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Transdifferentiation of somatotrophs to thyrotrophs in the pituitary of patients with protracted primary hypothyroidism

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Abstract In patients with protracted primary hypothyroidism, the pituitary is enlarged due to the lack of feedback inhibition by thyroid hormone. In the present work, adenohypophysial biopsies from three women with protracted primary hypothyroidism were investigated by routine histology, immunocytochemistry, double immunostaining, immunoelectron microscopy, and combined immunocytochemistry – in situ hybridization. These methods confirmed the presence of massive thyrotroph hyperplasia and the formation of "thyroidectomy" or "thyroid deficiency" cells. A number of thyroidectomy cells were found to be immunoreactive for growth hormone (GH). Double immunostaining and immunoelectron microscopy revealed the presence of bihormonal cells containing both GH and thyroid stimulating hormone (TSH). Immunostaining combined with in situ hybridization revealed GH immunoreactive cells expressing TSH mRNA as well as TSH immunopositive cells expressing GH mRNA. Our findings provide conclusive evidence that somatotrophs may transform to thyrotrophs. Thus, in addition to multiplication of thyrotrophs, transdifferentiation of GH cells to thyrotrophs contributes to the increase of TSH-producing cells. The presence of such bihormonal cells best termed "thyrosomatotrophs" supports the concept that adenohypophysial cells are not irreversibly committed to the production of one single hormone and that their phenotype can change in response to functional demand.

Key words Pituitary · Human · Somatotroph · Thyrotroph hyperplasia · Immunocytochemistry

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Introduction

With respect to the pituitary and other endocrine organs, the "one cell–one hormone" theory has been dogma for decades. Furthermore, it was claimed that the phenotype of adenohypophysial cells was unalterable, that they were irreversibly committed to the production of one single hormone [47]. The emergence of new technologies over the past decade have demonstrated exceptions to this concept and, thus, the "one cell–one hormone" theory has been abandoned. For instance, Childs et al. [12] found that individual cells of rodent pituitary can contain both adenocorticotropic hormone (ACTH) and follicle-stimulating hormone (FSH) simultaneously. An explanation may be found in a mechanism termed "transdifferentiation" [27], i.e., that, on demand, cells of one hormonal type may shift to another, often with respective morphologic alteration. Two of these transdifferentiation examples can be noted. Based on their studies in the rat, Frawley and associates [22] concluded that, depending on demand, somatotrophs can reversibly transform to lactotrophs. Similarly, in a prior study, our group demonstrated that in rats made hypothyroid by propylthiouracyl (PTU) administration, somatotrophs transform to stimulated thyrotrophs or so-called "thyroidectomy cells" [27]. This transdifferentiation was similarly reversible, since discontinuation of treatment led to the disappearance of thyroidectomy cells and the reappearance of somatotrophs.

Exploring the question of whether such transformations can occur in the human pituitary, Severinghaus [53] reported early studies claiming its occurrence with respect to various adenohypophysial cell types. However, conclusive evidence awaited new technologies. Our recent studies of the human pituitary in pregnancy indicated that somatotrophs are recruited to form lactotrophs [61]. The transformation was clearly reversible, since, after delivery, as the number of lactotrophs decreased, the number of cells both immunoreactive for growth hormone (GH) and expressing the prolactin (PRL) gene disappeared. Human pathology provides several other examples of cell transformation [1, 43].

A related process appears to underlie cell differentiation in pituitary adenomas of plurihormonal type, the cytogenesis of which may be explained by the hypothesis that, during neoplastic progression, the adenomatous cells produce other functionally and biochemically distinct hormones. A more dramatic example of transdifferentiation is metaplasia. It has been shown that the cells of pheochromocytoma [20] and various other neuroendocrine neoplasms, including pituitary adenoma [28], can undergo neuronal transformation.

While studying the morphology of enlarged pituitaries in patients with protracted primary hypothyroidism, we observed the transformation of somatotrophs to stimulated thyrotrophs called "thyroidectomy" or "thyroid deficiency" cells in a manner similar to the changes previously observed in hypothyroid rats. Herein, we describe for the first time the development of thyrosomatotrophs as well as the transformation of somatotrophs to thyrotrophs in the human adenohypophysis.

Material and methods

Pituitary tissue was obtained at transphenoidal surgery for suspected adenomas from three women. Case 1, aged 26 years, had elevated levels of blood thyroid-stimulating hormone (TSH) as well as hyperprolactinemia (73 ng/ml). Case 2, a 29-year-old woman, was not suspected of having hypothyroidism. Her PRL levels was elevated (50 ng/ml) and she was thought to have a PRL-producing adenoma. Case 3, a 51-year-old woman, had a 12-year history of primary hypothyroidism associated with multinodular goiter. She was undergoing thyroid hormone replacement (0.1 mg thyroxin daily); nevertheless, she developed pituitary enlargement. Imaging techniques documented an enhancing sellar mass in cases 1 and 2, whereas, in case 3, the enlarged gland extended into the suprasellar space. Tissue fixed in 10% buffered formalin, dehydrated in graded ethanol, paraffin-embedded, and sectioned at 5 µm was utilized for histology and immunocytochemistry. Additional specimens were fixed overnight at 4°C in 2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.4), osmicated for 1 h at room temperature with 1% OsO_4 in Millonig's buffer (pH 7.4), dehydrated in a graded ethanol series, embedded in an Epon-Araldite mixture, and examined on a Philips 410LS electron microscope.

