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Null Cell Adenoma of the Human Pituitary

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Summary. Among 343 surgically-removed pituitary adenomas, 56 tumors were unassociated clinically or biochemically with increased hormone secretion and contained no adenohypophysial hormones by the immunoperoxidase technique, except for 10 cases in which a few scattered cells showed positive immunostaining for β-TSH or β-FSH, β-LH, prolactin and/or α-subunit. These tumors were chromophobic adenomas with no PAS, lead hematoxylin or carmoisine positivity and electron microscopy failed to reveal their morphogenesis. The term null cell adenoma of the pituitary is proposed to designate this tumor type. This term recognizes the most obvious features of these tumors: the absence of markers which would permit the disclosure of their cellular origin. Null cells are also found in the nontumorous adenohypophysis, suggesting that null cell adenomas derive from preexisting nonneoplastic null cells. The question of whether pituitary null cells are hormonally inactive committed precursors, uncommitted stem cells or dedifferentiated cells remains to be elucidated.

Key words: Adenoma – Electron microscopy – Immunocytology – Pituitary – Pituitary tumor.

Introduction

In the course of a histologic, immunocytologic and fine structural study of 343 surgically-removed pituitary adenomas, 99 tumors were found to be unassociated with clinical and laboratory evidence of excessive hormone secretion. Two of these silent pituitary adenomas consisted of growth hormone cells, 22 of corticotroph cells and the immunoperoxidase technique revealed the pres-

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ence of growth hormone or ACTH, α-endorphin and β-LPH in the cytoplasm of adenoma cells. Nineteen tumors exhibited abundance of mitochondria and were regarded as oncocytomas. This work deals with the morphologic features of the remaining 56 tumors which constitute 16.5% of our material. We propose to introduce a new term: "null cell adenoma" to designate these neoplasms, since their structural characteristics are not sufficiently distinctive to permit the disclosure of their cellular origin.

Clinical Findings

The detailed description of case histories, clinical and laboratory investigations is beyond the scope of the present study.

The tumors were removed chiefly by transsphenoidal approach from 36 men and 20 women with an average age of 52 years, ranging from 34 to 83 years. The main complaints were visual disturbances of various degrees and headache. Endocrine investigations disclosed the lack of excessive adenohypophysial hormone secretion. In a few patients, blood prolactin levels were slightly above the normal levels. Hyperprolactinemia was interpreted as a "stalk section effect" and was presumably due to a decrease in the production or release of hypothalamic prolactin-inhibiting factors or to the suppression of their transport from the hypothalamus via the portal circulation to the adenohypophysis. In some patients, clinically or biochemically, various degrees of hypopituitarism were apparent. The time interval between the appearance of first clinical symptoms and operation varied from a few days to several years. The sella turcica was enlarged in every case and the radiologic diagnosis of pituitary adenoma was made with no major difficulty.

Morphologic Findings

Material and Methods. For light microscopy, tumor tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections of 4–6 μm thickness were stained with hematoxylin-phloxine-saffron, the PAS technique, lead hematoxylin and, in some cases, with Goldberg-Chaikoff's trichrome and Brookes' carmoisine techniques.

