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The pars tuberalis of the human pituitary

A histologic, immunohistochemical, ultrastructural and immunoelectron microscopic analysis

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Summary. Forty autopsy pituitaries were studied to elucidate the histology, immunocytology and ultrastructure of pars tuberalis in subjects with normal and abnormal endocrine homeostasis. Pars tuberalis consisted mainly of gonadotrophs interspersed with few corticotrophs and thyrotrophs, histologically resembling those of pars distalis. Somatotrophs and lactotrophs were not identified. There were no histologic differences attributable to age or sex. In cases of glucocorticoid excess, pars tuberalis corticotrophs showed Crooke's hyalinization. Following castration or hypophysectomy, pars tuberalis gonadotrophs exhibited more intense immunostaining for FSH[1] and LH than did normals. Ultrastructural analysis revealed gonadotrophs and corticotrophs showing no evidence of active secretion; immunoelectron microscopy demonstrated FSH, LH and ACTH in secretory granules. By light microscopy, squamous nests, often identified in pars tuberalis, were positive for immunoreactive keratin; cells at their periphery contained FSH, LH or ACTH, indicating derivation of nests by squamous metaplasia from gonadotrophs and corticotrophs. By electron microscopy, clusters of epithelial cells containing desmosomes and tonofilaments were surrounded by granulated gonadotrophs.

Human pars tuberalis cells represent mainly a subpopulation of gonadotrophs possessing all organelles required for synthesis and storage of hormones but showing ultrastructural features of functional inactivity; the reasons for this inactivity and for the formation of squamous nests remain unexplained.

Key words: Pituitary – Pars tuberalis – Gonadotrophs – Squamous nests – Ultrastructure

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1 Abbreviations used in this article include the following: FSH, follicle stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone; ACTH, adrenocorticotrophic hormone; LHRH, luteinizing hormone releasing hormone.

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The pars tuberalis of the human pituitary is composed of clusters of cells histologically similar to those of the pars distalis. Romeis (1940) regarded these cells as less differentiated than those of the adenohypophysis, since with the usual differential staining techniques they appear chromophobic.

The finding of basophils and the absence of acidophils was first noted in the distal portion of the pars tuberalis by Romeis (1940). With the advent of immunofluorescence, Midgley (1966) identified cells containing LH in the inferior pars tuberalis. Baker (1977), using the immunoperoxidase technique, found that gonadotrophs constitute the major cellular component; other adenohypophysial cell types, when present, appeared in small groups which Baker (1977) attributed to displacement of primordial tissue of pars distalis during embryogenesis. Osamura and Watanabe (1978) confirmed the predominance of gonadotrophs in the pars tuberalis using the immunoperoxidase method, however, they also found occasional thyrotrophs and corticotrophs. These authors suggested that squamous metaplasia of parenchymal cells in the pars tuberalis was associated with loss of hormone-secreting activity.

This portion of the adenohypophysis is well situated to receive portal blood from the hypothalamus, however, the functional activity and morphologic response of these cells have not been adequately documented. Moreover, the occurrence of squamous metaplasia in the cells of the pars tuberalis has been the subject of very limited investigation. Ours represents the first study of the human pars tuberalis which utilizes electron microscopy and ultrastructural immunohistochemistry to resolve these questions.

Materials and methods

The material consisted of the pars tuberalis of 40 pituitaries of adults taken from the autopsy files of St. Michael's Hospital. Equal numbers of males and females were examined and the ages ranged from 24 to 86 years.

Specimens for light microscopy included pituitaries of 3 patients with glucocorticoid excess of more than 1 year's duration, 6 patients with a history of diabetes mellitus of at least 8 years' duration, and 8 patients who had undergone castration at least 1 year prior to death following trauma or for treatment of endometrial hyperplasia, ovarian, breast and prostatic carcinoma. In 2 cases, the residual pars tuberalis following hypophysectomy was obtained; hypophysectomy was performed for the treatment of severe diabetes mellitus 1 month prior to death in one case, and for treatment of disseminated breast carcinoma 2 years before autopsy in the other. Pars tuberalis from 16 patients with no endocrine abnormality were examined. Tissues were obtained within 16 h of death in 26 cases, and between 16 and 30 h of death in 9 cases. They were fixed in 10% buffered formalin and embedded in paraffin. For routine examination, sections were stained with hematoxylin-eosin, hematoxylin-phloxine-saffron and the periodic acid Schiff (PAS) technique.