Histology and immunocytochemistry

For histology, the sections were stained with hematoxylin and eosin (H+E), periodic acid-Schiff (PAS), and the Gordon-Sweet silver method for reticulin. Immunocytochemical studies employed the streptavidin-biotin-peroxidase complex technique [29]. Details of immunocytochemistry for adenohypophysial hormones, including source of antibodies, dilutions, duration of exposure, and control procedures are described in previous publications [32, 33]. Double immunocytochemical staining was applied to paraffin or semi-thin plastic sections to identify the presence of bihormonal cells immunoreactive for GH and βTSH. Antibodies to both hormones were obtained form the National Pituitary Hormone Distribution Agency (Bethesda, Md.) and were used in specified maximal dilutions (human anti-βTSH 1:400 and human anti-GH 1:500). Semi-thin sections were etched for 15 min in a saturated concentration of potassium hydroxide in ethanol and then treated in 1% sodium metaperiodate for 5 min for removal of OsO_4 [4]. For double staining, paraffin and semi-thin plastic sections were incubated with βTSH antibody and then exposed to the streptavidin-biotin-peroxidase complex, diaminobenzidine serving as chromogen. Subsequently, the GH antibody was applied followed by the alkaline phosphatase/anti-alkaline phosphatase method. The reaction product was visualized using nitroblue tetrazolium/5-bromo-chloro-3-indolylphosphate (NBT/BCIP).

In situ hybridization

The oligonucleotide probes used were d(GAC TAT AGT GTC TGC ATA CAT GAA GCC ATC AAG) for human βTSH and d(GGC GCG GAG CAT AGC GTT GTC) for human GH [51]. These were synthesized by means of the solid-phase cyanoethyl phosphoramidiete method [7] on an automated DNA synthesizer (Gene Assembler) manufactured by Pharmacia, and were then purified by means of gel electrophoresis on 20% polycrylamide gels.

The in situ hybridization (ISH) reactions with radioactive probes were performed using ^{35}S-labeled oligonucleotide probes, labeled at their 3'-end as previously described [36, 37]. Hybridization of paraffin sections with 1×10^6–2×10^6 cpm per slide at 42°C for 18 h was followed by washings with 2× standard saline citrate (SSC) to 0.5×SSC and by autoradiography for 1–2 weeks. The combined ISH–immunocytochemistry procedures were carried out after washing with 2×SSC using h-βTSH and h-GH antisera (working dilution), a procedure described previously [35, 36, 39]. Controls for the double-labeling experiment with combined ISH–immunocytochemistry consisted of RNAse predigestion, omission of primary antibodies, and localization of the mRNAs and their respective products within the same cells.

Immunoelectron microscopy

To assess subcellular localization of βTSH and GH in thyrosomatotrophs, immunoelectron microscopy was performed using the double immunogold method of Bendayan [3]. Before labeling, ultra-thin sections were pretreated for 1 h in a saturated aqueous solution of sodium metaperiodate [4]. One side of the grids was then incubated at 37°C for12 h with specific antisera directed toward either GH or βTSH. Subsequently, the grids were treated at 37°C for 1 h with gold labeled, goat anti-rabbit IgG (Biocell Research Laboratories, Cardiff, UK), particle diameters being either 10 nm or 20 nm. Between each step, grids were washed in 0.2 M phosphate buffered saline (pH 7.5) admixed with 0.2% cold water fish gelatin (Sigma-Aldrich Ltd, Oakville, Ontario, Canada). For double immunostaining, the procedure was repeated on the other side of the grid using another specific antibody and a colloidal gold conjugate of a different size. After immunolabeling, sections were stained with uranyl acetate and examined on a Philips 410 LS electron microscope.

Fig. 1 Thyrotroph hyperplasia, causing enlargement, distortion and confluence of acini. Gordon-Sweet reticulin technique. Original magnification ×100