For immunocytologic localization of adenohypophysial hormones, the immunoperoxidase technique was used as described elsewhere (Kovacs et al., 1976; Kovacs et al., 1978). Paraffin sections of 4–6 μm thickness were immunostained, using the following antibodies: antigrowth hormone (Wellcome Research Laboratories, Beckenham, Kent, England), antiprolactin (donated by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada), anti 1–39 ACTH (Wellcome Research Laboratories, Beckenham, Kent, England), anti β-TSH, anti β-FSH, anti β-LH (donated by the National Pituitary Agency, University of Maryland, School of Medicine, Baltimore, Maryland, USA) and the α-subunits of the glycoprotein hormones (donated by Dr. I. Kourides, Department of Endocrinology, Sloan Kettering Institute, New York, N.Y., USA). The duration of exposure to the primary antibodies varied from 30 min to 3 h and the dilution of primary antibodies varied from 1:100 to 1:1,000. The immunologic reaction was visualized by using the horseradish peroxidase-antihorseradish peroxidase complex (Cappel Laboratories, Inc., Downingtown, Pennsylvania, USA) and 3,3′-diaminobenzidine. The specificity of immunostaining was verified by serial dilution of antibodies, by replacing the antibodies with normal rabbit serum as well as phosphate buffered saline and, where possible, antisera absorbed with excess antigen. For control purposes, several nontumorous pituitary glands obtained from surgical hypophysectomies and autopsies were also immunostained by using the same primary antibodies. Another control group included those surgically removed pituitary adenomas which were associated clinically with enhanced hormone secretion, i.e., growth hormone in patients with acromegaly, prolactin in patients with the amenorrhea-galactorrhea syndrome and ACTH in patients with Cushing's disease or Nelson's syndrome.

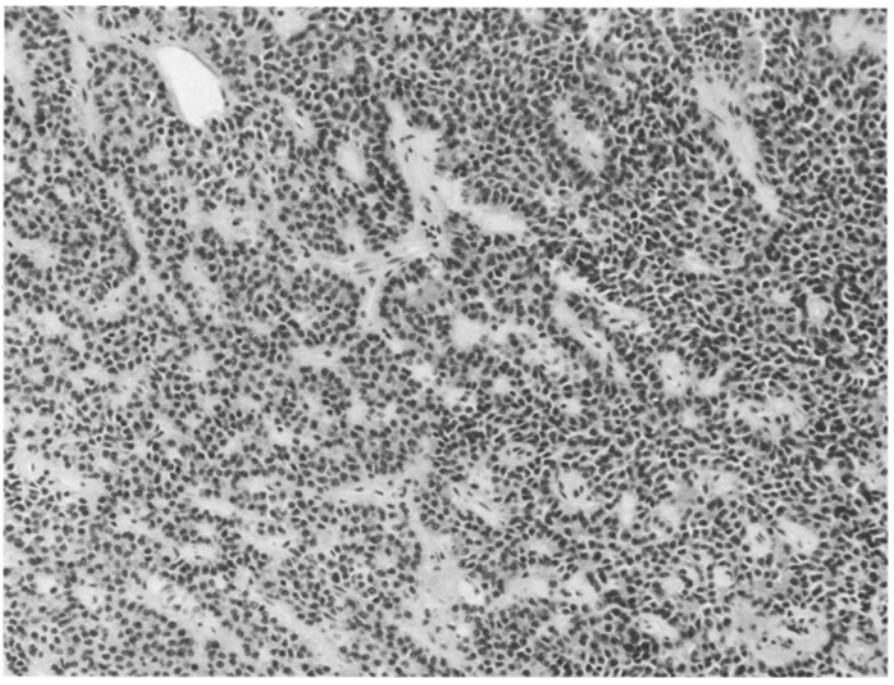

Fig. 1. Light microscopic appearance of a null cell adenoma containing no adenohypophysial hormones with the immunoperoxidase technique. Hematoxylin-phloxine-saffron stain, original magnification $\times 100$

For electron microscopy, pieces of tumor tissue were fixed in 2.5% glutaraldehyde in Sorensen's buffer, postfixed in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol, processed through propylene oxide and embedded in Epon 812 or in an Epon-Araldite mixture. Semithin sections were stained with toluidine blue and appropriate areas selected for the fine structural study. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a Philips 300 electron microscope.

Gross Findings. The tumors were removed in small pieces and often intermingled with blood. Gross inspection did not contribute to the diagnosis of the tumors.