The immunoperoxidase technique was performed for the localization of adenohypophysial hormones as described previously (Kovacs et al. 1981). Antibodies were obtained against human growth hormone[1], prolactin[2], 1-39 ACTH[3], α-endorphin[4], α-subunit of glycoprotein

1 (Wellcome Reagents Ltd., Beckenham, Great Britain)
2 (donated by Dr. H. Friesen, University of Manitoba, Winnipeg, Canada)
3 (Wellcome Reagents Ltd., Beckenham, Great Britain)
4 (donated by Dr. J. Polak, The Hammersmith Hospital, London, Great Britain)

hormones[5], β-FSH and β-LH[6], and β-TSH[7]. Immunohistochemical localization of keratin (donated by Dr. R. Schlegel, Peter Bent Brigham Hospital, Boston, Massachusetts, USA) was also performed (Schlegel et al. 1980; Asa et al. 1981). In each case, the immunologic reaction was visualized using the horseradish peroxidase-antihorseradish peroxidase complex (Cappel Laboratories Inc., Cochranville, Pennsylvania, USA), and 3,3′-diaminobenzidine (Sigma Chemical Company, St. Louis, MI, USA). Immunohistochemical localization of 2 hormones on the same histologic section utilized both 3,3′-diaminobenzidine, and 4-chloro-1-naphtol (DeLellis 1981). The specificity of immunostaining was verified by replacing antisera with normal rabbit serum as well as phosphate buffered saline. Absorption with excess antigen was performed for antisera against growth hormone, prolactin, 1-39 ACTH and α-endorphin. No crossreaction was found toward other pituitary hormones. Data on absorption studies are available for antisera against β-FSH, β-LH and α-subunit from their sources and for antikeratin antiserum in the literature (Schlegel et al. 1980).

Electron microscopy was performed on the pars tuberalis of 5 additional patients with normal endocrine equilibrium. Small pieces of tissue were obtained within 5 hours post mortem, fixed in 2.5% glutaraldehyde in Sorensen's buffer, postfixed in 1% osmium tetroxide in Millonig's buffer, dehydrated in graded ethanol, processed through propylene oxide and embedded in epoxy resin. Semithin sections were stained with toluidine blue and appropriate areas selected for fine structural study. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

For electron microscopic immunocytochemistry, tissues from pars tuberalis of 4 cases were fixed in 2.5% glutaraldehyde, dehydrated in graded ethanol and embedded in epoxy resin. Consecutive ultrathin sections were mounted on nickel grids and exposed to the appropriate antisera to detect immunoreactive 1-39 ACTH, β-FSH, β-LH, and β-TSH. The immunologic reaction was visualized using horseradish peroxidase-antihorseradish peroxidase and 3,3′-diaminobenzidine using the technique of Moriarty et al. (1973).

Hormone extractions from pituitary tissues were not performed. Blood hormone levels were not available for correlation with histological findings.

Results

By light microscopy, the pars tuberalis consisted of clusters and single chromophobic cells, scattered between longitudinally oriented portal blood vessels and resembling cells of the pars distalis (Fig. 1 a); most cells stained positively with PAS. In all pituitaries, immunocytochemistry revealed that the pars tuberalis population was composed predominantly of gonadotrophs which contained α-subunit, β-FSH and β-LH (Fig. 1 b). Occasional corticotrophs and thyrotrophs were also identified. Somatotrophs and lactotrophs were not found within the pars tuberalis in any case examined. Six cases of diabetes mellitus showed similar findings to those of normal controls.

Amongst normal controls and diabetics, the ages ranged from 34 years to 80 years; 13 patients were male and 9 were female. All showed predominance of gonadotrophs and similar intensities of immunopositivity and, therefore, no histologic differences between sexes or attributable to age differences were found.

