

LABORATORY 26 - ENDOCRINE SYSTEM

OBJECTIVES: LIGHT MICROSCOPY: Recognize the characteristics of the different endocrine organs, pituitary, pineal, thyroid, parathyroid and adrenal glands and be able to distinguish different regions and different cell types in each of the organs.

ELECTRON MICROSCOPY: Distinguish characteristic cell types according to nature of hormone secreted, i.e. protein versus steroid hormones. Also, unique characteristics, e.g. follicles of thyroid gland.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

[SL 121](#) Hypophysis. Mallory's stain

[SL 122](#) Hypophysis. PAS - Orange G stain

[SL 123](#) Hypophysis. H and E

[SL 171](#) (To be distributed in laboratory) Demonstration slide of pituitary immunohistochemical staining

[SL 128](#) Pineal gland

[SL 124](#) Thyroid gland

[SL 126](#) Parathyroid gland

[SL 127](#) and [52](#) Adrenal gland

POSTED ELECTRON MICROGRAPHS

28 Hypothalamus

S-32 Adrenal cortex

S-91 Thyroid

S-97 Adrenal medulla

[Lab 26 Posted EMs](#)

ELECTRON MICROGRAPHS

J. 20-12, 20-27, 20-28

W. 17.11, 17.18

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 12, Endocrine System

Frames: 779-825

EXTRA CREDIT CASE ON BLACKBOARD: PITUITARY TUMOR

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., [An Atlas of Histology](#)

Copies of this text are on reserve in the HSL.

Endocrine glands pp. 241 - 270

A. HYPOPHYSIS

There are numerous cytological and electron microscopic reports on the structure of the pituitary gland in the scientific literature. The study of the cells of the pars distalis is practically a specialty in itself. Our examination of this gland will be limited by necessity. Unfortunately, secretory granules that contain hormones are lost readily during the postmortem period. Some authors suggest that only specimens, removed from surgical patients or cases of sudden death and fixed immediately, permit valid cytological analysis. However, these stringent conditions are required only for precise studies of cell structure and identification. The sections of the glands on the following slides are suitable for routine study, but you should realize that the staining of the cell types is not necessarily consistent throughout the sections.

1. HYPOPHYSIS, Mallory's stain. SL 121 (W. 17.3). Scan the slide and identify the following anatomic divisions: the highly cellular pars distalis, the pars intermedia with large areas of blue staining colloid and the lightly stained pars nervosa (pars distalis, red arrow; pars intermedia, blue arrow, pars nervosa, green arrow). In the pars distalis find these identifying features: more than one cell type, a rich sinusoidal network and minimal stroma. Three cell groups should be identified. The staining on this slide is quite variable, as indicated by the variety of colors of the red blood cells in the sinusoids making cell identification difficult. However, for the most part acidophils are red (to orange-brown), the larger basophils are either blue or a very dark red in some slides and are more noticeably granular (granules are visible at high mag) and the chromophobes are unstained (red arrow) or are seen as clusters of nuclei (enclosed by red lines) with little surrounding cytoplasm evident. Figure W. 17.3b shows the acidophils staining orange. Also, observe that the various cell types are not evenly distributed in the gland; instead there are regions in which different cell types are concentrated. In the pars nervosa, identify pituicytes.
2. HYPOPHYSIS, PAS-Orange G stain SL 122 (pars distalis scan, high, and pars nervosa med.). Use this slide to study primarily the pars distalis. The purpose of these stains is to delineate cells broadly on the basis of the chemistry of their granules. The PAS positive granules are found in almost all of the basophils, since the hormones that they secrete are glycoproteins. Observe that basophils vary in their size, shape, and their staining intensity. These variations are related to the number of granules in the cell. The hormones liberated by this type of cell are FSH, LH, and TSH and ACTH. The granules of the acidophils stain with Orange G. The hormones in the granules of the acidophils have little, if any, carbohydrate component and therefore they are PAS negative. Orange G alone does not delineate the cells that secrete GH from those that secrete prolactin. With proper staining the two types of acidophils that produce either GH or prolactin can be distinguished from each other. The chromophobe is a third cell type. It has no granules and is distributed sparsely. Chromophobes are best identified as groups of closely packed nuclei, with indistinct cell outlines, having little, if any visible cytoplasm. Other structures staining PAS positive in the human pituitary are:
 - (a) Colloid - Areas of colloid can be found in the pars intermedia (W. 17.4) and to a more limited extent in the pars distalis. This colloid may represent a variety of proteins and is not necessarily associated with a particular hormone.
 - (b) Connective tissue stroma - collagen, reticular fibers, and basement membrane are all PAS positive to some degree.
 - (c) Herring bodies - nerve termini from neurons whose cell bodies are located in the hypothalamus, these are filled with secretory vesicles containing ADH and oxytocin.

