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Extracellular Growth Hormone Deposits in Pituitary Adenoma

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Summary. Ultrastructural examination of 184 pituitary adenomas demonstrated the presence of extracellular accumulations of electron dense material in 3 out of 64 cases with acromegaly. Fibrillary structures were seen in larger deposits of such material. This material was only observed in biopsies fixed directly with osmium tetroxide; initial fixation with glutaraldehyde did not retain the material and left empty spaces. Positive immuno-histochemical reaction with specific antibodies demonstrated that the extracellular material contained growth hormone (GH). The presence of this extracellular material could not be related to the age or sex of the patient nor to the duration of symptoms, size of the tumor, presence of diabetes mellitus, or concomitant secretion of prolactin. The pericapillary fibrous sheath was heavily thickened in the patient with the longest duration of symptoms, intermediate in thickness in the second and normal in the third.

Key words: Pituitary adenoma – Acromegaly – Hormone secretion – Electron microscopy – Immunologic techniques.

Introduction

The secretory process of normal and neoplastic pituitary cells is based upon formation and release of hormone granules (Farquhar, 1961, 1971; Farquhar and Smith, 1966; Farquhar et al., 1975; Howell and Whitfield, 1973; Kawarai and Nakane, 1970; Racadot et al., 1965). However, in end organ failure a direct release of pituitary tropic hormones occurs from the dilated cisternae of the endoplasmic reticulum (Contopoulos et al., 1958, Farquhar, 1971). The mechanisms regulating hormone synthesis, discharge, and destruction seem to work on the cellular level in normal and abnormal pituitary cells (Landolt and Hosbach, 1974; Landolt, 1975; Saeger, 1973a, b). The concentration of

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hormone found in the peripheral blood reflects the endocrine activity of the secretory cells; these show ultrastructural features (number of accumulated secretory granules, number of lysosomes, development of RER, degenerative changes in mitochondria) which reflect their functional state. No regulatory function has yet been assigned to the pituitary capillaries, but we have recently found evidence of a hormone pool in the intracellular space. The ultrastructure of the pituitary adenomas in these patients will be presented.

Material and Methods

Biopsy material from 184 pituitary adenomas (64 cases of acromegaly, 64 cases of adenomas without evidence of hormone secretion, 42 cases of prolactin-secreting adenomas, 6 oncocytomas, 4 cases with Cushing's syndrome, 3 cases of Nelson's syndrome, and 1 case with concomitant Addison's disease) was examined with the electron microscope. Positive findings, described below, were present in three patients whose pertinent clinical data are summarized in Table 1.

The biopsies were obtained during the trans-frontal or trans-sphenoidal excision of the adenomas. The specimens were immersed in fixative solutions within 30 s of their removal and were then minced into pieces of 1 mm^3. The following fixation, embedding, and staining procedures were used for light microscopy (for detailed description see Girod, 1976) and for electron microscopy (for detailed description see Landolt, 1975):

1. Light Microscopy

- 5% formaldehyde fixation, paraffin embedding, H&E, and PAS-Orange G staining (Pearse, 1949).
- Toluidine blue staining of semi-thin sections of material fixed and embedded for electron microscopy.

2. Electron Microscopy

- Direct osmium fixation, Durcupan® (Fluka AG, Buchs SG), Switzerland) embedding, uranyl acetate and lead citrate staining of ultrathin sections.
- Glutaraldehyde-osmium fixation, Durcupan embedding, uranyl acetate and lead citrate staining of ultrathin sections.

Table 1. Clinical data of the cases reported

Case No.	Sex	Age (years)	Syndrome	hGH Serum (normal < 5 ng/ml) fasting	Serum hPrl (normal < 15 ng/ml)	Duration of symptoms (years)	Adenoma diameter (mm)
1	F	59	Acromegaly[a]	78.5	12	12	5
2	F	30	Acromegaly[b]	39	not determined	5	17
3	M	53	Acromegaly[b]	31.6	1650	33[c]	22

[a] With manifest diabetes mellitus controlled by oral medication

[b] No diabetes mellitus, glucose tolerance test within normal limits

[c] This patient had undergone craniotomy and postoperative radiotherapy (8000 r/l) because of a chromophobe pituitary adenoma with bitemporal hemianopsia, adipositas, gynecomastia, impotence and slight acromegaly 33 years before this second operation