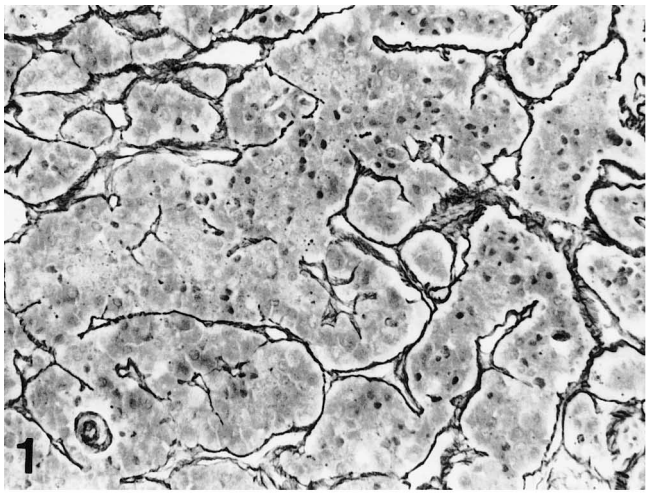
Fig. 2 In the area of the thyrotroph hyperplasia, the number of growth-hormone (GH) immunoreactive cells is markedly reduced. Immunostaining for GH. Original magnification ×400

Fig. 3 Hyperplastic thyrotrophs display varying degrees of immunoreactivity. Immunostaining for β-thyroid-stimulating hormone (TSH). Original magnification ×400

Fig. 4 Immunostaining for the α-subunit is evident in numerous adenohypophysial cells. Original magnification ×400

Fig. 5 Double immunostaining on semi-thin section shows co-expression (*arrow*) of growth hormone (GH) (*blue*) and β-thyroid-stimulating hormone (TSH) (*brown*). Original magnification ×1000

Fig. 6 Combined immunohistochemistry for growth hormone (GH) and in situ hybridization for β-thyroid-stimulating hormone (TSH) demonstrates the presence of silver grains representing β-TSH mRNA in GH immunopositive cells (*arrow*). Original magnification ×1000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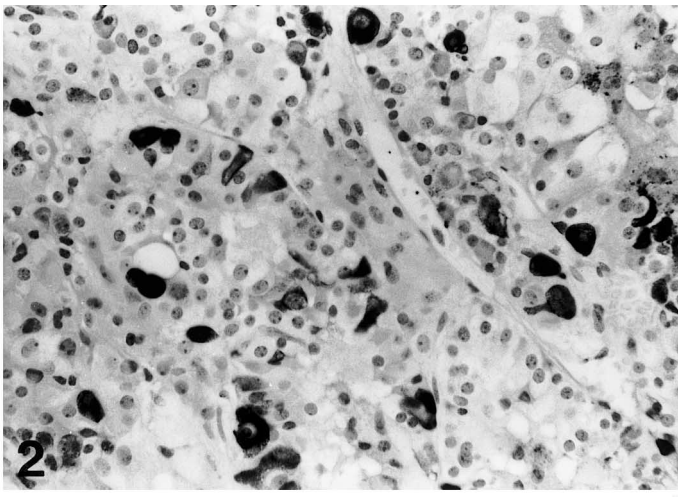
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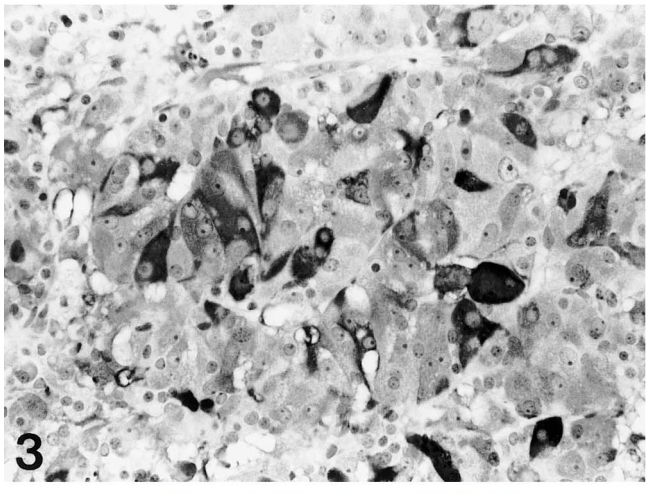
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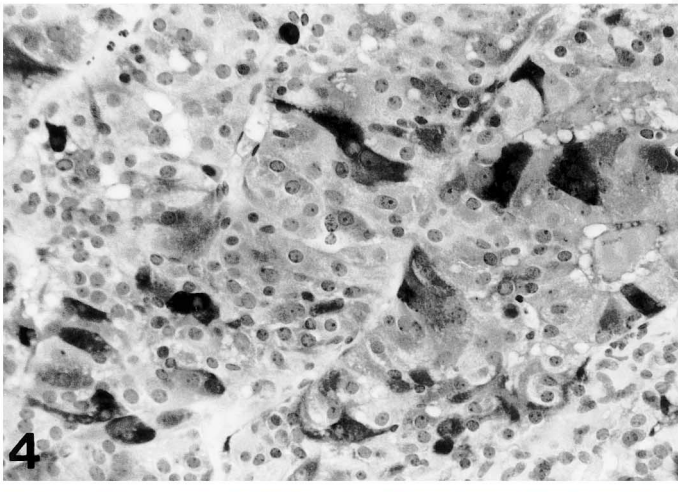
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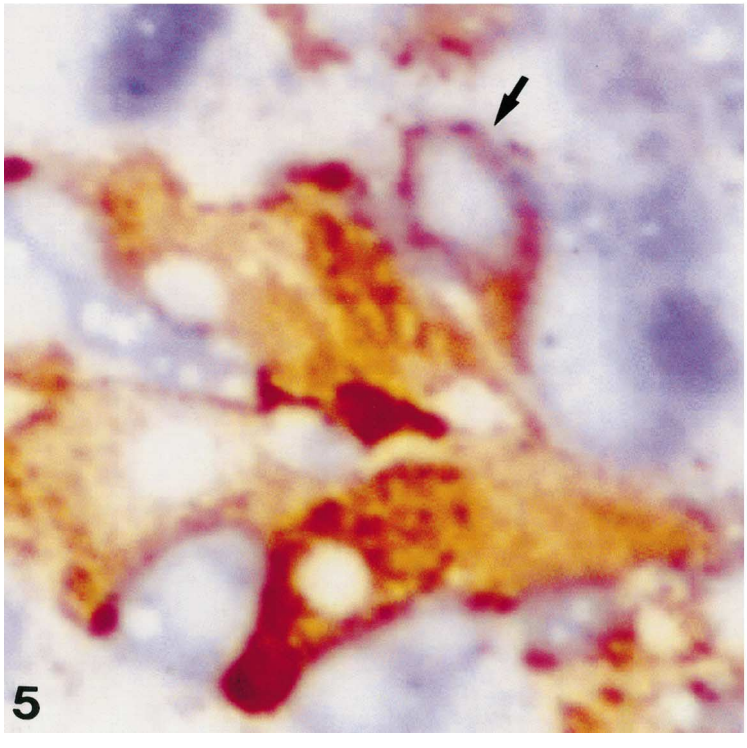
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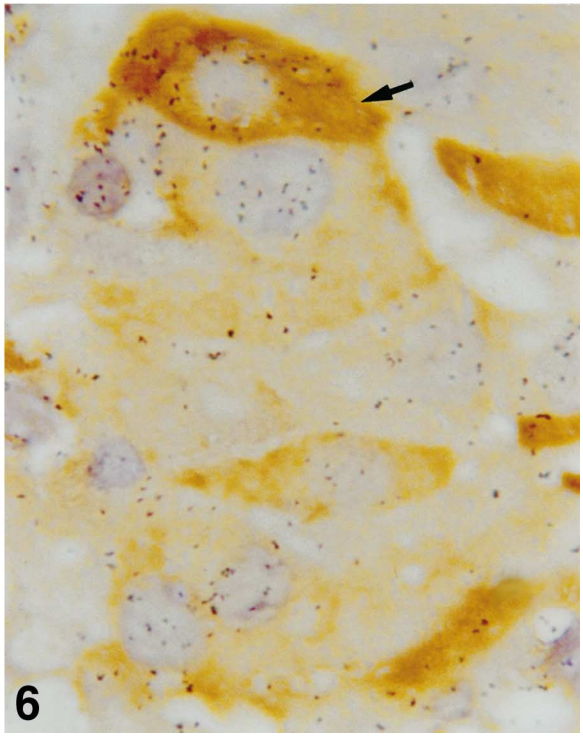
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Results

