Cytology of Pituitary Adenomas

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Recent gains in knowledge of cytology of the anterior pituitary and of factors that control secretion of its hormones warrant reconsideration of the nature of pituitary adenomas. The classical associations of acidophil adenoma with acromegaly, basophil adenoma with Cushing’s syndrome, and chromophobe adenoma with hypopituitarism still obtain. But exceptions, recorded in fair number, are the finding of cases of Cushing’s syndrome and of acromegaly associated with chromophobe rather than chromophil adenomas.

The cytoplasm of chromophobe cells of the human anterior pituitary is devoid of either intense red acidophil granules or blue basophil granules in sections stained by the trichrome methods of Heidenhain and Mallory. Pearse (1952) using the periodic acid-Schiff (PAS) technique that specifically stains glycoprotein, showed that many ‘chromophobes’ are in fact sparsely granulated cells, of the basophil series. These, together with totally nongranulated cells, all chromophobes in the original terminology, can be regarded as representing any one of three different states of cell activity: undifferentiated non-secreting stem cell, resting secretory cell, or very actively secreting cell with little or no storage of granules visible on light microscopy. The principle is now accepted that each pituitary hormone is secreted by a distinct non-interchangeable cell type (Benoit and Da Lage, 1963). Growth hormone (GH) and prolactin are secreted by acidophils; the existence of two distinct types of acidophil has recently been demonstrated in man (Herlant and Pasteels, 1971). Thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and the gonadotrophins are secreted by basophils. Separation of follicle stimulating (FSH) from luteinizing hormone (LH) cell types is not fully established in man. It is possible that more than one type of ACTH secreting cell exists in the human pituitary (Ezrin and Murray, 1963). Owing to species differences, variations of staining methods and designation of different cell types by the same Greek letters, the nomenclature of anterior pituitary cells is bewildering (Benoit and Da Lage, 1963). Specific identification of TSH, ACTH, FSH and LH basophils in the human pituitary is suggested by Pearse and van Noorden (1962) with a performic acid-Alcian blue PAS staining technique and by Ezrin and Murray (1963) with an aldehyde
thionin PAS technique. In addition to staining reaction the size, shape and situation of the cells help recognition of the different cell types. Immunohistological methods, using fluorescent antibody techniques, have identified the presence of growth hormone and ACTH in specific cells in normal human pituitaries and in selected functioning pituitary adenomas (Leznoff et al., 1962; Pearse and van Noorden, 1962; Phifer et al., 1970; Herlant and Pasteels, 1971).

Further advance in the identification of cell types has been made by electron microscopy, mostly in the study of the pituitary of experimental animals. The cells contain small membrane-bound dense secretory granules, rough-surfaced endoplasmic reticulum enclosing lamellae or cisternae, a Golgi complex, mitochondria, free ribosomes, and scattered large lipid pigment granules varying from 0.5 μ to a few microns in diameter (Figs 1 and 2). Intensity of secretory activity correlates directly with the quantity of rough-surfaces endoplasmic reticulum and the degree of expansion of the Golgi complex. Specific cell types are recognised chiefly by the average size and electron density of their secretory granules. The growth hormone granules of acidophil cells average 300 to 400 μ in most species; prolactin granules are larger, averaging 500 to 1,000 μ. The smallest secretory granules are found in TSH cells and average 100 to 150 μ. The granules of gonadotrophin cells are intermediate in size between those of TSH and GH cells. There is no firm agreement over identification of ACTH cells. There are only a few published reports of the ultrastructure of the normal human anterior pituitary (Luse, 1961; Foncin, 1966; Bergland and Torack, 1969). We have studied a human pituitary surgically removed in the treatment of breast cancer. Electron micrographs confirm that cell types can be fairly readily differentiated by measurement of the average diameter of secretory granules. It must be noted, however, that there is a very wide range of diameters of granules within any one cell and that the distribution is not necessarily Poisson in type. Variation in the size of granules associated with the same hormonal secretion might result from differences in the age of granules or the secretory state of the cell. We identified ACTH cells by the presence in some of them of cytoplasmic filaments typical of Crooke’s hyaline change (Porcile and Racadot, 1966) since the patient had been on large doses of prednisone. The granules were less electron dense than in other cell types and were intermediate in size between those of gonadotrophin and growth hormone cells (Fig. 2). A rough estimate of the average granule sizes in our preparations is: GH 270 μ (250 to 300 μ), Figs 1 and 2; ACTH 240 μ (200 to 300 μ), Fig. 2; gonadotrophins 200 μ (160–230 μ), Fig. 2; TSH 130 μ (100 to 150 μ), Fig. 1. We were unable to distinguish between FSH and LH cells.
The histology of anterior pituitary adenomas has been well documented by several authors (Dott and Bailey, 1925; Roussy and Oberling, 1933; Kernohan and Sayre, 1956; Russell, 1966), whose articles are recommended for detailed descriptions. Surgical material from 100 patients with non-

Fig. 1. Electron micrograph ×5,400 of normal anterior pituitary, woman aged 57 with carcinoma of breast. The nuclei of 7 cells can be seen, each cell outlined by a distinct fine plasma membrane and containing scattered electron dense secretory granules, sparse in the central cells where they are mostly distributed peripherally.