Ultrastructural immuno-histochemistry was performed according to a method adapted from Nakane (1971) on osmium fixed, Durcupan embedded material, since growth hormone (GH) antigen is not destroyed during this procedure (Nakane, 1971). Ultrathin sections were placed on gold grids which reduces non-specific precipitation of reaction product considerably, when compared with copper grids. The sections were then etched with xylene-saturated distilled water followed by 10% hydrogen peroxide, in order to expose to antigen. The sections were then incubated with rabbit anti-human GH serum (dilution 1:1000, Collaborative Research Inc., Waltham, Mass., USA) followed by washing with 0.01 M phosphate buffered saline (PBS) and incubation with sheep anti-rabbit Ig antibodies conjugated with horseradish-peroxidase (Institut Pasteur, Paris). The visualization of the peroxidase labeled antibody was done with 3,3′-diaminobenzidine (Sigma) as substrate (Graham and Karnovsky, 1966). The developed grids were then exposed to aqueous 1% copper sulphate followed by 2% osmium tetroxide and saturated aqueous uranyl acetate.

Results

1. Light Microscopy

Light microscopy demonstrated similar findings in all three cases which are therefore described together. The adenomas belonged to the diffuse type of Kernohan and Sayre (1956). Individual adenoma cells were of similar size and usually contained round nuclei with prominent nucleoli. The cytoplasm was rather scanty and contained eosinophilic (orangeophilic) granules in two cases (case 1 and 2). The capillary endothelium was thin in all three cases. The perivascular fibrous sheath was markedly thickened in case 3 and to a lesser degree in case 2. No changes were noted in case 1. The vascular lumen seemed to be compressed by the perivascular fibrous material which also extended between the surrounding epithelial cells. No fibrocytes or inflammatory cells were found. The perivascular material stained purple with PAS.

2. Electron Microscopy

Low power micrographs (Fig. 1) showed a dense accumulation of fairly uniform adenoma cells in all three cases. The nuclei were generally round or oval with occasional indentation, some cells contained two nuclei, and in general their chromatin was evenly dispersed. One or two nucleoli were present. The cytoplasm was rather light and contained a large number of different organelles. All three cases showed signs of increased protein synthesis, and the RER was particularly prominent in case 3. The large Golgi complexes contained secretory granules in different stages of development. The mitochondria were numerous and did not show oncocytic change. In addition, the cytoplasm contained numerous free ribosomes, smooth vesicles, lyosomes, and multivesicular lipid bodies. Intracytoplasmic spherical filamentous aggregates (for review see Landolt, 1975) were present in adenoma cells of case 1.

The osmiophilic secretory granules were particularly large and numerous in case 1. Their average diameter measured 320 nm. The granules were smaller but still numerous in case 2 where they showed an average diameter of 190 nm. Their diameter was similar in case 3 (180 nm), but they were much less numerous.

