Pituitary Tissues

Pituitary glands were collected from subjects 18 years of age and older in a series of 100 autopsies at the University of Michigan Medical Center from December 1982 to June 1983. The cases were not strictly consecutive but were otherwise unselected. Autopsies were generally performed 2–24 hours after death. In addition, 42 cases of surgically resected adenomas were selected for study. Each gland was fixed in 10% buffered formalin, weighed, and cut sagittally at 2-mm intervals. This usually resulted in 4–5 slices of pituitaries, which were embedded in paraffin. Two hematoxylin and eosin (H&E)-stained sections were prepared from each block. Adenomas were identified by their difference in pattern and cellularity in comparison with the surrounding gland. The maximum tumor diameters were estimated with an ocular micrometer. A routine silver impregnation method was used to show the reticulin network in normal and neoplastic pituitary glands.

Immunohistochemistry

Four-micron sections of each adenoma were cut and processed for immunohistochemistry with the use of the avidin-biotin complex (ABC)–peroxidase method as previously described.[6,14] Antisera to growth hormone (GH), PRL, ACTH, FSH, LH, and TSH were each used at a 1/1000 dilution. (gifts from the National Institute of Arthritis, Digestive, and Kidney Diseases). Antiserum to β-Endorphin (Immunonuclear Corp.) was also used at 1/1000 dilution. The monoclonal antibody to human chromogranin (see next section) was used at 1/10 dilution. The tissues were incubated with the primary antibodies for 1 hour at room temperature, then washed with phosphate-buffered saline (PBS) and incubated with biotinylated IgG and ABC–peroxidase complex (Vector Laboratories, Burlingame, Calif) for 30 minutes. Finally, the sections were treated with diaminobenzidine-HCl (20 mg/100 ml) with 0.05% H_2O_2 and counterstained with hematoxylin. Controls consisted of substituting normal serum for the primary antisera and of absorption of each antiserum with the appropriate antigen using 0.5 μg/ml of purified antigen (Sigma Chemical Co., St. Louis, Mo).

Production of Monoclonal Antibody (LK2H10) to Human Chromogranin

Antibody LK2H10 was produced by hybridoma-cell fusion technology as described in detail elsewhere.[13] Briefly, a Balb/c mouse immunized with 1-cu mm pieces of a human pheochromocytoma was sacrificed and the spleen cells fused with NS-1 mouse myeloma cells essentially as described by Galfre et al.[15]

Hybridoma clone LK2H10 was selected for subcloning and further testing because the antibody reacted strongly with tumor cells. After subcloning, antibody LK2H10 showed strong cytoplasmic reactivity in formalin-fixed paraffin-embedded sections of endocrine tissues when up to a 100-fold dilution of LK2H10 spent culture medium was used for peroxidase staining.

In order to assess the nature of the target molecule detected by monoclonal antibody LK2H10, a human pheochromocytoma, a normal human adrenal gland and a normal pituitary gland (each from different patients) were first extracted with 10 volumes of cold PBS in a Waring blender. The resulting soluble extract was applied to an LK2H10 antibody-Sepharose column, and the purified antigen was eluted from the affinity column with 0.5 N acetic acid. The purified antigen was then electrophoresed in a polyacrylamide slab gel containing sodium dodecyl sulfate (SDS) and the buffer system of Laemmli.[16] Proteins in the gel were electrophoretically transferred to nitrocellulose paper and then visualized by immunoperoxidase staining with antibody LK2H10. A reference preparation of human chromogranin A, purified from isolated catecholamine-containing granules of a human pheochromocytoma (a generous gift of Dr. Dan

