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Pituitary Chromophobe Adenomas Consisting of Prolactin Cells

A Histologic, Immunocytological and Electron Microscopic Study

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Summary. Morphologic studies of pituitary neoplasms removed by surgery from 36 human patients revealed 8 chromophobe adenomas which differed clearly from the remaining tumors. The cytoplasm of the adenoma cells failed to stain with PAS, aniline blue, aldehyde fuchsin, aldehyde thionin, orange G or light green, but positively stained granules were found by using erythrosine or carmoisine. Immunoperoxidase technique disclosed the presence of prolactin in the cytoplasm of some adenoma cells. The adenoma cells exhibited distinct ultrastructural features such as well developed rough surfaced endoplasmic reticulum with Nebenkern formation, prominence of Golgi apparatus, presence of misplaced exocytosis as well as pleomorphism of secretory granules with a considerable variation of size ranging from 130 to 500 nm in diameter. Thus, by electron microscopy the adenoma cells showed a close resemblance to prolactin cells of the non-tumorous pituitary glands except for the reduced size and number of secretory granules.

These chromophobe adenomas are regarded as representing a distinct pathological entity clearly distinguishable from other forms of pituitary neoplasms. In view of the morphologic findings and the elevation of blood prolactin level (measured in 3 patients) the term, "sparsely granulated prolactin producing pituitary adenoma", appears to be the most appropriate one to designate these tumors.

Key words: Pituitary Gland — Prolactin — Pituitary Adenoma — Electron Microscopy — Histology.

Prolactin producing tumors of the human pituitary gland were regarded morphologically as belonging to the acidophil adenoma group (Lewis and Van Noorden, 1974) whereas chromophobe adenomas were assumed to represent neoplasms with no hormonal activity (Russell, 1966). Recent findings, however, challenged these firmly held views. It was found that blood prolactin concentrations were abnormally high in a considerable number of patients with chromophobe adenomas (Tolis *et al.*, 1974) and furthermore, that chromophobe adenoma cells almost invariably contained some type of secretory granules in their cytoplasm (Schelin, 1962; McCormick and Halmi, 1971).

Increased blood prolactin levels are due to enhanced hormone secretion either from hypophysial adenomas or from non-tumorous pituitary glands (Turkington *et al.*, 1971; Nasr *et al.*, 1972; Boyar *et al.*, 1974; Tolis *et al.*, 1974). In order to distinguish between these two alternatives it is desirable to firmly establish the structural criteria allowing the identification of those pituitary neoplasms which are engaged in the production of prolactin. Forbes *et al.*, in 1954, were the first

to call attention to prolactin producing pituitary tumors associated with amenorrhea and galactorrhea. Although a few studies dealing with the histology (Herlant *et al.*, 1965; Lamotte *et al.*, 1966; Linquette *et al.*, 1967; Peake *et al.*, 1969; Friesen *et al.*, 1972; Linquette *et al.*, 1972; Guinet *et al.*, 1973; Lewis and Van Noorden, 1974; Zimmerman *et al.*, 1974), immunocytology (Zimmerman *et al.*, 1974), and electron microscopy (Mirouze *et al.*, 1969; Peake *et al.*, 1969; Racadot *et al.*, 1971; Guinet *et al.*, 1973; Lewis and Van Noorden, 1974) of these neoplasms were also reported, the available information is still limited and does not permit the reliable morphologic diagnosis of this entity.

In our material 3 chromophobe adenomas were found in association with hyperprolactinemia. Among 36 pituitary tumors 5 additional chromophobe adenomas were detected which exhibited identical structural features. Although in these 5 cases blood prolactin concentrations were not determined, we came to the conclusion that the 8 tumors represented a distinct pathological entity clearly distinguishable from those of other pituitary neoplasms. We describe here the structural features of these tumors including histology, tinctorial characteristics, immunocytology as well as electron microscopy with the aim to facilitate their separation and morphologic identification.