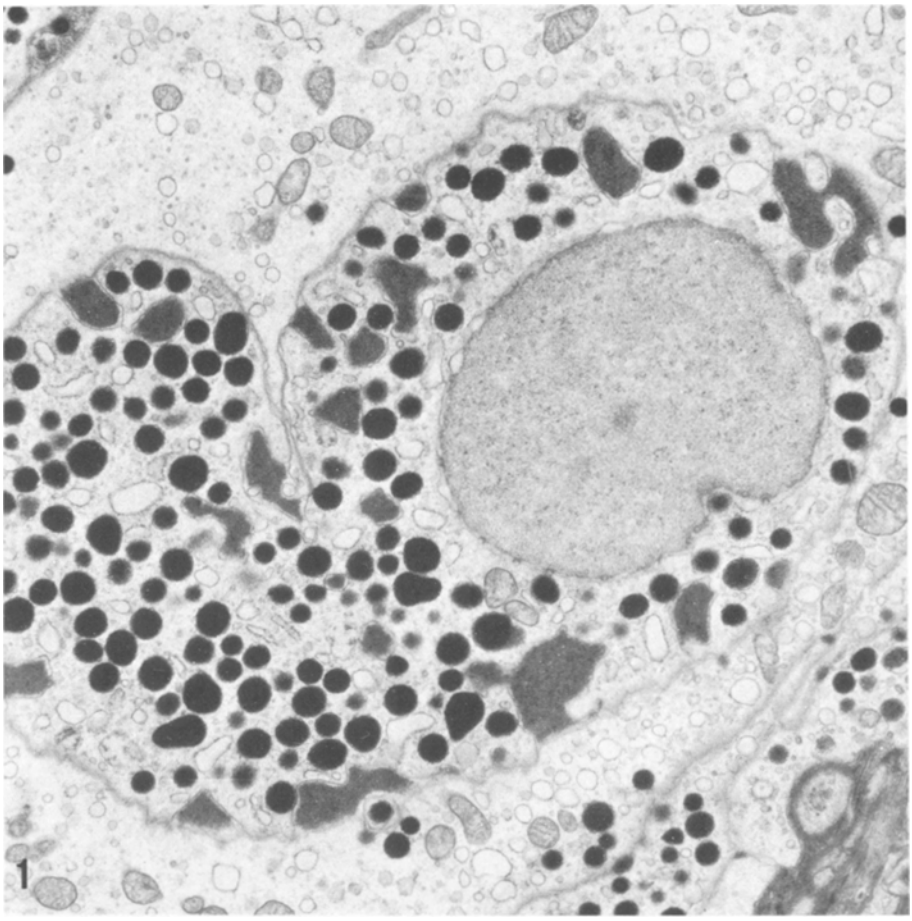


Fig. 1. Low power electron micrograph of a granulated adenoma cell with irregular extracellular electron dense deposits. Note decreased electron density of extracellular material compared with intracellular secretory granules. Case 1, osmium fixation, × 10,000

This confirms previous observations that the average granule size in acromegaly can vary considerably and that densely granulated adenomas usually contain larger granules than sparsely granulated ones (Landolt, 1975). The largest granules were often neither round nor oval but showed deformed contours. All three cases contained only a single cell type.

Numerous secretory granules could be seen in different stages of the releasing process (Figs. 2, 3) in which the granule membrane fused with the cell membrane and the electron dense core was discharged into the extracellular space. The original granule membrane then formed a "coated pit" (Douglas et al., 1971). The electron dense material in all three cases was not rapidly dissolved as is the case in normal pituitary and the large majority of adenomas, but remained visible as electron dense deposits in the intracellular space in biopsy material fixed with osmium tetroxide. These deposits seemed to enlarge with time (Fig. 1).