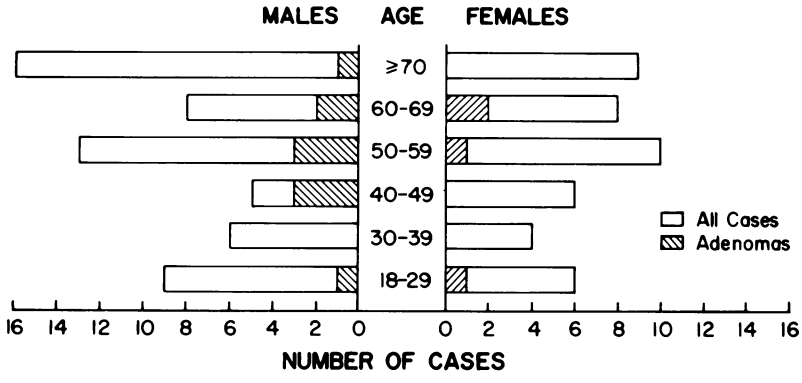


Figure 1—Age distribution of the autopsy patient population and the prevalence of pituitary adenomas in 100 autopsy pituitary glands. While many patients were over 70 years of age, the greatest numbers of adenomas were present in the fifth through the seventh decades of life.

O'Connor, Veterans Administration Hospital, San Diego, Calif) was similarly electrophoresed, and the mobility was compared with that of the purified antigen. The reference preparation of chromogranin A was also tested for its ability to block immunohistochemical staining of pituitary and adrenal tissues by antibody LK2H10.

Ultrastructural Immunohistochemistry

Ultrastructural immunohistochemistry was done with the ABC method. Sections of a surgically reacted GH adenoma were fixed in 4% paraformaldehyde, 1% glutaraldehyde in phosphate buffer, pH 7.2, for 1 hour. Sections were embedded in Polybed/Araldite. Ultrathin sections were placed on nickel grids for immunostaining. After etching in 5% H_2O_2 for 10 minutes and washing in PBS, the sections were treated with suppressor serum (5% normal horse serum) for 15 minutes and then incubated with antibody LK2H10 at 1/10 and 1/50 dilutions for 60 minutes at room temperature. After washes in PBS and incubation with biotinylated IgG and ABC-peroxidase complex treatments for 30-minute periods, the sections were stained with diamenobenzedine (DAB) for 5 minutes and then counterstained with 2% uranyl acetate for 30 minutes.

Controls for ultrastructural immunohistochemistry consisted of (1) omitting the primary and secondary antibodies and (2) substitution of antibody LK2H10 absorbed with chromogranin A in place of the primary antibody. Sections were viewed with a Zeiss 109 electron microscope.

Results

General Findings

The age and sex distribution of the autopsy series is depicted in Figure 1. Fifteen microadenomas were identified, with one gland having two adenomas. Four of these tumors occurred in females, and only one adenoma occurred in subjects 70 and older, despite the relatively large number of autopsies in this age group.

The maximum diameter of the majority of the adenomas was less than 2 mm (Figures 2 and 3). All adenomas were less than 4 mm except one, which was 6.8 mm. This was the only grossly evident tumor. There was no significant difference in pituitary weights between cases with and without adenomas. The mean pituitary weight for all females in the study was 640 ± 143 mg (SD), and for males, 593 ± 118 mg (SD). Reticulin stains demonstrated modification of the normal reticulin pattern in tumors greater than 2 mm (Figure 4).