Light Microscopic Findings. All the adenomas were well vascularized cellular tumors, consisting of spherical, elongated or slightly angular cells, often arranged radially around capillaries and forming rosette-like patterns (Fig. 1). No pleomorphism was evident and mitotic figures were rare. The adenoma cells exhibited no staining with acid or basic dyes except for a few cells which contained many large, slightly acidophilic granules in their cytoplasm. Subsequent electron microscopic studies disclosed an increase in the number and size of the mitochondria in some of the adenoma cells and it became apparent that the cytoplasmic acidophilia was due to staining of mitochondria and not of secretory granules. The cytoplasm of adenoma cells showed no PAS, lead hematoxylin or carmoisine positivity. Trichrome stains were noncontributory. Based on the tinctorial characteristics, all the tumors were diagnosed as chromophobic adenomas of the pituitary.

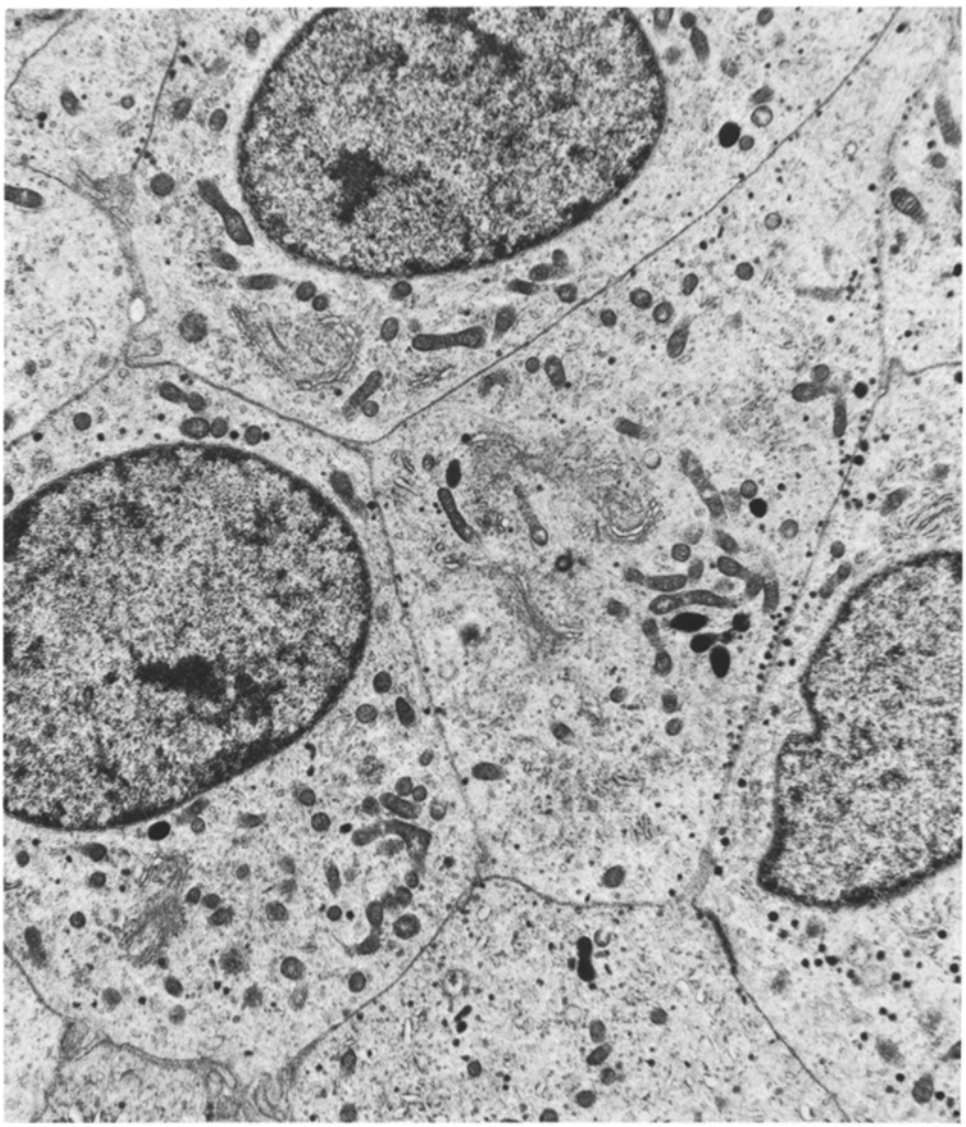

Fig. 2. Electron micrograph of a null cell adenoma showing poorly developed cytoplasmic organelles. Magnification $\times 7{,}300$