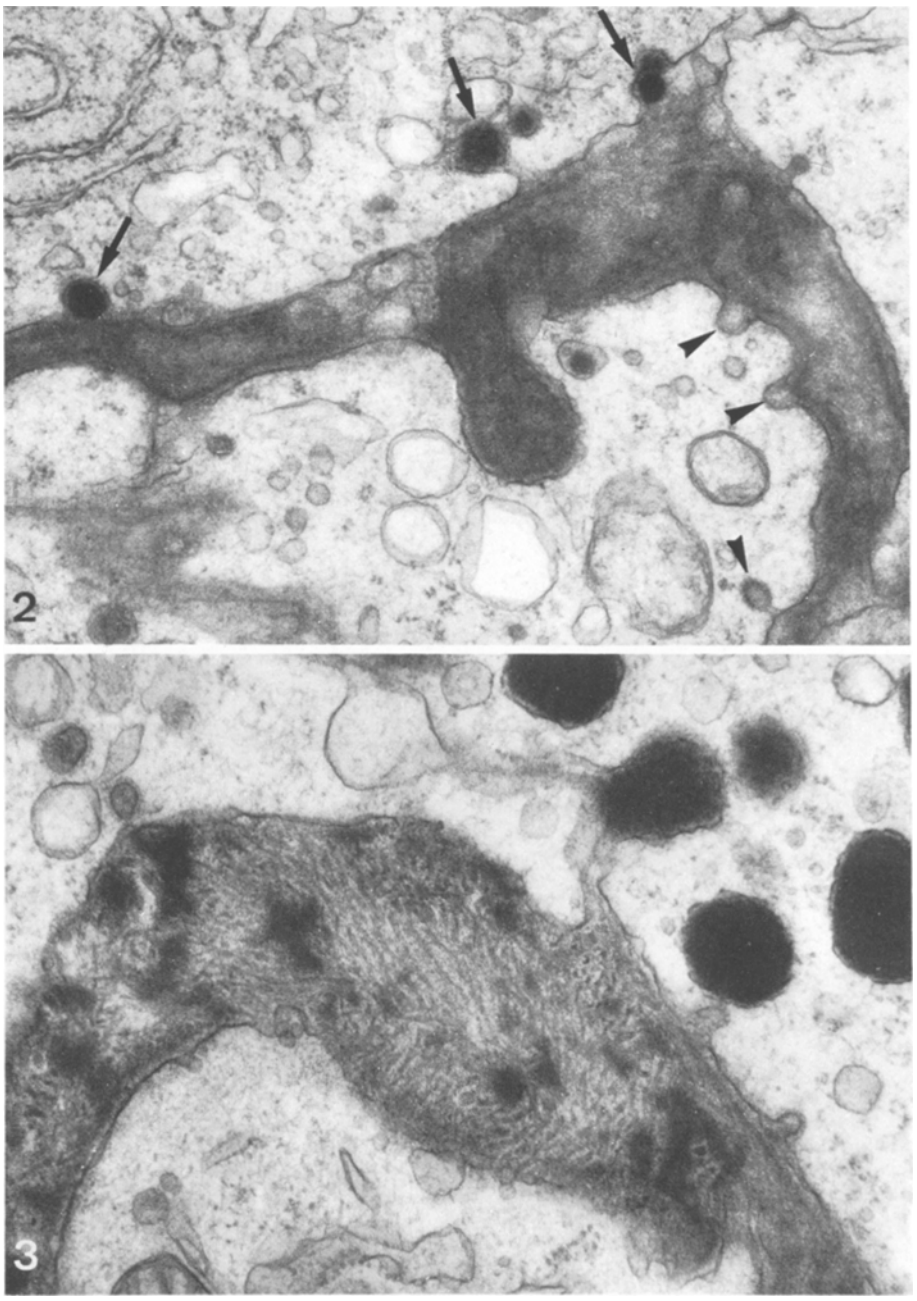
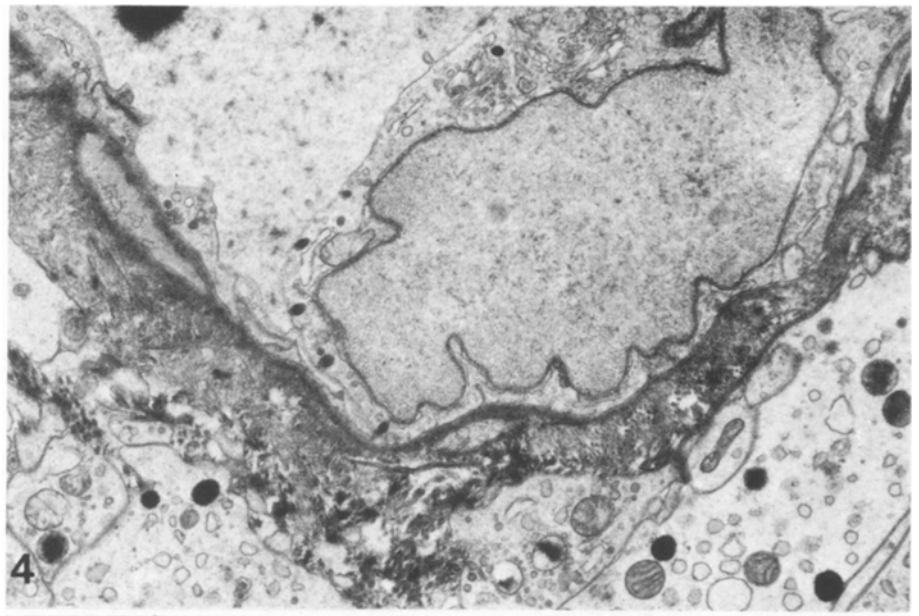


Fig. 2. Release of secretory granules (*arrows*) into the enlarged extracellular space which contains osmiophilic material. Some coated pits (arrowheads) can be seen. Case 3, osmium fixation, ×35,500

Fig. 3. Enlarged extracellular space containing ill defined osmiophilic material and fibrillary structures. Some coated pits are present. Case 1, osmium fixation, ×45,000