In 2 of 3 cases of glucocorticoid excess, pars tuberalis corticotrophs showed Crooke's hyalinization similar to that seen in corticotrophs of the

5 (Cambridge Nuclear Radiopharmaceutical Corp., Massachusetts, USA)

6 (prepared and donated by Dr. A.F. Parlow, National Institute of Arthritis, Metabolism and Digestive Diseases, Baltimore, Md, USA)

7 (donated by Biorad Laboratories, Richmond, California, USA)

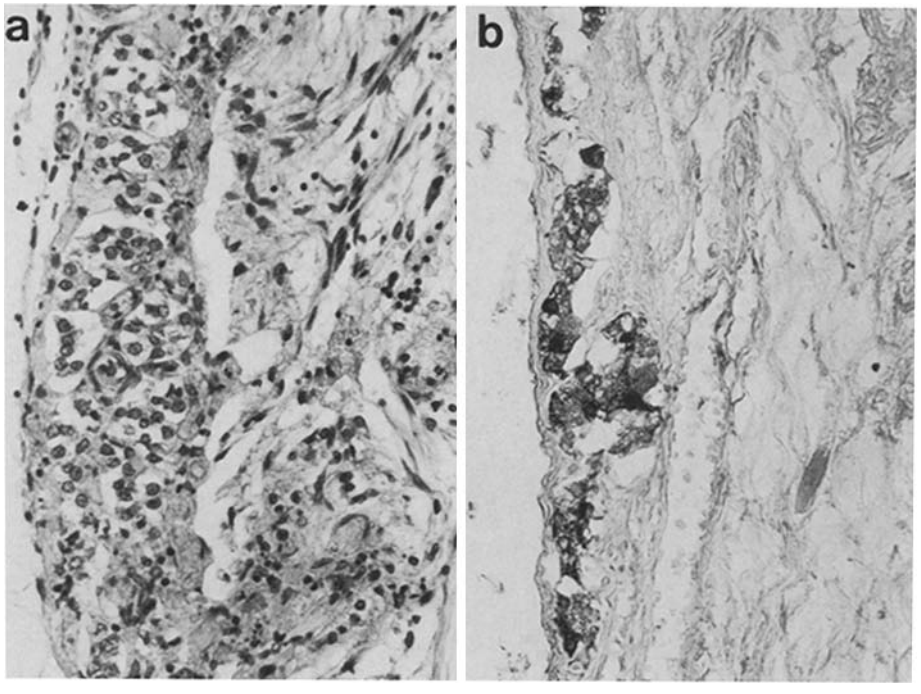

Fig. 1. **a** Pars tuberalis, caudal end, showing clusters of chromophobic cells amidst portal blood vessels. (Hematoxylin and eosin stain; original magnification ×80). **b** Many cells are positive for immunoreactive β-FSH. (immunoperoxidase technique for β-FSH; original magnification ×80)

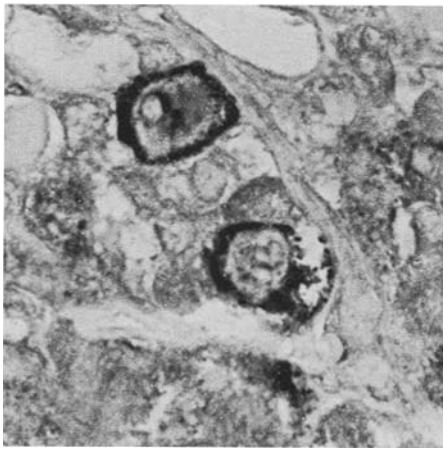

Fig. 2. Corticotrophs of pars tuberalis in a case of glucocorticoid excess show Crooke's hyalinization. (immunoperoxidase technique for 1-39 ACTH; original magnification × 320)

pars distalis (Fig. 2). In the third case, only gonadotrophs were seen in the pars tuberalis.

In all eight cases of previous castration, the cells of the pars tuberalis exhibited more intense positivity for immunoreactive FSH and LH than