Material and Methods

The material consisted of 8 surgically removed pituitary tumors. Tissue for morphologic investigation was immediately fixed after removal. The 8 patients were investigated preoperatively and their clinical histories were reviewed.

For light microscopy tumor tissue was fixed in 10 per cent neutral formalin and embedded in paraffin. Sections of 4–6 μ thickness were cut and stained with hematoxylin-phloxine-saffron, PAS, methyl green-pyronin, Goldberg-Chaikoff's trichrome, Masson's, Mann's stain, Herlant's erythrosine, Brookes' carmoisine, Gomori's aldehyde fuchsin and aldehyde thionin techniques.

For immunologic localization of prolactin the immunoglobulin-enzyme bridge method was used as described by Mason *et al.* (1969) with the following two modifications: (1) The duration of exposure to the specific antibody (anti-human prolactin donated by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada) and subsequent reagents was reduced to 5 minutes. (2) Instead of applying horseradish peroxidase and anti-peroxidase individually, the horseradish peroxidase-anti-horseradish peroxidase complex was used, as reported by Sternberger *et al.* (1970). (The peroxidase-anti-peroxidase complex was provided by Dr. L. A. Sternberger, Edgewood Arsenal, Maryland, U.S.A.). In control experiments the prolactin antibody was replaced by normal rabbit serum. For comparison the non-tumorous pituitary glands of two female subjects (one pregnant, one lactating) were removed at autopsy, fixed in neutral formalin, embedded in paraffin and immunostained for prolactin simultaneously with the tumors.

For electron microscopy pieces of tumor tissue were fixed in 2.5 per cent glutaraldehyde in 0.15 M Sorensen's buffer, post fixed in 1 per cent osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol and embedded in Epon 812. Thick sections were cut with a Porter-Blum MT-2 ultramicrotome and stained with toluidine blue. From selected areas ultrathin sections were cut and were stained with uranyl acetate and lead citrate and investigated with a Philips 300 electron microscope.

Results

Clinical Findings

The age of the patients ranged from 17–56 years. Five tumors occurred in male and 3 in female subjects. Headache and visual disturbances were the leading

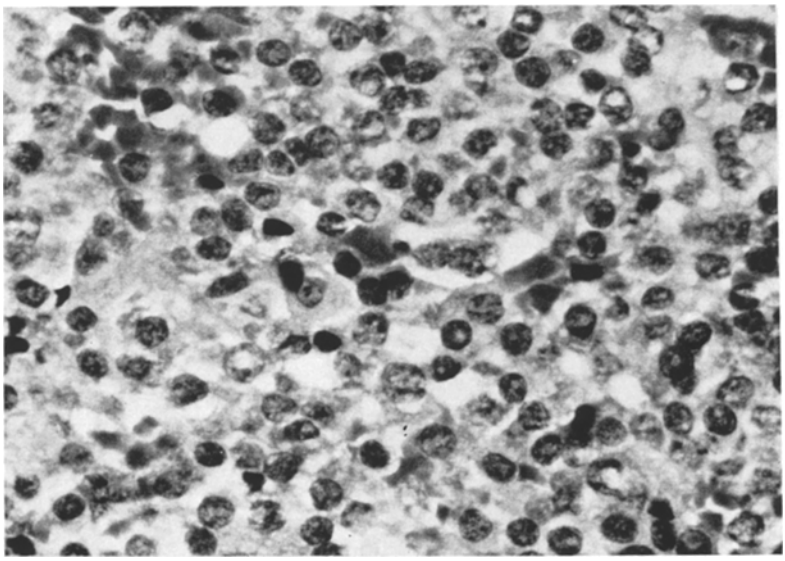

Fig. 1. Light microscopic features of the tumor are consistent with those of chromophobe adenoma. Hematoxylin-phloxine-saffron stain. Approximately × 400

clinical symptoms with or without mild or moderate hypopituitarism. X-ray examination showed enlargement of the sella turcica. In 1 female patient galactorrhea was observed. No other changes indicating enhanced pituitary hormone production were found. Blood prolactin concentrations were measured only in 3 cases and, compared with the control levels, a significant elevation was noted.