3. HYPOPHYSIS, H and E. SL 123 (scan) (W. 17.3a). This is a routine preparation. The acidophils appear more pink than red and do not show granularity. The basophils are blue on some slides. On other slides they have a dark reddish hue and granules are more evident. Chromaphobes lack stain and appear as clusters of nuclei without much cytoplasm (acidophils, red circles; basophils, blue circles; chromophobes, green circles). Again, note the uneven distribution of cell types. (pars distalis).
 - (a) Electron microscope (J. 20-5; W. 17.3d) - Note these cells have characteristics that suggest they secrete protein.
4. On all of the slides above, observe the pars nervosa (J. 20-3, 20-9; W. 17.5). No special staining procedure has been used for this region, but neurosecretory material is evident in many slides. Almost all the nuclei visible represent pituicytes that are the cells peculiar to this region. Some of the pituicytes may be pigmented. Distinctive histological features and cytological details are difficult to see in routine preparations. Study detailed histology and cytology of the pars nervosa and pars infundibulum in your text.
5. IMMUNOCHEMISTRY OF THE PITUITARY GLAND SL 171

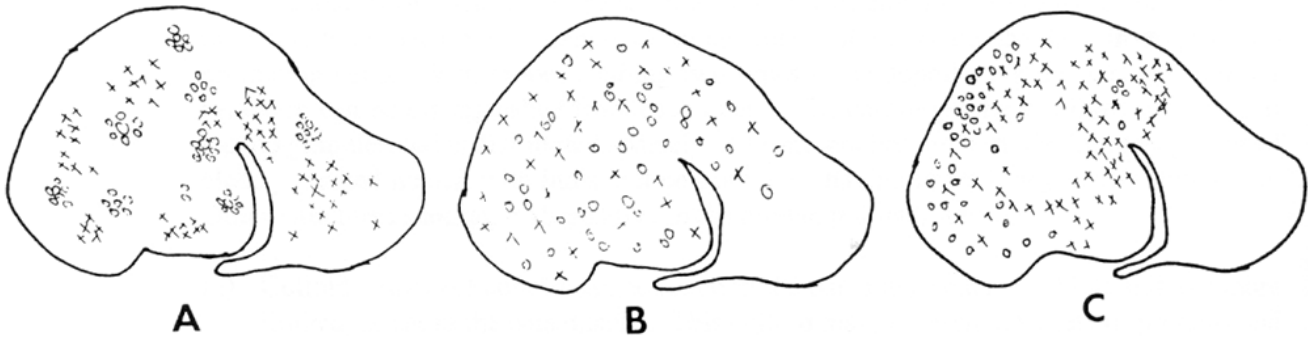
Although histological staining methods can be employed to differentiate acidophils, basophils and chromophobes into general categories within sections of the pars distalis, immunocytochemical methods must be used to differentiate between cells that secrete specific hormones. Slide #171, that will be distributed during the laboratory, contains a section of pituitary gland that has been stained with two antibodies to enable differentiation between basophils that secrete thyroid stimulating hormone (TSH) and acidophils that secrete growth hormone (GH). The two antibodies, each specific for one of these two hormones, bind to the hormones within secretory granules in the appropriate cell types. In order to visualize the distribution of both antibodies in a single section, two different methods were used:

1. Growth hormone antibody was localized by the peroxidase method that produces a brown precipitate wherever antibody is present (described in Lab.16, immunocytochemistry).
2. Thyroid stimulating hormone antibody was localized by an alkaline phosphatase method that produces a red precipitate to indicate the presence of antibody. Please note that in many of the slides the red precipitate is rather faint and relatively few cells show the presence of this hormone.

The section was counter-stained with hematoxylin to make the nuclei visible throughout the section.

Examine the slide and determine which regions of the pituitary gland (pars distalis, pars intermedia and pars nervosa) contain cells secreting GH and TSH.

On the next page, three outlines of the section show patterns of cell distribution in which "X" represents GH and "O" represents TSH. Which pattern (A, B or C) prevails in your slide?



1. Do the populations of cells overlap?
2. Do some cells express more intense staining than others?
3. Suggest how other acidophils and basophils would be distributed compared to these two cell types?
4. Are there some acidophils and/or basophils that express more than one hormone?
5. Could an immunocytochemical method, using antibodies for secretory products, be employed to determine the distribution of chromophobes? Or hormones in the pars nervosa?