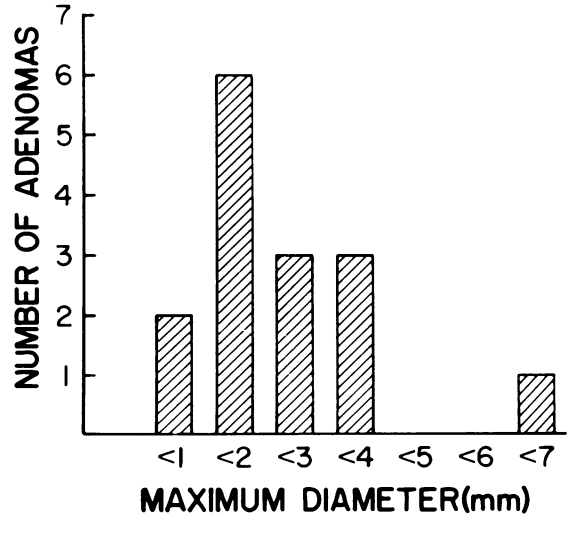
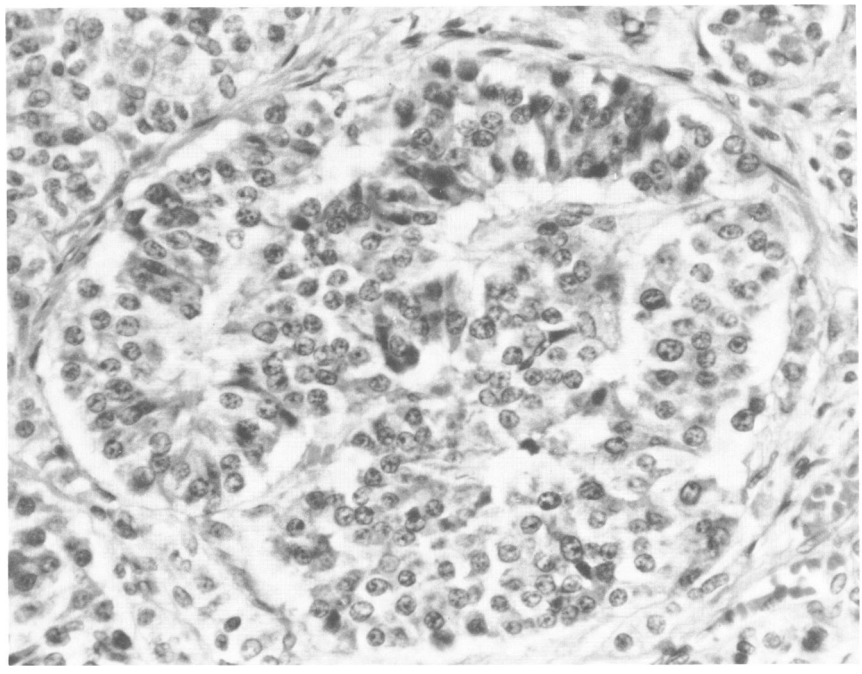


Figure 2—Size distribution of 15 pituitary microadenomas from 14 autopsy cases. The majority of tumors were less than 2 mm in maximum diameter, although one patient had an ACTH-producing tumor that was greater than 4 mm. The 7 null-cell adenomas and the 4 PRL-producing adenomas had mean diameters of 2.2 mm, while the TSH-producing micro-adenoma was 1.1 mm in diameter.



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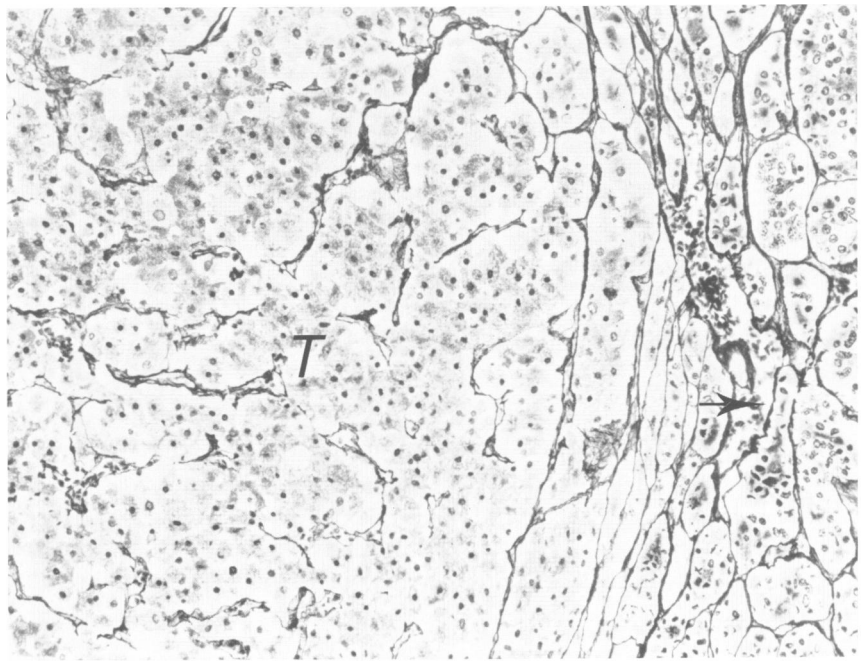

