Virchows Arch. A Path. Anat. and Histol. 381, 295–312 (1979)

Virchows Archiv A

Argyrophil Pituitary Tumors Showing TSH Cells or Small Granule Cells

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Summary. Among 74 histochemically and ultrastructurally studied pituitary adenomas, 12 apparently chromophobe tumors were characterized by the presence of numerous argyrophil cells. All these argyrophil adenomas failed to reveal presence of GH, prolactin or ACTH cells. Two tumors were found to consist of well granulated cells reacting intensely with anti-TSH antibodies and resembling TSH cells of the normal pituitary. The remaining argyrophil adenomas did not show TSH immunostaining and, with one exception, failed to react with an anti-HCG serum staining gonadotroph cells of human pituitary. They were composed of small, closely apposed cells with small compact or vesicular granules. These tumor cells seem to correspond to some small argyrophil cells found in non-neoplastic pituitary, which differ from TSH cells and from all other types of functionally identified adenohypophyseal cells.

Key words: Pituitary tumors – Argyrophil pituitary tumors – TSH tumors – Small granule tumors – Pituitary cells – Argyrophil pituitary cells – TSH cells – Small granule cells.

Introduction

It is evident from recent literature that many pituitary adenomas with signs of endocrine activity fail to show stainable granules by conventional histologic methods, and are thus classified by many pathologists as "chromophobe" adenomas. However, when these tumors are studied with more refined techniques using histochemical, immunocytochemical and ultrastructural methods, they show some secretory granules or hormone content (Schelin, 1962; McCormick and Halmi, 1971; Kuromatsu, 1968; Horvath and Kovacs, 1976; Saeger, 1977; Solcia et al., 1977).

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0340-1227/79/0381/0295/$03.60

These findings demonstrate that most "chromophobe" adenomas may secrete and may be classified into different functional types according to their ultrastructural morphology or immunostaining. Most of these adenomas, more appropriately called "sparsely granulated tumors" (Horvath and Kovacs, 1976) or "pseudocromophobe adenomas" (Solcia et al., 1977) have been shown to be composed of prolactin producing cells (Kovacs et al., 1975; Peillon et al., 1975; Saeger, 1975; Solcia et al., 1977) or GH cells (Landolt, 1975; Horvath and Kovacs, 1976). A few cases showing gonadotropin producing cells (Woolf and Schenk, 1974; Cunningham and Huckins, 1977) or thyrotropic (TSH) cells (Lamberg et al., 1969; Hamilton et al., 1970; Baylis, 1976) have also been reported.

Recently, among 74 pituitary adenomas we have found 12 clinically silent, histologically "chromophobe" tumors that were characterized by the presence of argyrophil granules when stained with the Grimelius' silver technique. Since preliminary histochemical and ultrastructural studies of such tumors (Solcia et al., 1977) failed to detect prolactin, GH or ACTH cells, in order to shed some light on the cellular origin of these tumors we started a thorough light and electron microscopic investigation of the argyrophil adenomas and of the argyrophil cells in non-tumor pituitaries.

Material and Methods

This report results from the histological, histochemical and ultrastructural study of 74 pituitary adenomas surgically removed between January 1973 and May 1978 at the Department of Neurosurgery of the Regional Hospital of Varese. Specimens of 12 pituitary chromophobe adenomas showing numerous argyrophil cells with Grimelius' stain were studied. Some GH-producing acidophilic tumors showing scattered argyrophil cells were not included in this study. The main clinical findings of the 12 cases are reported in Table 1. Cases 2, 3 and 9 were large intrasellar tumors, the remaining cases showed extrasellar extension.

For *light microscopy*, pieces of tumor tissue, 10 normal pituitary glands obtained at autopsy and 5 surgically removed non-tumorous pituitary glands were fixed with 10% formol, 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3, Bouin's fluid or Zamboni's buffered picric acid-formaldehyde mixture. Paraffin sections were stained with haematoxylin-eosin, Congo red, Grimelius' silver (1968), PAS-orange G and Gomori's aldehyde fuchsin.

An indirect immunofluorescence procedure (Coons et al., 1955) was applied to paraformaldehyde-, Bouin- or Zamboni-fixed sections using rabbit anti-human TSH serum (from Dr. A. Parlow, through the National Institute of Arthritis, Metabolism and Digestive Disease, Bethesda), rabbit anti-human ACTH serum (Wellcome), rabbit anti-human chorionic gonadotrophin (HCG) serum know to react with human pituitary LH in radioimmunoassay tests (Wellcome, England), rabbit anti-human growth hormone (GH) serum (Sorin, Saluggia, Italy) or a rabbit anti-human placental lactogen (HPL) serum detecting human prolactin in radioimmunoassay tests (Behringwerke, Marburg, West Germany), at dilutions 1:250 to 1:1,000, followed by goat (Hyland, England) or pig (Dakopatts, Denmark) anti-rabbit γ-globulin serum. The anti-TSH serum was applied directely or after addition with excess HCG (Serono, Rome) to remove α chain antibodies that would cross-react with gonadotrophins (Moriarty, 1973). The anti-HCG serum was also added, in some tests, with excess human TSH (Organon, Holland). Controls were performed: a) by adsorbing the specific antihormone serum with excess purified pituitary hormone b) by sustituting non-immune serum for the specific serum and c) by omitting the first step of the indirect test. Other sections, following incubation with the immune serum, were treated first with swine anti-rabbit serum IgG, then with peroxidase-antiperoxidase (P.A.P.) immune complex (Dakopatts) and finally with 3,3′-diaminobenzidine-H_2O_2 mixture (Sternberger, 1974). Controls were done as for immunofluorescence.

Parallel immunohistochemical tests were performed on sections of human non-tumor pituitaries; some of these sections showing GH, ACTH, TSH or HCG immunofluorescent cells were pho-

Table 1. Clinical findings in 12 patients with argyrophil adenomas

Case	Sex	Years	Endocrine sings and symptoms
1	M	47	Hypogonadism Slight hypothyroidism
2	M	47	Obesity Mild hypothyroidism and adrenocortical hypofunction
3	M	46	Loss of libido Cold intolerance
4	M	57	Previous removal of pituitary tumour Hypopituitarism
5	M	54	Loss of libido Weakness
6	M	53	Loss of libido Polyuria, polydipsia
7	F	46	Precocious menopause Slight adrenocortical hypofunction
8	F	65	Reduced energy Mild adrenocortical hypofunction
9	M	41	Obesity Loss of libido
10	F	54	Obesity Hirsutism
11	M	50	Loss of libido Reduced energy Mild hypothyroidism
12	M	65	Reduced energy and libido Mild hypothyroidism

tographed and then restained with Grimelius' silver. As noted by Woodtli and Hedinger (1976) immunofluorescence survived these procedures and allowed us to compare each immunofluorescent cell with argyrophil cells in the same section.

For *electron microscopy* small specimens of tumor tissue and of normal hypophysis were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. Specimens of all cases were post-fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and viewed in a Zeiss EM10 electron microscope. Some specimens of cases 2, 4, 5, 8, 11 and of normal hypophysis were cut with a Smith-Farquhar tissue sectioner (Sorvall), 100–150 µm sections were then stained with Grimelius' silver technique (Vassallo et al., 1971). They were then dehydrated and embedded in Epon. Sections of silver impregnated blocks were observed in the electron microscope with an without uranyl acetate counterstaining.

Results

Pituitary Adenomas

Light Microscopy. By the hematoxylin-eosin stains the tumors appeared to be mostly chromophobic. A number of cells showed varying degrees of cytoplasmic

eosinophilia in cases 4, 6, 7, 8, 9, and 10, due to the presence of oncocytes. All tumors investigated showed trabecular patterns of growth. Regular, long columns, few cells in thickness, and a papillary arrangement were prominent in cases 2, 10, 11 and 12. Branching, irregular, thick cords of small polygonal cells separated by sinusoidal channels and scanty connective tissue was the predominant appearance in the rest of the cases. Areas of diffuse growth were present in cases 1, 5 and 9. Amyloid, in form of stromal deposits, was found in case 8 only.

