

# Proliferative Lesions of the Pituitary in Rats

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## INTRODUCTION

In routine long-term rat studies commonly conducted in the field of toxicology, neoplasms of the pituitary gland are among the most commonly encountered in most strains (2,4,8). The vast majority of these neoplasms occurs in the pars distalis. Although various techniques are available to specifically identify the different hormonal cell types, routine diagnoses of proliferative changes in the rat pituitary in long-term studies are almost exclusively made from hematoxylin and eosin-stained preparations. The following information outlines a simplified classification system for the proliferative lesions seen in the pituitary of the rat.

## MORPHOLOGY

Although anatomically the pituitary of the rat is usually divided into three distinct zones - pars distalis, pars intermedia and pars nervosa - proliferative lesions in the latter two regions are rare. This classification system for proliferative lesions in the rat pituitary will focus primarily on changes in the pars distalis and include a brief discussion of lesions in the other zones of the pituitary.

Proliferative changes in the endocrine system, in general, and in the pituitary gland, in particular, tend to exhibit a narrow range of alterations in cellular morphology when progressing from normal to neoplastic cells.

## PARS DISTALIS

### *FOCAL HYPERTROPHY (Figures 1 , 2)*

These small noncompressive foci are poorly delineated from the normal parenchyma and have no alterations of the vascular network (Figure 1). Cells comprising this focus are larger due to more abundant cytoplasm, which is often pale or slightly eosinophilic (Figure 2) and may be vacuolated. The focus appears to reflect a reduction in numbers of cells per unit area and, therefore, may not represent a true proliferative increase in cell numbers seen in hyperplasia or neoplasia. The changes seen in these cells, which may represent the "nodule of hypertrophy" described by McComb (10), may reflect a "degenerative" exhaustion of a specific hormonal cell type in response to altered feedback due to senile changes in the target organs, e.g., ovarian atrophy.

### *HYPERPLASIA (Figures 3 , 4)*

This change is characterized by one or more focal proliferations of a single cell type (Figure 3). The focus is slightly delineated from the normal cell population with little or no evidence of compression. The vascular channels within the region are normal in comparison to the pattern seen in adenomas in which they are often ectatic. Portions of the margins of the focus may blend with the surrounding parenchyma (Figure 4). The uniform population of cells forming these foci vary little in size and character from normal but generally are pale. Normal-appearing individual eosinophilic or basophilic cells may be present in scattered regions of the focus.

## ADENOMA (FIGURES 5-12)

This change may range from a small microscopic focus seen as an incidental lesion to a large, 1 to 2 cm diameter, nodular or smooth, nonencapsulated mass obliterating the entire pituitary architecture. Prominent compression of the overlying brain parenchyma, often clinically manifested by severe signs (convulsions, head tilt, etc.), is common with large pituitary adenomas.

The hallmark of the adenoma is the presence of compression of the surrounding parenchyma with sharp delineation of the margins of the nodule (Figure 5). The cells comprising an adenoma are generally larger than normal with more abundant cytoplasm which is usually pale or faintly basophilic, but neoplasms composed of smaller than normal cells are occasionally seen. The nuclei are slightly larger than normal and round to oval and more vesicular. The mitotic index is usually low.

Various patterns of cellular arrangement may be seen from one neoplasm to another or within the same nodule (Figure 6). Many areas are composed of sheets of neoplastic cells (Figure 7). Often, adenomas possess prominently dilated vascular channels which may be lined by endothelial cells or neoplastic pituitary cells. The vascular network may be extensive and elicit an "angiomatous" or "cavernous" pattern, also called "hemorrhagic" pattern (Figure 8).

Certain neoplasms may display lobules, gland-like structures, or anastomosing cords of varying thickness (Figure 9). Occasional tumors may possess cells forming pseudofollicles which contain dense eosinophilic colloid-like material pooling between cellular cords (Figure 10).

Occasionally, an entire mass or a small focus within a mass is composed of highly pleomorphic cells of varying size and high mitotic index (Figure 11). Giant cells and areas of hemorrhage and necrosis may be present.