B. PINEAL GLAND.

1. [SL 128 \(high\)](#) (W. 17.24). This slide has sections of two pineal glands. In general, note the [capsule](#) and indistinct lobules of the gland. Insufficient detail has been retained to see entire cells. However, the majority of the clusters of plump oval nuclei set off by c.t. septa belong to the [pinealocytes](#) (chief cell). Scattered throughout the section, thin, more elongated nuclei are evident. These are the nuclei of [glial](#) cells which intermingle with the pinealocytes. Look for small, calcified bodies, [corpora arenacea](#) ([brain sand](#), [red circle](#)) (W. 17.24). These structures are characteristic of the pineal gland, but they are not always present. In the sections on this slide they may be small or absent, but in other specimens they may be very large and prominent.

C. THYROID AND PARATHYROID.

1. THYROID GLAND. [SL 124 \(low, high\)](#). (J. 20-25, 20-26; W. 17.6, 17.7). Observe that [follicles](#) vary with regard to the height of the epithelium ([follicular cells](#)) and appearance of the [colloid](#). Note thyroid [C cells](#) ([parafollicular cells](#)) are difficult to identify in routine H and E sections. Study this cell type in your text (J. p. 425, W. 17.7), know their function and embryological derivation. Augment this slide study by reviewing the ultrastructure, function and regulation of the principal (follicle) and C cell in your text (J. 21-20, 21-21; W. 17.8).
2. PARATHYROID GLAND. [SL 126 \(scan, high\)](#). (J. 20-31, 20-32; W. 17.12, 17.13). Observe that the [chief cells](#) form irregular cords. Single cells and clusters of [oxyphil](#) cells ([enclosed by red line](#)) are evident.
3. Electron microscope - From lecture, review involvement of cellular components in the entire secretory process for thyroxine. (J. 20-27; W. 17.11)

D. ADRENAL GLAND.

1. [SL 127 \(low\)](#) and [SL 52](#). (J. 20-13, 20-12; W. 17.14 to 17.20)
 - (a) Scan the slide and note the cortex ([1](#), [2](#)) and medulla and compare their appearance with the micrographs in the Atlas (W. 17.11). Identify the c.t. capsule, the zona glomerulosa, fasciculata and reticularis (the extent of the z. reticularis may be difficult to determine) ([z. glom. red line](#); [z. fascic. green line](#); [z. retic. blue line](#); [medulla left of Z. retic.](#)) in the cortex and the features that distinguish them (W. 17.15 - 17.17. Review the histophysiology of the cortex in your text (J. pp. 414-417).
 - (b) Observe the medulla noting that cellular detail is not well-preserved in routine preparations. Compare with figures J. 20-13D; W. 17.19 in your text and atlas. Note the central vein ([1](#), [2](#), [blue arrow](#)) or its branches within the medulla. Many of these vessels have characteristic thickenings of the smooth muscle of the media. Review the blood supply of the adrenal (J. 20-11; W. 17.20). Study the function of the medulla (J. pp. 389 to 394) noting the embryological origin of the medullary cells and the relationship to the autonomic nervous system.
 - (c) Electron Microscope - Review the characteristics of steroid secreting cells (J. 20-12; W. 17.18). Chromaffin cells from the medulla are shown in electron micrograph [# 97](#) that is posted.

REVIEW

1. What is the cell source of the following hormones: a) calcitonin? b) melatonin? c) cortisol? d) epinephrine?
2. Oxyphil cells are located in which organ?
3. Which cell type, identified in a section stained with H and E, would show an immunocytochemical localization for prolactin?

OBJECTIVES FOR LABORATORY 26: ENDOCRINE SYSTEM

1. Using the light microscope or digital slides, identify:

- Pituitary gland (for all stains used)
 - Adenohypophysis / pars distalis / anterior pituitary
 - Acidophils
 - Basophils
 - Chromophobes
 - Sinusoids
 - Neurohypophysis / pars nervosa / posterior pituitary
 - Pituicytes
 - Herring bodies (best seen with PAS)
 - Pars intermedia
 - Colloid
 - Immunocytochemistry
- Pineal gland
 - Pinealocytes
 - Glial cells
 - Corpora arenacea (brain sand)
- Thyroid gland
 - Follicle
 - Colloid
 - Follicular cells
 - Parafollicular cells (C-cells, difficult to identify with routine staining)
- Parathyroid gland
 - Chief cells
 - Oxyphil cells
- Adrenal gland
 - Cortex
 - Zona glomerulosa
 - Zona fasciculata
 - Zona reticularis
 - Medulla
 - Medullary cells (chromaphin cells)
 - Central vein

2. On electron micrographs, identify:

- Hypothalamus / pituitary
 - Secretory granules – do not have to recognize specific hormones or cell types
- Thyroid gland – understand production of thyroid hormone on EM
 - Follicular cell
 - Colloid
 - Parafollicular cells (C-cells)
- Adrenal gland
 - Cortex vs. medulla