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Figure 3 – Pituitary microadenoma from Case 3. This 1.25 mm PRL-producing adenoma is surrounded by a thin capsule of connective tissue. (H&E, ×330) **Figure 4** – Reticulin stain of a null-cell adenoma (3.5 cm in diameter) from Case 12 shows distortion of the reticulin network in the microadenoma (T) with preservation of the normal architecture in the adjacent normal pituitary tissue (*arrow*). (Reticulin, ×32)

Immunohistochemistry

Two adenomas could not be characterized because of insufficient material upon further sectioning. Of the remaining 13 adenomas (Table 1), there were 4 prolactinomas (23%), 1 TSH adenoma (7.7%), and 1 ACTH adenoma (7.7%). The ACTH tumor was also positive for β-endorphin. The remainder were negative for all seven hormones. We found no "mixed" adenomas. Although several adenomas of one cell type contained occasional cells positive for other hormones, these cells were entrapped nonneoplastic cells.

Specificity of Monoclonal Antibody LK2H10 for Human Chromogranin

Immunoelectroblotting of an adrenal and a pituitary gland extract and extracts from a pheochromocytoma combined with antibody LK2H10 peroxidase

Table 1—Immunohistochemical Staining of Pituitary Microadenomas From 12 Autopsy Cases With Polyclonal Antisera and With Monoclonal Antibody LK2H10 Against Human Chromogranin

Case	Age	Sex	Hormone	Staining with LK2H10
1	64	M	PRL	–
2	47	M	PRL	–
3	54	M	PRL	–
4	26	F	PRL	–
5	58	F	ACTH	+
6	49	M	Null	–
7	58	M	Null	+
8	40	M	Null	+
9	76	M	Null	+
10	59	M	Null	–
11	62	F	Null	+
12	60	F	TSH*	+
			Null*	+

* Two separate adenomas in one pituitary gland.

staining showed a reactivity with two large polypeptides (about 68,000 daltons) and a number of lower molecular weight polypeptides. The two largest polypeptides detected by LK2H10 were identified as chromogranin A, since identical mobility and LK2H10 reactivity were observed with the reference preparation of chromogranin A. The chromogranin specificity of antibody LK2H10 staining of tissue sections was confirmed by the ability of purified chromogranin A (0.5 μg/ml) to block peroxidase staining of pituitary and adrenal tissues by LK2H10.

Characterization of Pituitary Adenomas With LK2H10

The results of 55 adenomas stained with LK2H10 are summarized in Tables 1 and 2. Prolactinomas were consistently negative for LK2H10 staining in both autopsy and surgical material (Figure 5). Two oncocytomas, 1 TSH adenoma, and an FSH/LH adenoma were positive, whereas the immunoreactivity with ACTH adenomas was variable. All GH adenomas were focally positive. All 7 null-cell adenomas in the surgically resected pituitary tumors and 5 of 7 null-cell tumors from the autopsy series contained immunoreactive chromogranin detected by monoclonal antibody LK2H10 (Figure 6). The majority of the surrounding normal pituitary cells were positive for chromogranin, some with dense granular staining and others with lighter, more diffuse staining. A distinct population of cells failed to stain with the antibody. These corresponded to normal PRL cells when examined in serial sections stained for PRL and chromogranin. When fresh frozen sections of a normal human pituitary obtained at autopsy were stained with LK2H10 and PRL antiserum, the PRL cells were also negative with antibody LK2H10. Ultrastructural immunohistochemistry with a surgically resected GH adenoma showed that chromogranin was localized in cytoplasmic secretory granules (Figure 7A). The electron-dense material was present diffusely throughout the granule matrix of most secretory granules. Control sections were negative after ultrastructural immunohistochemistry (Figure 7B).

Clinical Correlation

Various diseases were observed in patients with adenomas, including pneumonia, pulmonary thromboembolus, myocardial infarct, carcinomatosis, and leukemia. Two subjects had traumatic deaths. In general, the diseases were representative of the autopsy series as a whole. Only one subject had clinical evidence of endocrine dysfunction. This subject (Case 5, Table 1), who died of atherosclerotic heart disease complicated by pneumonia, had had a diagnosis of Cushing's syndrome and adrenal cortical hyperplasia. An extensive workup, including computerized tomography scans, failed to demonstrate a pituitary lesion, and ACTH levels were within normal limits on several occasions. At autopsy she had Cushing's disease due to a 6.8-mm ACTH adenoma.