Pituitary adenomas grow by expansion, often proceeding dorsally against the parenchyma of the midbrain following lines of least resistance due to the incomplete development of the diaphragma sellae in the rat (Figure 12). Compression, edema, hemorrhage and necrosis may be seen in the brain adjacent to such large pituitary adenomas. These latter characteristics alone are not felt to constitute malignancy.

## CARCINOMA

As stated above, pituitary carcinomas in rats, as well as in man (12), cannot be distinguished readily from adenomas based on the nature of the cells and the architectural pattern of the neoplasm. The only criteria for differentiating adenomas from carcinomas are the presence of distant metastasis or aggressive local invasion of the adjacent brain or the adjacent sphenoid

bone. Local invasion must be clearly differentiated from "expansive" growth commonly seen with adenomas. Distant metastasis has not been described in the rat, but has been described for transplanted pituitary neoplasms (9).

## PARS INTERMEDIA

### HYPERPLASIA

This change is characterized by a diffuse, often irregular, broadening of the intermediate zone. Cellular morphology is not different from normal.

### ADENOMA (Figures 13, 14, 15, 16)

Adenomas of the pars intermedia are nonencapsulated and poorly circumscribed. Extension of tumor cells into the pars distalis and pars nervosa is a common feature (Figures 13, 14). Generally, tumor cells are larger than normal, have a slight basophilic agranular cytoplasm and vesicular nuclei with prominent nucleoli (Figure 13). Nuclear pleomorphism is common and varies from minimal to marked (Figures 13, 15, 16). Mitoses are rare. Lobular and whorled patterns of cellular arrangement have been observed. Tumors showing a lobular pattern are characterized by islands of cells separated from each other by thin strands of connective tissue and/or prominent vasculature (Figures 14, 15). Tumors showing a whorled pattern are characterized by cells which form ill-defined circular whorls (Figure 16). Prominent vascular spaces and areas of necrosis may be present; the latter particularly in the larger tumors.

## PARS NERVOSA

### PITUICYTOMA

This neoplasm arising from the pars nervosa and described in detail by Carlton and Gries (6), is rare in animals and man. The circumscribed, nonencapsulated mass is described as being composed of packets of small, uniform spindle cells arranged into cords and interlacing bundles. The cells have a minimal amount of cytoplasm and small, dark, round to ovoid nuclei which occasionally display pleomorphism. Areas of mineralization are sometimes seen.

## TUMOR OF EMBRYONIC REMNANT

### CRANIOPHARYNGIOMA

This rare neoplasm also described by Carlton and Gries (6), is believed to arise from embryonic epithelial

remnants of the craniopharyngeal duct. The neoplasm is composed of mixed regions of solid cords or nests and cystic spaces formed by keratinizing squamous epithelium. Papillary formations may be seen in the cystic regions and abundant keratin and cellular debris fill these cysts. The neoplasm has been noted to invade both brain and pituitary tissue.

## DISCUSSION

Few xenobiotics have been shown to induce increases in the incidence of pituitary neoplasms in rats. Some estrogenic agents and/or irradiation have been reported to increase the incidence of pituitary neoplasms (14). Compounds which alter the normal pituitary-target organ axis would be suspected to secondarily induce pituitary neoplasms by hyperstimulatory mechanisms due to altered feedback regulation (2, 8). Diet, in the form of high caloric and/or high protein levels, contribute to high incidences of pituitary neoplasms in rats not otherwise treated (3,8).

In the past, pituitary neoplasms have been classified primarily by the tinctorial characteristics exhibited by routine hematoxylin and eosin staining, i.e., acidophilic, basophilic, or chromophobic. Chromophobic neoplasms have, by far, been the most commonly diagnosed type found in all species having the highest incidences of pituitary neoplasms, i.e., man, dog, horse and rat (5,7,12). Unfortunately, commonly used histochemical stains have not been shown to be specific with regard to hormonal typing. Different histochemical staining properties may be seen for the same specific hormonal cell type in different species (8). Most current nomenclatures for pituitary neoplasms disregard the use of tinctorial properties derived from histochemical staining (6,8).