Immunocytologic Findings. The immunoperoxidase technique revealed the absence of adenohypophysial hormones in 46 tumors. However, a few scattered cells showed positive staining for α-subunit in 9 tumors. One of these tumors was also positive for β-TSH, 3 for β-FSH, 2 for β-LH and 2 for prolactin. In addition, 1 adenoma which was negative for α-subunit exhibited positive immunostaining for β-FSH and prolactin. The immunoreactive material was noted in the cytoplasm; its intensity varied and was limited only to a few

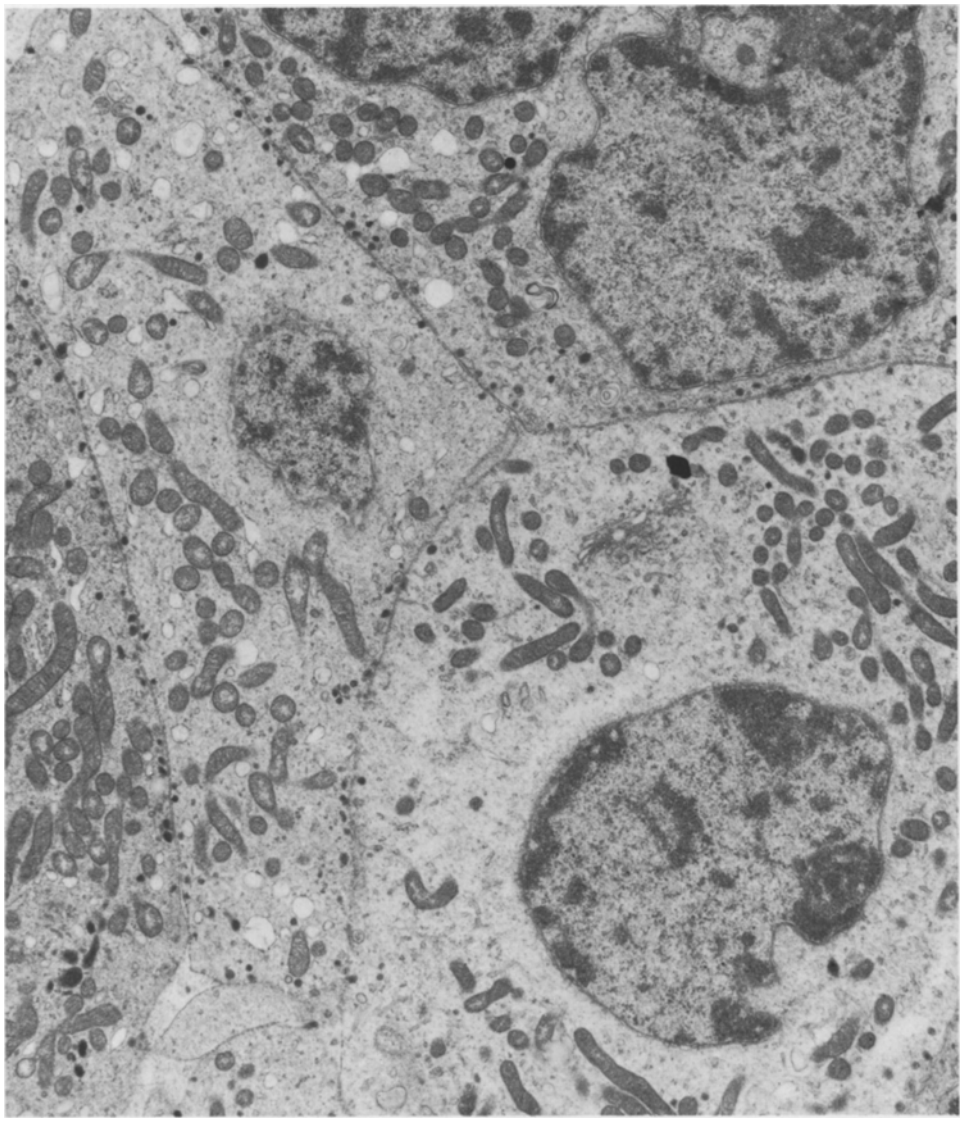

Fig. 3. Electron micrograph of a null cell adenoma showing some accumulation of mitochondria. Magnification ×9,200

cells. The substantial majority of adenoma cells showed no immunostaining in these cases.