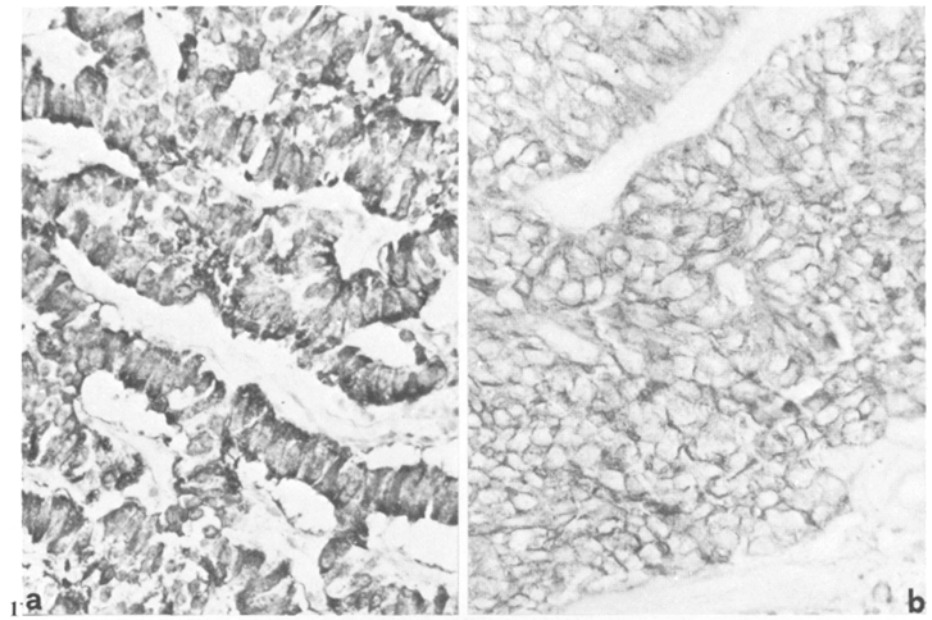
Cells reacting with Grimelius' silver technique were found to be numerous in all tumors, although various degrees of reactivity were seen in different cells and different tumors (Fig. 1). A few tumor cells, lightly stained with aldehyde fucsin and P.A.S. were seen in case 11 only.

Most tumor cells in case 11 and a large part of case 12 reacted with both anti-TSH and anti-HCG sera (Fig. 2). A few cells reacting with anti-HCG but none reacting with anti-TSH serum were found in case 2. The remaining tumors failed to react with both anti-TSH and anti-HCG sera. No GH, HPL or ACTH immunoreactive cells were found in the 12 adenomas investigated.

Electron Microscopy. Ultrastructural findings allowed us to distinguish tumors 11 and 12 from tumors 1 to 10.

In cases 11 and 12 elongated or polygonal, rather large, tumor cells were closely apposed and united by rare, incospicuous desmosomes. The cell contours were regular; the nuclei were ovoid or round with a small nucleolus. Common organelles were rather scarce in most of the cells. A crescent shaped Golgi complex could be distinguished; elongated and round mitochondria were rare in case 11 and numerous in case 12. The rough endoplasmic reticulum was represented by short, scattered cisternae which were often dilated. One or more large dense bodies (ceroid bodies) were often present. The secretory granules were abundant and diffusely distributed throughout the cytoplasm (Fig. 3). They were round or ovoid, small sized (case 11:141 $\pm$ 30 nm, 805 granules counted; case 12:147 $\pm$ 37, 284 granules), with a thin clear space underneath a limiting membrane, and an electron dense, homogeneous, core (Fig. 4). Such granules showed a moderate, diffuse reactivity with Grimelius' silver (Fig. 7a).

The tumor cells in cases 1 to 10 were small, closely apposed, with irregular shapes and frequently with some processes. The cell contours were irregular and showed complex interdigitations (Fig. 5). The nuclei were often irregular, sometimes with deep indentations; nuclear cytoplasmic intrusions were occasionally encountered. Mitochondria were numerous in all cases; they increased strikingly in number and appeared swollen (oncocytic transformation) in many cells in cases 4, 6, 7 and 9. The granular endoplasmic reticulum was relatively scarce and sometimes appeared in the form of parallel arrays of cisternae. Most of the cells contained abundant free ribosomes singly or in rosettes. The Golgi complex was well developed. In several cells whorled arrays of smooth endoplasmic reticulum were seen. Small secretory granules were found in every tumor, although their amounts varied greatly from cell to cell. Most cells contained only few granules concentrated near the cytoplasmic membrane. Two main kinds of granules were seen: 1) very small (90–120 nm), round, fairly



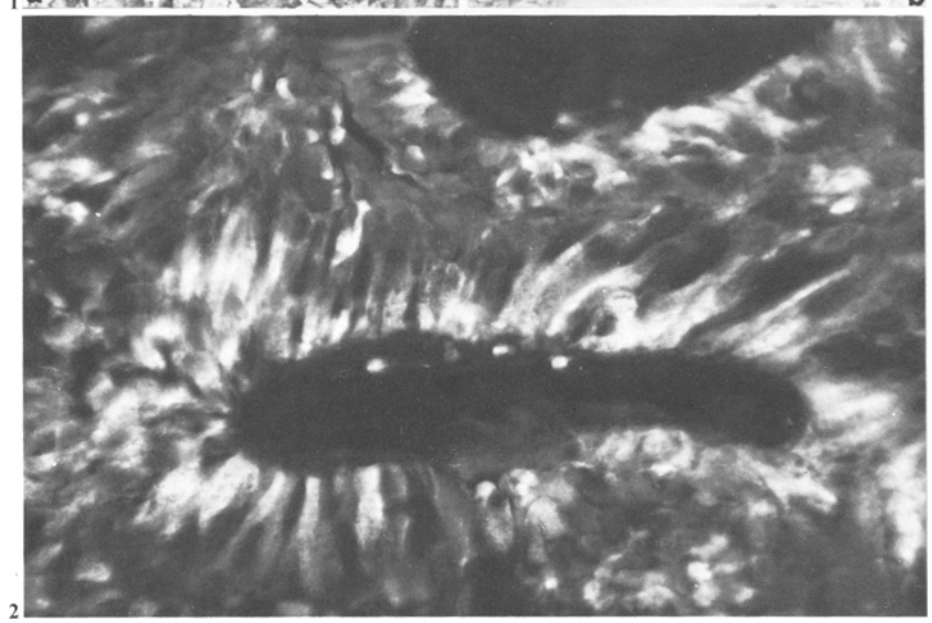


Fig. 1 a and b. Intense cytoplasmic argyrophilia and thin cords in tumor case 2 (**a**); weak, marginated argyrophilia and thick trabecular arrangement in case 7 (**b**). Grimelius' silver. ×280

Fig. 2. Intense TSH immunofluorescence of many elongated cells in tumor 11. Note palisading arrangement of tumor cells around fibrovascular structures. ×450