Light microscopy

Instead of the clinically suspected adenoma, histology revealed massive nodular thyrotroph cell hyperplasia in all three cases. The Gordon-Sweet silver method for reticulin showed that the characteristic acinar pattern of the human adenohypophysis was preserved in all samples analyzed. Acini were surrounded by a delicate capillary-containing reticulin-fiber network. Individual acini were, however, larger than usual and populated by larger numbers of adenohypophysial cells (Fig. 1). Neither cellular nor nuclear pleomorphism was evident and mitotic figures were lacking. The most striking finding was the accumulation of so-called thyroidectomy or thyroid deficiency cells, large pale cells with eccentric nuclei and abundant vacuolated cytoplasm. These cells are characteristic in the pituitaries of rats made hypothyroid by chemical, surgical or radioactive thyroidectomy and in those patients with untreated protracted primary hypothyroidism [19, 23, 27, 45, 48, 56]. Such cells were practically PAS negative, although large PAS-positive globules corresponding to lysosomes were often seen in their cytoplasm. In addition, they lay intermingled with other adenohypophysial cells, one distinctly PAS positive and negative as well as varying in immunotype, thus proving that the lesion under study was not monomorphous, i.e., adenomatous.

The streptavidin-biotin peroxidase complex method demonstrated that GH immunopositive cells appeared to be strongly decreased in number (Fig. 2). In contrast, many cells, including obvious thyroidectomy cells, were βTSH and α-subunit immunoreactive (Fig. 3 and Fig. 4). In one instance, multifocal lactotroph hyperplasia was also present, a finding not evident in other cases. The proportion of corticotrophs was within the normal range. Although gonadotrophs were not numerous, their apparent decrease could be due to the marked increase in thyrotrophs. The double-immunolabeling method showed some thyroidectomy cells to be immunoreactive for GH, thus suggesting a transformation of somatotrophs to thyroidectomy cells. Quantification confirmed the existence of a high proportion of bihormonal cells, 30% of βTSH immunopositive cells being GH immunoreactive as well. Such “thyrosomatotrophs” included not only cells with the appearance of thyroidectomy cells but also smaller cells with scant, nondescript cytoplasm (Fig. 5).