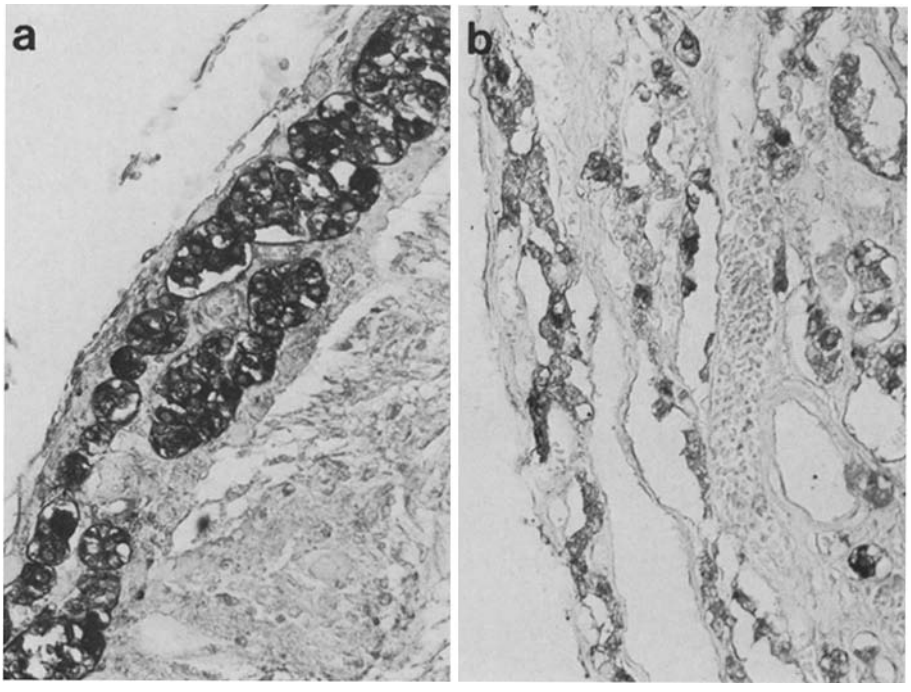

Fig. 3. a In a case of previous castration, positivity for immunoreactive β-FSH is more intense than **b** the positivity for the same antigen in a normal control. (immunoperoxidase technique for β-FSH; original magnification $\times 80$)

that seen in normals (Fig. 3). Four cases also contained occasional corticotrophs and 3 contained thyrotrophs.

Only gonadotrophs were identified in the pars tuberalis of two patients who had undergone previous hypophysectomy; these showed very intense immunostaining for FSH and LH similar to that of the castration cases.

Squamous cell nests were identified in the pars tuberalis in 15 cases, including 12 patients with normal hormonal environment, 2 patients with diabetes mellitus and 1 case of previous hypophysectomy. All of these were found to contain immunoreactive keratin (Fig. 4a), and all showed evidence of hormone production in cells at their periphery, most often FSH and/or LH or occasionally ACTH (Fig. 4b). In some cases, light microscopic double immunostain showed cells containing both keratin and hormone at the junction of the epithelial cells and hormone containing cells.

Ultrastructural analysis, performed on pars tuberalis of 5 patients with no endocrine abnormality, showed gonadotrophs and corticotrophs containing poorly developed rough endoplasmic reticulum and Golgi complexes and abundance of swollen mitochondria (Fig. 5). In many cells, large lysosomes were seen. Secretory granules were typical for the cell type in each case (Bloodworth et al. 1981); immunoelectron microscopy demonstrated FSH, LH and ACTH in secretory granules (Fig. 6).