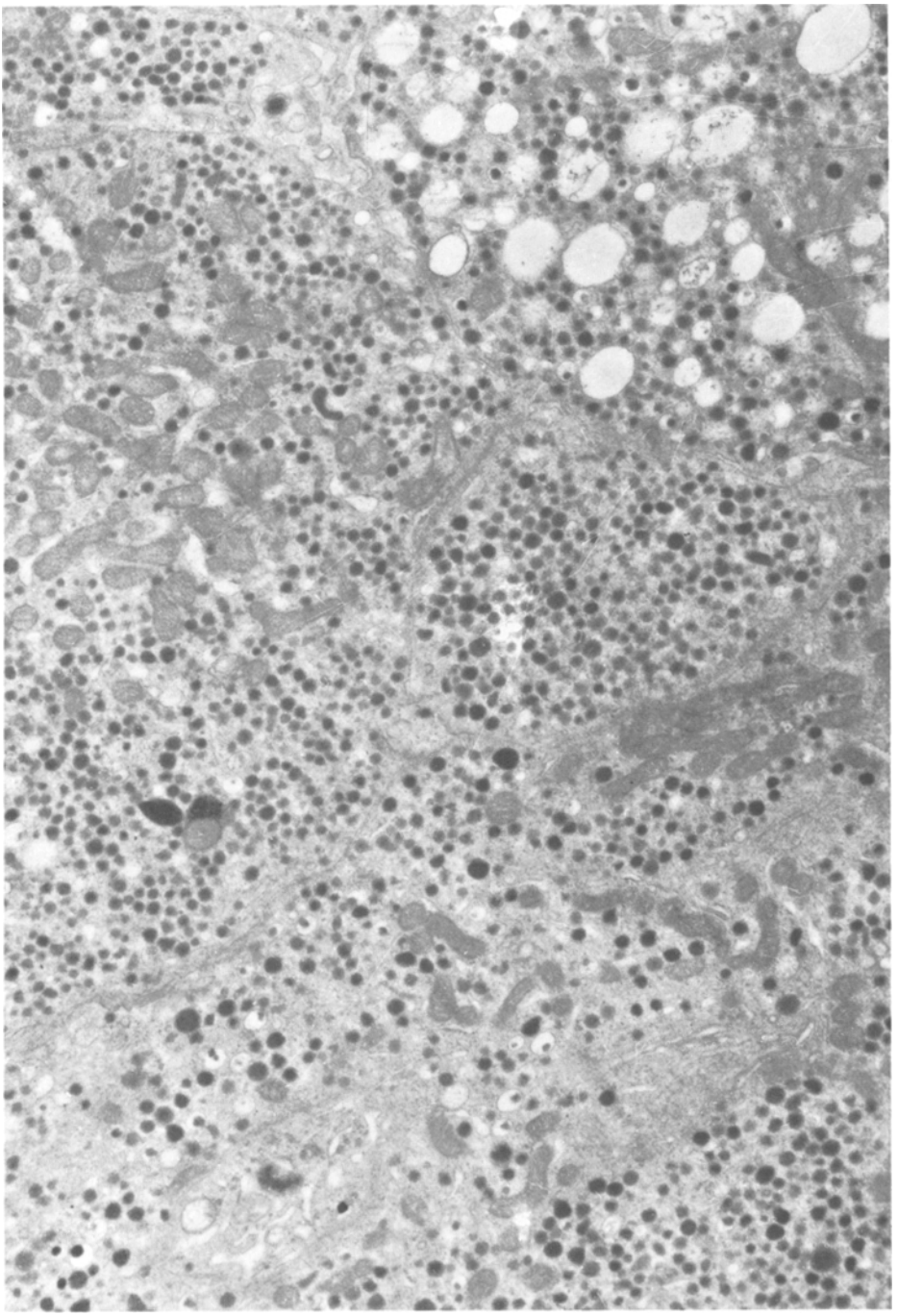


Fig. 3. Ultrastructure of tumor cells in TSH immunoreactive case 11. ×11,200

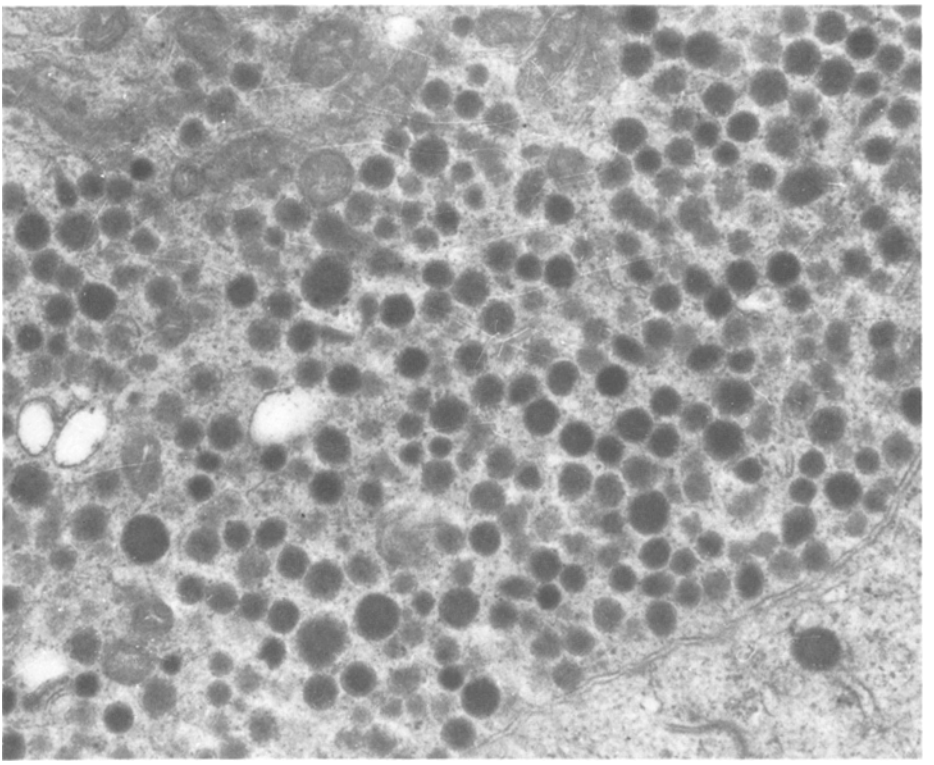


Fig. 4. Same tumor as in Figs. 2 and 3: enlargement of granules. ×28,000

and diffusely argyrophil granules showing a thin clear space in between the core and the enveloping membrane (Figs. 6a and 7b). 2) larger (120–170 nm) round, vesicular granules only partially filled with poorly osmiophilic and diffusely argyrophil cores, which were often eccentrically placed within the enclosing sac (Figs. 6b and 7c). The mean diameter of the granules varied from case to case (from 91 ± 22 nm, 151 granules counted, in case 1 to 153 ± 35 nm, 435 granules counted, in case 5) being larger in those cases in which the relative number of vesicular granules was greater. Vesicular granular in case 5 showed double inner structure characterized by a dense core surrounded by less dense, argyrophil matrix.

Non-Tumorous Hypophysis

We report here only those results which relate to the argyrohil cells of the normal human pituitary.

Light Microscopy. In non-tumorous anterior pituitaries, numerous cells showed fairly intense staining with the Grimelius' silver technique. The majority of these cells were located in the central mucoid wedge, sometimes grouped in