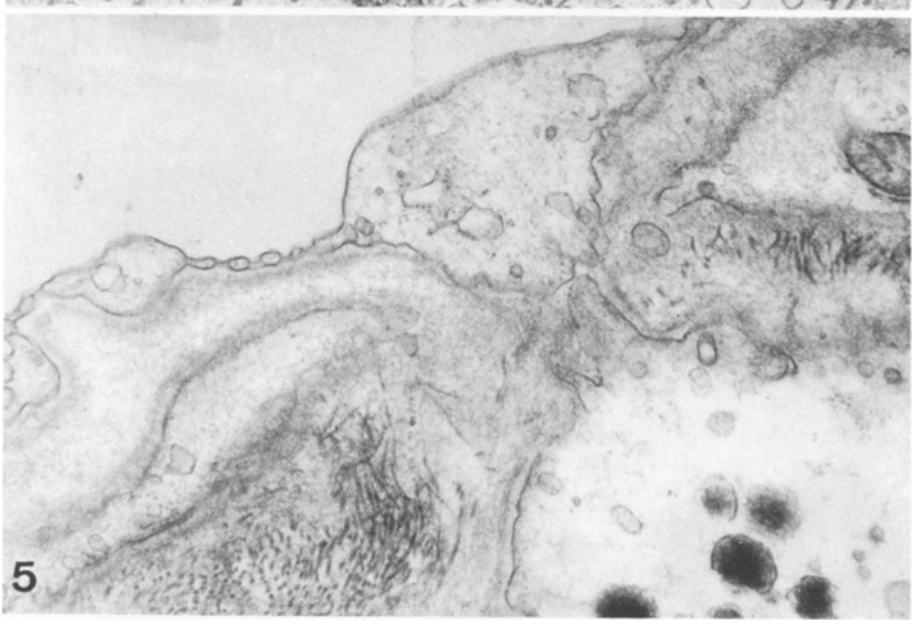


Fig. 4. Perivascular space with increased amount of collagen fibers. Case 1, osmium fixation, × 10,500

Fig. 5. Short segment of endothelial cell with typical fenestrations. Case 2, osmium fixation, × 34,000