Electron Microscopic Findings. The adenoma cells were polyhedral or elongated, closely apposed with irregularly outlined, deeply indented nuclei and inconspicuous nucleoli. The cytoplasm was electron lucent containing relatively few, poorly developed organelles (Fig. 2). The rough-surfaced endoplasmic reticulum

was sparse and consisted of a few scattered, short profiles studded with ribosomes on the external aspects of the membranes. Smooth-surfaced endoplasmic reticulum membranes were not apparent. The Golgi complexes, composed of flattened sacculi and a few smaller forming secretory granules, were inconspicuous in the majority of the cells. The mitochondria were small, rod shaped with transverse cristae and relatively electron dense matrix. In a varying number of cells, the number and size of mitochondria were increased (Fig. 3). In a few cells, almost the entire cytoplasmic area was occupied by mitochondria and the remaining organelles were obscured. Those adenomas, in which all the cells showed substantial oncocytic change, were classified as pituitary oncocytomas and omitted from this study. Here, only those tumors are included which showed slight or moderate oncocytic change not evident in every adenoma cell. It is pertinent to note, however, that there was a gradual transition, indicating that oncocytomas represent a variant of the tumor type dealt with in this work. The oncocytic change was accompanied by various degrees of mitochondrial abnormalities, such as gigantism, cavitation of the internal compartments, loss of cristae and their replacement with electron dense, granular material. Another characteristic feature was the abundance of cytoplasmic microtubules. They were especially numerous in the Golgi regions and in the long cytoplasmic processes. Secretory granules were spherical, usually sparse, and measured 100–250 nm in diameter. They had an easily noticeable limiting membrane which was separated from the electron dense core by an electron lucent halo. Secretory granules frequently lined up along the cell membranes, but no exocytosis was seen. Microfilaments were not apparent. Lysosomes were noted in the cytoplasm of a few cells, but they were not numerous. Junctions (zonulae adherentes) of various lengths were frequently observed between closely-apposed adenoma cells. Despite a detailed and careful electron microscopic study, the origin of adenoma cells remained unresolved.

Discussion

The tumors reported in this paper were unassociated clinically and biochemically with excessive hormone secretion. By histology, they were chromophobic adenomas with no PAS, lead hematoxylin or carmoisine positivity and in a substantial majority of cases the immunoperoxidase technique revealed no adenohypophysial hormones in the adenoma cells. By electron microscopy, they consisted of cells which invariably contained secretory granules and frequently showed oncocytic change but exhibited no specific ultrastructural features which would allow the disclosure of their origin and would link them with any hormone-producing adenohypophysial cell type known to occur in the human anterior lobe. The fine structural features of hormonally inactive pituitary adenomas were investigated by several authors (Schelin, 1962; Kuromatsu, 1968; Zambrano et al., 1968; Doniach, 1972; Schechter, 1973; Tomiyasu, 1973; Lewis and Van Noorden, 1974; Trouillas et al., 1974; Gray et al., 1975; Landolt, 1975; Oliver et al., 1975; Saeger, 1975; Horvath and Kovacs, 1976; Saeger

et al., 1976; Kovacs et al., 1977; Roy, 1977; Landolt, 1978a and 1978b; McCarty et al., 1978).