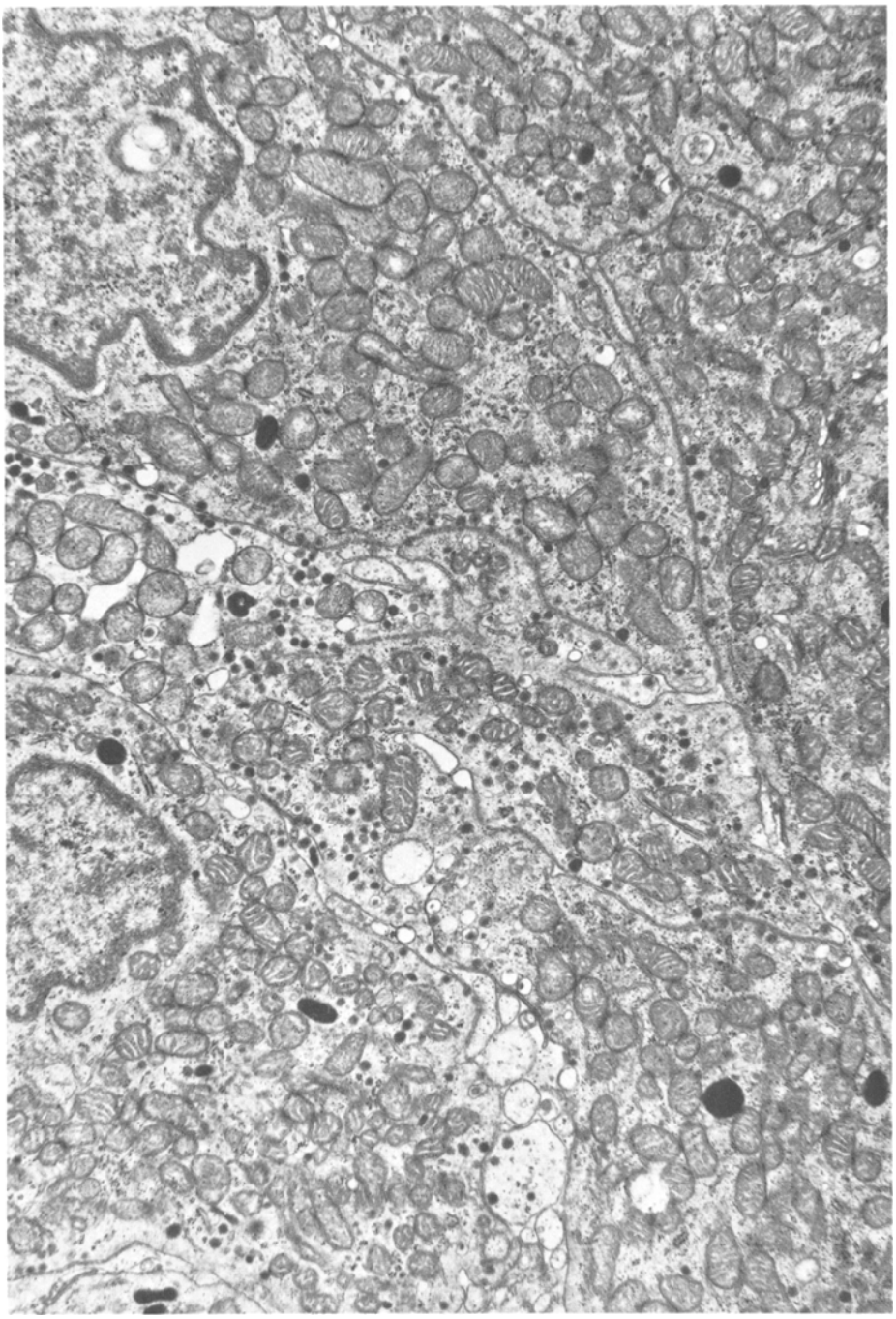


Fig. 5. Ultrastructure of tumor cells with small granules in case 4. Note margination of granules. ×14,200

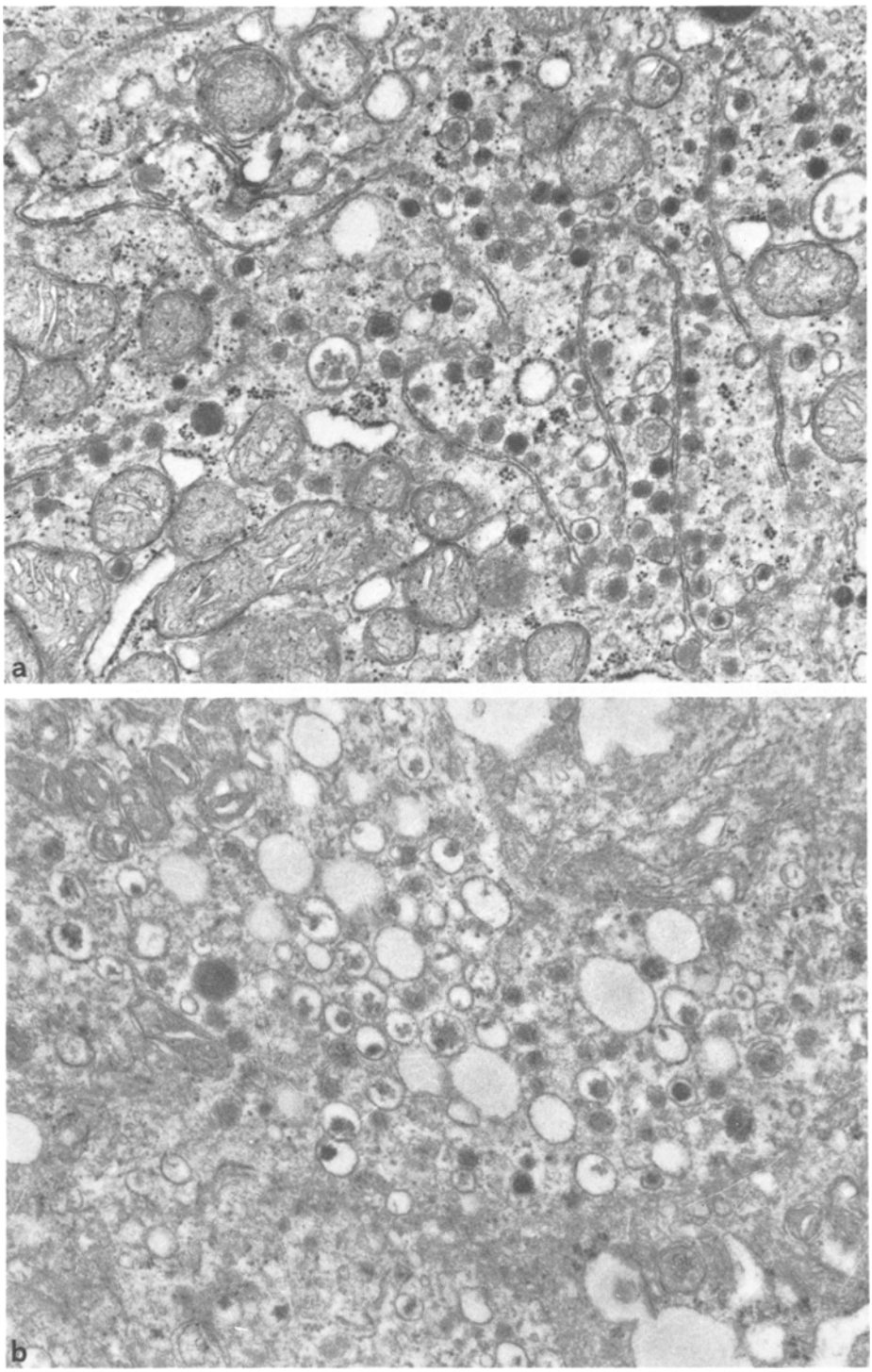


Fig. 6a and b. Small, cored granules (**a**, case 4) and slightly larger, vesicular granules (**b**, case 8) in two argyrophil tumors unreactive with all hormone antisera. ×28,000

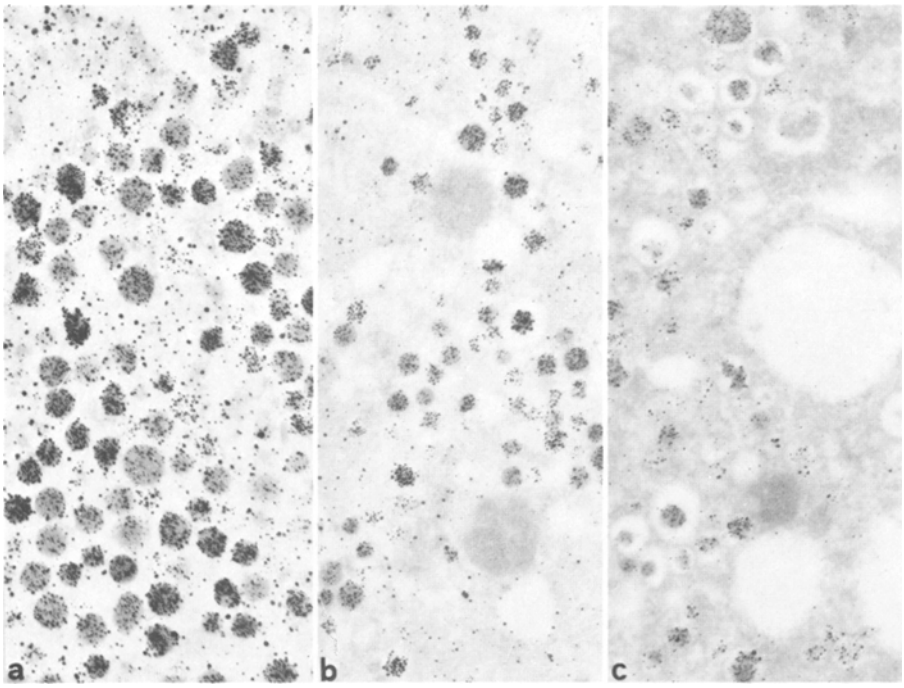


Fig. 7a–c. Granules of tumors 11 (**a**), 4 (**b**) and 8 (**c**) stained with Grimelius' silver. Compare with figures 4, 6a and 6b, respectively. ×28,000