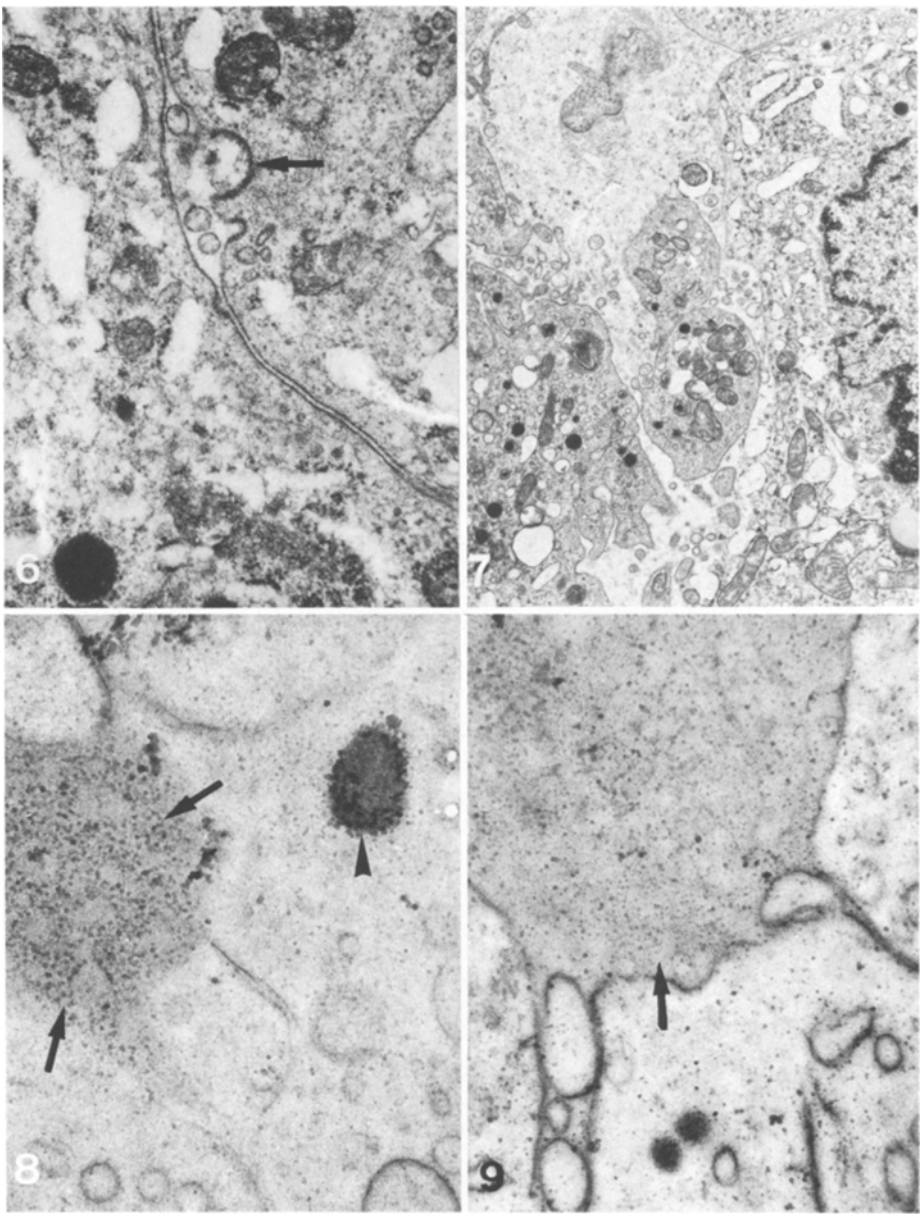


Fig. 6. Only traces of extracellular electron dense material can be found in the region of coated pits after glutaraldehyde-osmium fixation. Compare with Figure 2 which was obtained from the same patient (case 3). ×27,000

Fig. 7. Some fibrous material remains in larger intercellular lacunae after glutaraldehyde-osmium fixation. Case 3, ×10,000

Fig. 8. The fine granular reaction product of the immunoperoxidase technique localizes the human growth hormone in the extracellular lacunae (marked by *arrows*) and a secretory granule (*arrowhead*). Case 1, osmium fixation, anti-hGH reaction, ×26,000

Fig. 9. Histo-immunologic localization of growth hormone in intercellular electron dense material in case 3. Osmium fixation, anti-hGH reaction, ×24,000

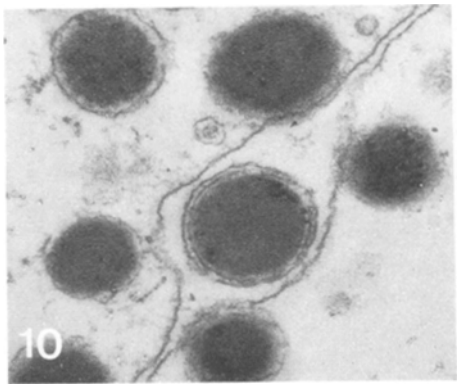


Fig. 10. Intercellular secretory granule surrounded by two membranes in a case of acromegaly without intercellular electron dense deposits. (For further discussion see text). Osmium fixation, × 43,500

Their electron density diminished as compared to the original granule cores but remained relatively high. The electron dense material was unevenly distributed in these deposits. Larger areas showed fibrillary substructures which seemed to emerge from irregularly delineated dense particles (Fig. 2). The enlarged extracellular spaces often communicated directly with the perivascular space (Fig. 4) in which typical collagen fibres could be observed. In the case 1 and 3 the amount of perivascular collagen in many capillaries was abnormally increased. The capillary walls consisted of normal endothelial cells resting on a normal looking basement membrane which enclosed occasional pericytes. Endothelial fenestrations were present in all three cases (Fig. 5). The perinuclear cistern of some endothelial cells in case 1 contained tubular inclusion typical for acromegaly. (Landolt et al., 1976).

Glutaraldehyde-osmium fixed tissue biopsies did not show the electron dense extracellular material described above. The contents of the secretory granules dissolved leaving only traces of floccular material in the area of the coated pits (Fig. 6). Some fibrous material could be seen in larger but otherwise apparently empty extracellular spaces (Fig. 7). The intracellular membrane surrounded granules kept their normal electron density after glutaraldehyde-osmium fixation.

3. Ultrastructural Immuno-Histochemistry

Immuno-histochemical staining with anti-human GH was performed on osmium fixed, plastic embedded material in order to obtain further information concerning the nature of the electron dense extracellular material. The fine granular product of the peroxidase reaction could be seen in the region of the intracellular intact secretory granules and in the enlarged extracellular space of all three cases (Figs. 8, 9). The reaction product was denser in the region of the intact secretory granules than in the area of the extracellular electron dense material.