A number of review articles (8, 11) have outlined techniques presently available to specifically identify nearly all hormonal cell types in normal and neoplastic rat pituitary glands. These techniques include immunohistochemistry, fluorescent antibody staining and electron microscopy. Utilization of one or more of these methods will aid in delineation of the specific hormonal cell types present in the rat pituitary. Although many of these techniques have been standardized to a point beyond the basic research laboratory, adaptation to routine long-term rat studies may still, for most practical purposes, be difficult. The large numbers of animals utilized in bioassay studies and the variability in the level of preservation of the pituitary from animals which die unexpectedly during these studies does not allow for a reliable and consistent identification of pituitary cell types in all animals on a routine basis.

Presently, routine diagnosis of proliferative lesions

in the rat pituitary in the field of toxicologic pathology is performed almost exclusively from formalin-fixed, paraffin-embedded, and hematoxylin and eosin-stained sections, thus requiring a more simplified approach to classification systems. This simplified philosophy with regard to proliferative lesions in the pituitary of the rat was adopted.

As stated previously, the use of terminology describing tinctorial characteristics, i.e., chromophobic, has been abandoned by most classification systems and is not recommended.

Morphologic modifiers, i.e., angiomatous, should not be used directly in the tabulation of pituitary neoplasms in the rat, but left for textual comments concerning the specific lesion.

One of the most controversial topics associated with pituitary neoplasms in the rat has been those lesions manifesting a "pleomorphic" cell pattern. Some authors have considered these neoplasms to be "adenomas with malignant potential"(7) or areas representing "carcinomatous transformation"(8). Others have described these features but have determined that they do not represent the presence of a malignancy (2,6,13). The term carcinoma in our classification is restricted to neoplasms showing evidence of metastasis or local invasion. With regard to local invasion, which in nearly all cases proceeds into the brain, clear-cut evidence of aggressive growth through the meninges is considered evidence of malignancy.

## NOMENCLATURE AND DIAGNOSTIC CRITERIA PARS DISTALIS

### FOCAL HYPERTROPHY

1. Slightly delineated focus or area
2. No apparent compression
3. Indistinct borders
4. Cells enlarged due to increase in eosinophilic cytoplasm-droplets or vacuoles
5. Nuclei normal
6. Reduction in cells per unit area - nonproliferative

### FOCAL HYPERPLASIA

1. Focal proliferation of single cell type
2. Little or no compression
3. Relatively indistinct borders
4. Cells slightly larger and paler than normal
5. Nuclei often similar to normal cells
6. Occasional "normal" cell types interspersed in focus

### ADENOMA

1. Growth by expansion with prominent compression

2. Sharp delineation from normal areas
3. Cells usually larger with abundant, pale cytoplasm
4. Nuclei more vesicular
5. Variety of architectural patterns — diffuse, angiomatous, glandular, pseudofollicular, pleomorphic

#### CARCINOMA

1. Morphologically similar to adenoma
2. Rarely metastasize
3. Local invasion into brain

#### PARS INTERMEDIA

##### HYPERPLASIA

1. Diffuse broadening of intermediate zone
2. Normal cell morphology

##### ADENOMA

1. Nonencapsulated and poorly circumscribed
2. Cells extend into other zones of gland
3. Cells larger and more basophilic than normal
4. Nuclear pleomorphism common
5. Lobular or whorled pattern

#### PARS NERVOSA

##### PITUICYTOMA

1. Circumscribed, nonencapsulated
2. Dense packets of spindle cells
3. Indistinct cords and interlacing bundles
4. Dark ovoid to round nuclei and minimal indistinct cytoplasm
5. Nuclear pleomorphism seen in some regions
6. Foci of mineralization sometimes present

#### TUMOR OF EMBRYONIC REMNANT

##### CRANIOPHARYNGIOMA

1. Keratinizing squamous epithelium
2. Cells arranged in cords, columns, nests
3. Cystic spaces filled with keratin and debris
4. Invasion of brain or pituitary tissue