small clusters; a moderate number of single, small scattered cells was also observed in the lateral acidophilic wings. Cells reacting specifically with anti-GH serum, anti-HPL serum (interpreted as prolactin cells), anti-ACTH serum, anti-HCG serum added with excess human TSH (interpreted as gonadotroph cells) and anti-TSH serum added with excess HCG (interpreted as TSH cells) were detected with both immunofluorescence and immunoperoxidase tests. A large majority of HCG and TSH immunofluorescent cells, when restained with Grimelius' silver, showed various degrees of argyrophilia. The cells reacting with anti-ACTH, anti-HPL or anti-GH serum failed to stain with Grimelius' silver, apart from a very weak, somewhat equivocal staining of a few GH cells.

Electron Microscopy. In silver stained sections three types of cells containing numerous reactive granules could be distinguished. *Type 1 cells* had either very small (90–120 nm) granules with fairly intense diffuse reactivity of their core (Fig. 8a) or larger granules (120–170 nm), which often presented an intensely argyrophil halo surrounding a negative core. *Type 2 cells* had medium sized (150–200 nm) round or ovoid granules with fairly reactive core (Fig. 8b). *Type 3 cells* had rather large (200–280 nm) granules with variable, diffuse positivity of their cores. Most cells identified as GH cells according to Li et al. (1977) and Pelletier et al. (1978), and all the cells identified as prolactin cells according to Pelletier et al. (1978) or as ACTH-MSH cells according to Halmi and Moriarty

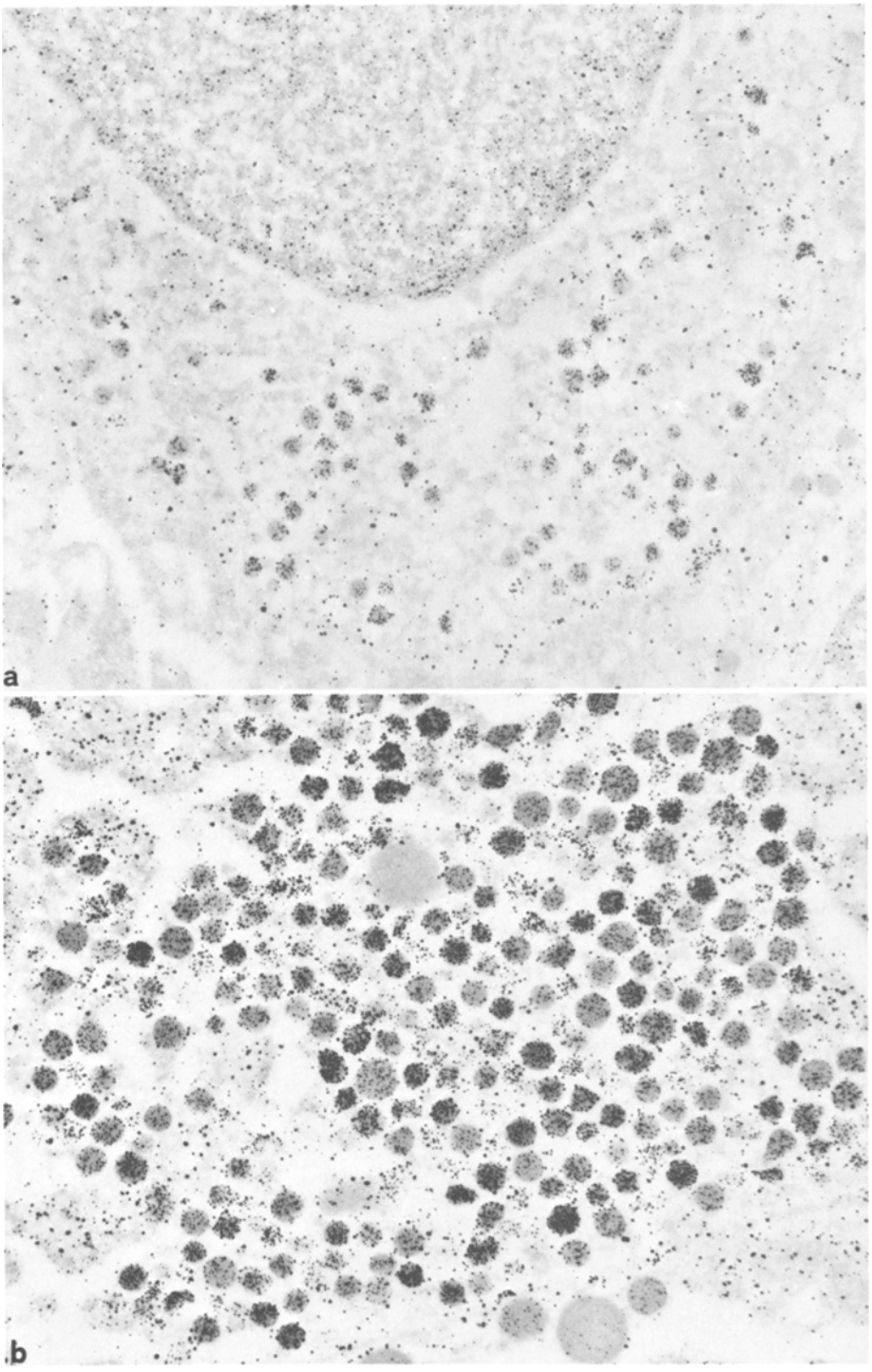


Fig. 8a and b. Granules of type 1 (**a**) and type 2 (**b**) cells of non-tumorous hypophysis. Compare with Figs. 9a and 10, respectively. ×28,000