Morphologic Findings

Gross Findings. Tumor tissue was removed in pieces of different size and was mixed with various amounts of blood. Gross examination failed to yield any valuable information as regards the type of tumor.

Light Microscopic Findings. Sections stained with the hematoxylin-phloxine-saffron technique were consistent with the diagnosis of chromophobe pituitary adenoma in all 8 cases. The tumors were cellular, consisting of round or irregular cells, commonly intermingled with blood and occasionally arranged in cords or acinuslike structures (Fig. 1). The nuclei of the adenoma cells were relatively large and moderately rich in chromatin. The cytoplasm was unremarkable with no or only very few granules. Routine sections failed to establish the cell type from which the tumor derived, the rate of secretory activity or the nature of hormones produced by the adenoma cells.

Tinctorial Characteristics. The most conspicuous feature of the tumor cells was the lack of staining with the conventional dyes. The cytoplasm of the tumor cells was PAS negative, and failed to stain with aldehyde fuchsin, aldehyde thionin, or with various acid dyes, such as Orange G or light green. Trichrome stains were also not contributory. Thus, based on tinctorial properties the tumors

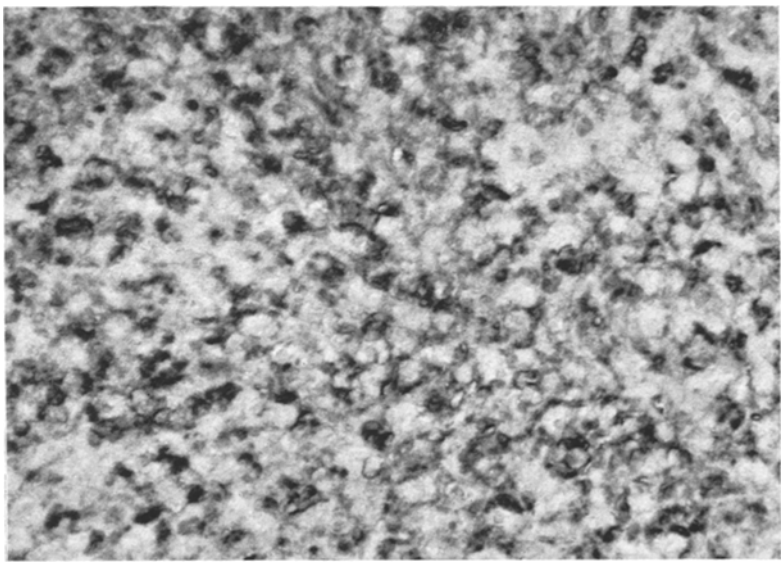

Fig. 2. Immunoreactive prolactin is evident in the form of dark reaction product in the cytoplasm of many adenoma cells. Immuno-peroxidase technique. Approximately $\times 400$

corresponded to chromophobe adenomas in all 8 cases. However, when Herlant's erythrosine or Brookes' carmoisine methods were applied, a few small, bright red granules were detected in the cytoplasm of some adenoma cells by high power oil immersion. Red cytoplasmic coloration was obtained by the methyl green-pyronin procedure. As the tumor cells contained a large number of membrane bound and free ribosomes the pyroninophilia may have been due to the presence of ribonucleic acid. Ribonuclease digestions were, however, not carried out, thus this possibility was neither proved nor disproved.

Immunocytologic Findings. By using the immunoperoxidase technique definite positive reaction was obtained in 4 out of 5 examined tumors. The brown reaction product was clearly recognizable in the cytoplasm of the tumor cells (Fig. 2), whereas appropriate control sections failed to show any positivity. The brown deposits were, however, not evenly distributed. The cytoplasm of some tumor cells exhibited strong positivity, while many adenoma cells showed only weak staining and others did not seem to stain at all. Our findings with anti-human prolactin are in agreement with those of Zimmerman *et al.* (1974), who used anti-ovine prolactin for immunoreactive prolactin localization in human pituitary adenomas.