Results of combined immunocytochemistry–ISH corroborated the findings of double immunocytochemistry in that cells expressing βTSH and GH mRNA as well as GH and βTSH mRNA were identified, the former being greater in number (Fig. 6).

Electron microscopy

Sampled acini were expanded and contained large polar cells of low electron density, displaying all the characteristics of thyroidectomy cells, in addition to spherical, euchromatic nuclei and small nucleolus, their abundant cytoplasm possessing dilated profiles of rough endoplasmic reticulum (RER), which varied greatly from one cell to another. Golgi complexes were large and harbored relatively few forming secretory granules. Spherical, drop-shaped, or slightly irregular secretory granules, measuring up to 200 nm, were relatively few in number in most thyroidectomy cells, thus explaining their rather modest βTSH immunoreactivity. Heterogeneous electron-dense lysosomes were frequently encountered. Although stimulated thyrotrophs represented the large majority of cells, other cell types also lay intermingled. Considerably smaller, well-granulated TSH cells were far less common and showed only mild RER dilation. Scattered middle-sized cells harboring large, electron-dense spherical granules as well as small variably electron-dense secretory granules were also encountered (Fig. 7). Somatotrophs appeared reduced in number and varied in size, shape, and granularity. One contingent of somatotrophs appeared normal in size, electron density, and cytoplasmic features. A large number of small somatotrophs were characterized by a more heterochromatic nucleus, a narrow rim of cytoplasm, and a single layer of secretory granules beneath the plasmalemma. Yet another and sizeable group of somatotrophs was reduced in size and showed markedly increased electron density (Fig. 8). Scattered lactotrophs were large, their abundance of RER signifying a stimulated state. In contrast to the histologic specimen, samples examined by electron micros-

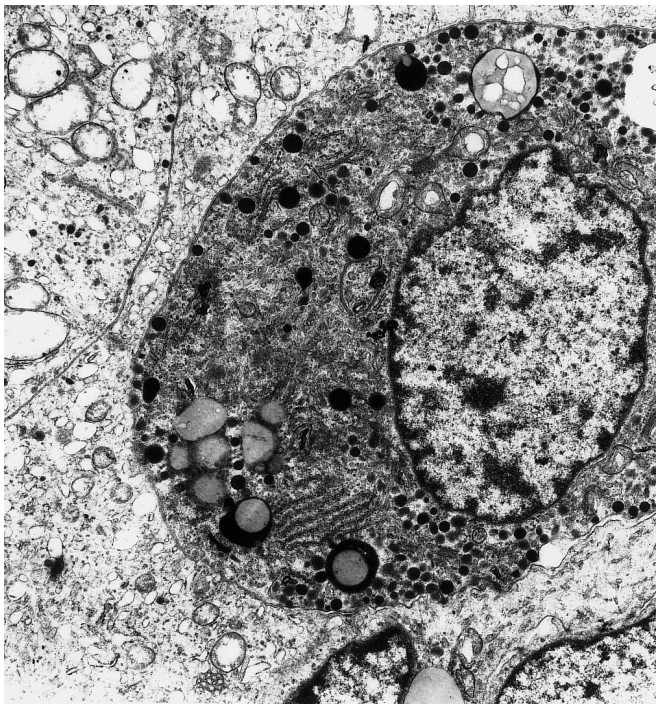

Fig. 7 Thyrosomatotroph in early phase of transformation is depicted. Large somatotroph-type granules are still present, but the majority of secretory granules are small, similar to those of thyrotrophs. Original magnification ×5720