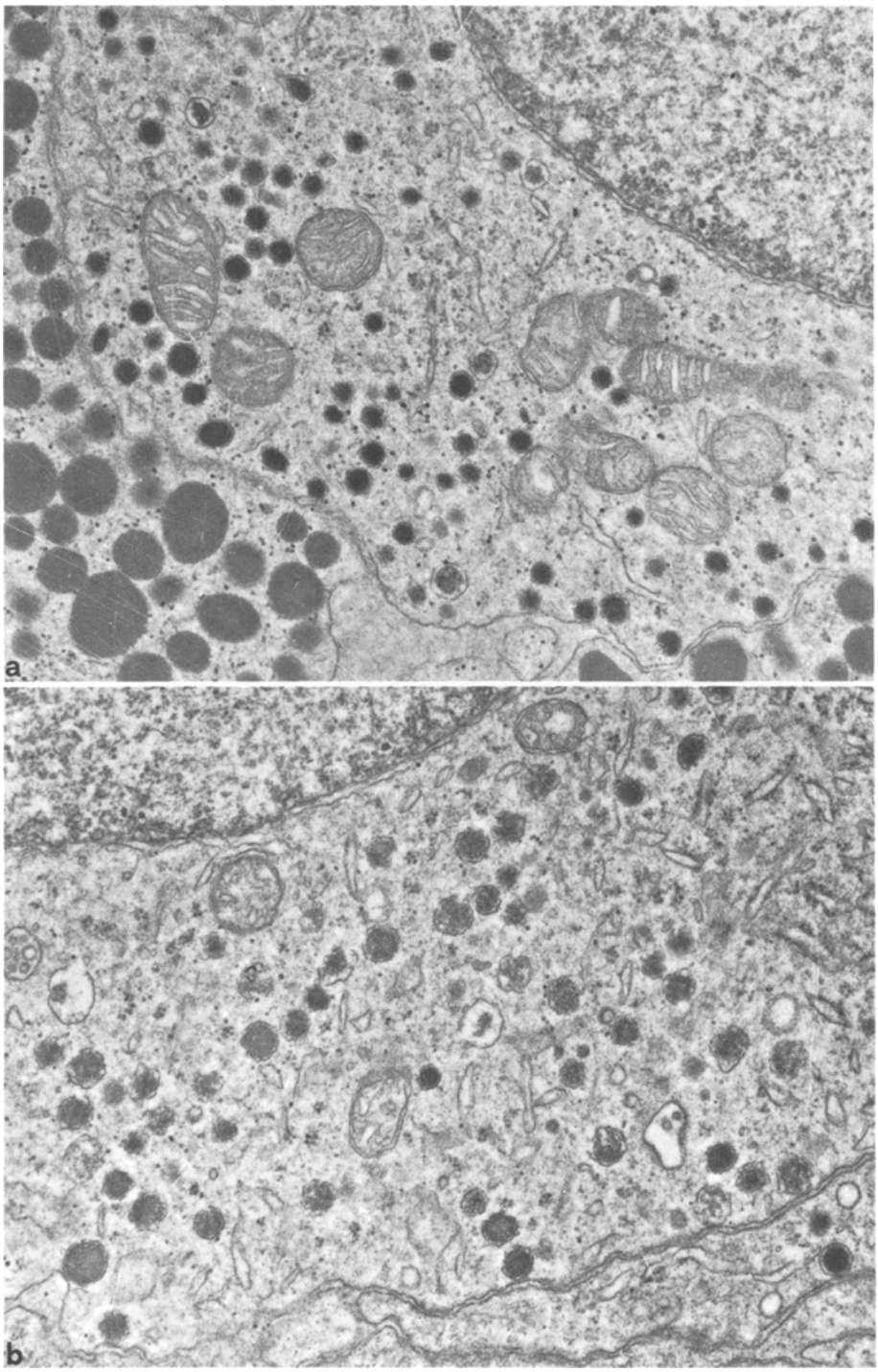


Fig. 9a and b. Small, cored granules (**a**) and slightly larger, partly vesicular granules (**b**) of two type 1 cells in non-tumorous hypophysis. Compare with Figs. 6a and 6b, respectively. ×28,000

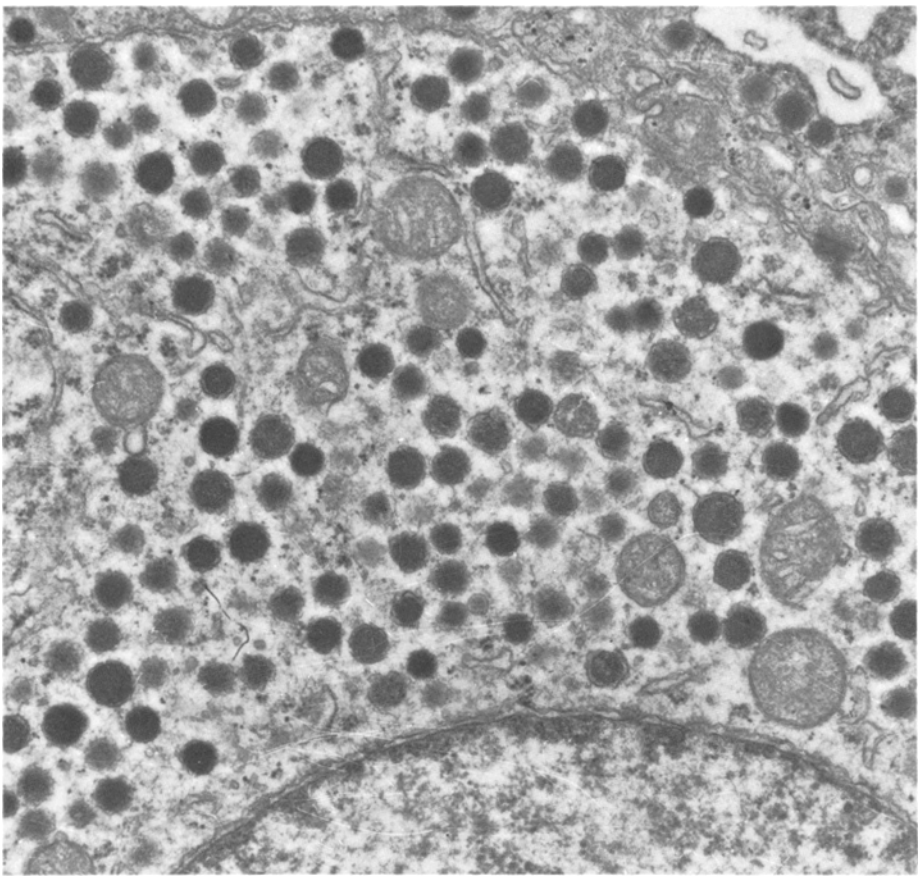


Fig. 10. Dense, homogeneous granules of a type 2 cell (presumed TSH cell) in non-tumorous hypophysis. Compare with granules of the TSH immunoreactive tumor depicted in Fig. 4. ×28,000

(1977) and Pelletier et al. (1978) had granules negative with Grimelius' silver. Some cells with large, round, argyrophobe granules closely resembling those of GH cells, also showed small (around 140 nm) scattered granules with an argyrophilic halo.

In conventional electron microscopy cells corresponding to the above types could be easily recognized. *Type 1 cells* were small, ovoid with irregular contours and poorly developed cell organelles. In some cells the granules were very small (119 ± 20 nm, 421 granules counted), few in number and often concentrated near the plasma membrane. They generally consisted of a round, rather dense, core surrounded by a thin clear halo (Fig. 9a). Other cells contained round or ovoid granules (150 ± 29 nm, 494 granules counted) with variable appearances, from relatively dense to vesicular (Fig. 9b). Vesicular granules, often showing an eccentric core, probably correspond to the granules with an argyrophil halo observed in silver stained specimens. These cells closely resembled the cells tumors 1 to 10.

Type 2 cells were larger than type 1 cells, polyhedral, with smooth contours, well developed Golgi and endoplasmic reticulum and abundant lysosomes. Their granules (176 ± 28 nm, 391 granules counted) were round or ovoid, with marked electron density of the core and a narrow clear space between the core and the surrounding membrane (Fig. 10). These cells closely resembled both TSH cells of human pituitary as identified immunocytochemically by Moriarty and Tobin (1976) and by Pelletier et al. (1978) and the cells observed in our tumors 11 and 12.

Type 3 cells were rather large, round cells, with secretory granules randomly distributed throughout the cytoplasm. The granules, measuring 243 ± 48 nm in diameter (268 granules counted), were round, varied considerably in their electron density and seldom showed an halo between the core and the enveloping membrane. These cells resembled FSH cells as described by Kurosumi (1968), type 1 gonadotrophs of Moriarty (1976) and gonadotroph cells of Pelletier et al. (1978). Type 3 cells were not seen in any of the tumors studied.

Discussion

Present histological results show that among pituitary adenomas a group of tumors, characterized by trabecular structure and diffuse argyrophilia, can be identified. As in other endocrine cells and tumors, the argyrophilia has been shown to be a property of secretory granules. In previous studies, the argyrophil material has been interpreted as acid glycoproteins stored in secretory granules together with peptides and amines (Solcia et al., 1976).