Findings on Epon-Embedded Toluidine Blue Stained "Thick" Sections. All adenomas were composed of closely apposed polyhedral cells with relatively large oval or pleomorphic nuclei. The chromatin was either light and evenly distributed or appeared in large clumps. The nucleoli were round and prominent. At the light microscopic level there were only a few dark blue-stained secretory granules discernible in the translucent cytoplasm.

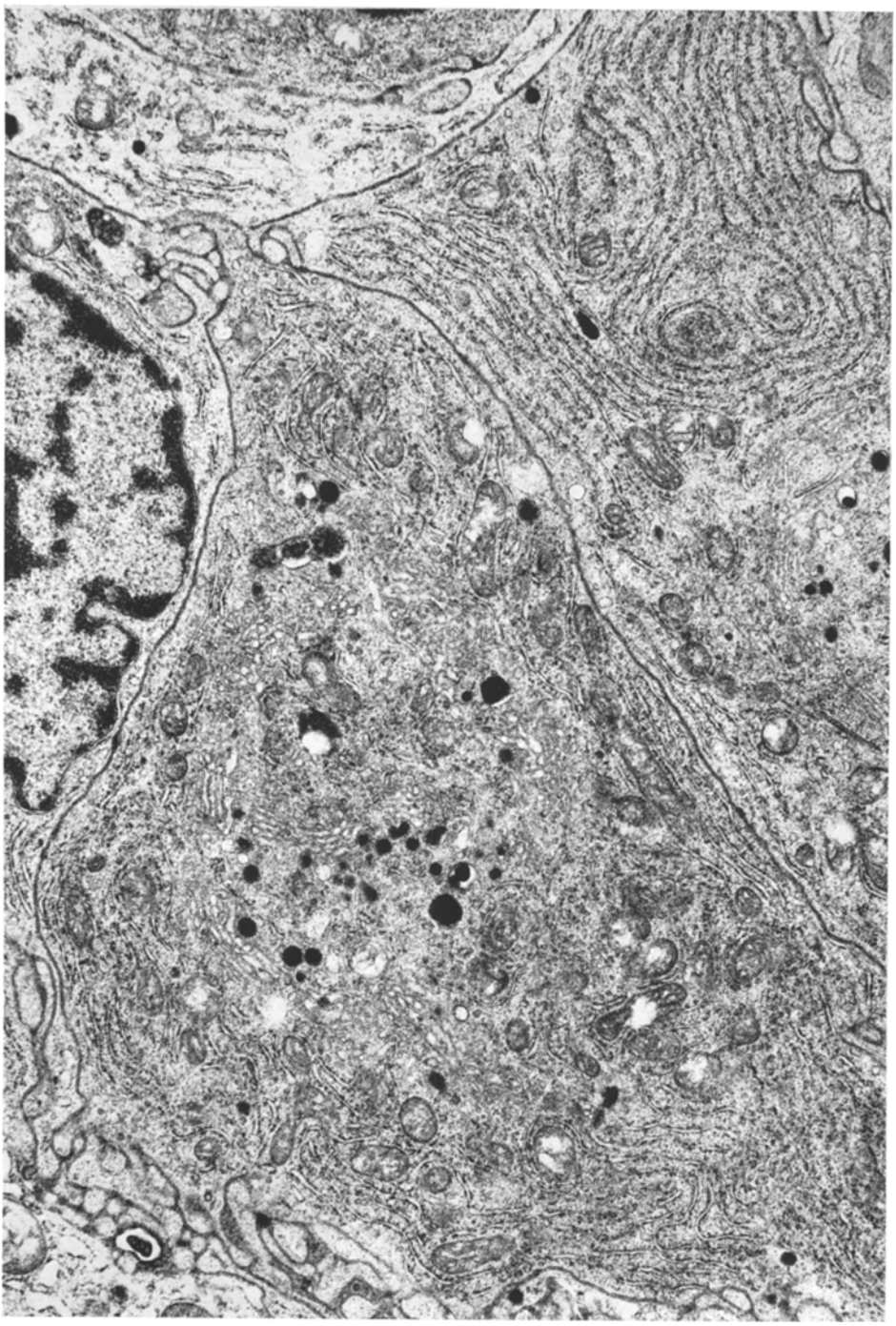

Fig. 3. Electron micrograph of adenoma cells showing prominent Golgi apparatus and extensively developed RER with Nebenkern formation. × 12750

Electron Microscopic Findings. The 8 adenomas exhibited similar ultrastructural characteristics with very little or no variation from one case to another (Figs. 3 and 4). The oblong or polyhedral cells had large, centrally located, oval, kidney-shaped or irregular nuclei containing various amounts of chromatin and a relatively sparse cytoplasm. At one side of the nucleus most of the cytoplasmic space was occupied by several parallel rows of the extensively developed rough surfaced endoplasmic reticulum (RER). Formation of Nebenkerns (concentric whorls of RER) was common. Numerous free ribosomes and polysomes were also seen. At the opposite side of the nucleus a prominent crescent shaped Golgi apparatus was situated with moderately dilated sacculi and several vesicles. The sacculi regularly contained varying numbers of immature secretory granules. The rest of the cytoplasm around the Golgi region was mainly occupied by profiles of RER and mitochondria. The latter were spherical or oblong, had a moderately dense matrix and lamellar cristae. Swollen forms were infrequently encountered. All adenoma cells contained spherical or pleomorph secretory granules. There were, however, considerable cell-to-cell variations in size and number. The majority of cells possessed secretory granules measuring 130–300 nm, but some contained large granules with a diameter of 500 nm and over, as