Part 6: Pituitary Gland

Normal Physiology and Structure

The pituitary gland comprises the adenohypophysis, which is made up of the pars distalis, pars intermedia and pars tuberalis and the neurohypophysis which includes the pars nervosa, infundibular stem and median eminence. The pars distalis forms the largest proportion of the gland and functions as the overall regulator of peripheral endocrine function by synthesizing and secreting at least 6 major trophic hormones. These include growth hormone (GH), prolactin (PrL), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Since this is the important area of the pituitary with respect to detecting endocrine active compounds, the rest of this section will concentrate only on this part of the pituitary. For reviews see (Page, 1994; Tucker, 1999; Greaves, 2007).

Each hormone of the pars distalis is generally secreted by a seperate cell type, but some cells are able to secrete two hormones. The different hormones impart different staining properties to the cells. Using histological stains based on Orange G and periodic acid-Schiff (PAS), the cells of the pars distalis have been divided into acidophils (orange G positive), basophils (PAS positive) and chromophobes (absence of staining). In the rat, these have been reported to constitute 40, 10 and 50% respectively of the cell population of the pars distalis. The staining characteristics are dependent on the level of secretory activity, and when the cells have just secreted their granules or when secretory activity is increased, all the cells take on chromophobic characteristics due to the relative abundance of secretory organelles (endoplasmic reticulum and Golgi) and relative lack of secretory granules. Although, a similar differentiation of acidophils, basophils and chromophobes can be made using conventional H&E staining (see Table 1), the distinction between the different cell types is less clear, but the following broad categorizations apply:

Acidophils stain with eosin and secrete GH (somatotrophs), PrL (lactotrophs) and some cells are able to secrete both hormones (somatomammotrophs). PrL secreting cells may be acidophilic or chromophobic depending on the size and number of secretory granules. Acidophils are located predominantly in the lateral aspects of the lobes.

Basophils stain relatively poorly, but have a slightly basophilic appearance in H&E stained sections. They generally reflect TSH (thyrotrophs) or FSH and LH (gonadotrophs) secreting cells and to a lesser extent ACTH (adrenocorticotrophs) secreting cells. These cells are more concentrated in the central portion of the pars distalis.

Chromophobes stain poorly with hematoxylin and eosin and are characteristic of ACTH (corticotroph) secreting cells. ACTH secreting cells are also present in the pars intermedia. In addition, basophils and eosinophils that have recently degranulated or are in the process of active synthesis of hormone will appear chromophobic.

The relative proportions of the different cell types in the pars distalis varies with species and staining methodology employed. Evaluation of the gland with respect to changes in hormone-secreting cell populations is best conducted using specialized immunocytochemical techniques and may also require quantification to detect more subtle changes. Using immunocytochemical techniques on the rat pituitary, Dada et al (1984) reported that prolactin secreting cells represent 30-50% of cells, GH

secreting cells are 20% of the population and the other types of hormone secreting cells each constitute 2-6% of cells. As can be appreciated, this categorization bears little resemblance to the categorization of cells based on acidophil, basophil and chromophobe status.

Table 1: Categorization of cells of the pars distalis using hematoxylin and eosin stain

Cell type	**Staining properties (H&E)**	**Hormone secreting cell**	**Secreted hormone**
Acidophils (40%)	Eosinophilic	Somatotrophs Mammotrophs (lactotrophs) Somatomammotrophs	GH Prl GH + Prl
Basophils (10%)	Weakly basophilic	Thyrotrophs Gonadotrophs	TSH FSH + LH
Chromophobes (50%)	Pale staining	Adrenocorticotrophs Mammotrophs Recently degranulated cells Actively secreting cells	ACTH Prolactin All hormones All hormones

Hormonal Regulation of Pituitary Function

Regulation of the various trophic hormones from the cells of the pars distalis is through a complex interplay of different inputs. These include positive and negative feedback from the peripheral target tissues, stimulation or inhibition by hormones secreted from the hypothalamus and also modulation through hormones secreted from other organs. The major regulatory hormones are listed in Table 2, but there are many other hormones that modulate pituitary function. For a comprehensive review of various aspects of pituitary regulation see (Knobil, 1994).

Table 2: Major regulatory hormones of pituitary function

Pituitary hormone	**Major releasing factors**	**Major inhibitory factors**
Prolactin	Prolactin releasing factor	Dopamine
Growth hormone	GH releasing hormone	Somatostatin
Thyroid stimulating hormone	TSH releasing hormone	T3 and T4 (thyroid hormones), prolactin, somatostatin
LH and FSH	Gonadotrophin releasing hormone (GnRH), activin	Estrogen, androgens, inhibin
Adrenocorticotrophic hormone	Corticotrophin releasing hormone	Cortisol, corticosterone (adrenal cortical hormones)

Normal Background Variation of Structure

The relative numbers of acidophils, basophils and chromophobes will vary depending on the quality of staining and the precise level of sectioning through the pituitary. Certain cell types are distributed in specific areas of the pars distalis, which may or may not be sectioned in different animals.