Immunohistochemical and ultrastructural findings suggest the occurrence of at least two types of such tumors. The first type (cases 11 and 12) is composed of cells exhibiting a specific immunoreactivity for TSH. Ultrastructurally these tumor cells are found to be rich in rather small secretory granules closely resembling those of normal human TSH cells as revealed by electron immunocytochemistry (Moriarty and Tobin, 1976 and Pelletier et al., 1978). It seems worthwhile to point out that in the latter cells the granules ranged in diameter from 150 to 300 nm (Moriarty and Tobin, 1976) and from 125 to 200 nm (Pelletier et al., 1978), a size greater than that previously attributed to the granules of presumed human TSH cells (100 nm, Bergland and Torack, 1969; 80–150 nm, Lawzewitsch et al., 1972). In this respect the type 2 cell we found in non tumorous hypophysis corresponds much better to the TSH cell described by Moriarty and Tobin (1976) and Pelletier et al. (1978) than to those reported by Bergland and Torack or Lawzewitsch and coworkers.

In the literature, the few cases of TSH producing adenomas reported are of two kinds, the first being associated with hyperthyroidism, the second one with long standing hypothyroidism (Mösli and Hedinger, 1968; Linquette et al., 1971; Duello and Halmi, 1977). The presence, in both our cases of low serum thyroid hormone levels places them both into the latter group of TSH tumors. Previous ultrastructural studies of TSH adenomas are scarce (Mornex et al., 1972; Horn et al., 1976; Samaan et al., 1977; Duello and Halmi, 1977) and electron micrographs were shown only in the two latter papers. The tumors

studied by Duello and Halmi (1977) seems to have several ultrastructural features in common with our cases.

The second type of argyrophil adenoma (cases 1 to 10) is composed of cells which, with the exception of one tumor showing a few anti-HCG reactive cells, fail to react with the antisera used. The presence of small, poorly osmiophilic, haloed or vesicular granules, the abundance of mitochondria (oncocytic transformation) and the irregularity of cell contours characterize these tumors ultrastructurally and allow their identification in the electron microscope. These adenomas appear to correspond, at least in part, to "tpye 2 chromophobe adenomas" of Kuromatsu (1968), "chromophobic adenomas" of Schechter (1973), "chromophobe adenomas" of Racadot et al. (1975), some of the "endocrine inactive adenomas with secretory granules" of Landolt (1975), and "undifferentiated cell adenomas" of Horvath and Kovacs (1976). The origin of these tumors has been variously interpreted. Kuromatsu (1968) presumes that they derive from corticotrophs, because the size and appearance of their granules are similar to the rat cells reputed to be corticotrophs by Kurosumi and Kobayashi (1966). Schechter (1973) considers the tumor granules to be similar to those found in pituitary basophils. Horvath and Kovacs (1976) suppose that the adenomas arise from a common precursor of basophil cells. These suggestions are not substantiated by our immunohistochemical data. Further work with more sera and different technical approaches are needed before we can safely exclude that any of the known pituitary peptides are produced by such tumours. However, our negative immunohistochemical findings might be explained by the production of a peptide which has not yet been detected in the adenohypophysis or not yet localized at cellular level. In this connection it seems interesting that recently a distinct population of cells exhibiting cytoplasmic neurotensin-like immunofluorescence has been reported in the anterior pituitary of the rat (Uhl et al., 1977).

In any case, the ultrastructural observations of these authors, together with our own, indicate a secretory function of the cells composing these tumors, despite lack of immunostaining. Our ultrastructural data suggest origin of this type of argyrophil adenoma from a cell with small argyrophil granules (type 1 cell of this paper) found in normal human pituitary. Such cells appear to be ultrastructurally distinguishable from TSH cells, their granules being more vesicular, less osmiophilic, with different silver reactivity and usually smaller. Our type 1 cell seems to correspond to the "adrenocorticotropic cells" of Foncin and Le Beau (1974), the "ACTH cells" of Lawzewitsch et al. (1972) and the "ACTH cells" described by Kurosumi and Kobayashi (1966) in the rat pituitary, later shown to differ from true ACTH cells of the same species (Siperstein and Miller, 1970; Bowie et al., 1973). A relationship might also exist between type 1 cells and some cells with very small granules described in the rostral zone of the rat and mouse pituitary which are not stained immunohistochemically for ACTH (Moriarty, 1973) and are not affected by a propylthiouracil treatment known to modify TSH cells (Stoeckel et al., 1973). Furthermore, type 1 cells should be linked with the small granulated cells described by Pelletier et al. (1978) in the human pituitary, which failed to react with anti-GH, -prolactin, -ACTH, -LH, -FSH, and -TSH sera.

It is interesting to recall that cells with small haloed granules (P cells) resembling those of the present type 1 cells ultrastructurally were seen in human bronchial and gastric mucosa (Bensch et al., 1965a; Capella et al., 1978), many bronchial carcinoids (Bensch et al., 1965b; Hage, 1973; Capella et al., 1979), thymic carcinoids (Rosai and Levine, 1976) and some pancreatic tumors (Capella et al., 1977). Since the function of all these cells from different sites is still obscure, it remains uncertain whether or not they represent a morphologically and functionally homogeneous cell population.

Acknowledgements. The authors are greatly indebted to the National Institute of Arthritis, Metabolism and Digestive Disease (NIAMDD) for the gift of anti-TSH serum, and to the SORIN, Saluggia (Italy) for the gift of anti-GH serum. P.F. is a fellow of the Fondazione Anna Villa Rusconi, Varese.

References

Baylis, P.H.: Case of hyperthyroidism due to a chromaphobe adenoma. Clin. Endocrinol. **5**, 145–150 (1976)

Bensch, K.C., Gordon, G.B., Miller, L.R.: Studies on the bronchial counterpart of the Kultschitzky (argentaffin) cell and innervation of bronchial glands. J. Ultrastruct. Res. **12**, 668–686 (1965a)

Bensch, K.G., Gordon, B., Miller, L.R.: Electron microscopic and biochemical studies on the bronchial carcinoid tumor. Cancer **18**, 392–602 (1965b)

Bergland, R.M., Torack, R.M.: An ultrastructural study of follicular cells in the human anterior pituitary. Am. J. Path. **57**, 273–297 (1969)

Bowie, E.P., Williams, G., Shiino, M., Rennels, E.G.: The corticotroph of the rat adenohypophysis: a comparative study. Am. J. Anat. **138**, 499–520 (1973)

Capella, C., Solcia, E., Frigerio, B., Buffa, R., Usellini, L., Fontana, P.: The endocrine cells of the pancreas and related tumours. Ultrastructural study and classification. Virchows Arch. A Path. Anat. Histol. **373**, 327–332 (1977)

Capella, C., Hage, E., Solcia, E., Usellini, L.: Ultrastructural similarity of endocrine-like cells of the human lung and some related cells of the gut. Cell Tiss. Res. **186**, 25–37 (1978a)

Capella, C., Gabrielli, M., Polak, J.M., Buffa, R., Bordi, C., Solcia, E.: Ultrastructural and histological study of 11 bronchial carcinoids. Evidence for different types. Virchows Arch. A Path. Anat. Histol. (1979)

Coons, A.H., Leduc, E.H., Connolly, J.M.: Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J. exp. Med. **102**, 49–63 (1955)

Cunningham, G.R., Huckins, C.: An FSH and prolactin-secreting pituitary tumor: pituitary dynamics and testicular histology. J. Clin. Endocrinol. Metab. **44**, 248–253 (1977)

Duello, T.M., Halmi, N.S.: Pituitary adenoma producing thyrotropin and prolactin. An immunocytochemical and elctron microscopy study. Virchows Arch. A Path. Anat. Histol. **376**, 255–265 (1977)

Foncin, J.F., Le Beau, J.: Identification au microscope électronique des cellules adrénocorticotropes de l'hypophyse humaine. C.R. Soc. Biol. (Paris) **158**, 2276–2279 (1964)

Grimelius, L.: A silver nitrate stain for α_2 cells in human pancreatic islets. Acta Soc. Med. upsalien **73**, 243–270 (1968)

Hage, E.: Histochemistry and fine structure of bronchial carcinoid tumors. Virchows Arch. Abt. A Path. Anat. **361**, 121–128 (1973)