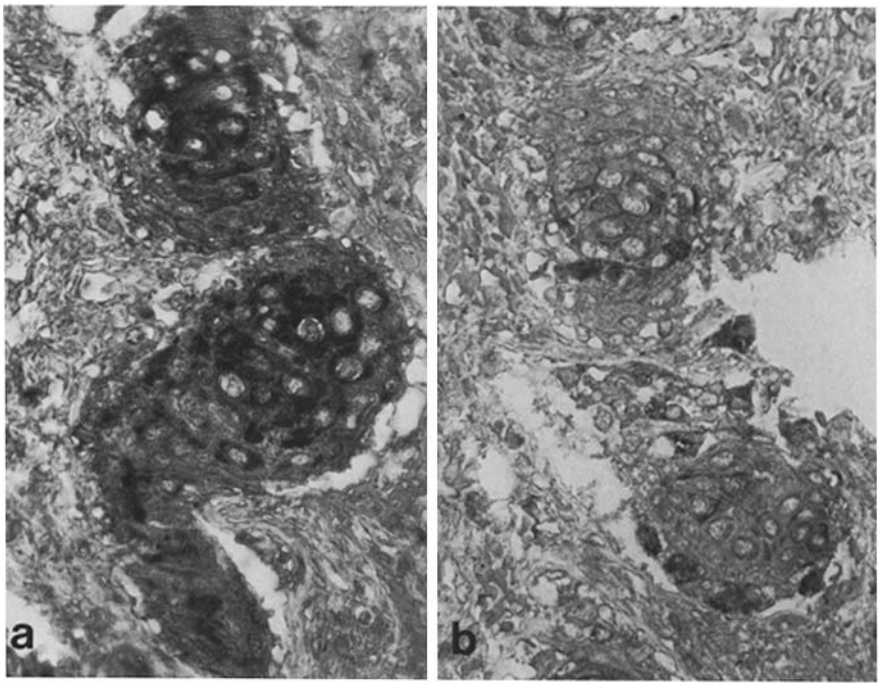

Fig. 4. a Squamous cell nests in pars tuberalis contain keratin. (immunoperoxidase technique to detect keratin; original magnification ×128). **b** Cells at their periphery contain β-FSH. (immunoperoxidase technique for β-FSH; original magnification ×128)

Squamous nests, identified in the ultrastructural examination of 2 cases, consisted of clusters of epithelial cells containing prominent desmosomes and conspicuous tonofilaments surrounded by densely granulated gonadotrophs. Transition forms, that is, cells containing both tonofilaments and secretory granules, were not identified conclusively by electron microscopy, due to discontinuous cell membranes in autopsy specimens.

Discussion

The pars tuberalis is a structurally distinct component of the anterior pituitary gland. By virtue of its position, pars tuberalis secretory products may flow directly into the hypophysial portal system and reach the pars distalis. Moreover, hypothalamic hormones from the median eminence which regulate pars distalis function must reach the pars tuberalis first.

Morphologic studies of the pars tuberalis in animals have shown the presence of gonadotrophs in several mammals (Baker and Yu 1975; Baker et al. 1977; Gross 1978; Girod et al. 1980). This has been confirmed by biochemical and physiological data (Reichlin 1963; Legait 1969). Other cell types, including corticotrophs, thyrotrophs, somatotrophs and lactotrophs, have been found in smaller numbers in various species (Baker et al. 1977; Girod et al. 1980).