well. The most distinguishing feature of the 8 tumors was the presence of misplaced exocytosis (Horvath and Kovacs, 1974), i.e. the extrusion of secretory granules by reverse pinocytosis along the lateral cell membranes into the intercellular space, remote either from capillaries or from the intercellular extensions of basement membrane. Numerous such granule extrusions had readily been identified in all 8 cases. Exocytosis of more granules at the same site also occurred.

Less conspicuous but regular components of adenoma cells were the centrioles and cytoplasmic microtubules, usually situated in or around Golgi areas, as well as a few lysosomal dense bodies.

In one case additional cytoplasmic features were mitochondrial gigantism with close association of RER and production of an amyloid-like substance. This case is reported in detail elsewhere (Bilbao *et al.*, accepted for publication).

The adenoma cells were closely apposed and the cell membranes smooth except for the pits of extruding granules. At the junctions of 3 or more cells, however, the cell membranes formed several interdigitating microvilli. Intercellular junctions of the adherent type were common findings in all adenomas. The vessels of the adenomas did not exhibit striking changes. In some cases thickening of the endothelium was seen with decrease in the number of fenestrations.

Discussion

Present results clearly show that the 8 adenomas described above belong to a distinct entity which should be recognized by practicing pathologists and distinguished from other tumors of the pituitary gland. At present the diagnosis can only be established conclusively on the basis of electron microscopic studies. The well developed rough surfaced endoplasmic reticulum with Nebenkern formation, the prominence of the Golgi apparatus, the presence of misplaced exocytosis, the pleomorphism of the secretory granules with considerable variation in size, ranging from 130–500 nm in diameter, are fine structural features which are