Halmi, N.S., Moriarty, G.C.: The cells of origin of ACTH in man. Ann. N.Y. Acad. Sci. **297**, 167–182 (1977)

Hamilton, C.R., Adams, L.C., Maloof, F.: Hyperthyroidism due to thyrotropin-producing pituitary adenoma. N. Engl. J. Med. **287**, 1077–1080 (1970)

Horn, K., Erhardt, F., Fahlbusch, R., Pickardt, C.R., Werder, K.V., Scriba, P.C.: Recurrent goiter, hyperthyroidism, galactorrhea and amenorrhea due to a thyrotropin and prolactin-producing pituitary tumor. J. Clin. Endocrinol. Metab. **43**, 137–143 (1976)

Horvath, E., Kovacs, K.: Ultrastructural classification of pituitary adenomas. Canad. J. Neurol. Sci. **3**, 9–21 (1976)

Kovacs, K., Horvath, E., Corenblum, B., Sirek, A.M.T., Penz, G., Ezrin, C.: Pituitary cromophobe adenomas consisting of prolactin cells: a histologic, immunocytological and electron microscopic study. Virchows Arch. A Path. Anat. and Histol. **366**, 113–123 (1975)

Kuromatsu, C.: The fine structure of the human pituitary chromophobe adenoma with special reference to the classification of this tumor. Arch. Histol. Jap. **29**, 41–61 (1968)

Kurosumi, K.: Functional classifixation of cell types of the anterior pituitary gland accomplished by electron microscopy. Arch. Histol. Jap. **29**, 329–362 (1968)

Kurosumi, K., Kobayashi, Y.: Corticotrophs in the anterior pituitary glands of normal and adrenalectomized rats as revealed by electron microscopy. Endocrinology **78**, 745–758 (1966)

Lamberg, B.A., Ripatti, J., Juustila, H., Sivula, A., Björkesten, G.: Chromophobe pituitary adenoma with acromegaly and TSH-induced hyperthyroidism associated with parathyroid adenoma. Acta endocr. (Kbh.) **60**, 157–172 (1969)

Landolt, A.M.: Ultrastructure of human sella tumours. Acta Neuroch. Suppl. **22**, 1–167 (1975)

Lawzewitsch, I., Dickmann, G.H., Amezua, L., Pardal, C.: Cytological and ultrastructural characterization of the human pituitary. Acta anat. **81**, 286–316 (1972)

Li, J.Y., Dubois, M.P., Dubois, P.M.: Somatotrophs in the human fetal anterior pituitary. An electron-microscopic-immunocytochemical study. Cell Tiss. Res. **181**, 545–552 (1977)

Linquette, M., Fossati, P., Decoulx, M., Lefebvre, J., Lainé, E.: Adénomes thyréotropes. In: Les adénomes hypophysaires sécrétants, p. 155. Paris: Masson 1971

McCormick, W.F., Halmi, N.S.: Absence of chromophobe adenomas form a large series of pituitary tumors. Arch. Path. **92**, 231–238 (1971)

Moriarty, G.C.: Adenohypophysis: ultrastructural cytochemistry. A review. J. Histochem. Cytochem. **21**, 855–894 (1973)

Moriarty, G.C.: Immunocytochemistry of the pituitary glycoprotein hormones. J. Histochem. Cytochem. **24**, 846–863 (1976)

Moriarty, G.C., Tobin, R.B.: Ultrastructural immunocytochemical characterization of the thyrotroph in rat and human pituitaries. J. Histochem. Cytochem. **24**, 1131–1139 (1976)

Mornex, R., Tommasi, M., Curé, M., Farcot, J., Orgiazzi, J., Rousset, B.: Hyperthyroidie associée à un hypopituitarisme au cours de l'évolution d'une tumeur hypophysaire sécrétant TSH. Ann. Endocr. (Paris) **33**, 390–396 (1972)

Mösli, P., Hedinger, Chr.: Nodulaire Hyperplasie und Adenome des Hypophysenvorderlappens bei Hypothyreose. Acta. Endocr. (Kbh.) **58**, 507–520 (1968)

Peillon, F., Racadot, J., Oliver, L., Racadot, O., Vila-Porcile, E.: Les adenomas à prolactine. Structure et corrélations anatomo-cliniques dans 91 cas. Ann. Endocr. (Paris) **36**, 277–278 (1975)

Pelletier, G., Robert, F., Hardy, G.: Identification of human anterior pituitary cells by immunoelectron microscopy. J. Clin. Endocrinol. Metab. **46**, 534–542 (1978)

Racadot, J., Vila-Porcile, E., Oliver, L., Peillon, F.: Electron microscopy of pituitary tumors. Progr. Neurol. Surg. **6**, 95–141 (1975)

Rosai, J., Levine, G.D.: Tumors of the thymus. In: Atlas of tumor pathology, Fasc. 13. Washington: Armed Forces Institute of Pathology 1976

Saeger, W.: Hypophysenadenome bei Galactorrhoe. Licht- und elektronenoptische Untersuchungen. Virchows Arch. A Path. Anat. and Histol. **368**, 123–139 (1975)

Saeger, W.: Die Hypophysentumoren. Veröffentlichungen aus der Pathologie, Heft 107. Stuttgart-New York: G. Fischer 1977

Samaan, N.A., Osborne, B.M., Mackay, B., Leavens, M.E., Duello, T.M., Halmi, N.S.: Endocrine and morphologic studies of pituitary adenomas secondary to primary hypothyroidism. J. Clin. Endocrinol. Metab. **45**, 903–911 (1977)

Schelin, U.: Chromophobe and acidophil adenomas of the human pituitary gland. A light and electron microscopic study. Acta pathol. microbiol. scand. Suppl. **158**, 5–80 (1962)

Schechter, J.: Electron microscopic study of human pituitary tumors. I. Chromophobic adenomas. Am. J. Anat. **138**, 371–386 (1973)

Siperstein, E.R., Miller, K.J.: Further cytophysiologic evidence for the identity of the cells that produce adrenocorticotrophic hormone. Endocrinology **86**, 451–486 (1970)

Solcia, E., Capella, C., Buffa, R., Frigerio, B.: Histochemical and ultrastructural studies on the argentaffin and argyrophil cells of the gut. In: Chromaffin, enterochromaffin and related cells, (R.E. Coupland and T. Fujita, eds.), pp. 223–225. Amsterdam: Elsevier Scientific Publishing Company 1976

Solcia, E., Capella, C., Buffa, R., Frigerio, B., Fontana, P., Usellini, L.: Tumori dell'adenoipofisi: diagnosi morfologica e classificazione. Pathologica **69**, 333–346 (1977)

Sternberger, L.A., Hardy, P.H., Jr., Cuculis, J.J., Mayer, H.G.: The unlabeled antibody enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antiperoxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. **18**, 315–333 (1970)

Stoeckel, M.E., Dellmann, H.D., Porte, A., Klein, M.J., Stutinsky, F.: Corticotrophic cells in the rostral zone of the pars intermedia and in the adjacent neurophypophysis of the rat and mouse. An electron microscopic study. Z. Zellforsch. **136**, 97–110 (1973)

Uhl, G.R., Kuhar, M.G., Snyder, S.H.: Neurotensin: immunohistochemical localization in rat central nervous system. Proc. Natl. Acad. Sci. USA **74**, 4059–4063 (1977)

Vassallo, G., Capella, C., Solcia, E.: Grimelius' silver stain for endocrine cell granules, as shown by electron microscopy. Stain Technol. **46**, 7–14 (1971)

Woodtli, W., Hedinger, Chr.: Histologic characteristics of insulinomas and gastrinomas. Virchows Arch. A Path. Anat. and Histol. **371**, 331–350 (1976)

Woolf, P.D., Schenk, E.A.: An FSH-producing pituitary tumor in a patient with hypogonadism. J. Clin. Endocr. Met. **38**, 561–568 (1974)

Received July 11, 1978