Previously, nonfunctioning pituitary adenomas were classified as chromophobic adenomas. The term chromophobic adenoma, which gained widespread acceptance and was used for several decades, however, is inappropriate because these tumors always contain secretory granules (McCormick and Halmi, 1971) and are in many cases accompanied by increased secretion of different hormones (Kovacs et al., 1977). Other names, such as undifferentiated cell or precursor cell adenoma are ambiguous. The former may suggest a more aggressive behavior of these tumors. However, this is not the case; these adenomas usually show no rapid growth rate, are not pleomorphic and only occasionally contain mitotic figures. The latter is not firmly based since the question – whether the cells constituting the tumors are progenitors of more mature cell types – remains unresolved.

Recently, it became apparent that lymphocytes can be divided into bone marrow derived (B) and thymus dependent (T) types. Some lymphocytes, however, cannot be classified as B or T cells using the currently available methods. Those cells which lack specific markers are called null lymphocytes, and their tumors, null cell leukemias and lymphomas (Berard et al., 1978; Hoffman-Fezer et al., 1978; Forbes et al., 1979; Mann et al., 1979; Reddy and Goh, 1979). Based on this analogy, we wish to introduce the term null cell adenoma of the pituitary to designate those adenohypophysial tumors which are described in the present paper. This name pinpoints the principal characteristics of these tumors, i.e. the absence of histologic, immunocytologic or ultrastructural markers which would permit the disclosure of their derivation.

What is the cell type from which null cell adenomas derive? Are null cells present in the nontumorous adenohypophysis and if so, what is their origin and function? What mechanisms regulate their formation, maturation and activity? Are they capable of transforming to other cell types? In an ultrastructural study of 32 surgically-removed nontumorous human adenohypophyses, not reported here in detail, several null cells were detected in every gland (Fig. 4), suggesting that they may serve as the source from which null cell adenomas arise. A few null cells were also noted in adenomas composed of growth hormone cells or corticotroph cells.

The genesis of null cells in the nontumorous adenohypophysis is not known. They may represent undifferentiated progenitors of mature adenohypophysial cells, either committed stem cells or, more primitive, uncommitted ones, in which specific morphologic markers, because of immaturity, had not yet developed. They may well be pluripotent cells which may differentiate and give rise to one or more hormone-producing adenohypophysial cells. Although the presence of stem cells in the human adenohypophysis has yet to be proven, animal experiments seem to support their existence (Watanabe et al., 1973; Watanabe and Daikoku, 1976; Gash et al., 1977; Ishikawa et al., 1977; Shiino et al., 1977; Bowie et al., 1978; Shiino et al., 1978a, 1978b). Rathke pouches, isolated from rat fetuses and maintained in tissue culture or grafted into the hypophysiotrophic area of the hypothalamus or under the kidney capsule were