The pituitary is a homeostatic tissue that is continually responding to the environmental and physiologic status of the animal. For example, the number of acidophils varies with the stage of the estrous cycle in the rat, presumably as a result of changes in prolactin secretion. In the rat, decreased food intake, decreased body weight gain or body weight loss are associated with decreased gonadotroph activity due to reduced GnRH release. This is a normal physiologic response to non-specific stress.

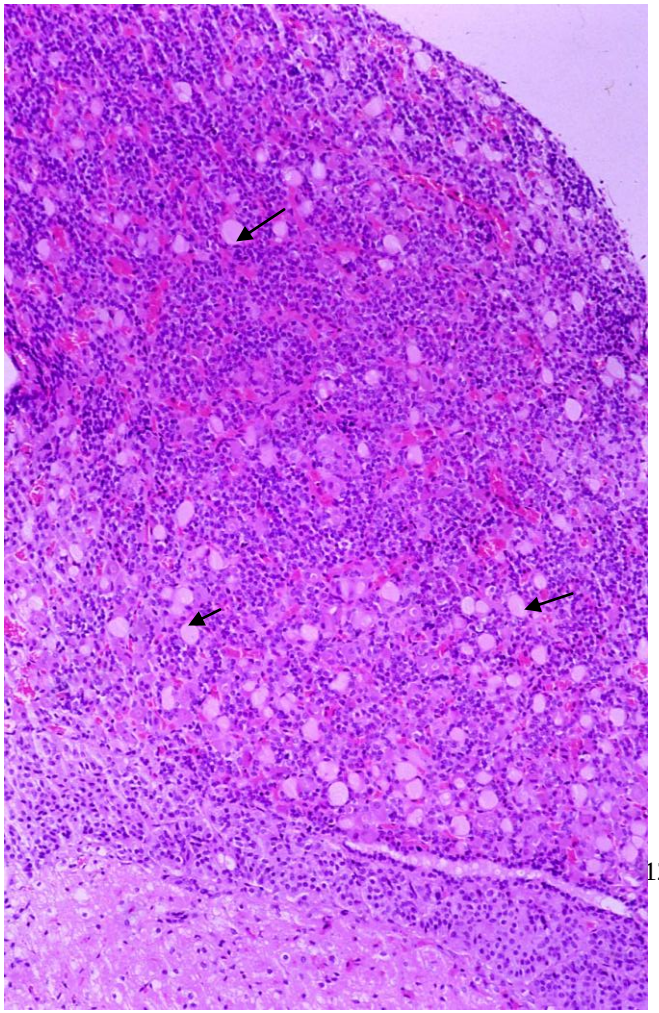
Morphologic Patterns of Hormone Disruption

In a 28 day study such as the TG407, very few changes are likely to be detected in the pituitary in response to endocrine disruption. The most common responses of the pituitary to endocrine disrupting toxicants are cellular hypertrophy, hyperplasia or atrophy but these are generally only detected after long term responses to prolonged hormonal imbalance (for review see (Greaves, 2007).

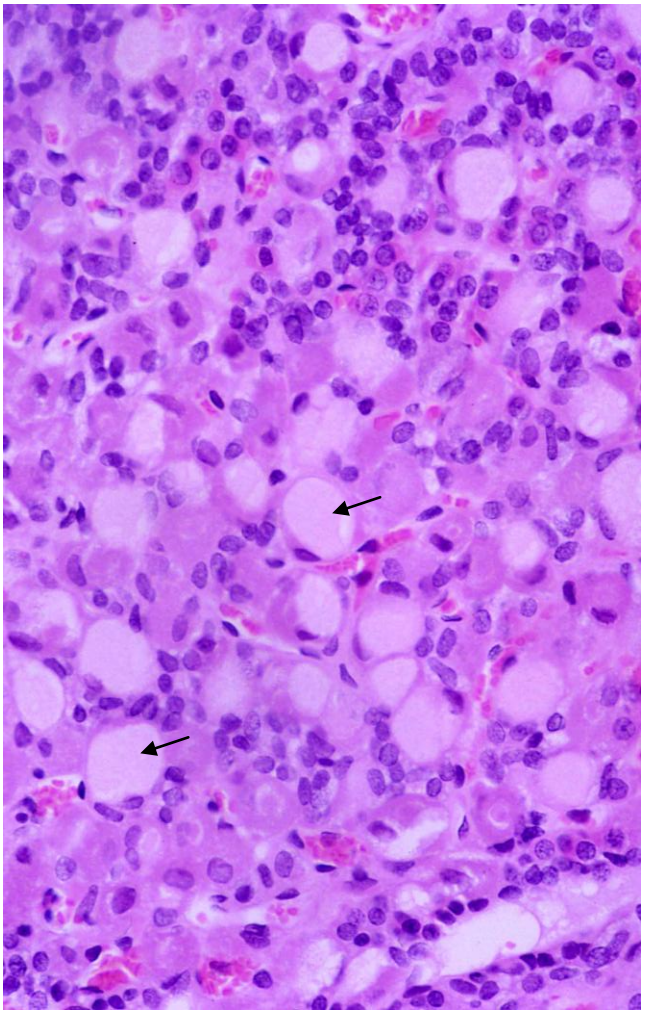
Changes in pituitary cells are generally secondary responses to altered feedback pathways to the pituitary caused by hormone imbalance elsewhere. The cell type affected in the pituitary will be dependant on the endocrine organ that has been affected. In the rat, prolactin secreting cells (lactotrophs) are the most commonly affected pituitary cell to xenobiotics, and prolactin secreting tumors of the pars distalis are one of the most common incidental and toxicant induced tumors in this species. Diffuse hyperplasia, or more commonly focal hyperplasia of chromophobe cells is a common precursor lesion to the prolactin secreting adenoma, but such changes are only seen following long term hormonal imbalance.