Discussion

The detection of chromogranin by monoclonal antibody LK2H10 was a helpful diagnostic aid in the immunohistochemical characterization of null-cell adenomas in this study. The chromogranins, which were first identified many years earlier in the adrenal glands, comprise a group of acidic proteins which make up most of the soluble proteins of catecholamine storage vesicles.[17] Recent data from our laboratory and from others indicate that chromogranin can be found in subsets of cells and tumors of the diffuse

Table 2—Immunohistochemical Localization of Chromogranin by Monoclonal Antibody LK2H10 in Surgically Resected Pituitary Adenomas

Type of adenoma*	Positive staining with LK2H10
PRL	0/15
GH	9/9†
ACTH	6/8
FSH/LH	1/1
Null-cell	7/7
Oncocytoma	2/2

* Immunohistochemical localization in formalin-fixed paraffin-embedded tissues.

† Tumors focally positive.

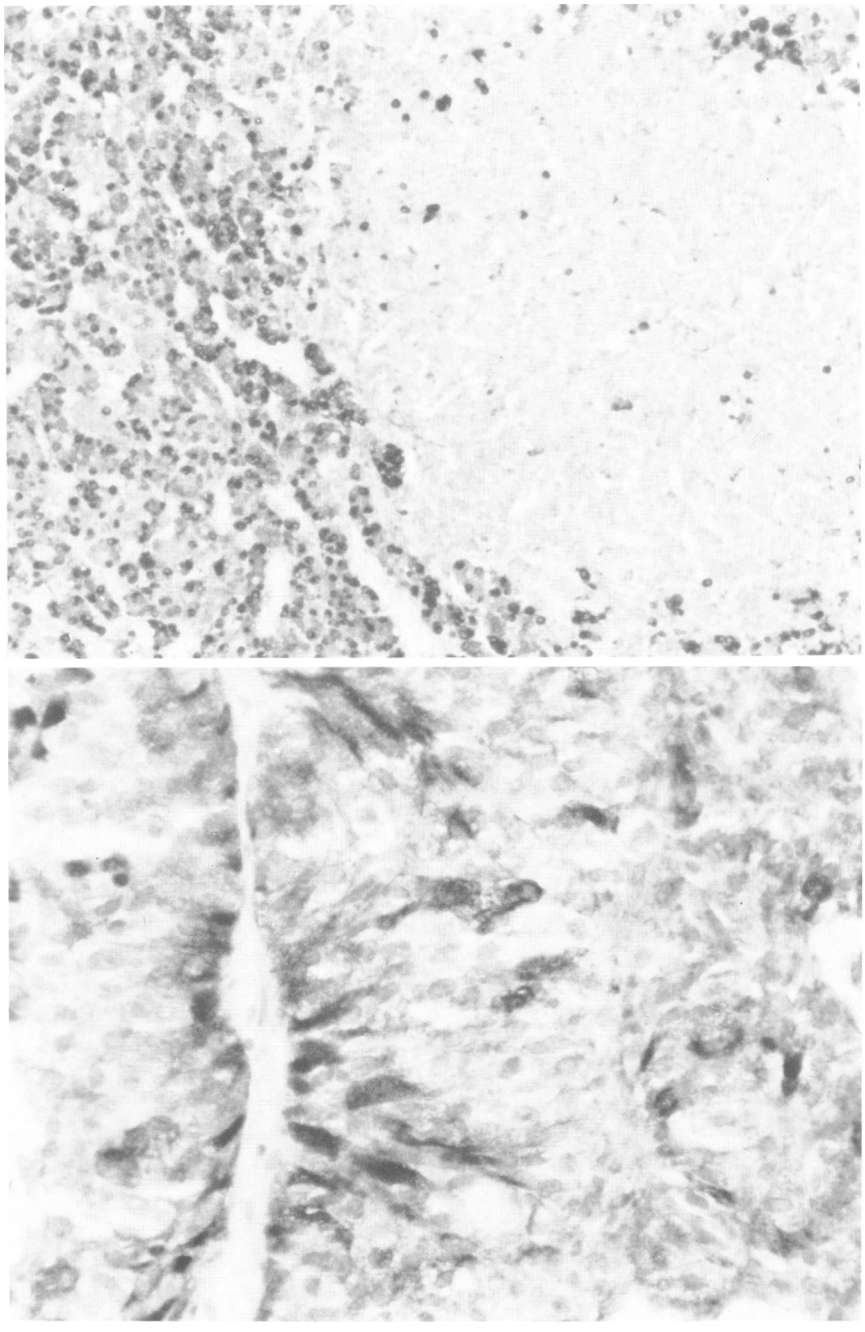

Figure 5 – PRL-producing adenoma from Case 3 stained for chromogranin with monoclonal antibody LK2H10. Most of the surrounding normal pituitary cells are positive, whereas the PRL-adenoma shows negative immunoreactivity. A few entrapped normal pituitary cells within the adenoma are also positive. (Immunoperoxidase, ×132) **Figure 6** – Null-cell adenoma