#### REFERENCES

1. Barsoum NJ, Moore JD, Gough AW, Sturgess JM, and DeLa Iglesia FA (1985). Morphofunctional investigations on spontaneous pituitary tumors in Wistar rats. *Toxicol. Pathol.* 13: 200-207.
2. Berkvens JM, Van Nesselrooy JH, and Kroes R (1980). Spontaneous tumors in the pituitary gland of old Wistar rats. A morphologic and immunocytochemical study. *J. Pathol.* 130: 179-191.
3. Berry PH (1986). Effect of diet or reproductive status on the histology of spontaneous pituitary tumors in female Wistar rats. *Vet. Pathol.* 23: 606-618.
4. Burek JD (1978). *Pathology of Aging Rats*. CRC Press, Inc., West Palm Beach, FL, pp. 54-58.
5. Capen CC (1983). Functional and pathologic interrelationships of the pituitary gland and the hypothalamus. In: *Endocrine System-Monographs on Pathology of Laboratory Animals*, TC Jones, U Mohr, and RD Hunt (eds). Springer-Verlag, Berlin, pp. 101-120.
6. Carlton HW and Gries CL (1983). Adenoma and carcinoma in pars distalis and pars intermedia. In: *Endocrine System-Monographs on Pathology of Laboratory Animals*, TC Jones, U Mohr, and RD Hunt (eds). Springer-Verlag, Berlin, pp. 134-153, 156-160.
7. Crocker DW (1988). The pituitary gland. In: *Surgical Pathology*, WF Coulson (ed). JB Lippincott, Philadelphia, pp. 956-982.
8. Furth J, Nakane PK, and Pasteels JL (1976). Tumors of the pituitary gland. In: *Pathology of Tumors in Laboratory Animals, Vol I, Part 2*, VS Turusov (ed). IARC Sci. Publ. 6: 201-238.
9. Ito A, Moy P, Kaunitz H, Kortwright K, Clark S, Furth J and Meites J (1972). Incidence and character of the spontaneous pituitary tumors in strain CR and W/Fu male rats. *J. Natl. Cancer Inst.* 49: 701-711.
10. McComb DJ, Kovacs K, Beri J, and Zak F (1984). Pituitary adenomas in old Sprague-Dawley rats. A histologic, ultrastructural, immunocytochemical study. *J Natl Cancer Inst.* 73: 1143-1166.
11. Osamura RY (1983). Histology, ultrastructure and immunocytochemistry in the rat pituitary. In: *Endocrine System - Monographs on Pathology of Laboratory Animals*, TC Jones, U Mohr, and RD Hunt (eds). Springer-Verlag, Berlin, pp. 121-129.
12. Robbins SL, Cotran RS, and Kumar V (1984). *Pathologic Basis of Disease*. WB Saunders Company, Philadelphia, PA, pp. 1192-1200.
13. Sandusky GE, VanPelt CS, Todd GC, and Wightman K (1988). An immunocytochemical study of pituitary adenomas and focal hyperplasia in old Sprague-Dawley and Fisher 344 rats. *Toxicol. Pathol.* 16: 376-380.
14. van Zwieten MJ (1984). The Rat as Animal Model in Breast Cancer Research. Martinus Nijhoff Publ., Boston, pp. 159-182.

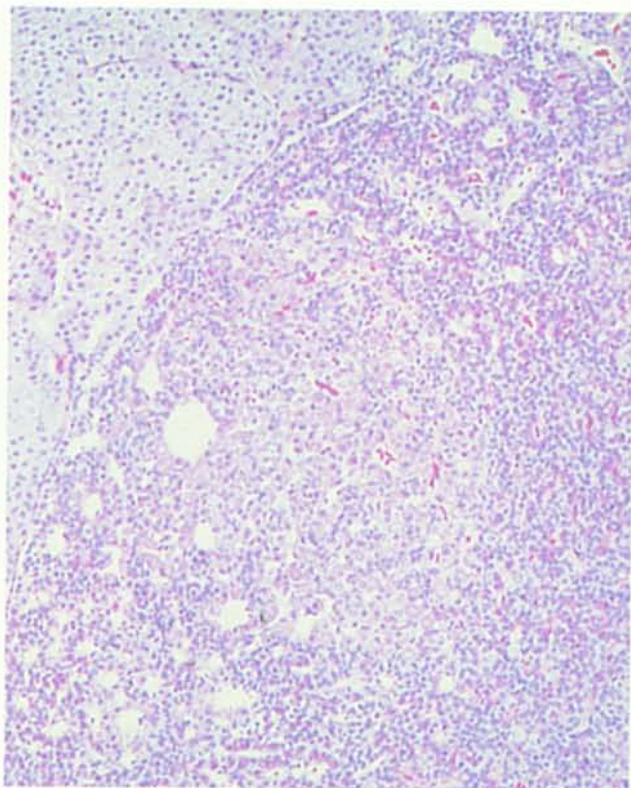


Fig. 1. Pars Distalis, Focal Hypertrophy - Noncompressive focus of cells with more abundant pale, eosinophilic cytoplasm. Considered nonproliferative with reduced number of cells/unit area. (H&E, 40x)