Hypertrophy and vacuolation of pituitary cells

(Capen, 1983) described the time dependant effects seen in the hormone secreting cells of the pars distalis following ablation of the corresponding target tissues (gonadectomy, thyroidectomy and adrenalectomy). Initially, there is a rapid release of the preformed storage granules of the affected trophic hormone. Over the course of the next few days the degranulated cells become larger and pale staining (chromophobic) due to expansion of the cytoplasm. With sustained stimulus over the next few weeks, the subtype of cells relating to the hormone affected will increase in number. These will be distributed as small groups of cells scattered throughout the pars distalis. After many weeks or months of strong stimulation, these affected cells become finely vacuolated due to cystic dilation of the rough endoplasmic reticulum profiles. Following chronic stimulation the small vacuoles will finally coalesce into a single large clear vacuole that often displaces the nucleus peripherally and forms the characteristic "signet ring" cell, sometimes called castration cells, thyroidectomy cells, adrenalectomy cells, depending on the underlying condition that has caused the change.



Although it is not possible to identify the secretory product in enlarged pale staining cells without conducting immunocytochemical stains, an assumption can generally be made based on the target peripheral tissue that has been affected.



Pituitary from a male rat treated with an agent that caused castrate levels of testosterone. The pale staining "signet ring" cells (arrows) in the pars distalis reflect gonadotrophs that contain large quantities of LH. These cells are often called "castration cells". However, immunocytochemistry is required to positively identify the secretion, since these cells could just as easily be thyrotrophs (secreting TSH) or lactotrophs (secreting prolactin) or any of the other trophic secretory cells in the pars distalis

Diffuse hyperplasia or atrophy of the pars distalis

This is unlikely to be seen in a short term 28 day exposure. It is possible that weight changes in the pituitary might reflect diffuse hyperplasia or atrophy of cells in the pars distalis, but the change would need to be quite marked before this endpoint reflected the change.

Recommended Terminology and Severity Grading for Histopathological Findings

Pars distalis: enlarged pale staining cells: presence of large pale staining cells **(**enlarged chromophobes) which are generally distributed throughout the pars distalis.

Pars distalis: enlarged cells with vacuoles: presence of large cells generally containing several or a large centrally located vacuole that displaces the nucleus peripherally

Due to the variability in the staining characteristics of cells of the pars distalis and the variability in the plane of section through the pituitary, it is unlikely that changes in the relative numbers of acidophils, basophils or chromophobes will be reliably detectable. However, if such changes are suspected, it is recommended that additional sections be stained using immunocytochemical stains to confirm and investigate potential increases or decreases in hormone secreting cells.

Severity Gradings:

Severity gradings ranging between minimal: "smallest degree of change that can be consistently distinguished from normal background variation" and severe: "greatest degree of change that is likely to occur" are subjective. The following gradations are generally used to define a 5 grade severity system.

Minimal = very few/very small
Slight = few/small
Moderate = moderate number/moderate size
Marked = many/large size
Severe = very many/very large size

Critical aspects of histopathological evaluation

A good knowledge of the variation in staining characteristics of the cells of the pars distalis is needed.

The most likely change will be the appearance of large pale staining cells reflecting increased secretory activity in one or more subpopulations of the hormone secreting cells. Although this change is likely to ultimately lead to the presence of large vacuoles, (signet ring cells), this is unlikely to be seen in a 28 day study except with very severe hormone disruption. Similarly, it is extremely unlikely that any degree of hyperplasia will be detectable with such a short term exposure.

Any apparent changes in the relative numbers of acidophils, basophils and chromophobes should be investigated using immunocytochemical stains.

References

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