from a surgically resected pituitary tumor stained with antibody LK2H10. Most of the tumor cells stain positively with this antibody, whereas all cells were negative after staining with antisera to 7 pituitary hormones. (Immunoperoxidase, ×330)

neuroendocrine system, including pheochromocytomas, medullary thyroid carcinomas, thyroid C-cell hyperplasia, parathyroid adenomas, and pancreatic islet adenomas.[13,18] This is in contrast to other endocrine markers, such as neuron-specific enolase, which are present in all cells of the diffuse neuroendocrine system.[12] All of the surgically removed null-cell adenomas and most of the autopsy-derived null-cell adenomas were positive when stained with LK2H10. Since ultrastructural studies were not done on the autopsy cases, the ultrastructural features of the two null-cell tumors that were negative for all 7 anterior pituitary hormones and for chromogranin immunoreactivity remain unknown. Prolactinomas were consistently negative with antibody LK2H10, whereas a variety of adenomas of other cell types were predominantly positive. The failure of LK2H10 to stain normal and neoplastic PRL cells may indicate that these

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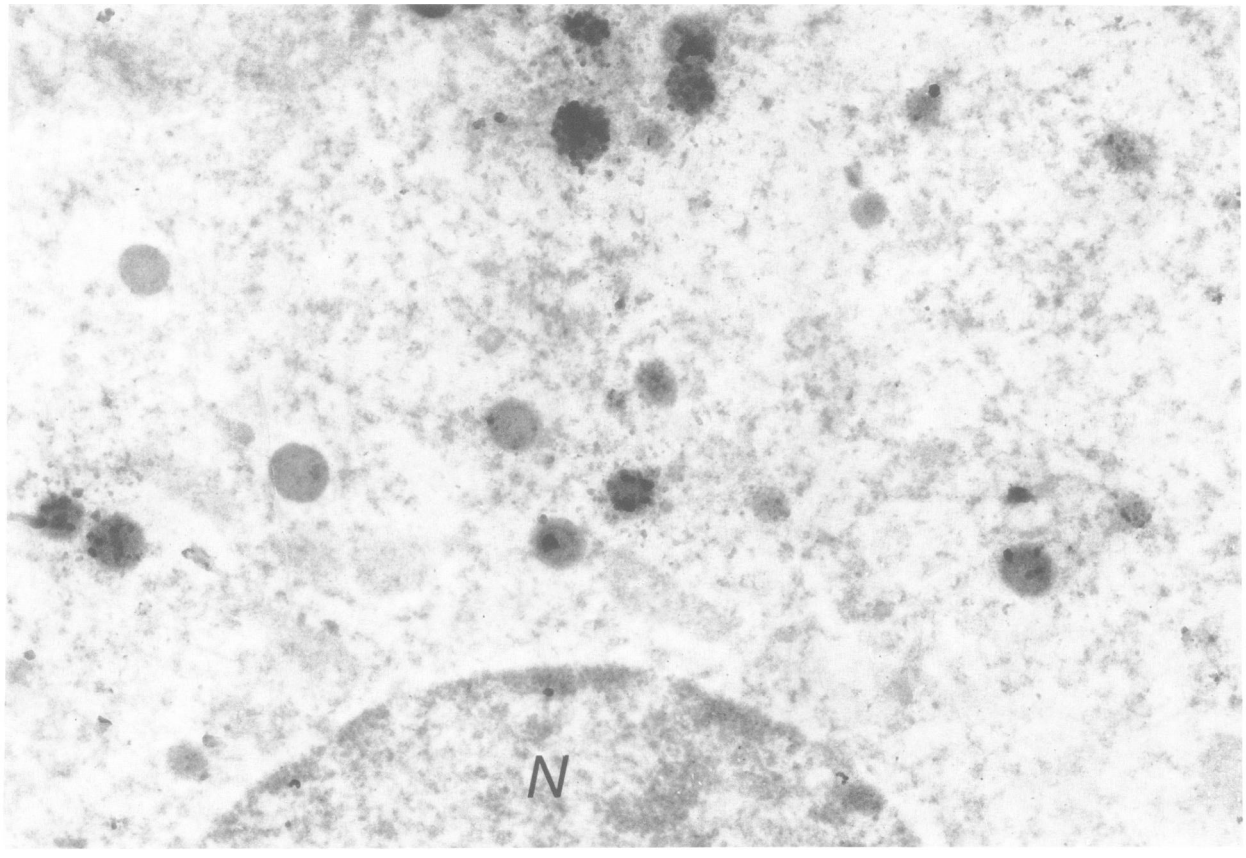