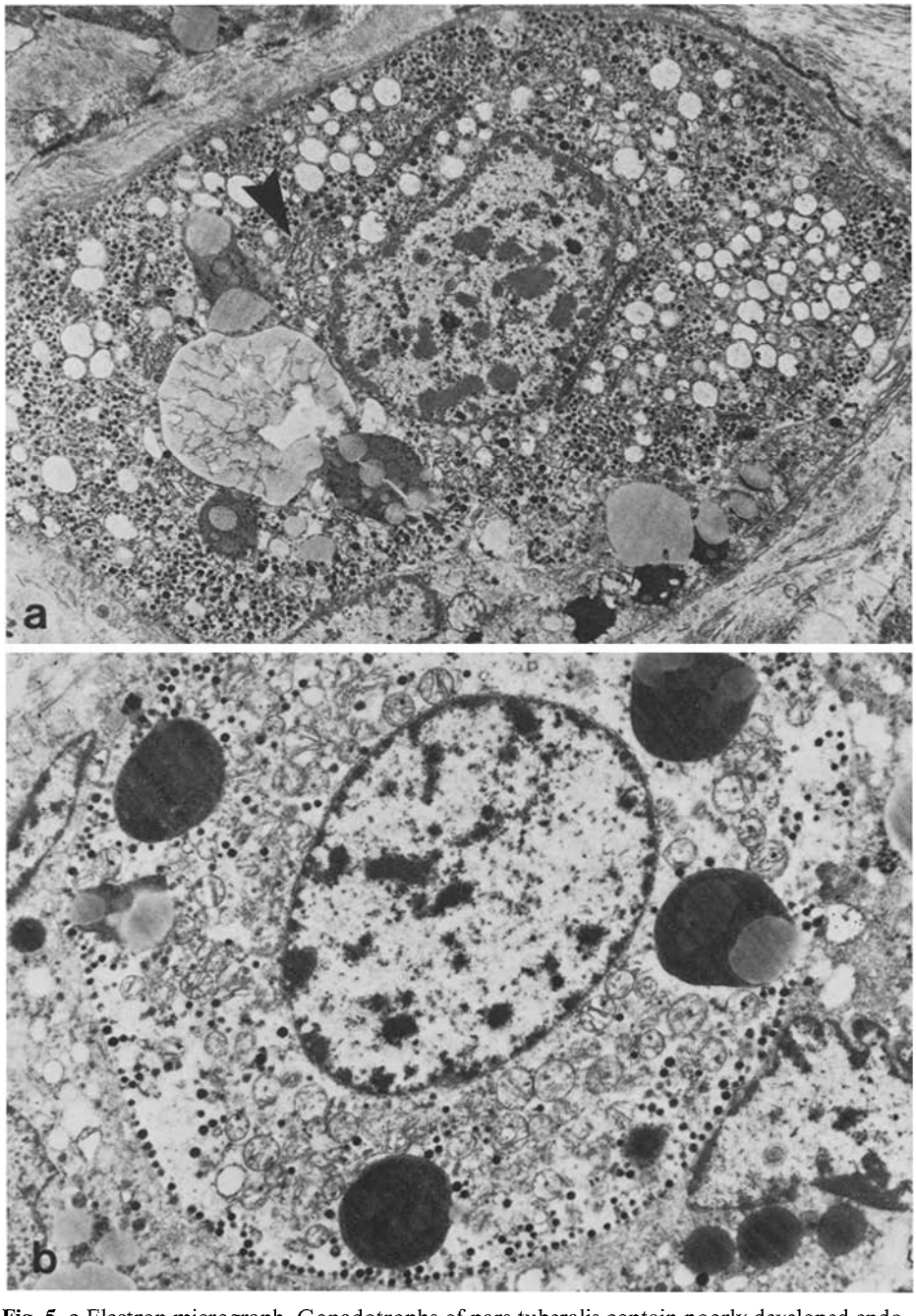

Fig. 5. a Electron micrograph. Gonadotrophs of pars tuberalis contain poorly developed endoplasmic reticulum and Golgi complex (*arrowhead*) and swollen mitochondria. (magnification ×5,000). **b** Mitochondrial dilatation is accompanied by large lysosomes. (magnification ×7,000)

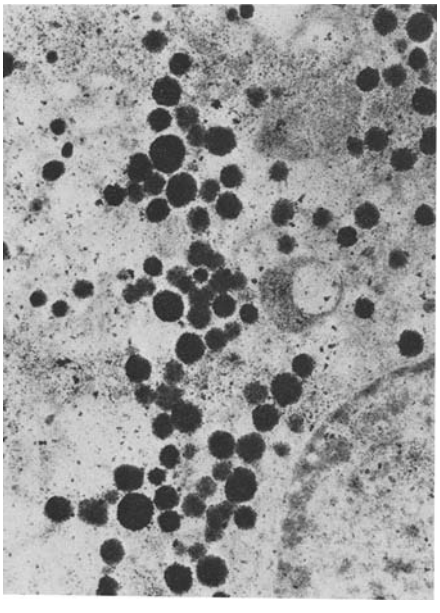

Fig. 6. Immunoelectron microscopy of a gonadotroph cell in the pars tuberalis showing immunoreactive β-LH in secretory granules. (magnification $\times 12{,}000$)

Gonadotrophs of the rat pars tuberalis appear to respond to castration and steroid hormone replacement therapy (Gross 1978). Hypertrophy of pars tuberalis follows hypophysectomy in rat, dog and rabbit (Girod et al. 1980). Studies of pars tuberalis following hypophysectomy in rats have shown hypertrophy and hyperplasia of gonadotrophs (Baker and Yu 1975; Gross 1978; Gross and Page 1979), as well as corticotrophs (Baker and Yu 1975). Hypophysectomy, not including removal of pars tuberalis, does not totally suppress secretion of adenohypophysial hormones in rats and monkeys (Gross and Page 1979; Girod et al. 1980; Ordronneau and Petrusz 1980). All the other cell types have been identified by histochemistry and immunocytochemistry following hypophysectomy in rats (Ordronneau and Petrusz 1980). It was suggested that, in the intact animal, gonadotrophs may predominate in the pars tuberalis, however, after hypophysectomy, other cell types are activated, thus the pars tuberalis may be a reserve pool of cells capable of producing several adenohypophysial hormones (Ordronneau and Petrusz 1980).