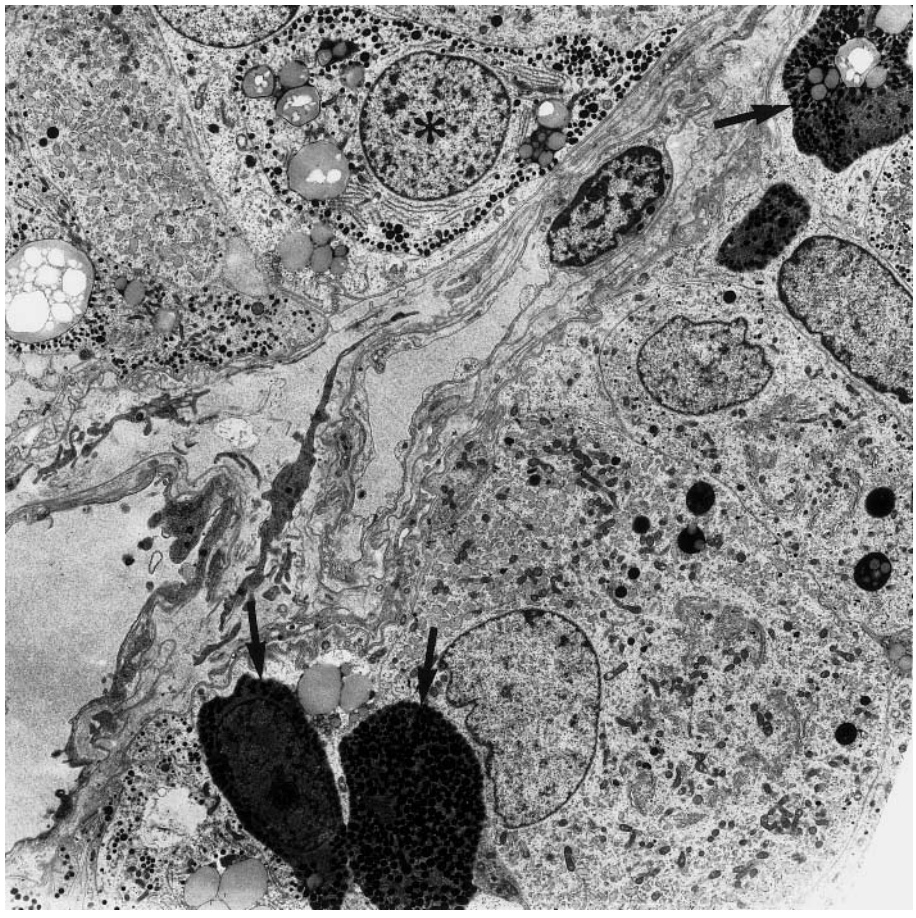

Fig. 8 Low-power electron micrograph of thyrotroph hyperplasia. The majority of cells represent thyroidectomy cells. Note the three electron-dense growth-hormone (GH) cells undergoing involution (*arrowheads*) and one displaying regular features (*asterisk*). Original magnification ×4000

copy contained no nodules of hyperplastic lactotrophs. Corticotrophs appeared normal in frequency, size, and morphology. Cells readily identifiable as gonadotrophs were much less numerous than usual.

The double immunogold method confirmed the presence of bihormonal cells containing both GH and βTSH. Furthermore, based on morphologic characteristics and immunogold labeling of the secretory granules, three types of thyrosomatotrophs could be identified. Of these, the first and most numerous type of thyrosomatotroph contained small secretory granules (approximately 180 nm diameter) primarily bihormonal, labeled for both GH and βTSH, and lesser numbers immunoreactive for either GH or βTSH alone (Fig. 9). The other two types were least frequent. These cells possessed two varieties of large, approximately 300-nm diameter, secretory granules, the majority containing growth hormone alone and the remainder both βTSH and GH (Fig. 10). Cells belonging to the third cell type were more numerous than the second and contained two kinds of secretory granules. Large, approximately 300-nm diameter secretory granules immunolabeled for GH were only few, whereas small, approximately 180-nm diameter secretory granules possessed only βTSH or were bihormonal and reactive for both GH and βTSH (Fig. 11).

Since the thyrotrophs and somatotrophs encountered showed very different and distinctive morphologic characteristics, the identification of thyrosomatotroph variants was regarded as the morphological manifestation of the steps in the transdifferentiation of somatotrophs to thyrotrophs.

Discussion

The mechanisms regulating pituitary cytogenesis and cell proliferation are complex and poorly understood. Different hypotheses have been introduced to explain the nature and origin of cells contributing to pituitary hyperplasia in such varied settings as pregnancy and lactation [58], estrogen treatment [2, 57], including protracted primary hypothyroidism [31, 56], and Addison's disease [55], as well as in rare instances of GH-releasing hormone [50], or corticotropin releasing hormone (CRH)-producing extrapituitary tumors [9]. Principal suggestions centered upon an increase in cell number due to multiplication of the stimulated cell type [62] and a differentiation of stem cells into mature cells of that type cells [47, 53, 67]. The presence of mitoses in hyperplastic pituitary cells [62] and evidence suggesting that most pituitary adenomas are monoclonal [24, 59] led to the assumption that hyperplasia of a pituitary cell type can only be attributed to proliferation of such cells. In contrast, others have suggested that cells of one line might trans-