Discussion

Secretory products are usually released by exocytosis from normal and adenomatous pituitary cells (Lever and Peterson, 1960; Farquhar, 1961). The electron dense granule contents dissolve rapidly in the extracellular space and can no longer be seen after their emergence from the secretory pit. It is therefore assumed that the substance undergoes some physiochemical change rendering it invisible on its way to the blood stream (Farquhar, 1961). Granule discharge occurs in those parts of the normal pituitary which are in contact with the vascular basement membrane (Pelletier et al., 1971). The observation that in certain adenomas granules are released from parts of cell membrane which are distant from the nearest capillary has been termed "misplaced exocytosis" (Horvath and Kovacs, 1974).

Some authors have described intact extracellular secretory granules in the normal (Salazar and Peterson, 1964) and adenomatous pituitary gland (Hirano et al., 1972; Tomiyasu et al., 1973; Zambrano et al., 1968). These findings are probably artefacts, caused by trauma to the tissue before satisfactory fixation is achieved (Foster, 1971). A release mechanism for secretory granules from intact cells which allows them to retain their membrane has not been described. A microapocrine secretory process during which small cellular processes containing one or more secretory granules are pinched off would leave the granule cores surrounded by two membranes, one originating from the Golgi saccule, the other from the cell surface (Fig. 10). Serial sections would be needed to prove that this particular granule with two membranes is not only situated in a slender cell process but also has been released into the intercellular space.

The presence of extracellular electron dense material in some pituitary adenomas, identified by immuno-histochemical methods as growth hormone has not to our knowledge been described in the literature. It may be due to the fact that this is a rare finding only present in 1.6% of all our adenomas and in 4.7% of our patients suffering from acromegaly. A second reason however, may be that a particular and uncommon fixation technique is used in our laboratory. Neither formaldehyde nor glutaraldehyde used as a prefixation medium can stabilize this material sufficiently when it is free in the extracellular space, only direct osmium tetroxide treatment can precipitate the hormone containing material.

Extracellular pituitary hormone deposits are only present in cases with abnormal GH secretion. Prolactin secretion is apparently normal since no similar findings were present in 42 prolactinomas. Prolactin levels in the serum of the cases presented here were elevated in one case, normal in a second one and were not determinated in the third (Table 1). No relationship to other clinical data, sex and age of the patients, duration of symptoms, previous treatment, or adenoma diameter can be seen. One patient suffered from manifest diabetes mellitus whereas the preoperative glucose tolerance was normal in the two other cases.

The ultrastructure of the blood vessels in pituitary adenomas has been described in several papers (Hirano et al., 1972; Kovacs and Horvath, 1973;

Schechter, 1972). Ultrastructural abnormalities are infrequent in these circumstances. They consist of thickening of the endothelial lining, endothelial swelling, endothelial blebbing, accumulation of microfilaments in the cytoplasm of the endothelial cells, partial or total loss of fenestrations, thickening of the basement membrane, and disorganization of the parenchymal-pericapillary interface. An accumulation of an intra- and perivascular, electron dense but unidentified material was demonstrated by Hirano and collaborators (1972) in non functioning, so called "chromophobe" adenomas.

The ultrastructure of the blood vessels present in our three cases is consistent with the findings cited above. But it is important to note that the structural alterations vary from vessel to vessel even within the same biopsy. The extent of these changes seems to be related to the duration of symptoms preoperatively (Table 1). They are not related to diabetic basement membrane hypertrophy (Siperstein et al., 1968). Case 1, with manifest diabetes mellitus and severe basement membrane involvement in a muscle biopsy, showed only intermediate changes in the pituitary adenoma vessels, whereas case 3 after a course of 33 years with severe vascular changes in the pituitary adenoma biopsy, did not show even latent diabetes in the glucose tolerance test. Endothelial fenestrations are present in all three biopsies but they are less frequent if compared to the normal pituitary adenomas lacking extracellular hormone deposits. Vascular changes therefore seem not to represent the crucial factor causing the abnormal hormone accumulation. – Complex formation has been described in human GH, giving rise to "little" and "big" GH (Gorden et al., 1973). This complex formation might affect the passage of GH into the capillaries.

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