TSH = thyrotrophic hormone secreting cell with secretory granules arrowed averaging 130 mµ in diameter. GdTr = gonadotrophin secreting cell with secretory granules arrowed averaging 200 mµ in diameter. GH = growth hormone secreting cell with secretory granules arrowed averaging 270 mµ in diameter. Mi = mitochondria; Go = Golgi complex; Er = rough surfaced endoplasmic reticulum; Li = lipid granules.
secretory chromophobe adenoma seen at The London Hospital between 1950 and 1970 was reviewed histologically and special points noted are as follows. Many neoplastic cells in the majority of chromophobe adenomas contain scattered fine granules that stain weakly acidophil or basophil or indeterminately. Dott and Bailey (1925) noted these granules and considered them to be mitochondria, an interpretation now confirmed by electron microscopy (Meneghelli et al., 1964). A rare finding is an intense diffuse

Fig. 2. Electron micrograph × 5,400 of same pituitary as in Fig. 1. 
ACTH = corticotrophin secreting cell with secretory granules arrowed averaging 240 mμ in diameter. Note that they are less electron dense than the GH secretory granules arrowed. Er = rough-surfaced endoplasmic reticulum arranged in distended lamellae. The Golgi complex (Go) is expanded.
acidophilic staining of the cytoplasm of chromophobe adenoma cells. The acidophilic material is histochemically tyrosine-negative, in contrast to the strongly positive reaction given by GH granules (Pearse and van Noorden, 1962). It is likely that the diffuse tyrosine-negative acidophilia is a result of damage to cytoplasmic proteins during operative removal of the tumour since it is not seen in post-mortem specimens of pituitary. The variations in histological arrangement of cells in pituitary adenomas are usually categorised as diffuse, or sinusoidal (layers of cells apposed to sinusoidal blood-vessels) or papillary. The papillae mostly appear artefactual, produced by separation of sinusoidal elements either by trauma or by anoxic degeneration of the cells furthest away from blood-vessels (Schnitker et al., 1963).

There are few reports to date on the ultrastructure of human pituitary adenomas. Schelin (1962) described the electron microscopic appearances of surgical material from 13 chromophobe adenomas not associated with any hormonal hypersecretion. The neoplastic cells were well differentiated. Some contained secretory granules about 100 mμ in diameter, others 300 mμ; the former predominated. The presence of only moderate amounts of rough-surfaced endoplasmic reticulum and a compact Golgi complex indicated that the cells were not actively secreting. Similar findings were described by Meneghelli et al. (1964) in five chromophobe adenomas. An additional feature noted in both reports was the presence of numerous mitochondria in many of the neoplastic cells.

Electron microscope studies of surgical material removed from four recent cases of chromophobe pituitary adenomas at The London Hospital confirm the differentiated nature of the neoplastic cells, the presence of secretory granules of about 100 mμ or 200 to 300 mμ in diameter (Figs 3 and 4) and the frequent marked proliferation of mitochondria (Fig. 4). Though profiles of rough-surfaced endoplasmic reticulum (Fig. 3) are on the whole reduced in quantity, and the Golgi complex (Fig. 4) is small, occasional cells contain prominent rough-surfaced endoplasmic reticulum and others an expanded Golgi complex. A few cells are poorly differentiated in that they are almost devoid of cytoplasmic organelles.

Schelin (1962) also studied the ultrastructure of ten surgically removed acidophil adenomas associated with acromegaly. The neoplastic cells contained typical GH secretory granules averaging 300 mμ in diameter. The degree of granularity varied considerably within the cells of each tumour and between tumours, correlating with intense and sparse granularity of the cells seen with light microscopy. Marked storage of secretory granules was associated with reduction in rough-surfaced endoplasmic reticulum and a small Golgi complex. In poorly granulated cells, rough-surfaced endoplasmic
reticulum was prominent, the Golgi complex was expanded and secretory granules were mostly confined to the cell periphery aligned to the plasm membrane. Predominence of these actively secreting cells in a tumour correlated both with the presence of ‘chromophobes’ in light microscopy and clinical evidence of active acromegaly. Predominence of storage acidophils correlated with clinical inactivity.