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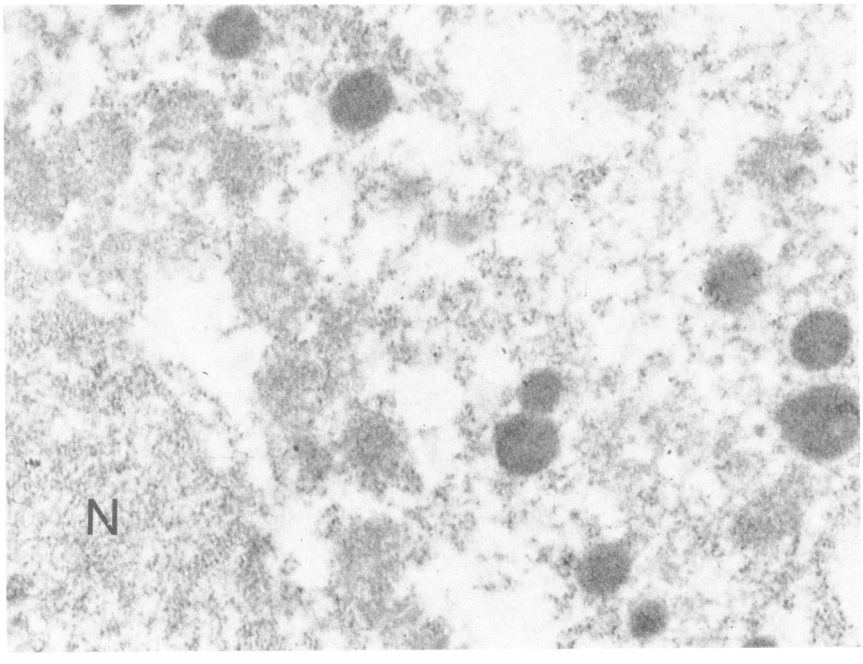

Figure 7A — Ultrastructural immunohistochemical localization of chromogranin in cytoplasmic secretory granules of a GH-producing adenoma using the avidin–biotin complex–peroxidase method with DAB-HCl. The nucleus (*N*) and most of the cytoplasm are negative, whereas granule matrix shows a diffuse positive immunoreactivity to antibody LK2H10. (×28,300) **B** — Control section for ultrastructural immunohistochemical localization of chromogranin. Antibody LK2H10 was incubated with 0.5 μg/ml of purified chromogranin A before immunostaining, and this abolished staining of secretory granules. (×24,800)

Table 3—Reported Prevalence of Pituitary Adenomas at Autopsy

Reference	Number of autopsies	% with adenomas
Susman, 1933[8]	260	8.4
Costello, 1936[4]	1,000	22.5
McCormick and Halmi, 1971[9]	1,600	9.1
Kovacs et al, 1980[1]	152*	13.0
Parent et al, 1981[7]	500	8.5
Burrow et al, 1981[3]	120*	27.0
Present study	100*	14.0

* Prospective studies.

cells have smaller amounts of chromogranin or that the chromogranin in this cell type may not be detected by antibody LK2H10.