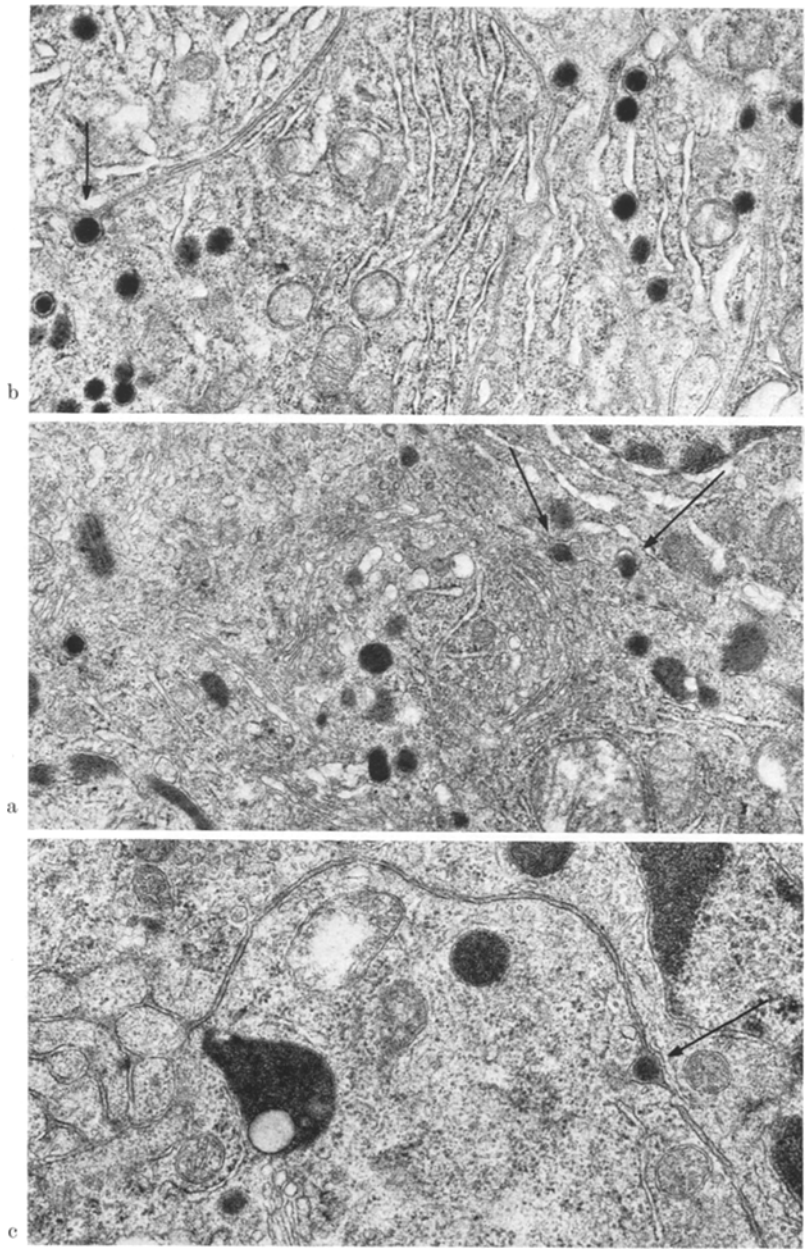

Fig. 4a—c. Misplaced exocytosis (arrows) in different prolactin-cell adenomas. (a) × 21600 (b) × 17300. (c) × 25900

sufficiently characteristic and distinctive to allow identification of the adenoma cells as prolactin cells.

The various staining procedures at the light microscopic level failed to provide convincing evidence which would permit classification of the cell type constituting the tumor. The fact that no positive staining was obtained with PAS, aniline

blue, aldehyde fuchsin, aldehyde thionin or with other basic dyes eliminates the possibility that the tumors are composed of melanocorticotroph, gonadotroph (FSH, LH) or thyrotroph cells. Non-tumorous prolactin cells are assumed to belong to the acidophil series, and some pituitary adenomas associated with galactorrhea, amenorrhea and hyperprolactinemia exhibit various degrees of cytoplasmic acidophilia also (Peake *et al.*, 1969; Lewis and Van Noorden, 1974). In our cases the cytoplasm of the tumor cells contained only few and small granules which were not capable of taking up the dyes in sufficient amount to allow their identification as acidophil cells at the light microscopic level. The marked reduction in size and number of secretory granules may also account for the finding that only few and small granules were detected with erythrosine or carmoisine in the cytoplasm of some adenoma cells claimed to selectively stain prolactin cells (Herlant and Pasteels, 1967; Peake *et al.*, 1969; Pasteels *et al.*, 1972; Guyda *et al.*, 1973; Zimmerman *et al.*, 1974).