There have been two reports of the ultrastructure of a pituitary tumour

Fig. 3. Electron micrograph ×9,000 of chromophobe adenoma removed from a woman aged 26. Secretory granule 230 mμ in diameter arrowed at top of figure, another granule 130 mμ arrowed on the right.

Er = rough-surfaced endoplasmic reticulum arranged in lamellae. Note the marked nuclear indentations.
associated with Cushing's syndrome (Foncin and LeBeau, 1963; Bergland and Torack, 1969). Both were examples of ACTH secreting 'chromophobe' adenomas that presented clinically after bilateral adrenalectomy for Cushing's syndrome. The neoplastic cells showed the cytology of active secretion and contained sparse secretory granules ranging from 100 to 400 m\(\mu\) in diameter.

In summary, electron microscope studies show that non-secreting chromophobe adenomas are made up of differentiated secretory cells in an inactive state whereas chromophobe adenomas associated with acromegaly or

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**Fig. 4.** Electron micrograph \(\times 9,000\) of chromophobe adenoma removed from a woman aged 60. Secretory granules ranging from 100 m\(\mu\) to 150 m\(\mu\) in diameter at upper arrow. Note the numerous mitochondria (Mi) and the Golgi complex (Go).
Cushing's syndrome consists respectively of very actively secreting GH acidophils or ACTH basophils. The degree of secretory activity of anterior pituitary cells is controlled by specific peptide releasing factors. These are neurohumoral secretions synthesised by hypothalamic neurones and reach the anterior pituitary via the portal blood-vessels in the pituitary stalk. The development of hypopituitarism in patients with chromophobe adenomas may be due therefore to mechanical interference with portal blood flow from compression or distortion of the stalk, to compression of hypothalamic structures, to compression of residual anterior pituitary cells, and to distortion of blood flow within the pituitary gland. Similar complications may arise in cases of acromegaly due to a large acidophil adenoma. Basophil adenomas associated with Cushing's syndrome tend to be small, but the ACTH secreting tumours that present after adrenalectomy tend to be larger.

Prolactin secretion is normally suppressed by hypothalamic prolactin inhibiting factor (PIF). The syndrome of amenorrhoea and galactorrhoea associated with chromophobe adenoma might be considered to be a consequence of mechanical interference with the production or passage of PIF to the anterior pituitary. However, a number of authors have demonstrated secretory granules greater than 500 mμ in diameter of prolactin type in electron microscopy of chromophobe adenomas associated with this syndrome (Mironze et al., 1966; Peake et al., 1969; Racadot et al., 1971).

Gonadotrophin secreting pituitary adenomas can be readily induced in rats by removal of the gonads (Griesbach and Purves, 1960) and TSH secreting tumours by ablation of the thyroid in mice (Furth and Clinton, 1958) or subtotal thyroidectomy in rats (Doniach and Williams, 1962). Removal of these target organs leads to maintained excessive output of hypothalamic releasing factors, consequent hyperplasia of gonadotroph or TSH secreting cells respectively and the eventual development of gonadotrophic or thyrotrophic adenomas. The latter situation has been recorded in untreated primary myxoedema in man (Bimes et al., 1966; Mösl and Hedinger 1968). Histological study of surgical material from 100 cases of chromophobe adenoma and 11 cases of acidophil adenoma removed at The London Hospital does not reveal any evidence of hyperplasia in fragments of surviving non-neoplastic anterior pituitary gland. It would appear that in man most secretory adenomas arise autonomously.

Subclinical chromophobe adenomas in man are remarkably common. Costello (1936) found 225 examples in a post-mortem series of 1,000 semi-serially sectioned human pituitaries. Their pathogenesis is unknown. In Costello's series they were twice as common in males as in females; the age range was 2 to 86 years. The lack of secretory activity of chromophobe
adenomas might indicate incomplete or abnormal functional differentiation in spite of a comparatively normal ultrastructural cell structure. An alternative speculation is that their blood supply might be derived directly from capsular arteries instead of portal vessels, with consequent deprivation of hypothalamic releasing factors. This leads to two further speculations: first, that secretory adenomas differ from non-secretory in that the former might be supplied by portal vessels, and second, that some secretory adenomas might represent chromophobe adenomas that have picked up a portal blood supply at some period after their formation. It must be emphasised that, as yet, there is no supporting evidence for any of these speculations. For the present, it appears that non-functioning chromophobe adenomas are made up of differentiated but inactive secretory cells and that secretory adenomas, both chromophil and chromophobe, are autonomous tumours consisting of well-differentiated cells of acidophil or basophil origin.

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