The 14% prevalence of pituitary adenomas in this autopsy series is in general agreement with previous studies (Table 3). It is likely that extensive serial sectioning would have revealed additional adenomas, because one study showed a 27% prevalence of tumors.[3] However, their small sizes would probably have precluded complete immunohistochemical characterization.

PRL was the most commonly identified hormone in the autopsy-derived adenomas, although the 23% prevalence is somewhat lower than that reported in another autopsy series (41%).[3] Over half of the pituitary adenomas were of the null-cell type, and these did not stain with any of the known major pituitary hormones. The predominance of null-cell adenomas is in marked constrast to surgically resected adenomas, which in this series and in most other series are predominantly prolactinomas.[19-22] The presence of an asymptomatic TSH microadenoma in this series was unusual. Most TSH tumors are large and usually produce symptoms of hyperthyroidism. However, TSH microadenomas have been reported recently.[23,24] Since the patient in our series did not have an elevated T_4 level and the thyroid gland was not enlarged at autopsy, this tumor was probably asymptomatic. The occurrence of a TSH adenoma in a patient with no known endocrine dysfunction suggests that small asymptomatic adenomas other than null and PRL cell types may occur occasionally. A recent report of 107 incidental pituitary adenomas at autopsy, which included 1 TSH and 4 ACTH tumors, supports this concept.[25] Although the definition of adenomas include compression of adjacent normal tissue and distortion of the normal architecture by a homogeneous cell population,[25] small adenomas (less than 2 mm) did not show some of these changes in this study, especially after reticulin staining, and it is not known whether these areas represent true adenomas or merely hyperplastic nodules.

Incidental pituitary adenomas that are found at autopsy may have developed several years before death, and a slow growth rate could explain their generally small size and failure to produce symptoms.[1,25,26] A recent longitudinal study of patients with PRL microadenomas indicated that only in a few patients (3/27) did the tumors grow during a 6-year follow-up period.[27] Additionally, in experimental animals, an appropriate endocrine milieu such as elevated estrogen levels in rats with prolactinomas appears to be necessary for proliferation of certain tumors.[28] In the absence of such a milieu, a decreased growth rate or regression may occur.[1] Parent et al[7] also suggested that some pituitary adenomas may regress spontaneously, on the basis of the presence of infarcts and cystic changes proximal to some adenomas, as well as resolution of neuroendocrinopathy. We are uncertain whether the apparent lower prevalence of pituitary adenomas in the older (greater than 70) age group in this series is a reflection of regressive phenomena, although histopathologic changes associated with spontaneous regression, ie, infarcts and cystic changes, were not noted. Alternatively, it could reflect sampling error or other factors such as medications, antimetabolite therapy, irradiation, etc.; however, we were unable to implicate such factors. Unfortunately, whereas most series report a higher number of adenomas in the sixth and seventh decades than in older patients,[4,7,25] the relative prevalence in each age group is not clear, because the patient populations have been relatively small.

The term "null-cell adenoma" has been used to designate pituitary adenomas lacking histologic, immunohistologic, or ultrastructural features which would permit the disclosure of their origin.[10] This designation is based on an analogy to classical lymphocyte markers, ie, the designation of cells lacking specific T- and B-cell markers as null lymphocytes. However, with the advent of hybridoma technology, it has become possible to characterize the original "null" lymphocytes with monoclonal antibodies directed against specific antigens, for example, the common acute lymphoblastic leukemia antigen (CALLA), among others.[29] Kovacs et al[10] recognized that the number of tumors diagnosed as null-cell adenomas would diminish with the discovery of new markers.

Because chromogranin may have a widespread but so far undefined role in endocrine secretory processes,[13] the characterization of adenohypophyseal cell types with this marker may help to define basic storage and secretory mechanisms in this endocrine organ. Additionally, such studies may help to define the origin, function, maturational stages, and activity of normal and neoplastic null cells in the adenohypophysis.

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