Immunoperoxidase techniques seem at present to be most promising for the localization of hormones in specific adenohypophysial cells. However, more basic work and more readily available specific antisera are required before these techniques can be routinely applied. In some tumors from our material prolactin was convincingly localized in the cytoplasm of the adenoma cells, while in others the positivity was patchy and weak. No definite explanation can be given which could account for the inconsistency of staining. It might be that the antigenic determinants of prolactin were altered or destroyed during the fixation or embedding, or that the reacting groups of the antigen were not free or became bound in the adenoma cells, or that the antigenicity of prolactin in the tumors was not the same as in the blood, or in non-tumorous pituitary tissues, or finally, that prolactin was not stored in these tumors in sufficient amounts to allow their identification by the immunoperoxidase technique.

Some prolactin producing adenomas were found, by electron microscopy to consist of cells with large secretory granules similar to those of the non-tumorous prolactin cells (Peake *et al.*, 1969; Lawzewitsch *et al.*, 1972; Lewis and Van Noorden, 1974). In our material, however, the tumor cells contained fewer and smaller granules compared with the prolactin cells of the non-adenomatous pituitary and closely resembled those prolactin cells which are abundant in the estrogen stimulated animal pituitaries or in estrogen-induced pituitary neoplasms (Hymer *et al.*, 1961; Lundin and Schelin, 1962; Schelin *et al.*, 1964; Schelin and Lundin, 1971). Reduction in number and size of secretory granules may be due to enhanced prolactin release, i.e. granules are extruded prematurely from the adenoma cells at an accelerated rate, thus they decrease in number and fail to reach normal size. It is also possible, however, that the principal mechanism leading to a decrease in number and size of secretory granules is an inherent defect in granule storage, i.e. prolactin is secreted from the adenoma cells without being previously stocked in a form of ultrastructurally detectable granules. The details of granule production are not yet fully elucidated. Hence, no firm explanation can be offered as to which steps of this process are bypassed and/or impaired when there is a failure of granule accumulation antecedent to prolactin release.

More cases of prolactin producing adenomas will have to be investigated with special emphasis on correlations between clinical history, hormone determinations

and tumor morphology, including electron microscopy before a definite answer can be given as to whether tumors composed of sparsely or of densely granulated prolactin cells are more active endocrinologically. In our material some tumors with ultrastructural features of high secretory activity and with marked reduction in size and number of secretory granules were unaccompanied by prominent clinical symptoms. It must be stressed, however, that more than half of the tumors investigated by us were removed from male patients and it is known that the consequences of enhanced prolactin secretion are not as obvious in men as in women. In cases of growth hormone producing tumors associated with acromegaly it has been claimed that adenomas composed of sparsely granulated cells are endocrinologically more active than those consisting of densely granulated cells (Schelin, 1962; Lewis and Van Noorden, 1972; Saeger, 1973). Other reports (Kinnman, 1973), however, have failed to confirm this view. In our unpublished study of pituitary adenomas removed from 13 patients with acromegaly no close correlations were found between clinical features and number and size of secretory granules.

It also remains to be determined whether the densely and sparsely granulated forms of prolactin producing adenomas represent two variants of the same tumor, or two distinct entities. At present there is no convincing evidence which would indicate that the densely granulated form could transform into the sparsely granulated form or vice versa. It may well be that number and size of secretory granules are not fixed in hormone-dependent tumors; they may reflect only rates of secretory activity and may change as a result of functional demand. The possibility, however, cannot be excluded that some adenomas have a genetically predetermined pace of hormone synthesis and storage, which may remain unaltered by stimulating or suppressing hormone production or release.

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