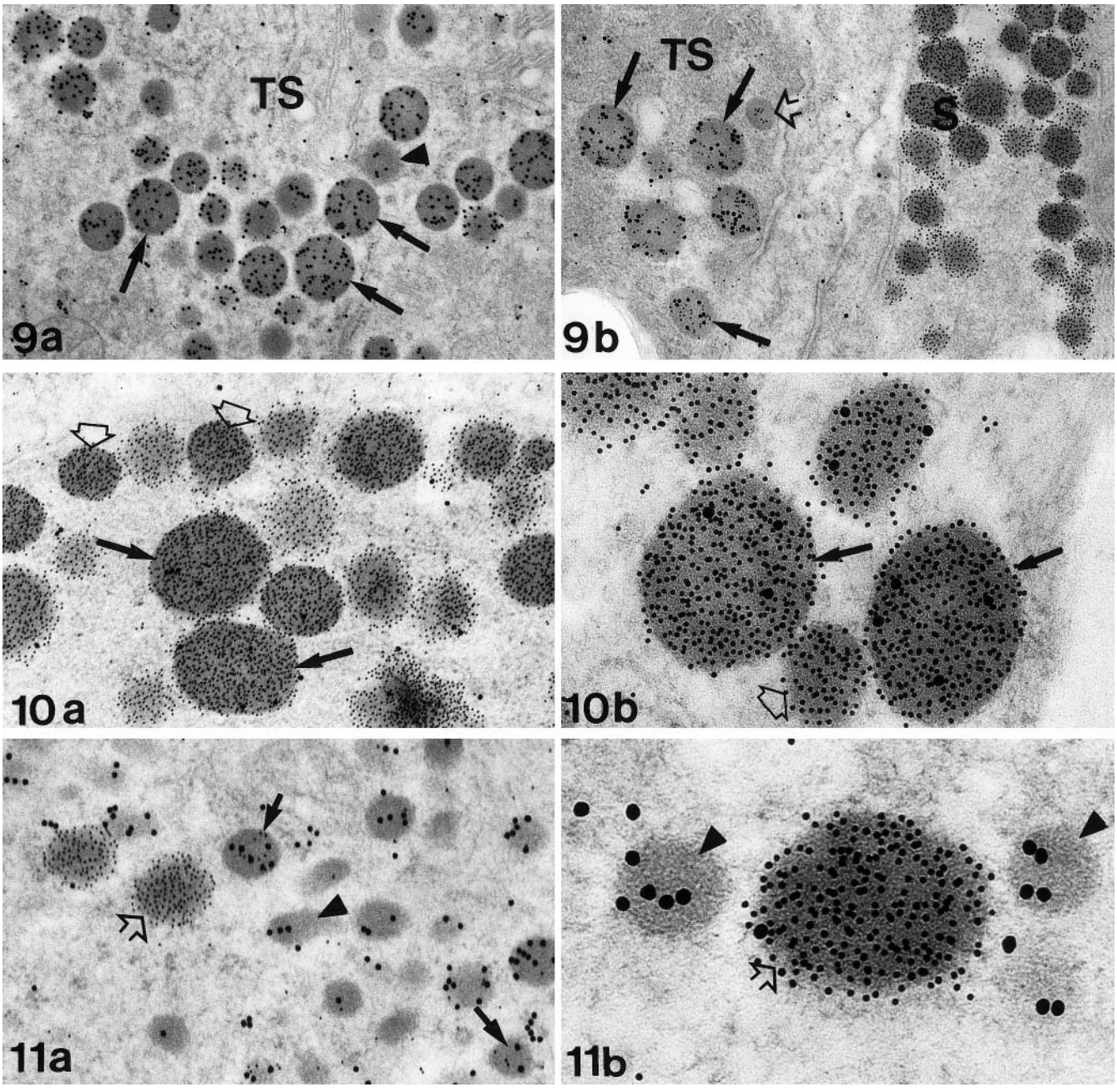

Fig. 9 Double immunogold labeling shows thyrosomatotrophs (*TS*) containing bihormonal granules (*arrows*) as well as monohormonal granules labeled either for growth hormone (GH) (*open arrows*) or β-thyroid-stimulating hormone (TSH) (*arrowheads*). Somatotroph (*S*). GH 10 nm, β-TSH 20 nm. Original magnification: **a** ×35,500; **b** ×27,690

Fig. 10 Double immunogold labeling demonstrates thyrosomatotrophs containing bihormonal granules (*arrows*) and growth hormone (GH) monohormonal granules (*open arrows*). GH 10 nm, β-TSH 20 nm. Original magnification: **a** ×22,000; **b** ×96,600

Fig. 11 Double immunogold labeling shows thyrosomatotrophs containing bihormonal granules (*arrows*) as well as growth hormone (GH) (*open arrows*) and β-thyroid-stimulating hormone (TSH) (*arrowheads*) monohormonal granules. GH 10 nm, β-TSH 20 nm. Original magnification: **a** ×36,920; **b** ×94,500

form to those of another, thus acquiring their morphologic features and secretory capacity – a process termed "transdifferentiation" [27]. Such an interconversion is viewed not as a direct process but as occurring through bihormonal transitional cells exhibiting functional components common to both cells [21, 44]. The occurrence in different species of adenohypophysial cells producing more than a single hormone, e.g., GH/PRL [21], GH/luteinizing hormone (LH) [11, 15, 16], GH/TSH [27], ACTH/PRL [42], ACTH/FSH [41]or ACTH/TSH [13], suggests that bihormonal cells play an adaptive role in situations of physiologic demand for hypersecretion of a specific hormone. Indeed, it was Childs [10] who introduced the concept that monohormonal cells may be "reserve cells" and are, in fact, multipotential with respect to hormonogenesis.