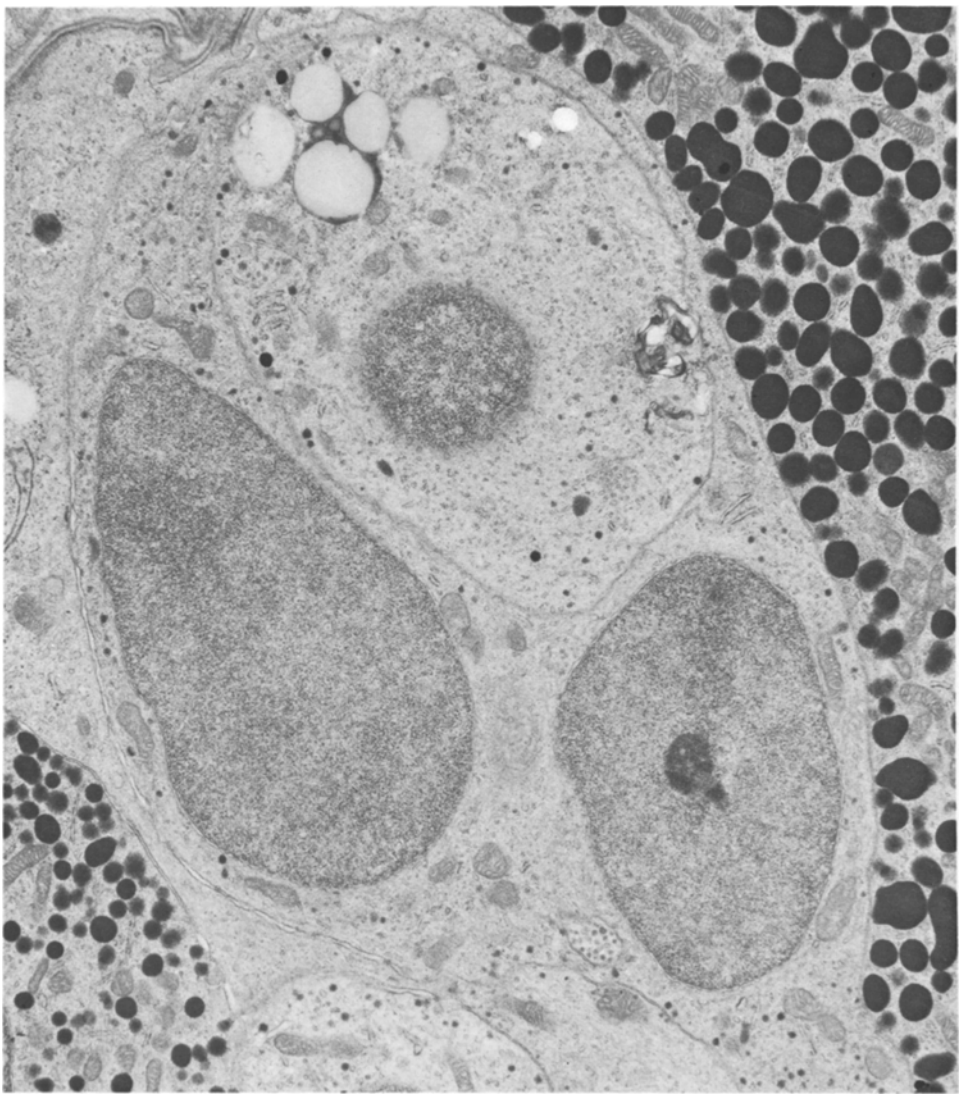

Fig. 4. Electron micrograph of null cells among densely granulated adenohypophysial cells in the nontumorous anterior pituitary. Magnification ×8,600

shown to undergo cytodifferentiation. Light and electron microscopic investigation disclosed the formation of various adenohypophysial cells from the primitive primordia. Secretory granules began to appear and production of hormones was demonstrated by radioimmunoassay and immunocytology. Alternatively, null cells may derive from mature adenohypophysial cells in which, as a result of dedifferentiation, hormone synthesis is halted and specific markers allowing for their identification are lost. There is some experimental evidence which supports this interpretation. It has been shown (Corenblum et al., 1977) that growth hormone cells of rats made hypothyroid with propylthiouracil feeding

undergo dedifferentiation; they lose their secretory granules and hormone content and become unrecognizable morphologically. This process is reversible, since after withdrawal of propylthiouracil from the diet, growth hormone cells regranulate and become identifiable again by immunostaining (Corenblum et al., 1977).

Ten cases of clinically and biochemically nonfunctioning adenomas exhibited identical histology and ultrastructure to null cell adenomas except that a few scattered cells showed positive immunostaining for β-TSH or β-FSH, β-LH, prolactin and/or α-subunit. In our view, these tumors should be included in the null cell adenoma group. Tumors are usually composed of several subclones in various phases of differentiation and tumor cell heterogeneity is consistent with the interpretation that null cells are capable of differentiating into hormone-producing specific cell lines or that some null cells in the adenomas are not dedifferentiated and their markers are not completely lost.

In our material of 343 surgically-removed pituitary adenomas, 56 tumors (16.5%) were classified as null cell adenomas, indicating that their occurrence is not rare. We believe, however, it is not too optimistic to anticipate that, with increasing knowledge, appropriate markers will be found and, with the use of more sophisticated methodology currently unavailable, the number of diagnosed null cell adenoma cases will diminish.

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