Human studies have also documented the predominence of gonadotrophs in the pars tuberalis (Midgley 1966; Baker 1977; Osamura and Watanabe 1978). Our findings confirm this and the occasional presence of corticotrophs and thyrotrophs in this region. Acidophils were not found in any specimen in our study. The ultrastructure and electron microscopic immunocytochemistry of the human pars tuberalis shows that these cells possess all organelles required for hormone synthesis and contain immunoreactive FSH, LH and ACTH. However, in our patients with normal endocrine environments, they contain only scant amounts of rough endoplasmic

reticulum and poorly developed Golgi complexes and, therefore, appear to remain functionally inactive.

This morphologic study of the human pars tuberalis in abnormal endocrine conditions indicates that corticotrophs in the pars tuberalis react to glucocorticoid excess, as do those of the pars distalis, with Crooke's hyalinization. Castration seems to induce increased storage of FSH and LH as reflected by increased intensity of immunostaining for these hormones. The same is true following hypophysectomy. Hypertrophy of pars tuberalis has been reported following hypophysectomy in man (LeBeau et al. 1963). Moreover, there has been clinical evidence of incomplete hormone suppression following this procedure (Conway and Collins 1969; Lachelin et al. 1977); while enhanced hormone synthesis by pars tuberalis may explain failure of this procedure to eliminate hormone production (Gross and Page 1979), this may be due to a remnant of pars distalis in cases of incomplete hypophysectomy. Unlike the animal studies cited above, our study shows that hypophysectomy does not activate reserve cells of other types in man.

The apparent functional inactivity of pars tuberalis gonadotrophs at the ultrastructural level in patients with normal endocrine homeostasis, raises the possibility that they lack receptors for LHRH. There has not yet been a study of receptor structures and effect of this hypothalamic hormone on pars tuberalis cells. However, castration or hypophysectomy resulted in increased intensity of immunostaining for FSH and LH in our study. Perhaps an inhibitory factor is responsible for the functional inactivity of gonadotrophs under normal conditions. There is reported to be an anatomical connection between pars tuberalis cells and tanycytes (Knowles and Anand Kumar 1969), which may detect and monitor changes in concentrations of gonadal hormones and LHRH in cerebrospinal fluid of the third ventricle (Heller et al. 1968; Knowles 1972; Uemura et al. 1975; Uemura and Kobayashi 1977); thus, tanycytes may provide the controlling stimulus or inhibition to pars tuberalis gonadotroph activity in normal and abnormal conditions.

The etiology of squamous nests in the pars tuberalis is another issue which has been unresolved to date. The earliest speculation regarding clumps of squamous epithelium in this region considered these structures to be remnants of the embryonic hypophysial duct (Luschka 1860; Erdheim 1904; Kiyono 1924; Carmichael 1931). Others have raised the possibility that squamous nests arise from metaplasia of pituitary cells of pars tuberalis (Susman 1931–32; Biggart 1949; Hunter 1955; Luse and Kernohan 1955; Goldberg and Eshbaugh 1960). The evidence was indirect and presumptive: (1) squamous nests are rarely found in patients under 20 years of age and increase in frequency with age; (2) they are not found in loci explained anatomically as derivation from the obliterated connection with the pharynx; (3) the hypophysial duct is lined by low cuboidal epithelium, not by squamous epithelium; and (4) the size and shape of the cells and squamous nests resemble those of clusters of pituitary cells in that region.

The demonstration of granulated hormone-containing gonadotrophs and corticotrophs at the periphery and within squamous nests of pars tuber-

alis indicates the derivation of the epithelial cells by metaplasia. However, the reason for this metaplasia remains unknown. Several authors have suggested that it occurs secondary to irritative phenomena or accumulation of colloid (Luse and Kernohan 1955; Cheetham 1963); others have proposed vitamin A deficiency in some cases (Luse and Kernohan 1955). In our study, none of these reasons can be implicated. Moreover, the change is not related to castration. It thus remains unclear what the etiology of this change is and why it occurs in gonadotrophs and corticotrophs of pars tuberalis.

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