To our knowledge, the present study is the first to document the formation of thyrosomatotrophs in the human pituitary. Under normal conditions, both somatotrophs and thyrotrophs are distinct and exhibit marked differences in their histologic appearance, and in immunocytochemical as well as ultrastructural features [26]. Furthermore, they produce two very distinct hormones, ones differing in chemical composition, immunoreactivity and biologic action [26]. Recent advances in molecular genetics and endocrinology lead to a deeper insight, however. The facts that Pit-1, a transcription factor necessary for the expression of both the GH and βTSH-subunit genes [5, 30, 34, 60], is produced in both somatotrophs and thyrotrophs [38, 60] and thyroid hormones exert their feedback effect (either directly upon the pituitary or via the hypothalamus) upon both thyrotrophs and somatotrophs [49] makes the existence of thyrosomatotrophs easier to understand and accept.

Although division of pre-existing thyrotrophs and differentiation of stem cells most likely contribute to the development of new thyrotrophs, our results indicate that transdifferentiation also plays a role in thyrotroph hyperplasia as seen in protracted primary hypothyroidism. The present results are in agreement with those previously reported in experimental rat studies of Horvath et al. [27]. Studying PTU-induced hypothyroidism, these authors clearly demonstrated transdifferentiation of somatotrophs to thyrotrophs. The process was reversible, since thyrotrophs reverted to somatotrophs after discontinuation of PTU administration. These findings are in keeping with endocrine data in patients with primary hypothyroidism wherein thyroxin replacement causes not only a decrease in plasma TSH levels but an enhanced GH response to GRH stimulation as well [64, 65]. Indeed, numerous studies have concluded that thyroid hormones are important regulators of both GH and TSH gene expression. It was found that triiodothyronine (T3) stimulates GH and inhibits TSH gene expression [40]. Rodriguez-Garcia et al. [46] reported that the mechanisms underlying T3 action on somatotrophs and thyrotrophs are operational at early phases of fetal development, thus suggesting that T3 is involved in the formation and/or maintenance of somatotroph and thyrotroph phenotypes. T3 regulates GH and TSH gene expression by interacting with nuclear T3 receptors (TR) which then bind to TR responsive elements in the 5'-flanking regions of the GH and TSH genes [6, 8, 17]. Increased TR-mRNA expression has been reported in the pituitary of hypothyroid rat [14]. Collectively, these findings may correlate with the pituitary's vigorous response to thyroid hormone replacement in patients with primary hypothyroidism [52].

Demonstration of the presence of thyrosomatotrophs in the human pituitary supports the concept that somatotrophs exhibit a certain "plasticity". This is perhaps linked to the well-known fact that somatotrophs are the most abundant cell in the human pituitary. Somatotroph plasticity may well explain the co-expression of GH with PRL [21], LH [11, 15, 16] or TSH [27] within the same cell, as well as the transformation of adenomatous somatotrophs to nerve cells [28]. In pituitary tumors, changes in phenotype are irreversible in that the development of new characteristics leads to tumor cell heterogeneity, a process likely due to mutation or gene deletion. Such "lineage infidelity" does not occur in nontumorous cells. By contrast, transdifferentation in the nontumorous pituitary is a reversible process apparently due to transcriptional or post-transcriptional changes.

As previously noted, Pit-1 is a pituitary-specific transcription factor necessary for the activation of GH and βTSH-subunit genes [5, 30, 34, 60]. However, Pit-1 alone cannot explain why GH and βTSH are expressed in two distinct, Pit-1-containing pituitary cell types. Different GH and βTSH promoter activities arise from synergistic interactions between Pit-1 and numerous other promoter-specific transcription factors. Of these, such interactions as those between Pit-1 and TR or estrogen receptor are strongly dependent on T3 and estrogen concentration [25, 54, 60]. Thus, hypothyroidism may cause changes in Pit-1 synergies that influence GH and βTSH transcription. Such a mechanism could explain transdifferentiation of somatotrophs to thyrotrophs, a process wherein thyrosomatotrophs represent an obligatory transitional cell.

In addition to Pit-1 transcription factor, several other homeobox genes including Prop1, LH×3, LH×4, and Pt×1 are required for orderly pituitary cell lineage development [18, 66]. These transcription factors are dependent on each other for development, i.e., Prop1 is required for determination of Pit-1 lineage. Recent studies have also provided data on the molecular basis for generating various pituitary cell phenotypes from common precursors by sequential phases of signaling events [63]. The interaction of various gene products such as BMP4, Wnt5a and FGF8 represent specific signaling in pituitary cell development. Alterations in these interacting genes and their gene products could contribute to altered differentiation or transdifferentiation with proliferative stimuli such as TSH cell hyperplasia in human pituitaries.

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