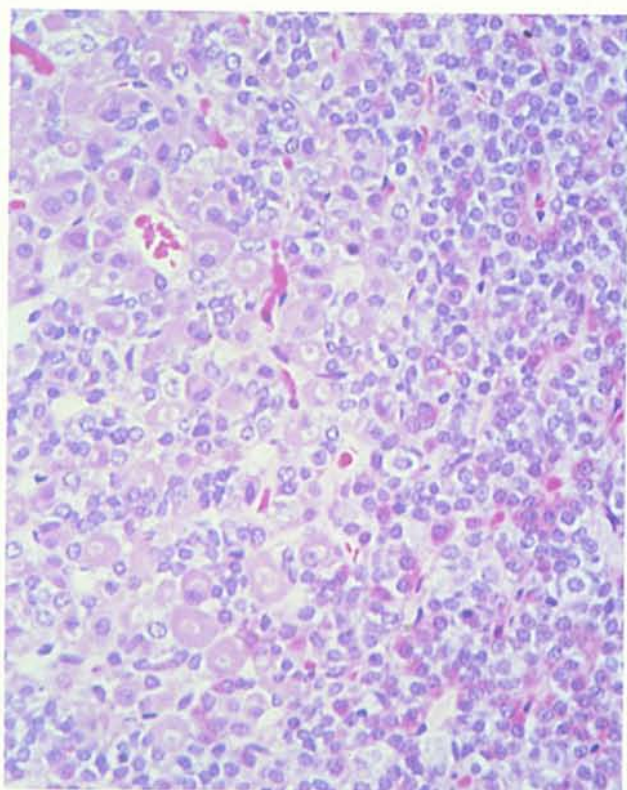


Fig. 2. Focal Hypertrophy - Higher magnification of Figure 1. (H&E, 200x)

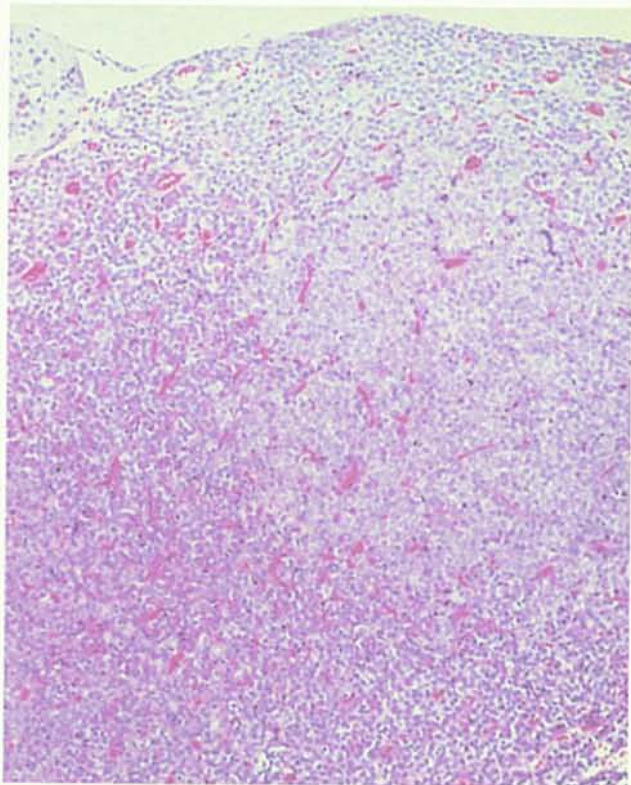


Fig. 3. Pars Distalis, Focal Hyperplasia - Focus of cellular proliferation with little or no compression; cells near normal in appearance. (H&E, 40x)

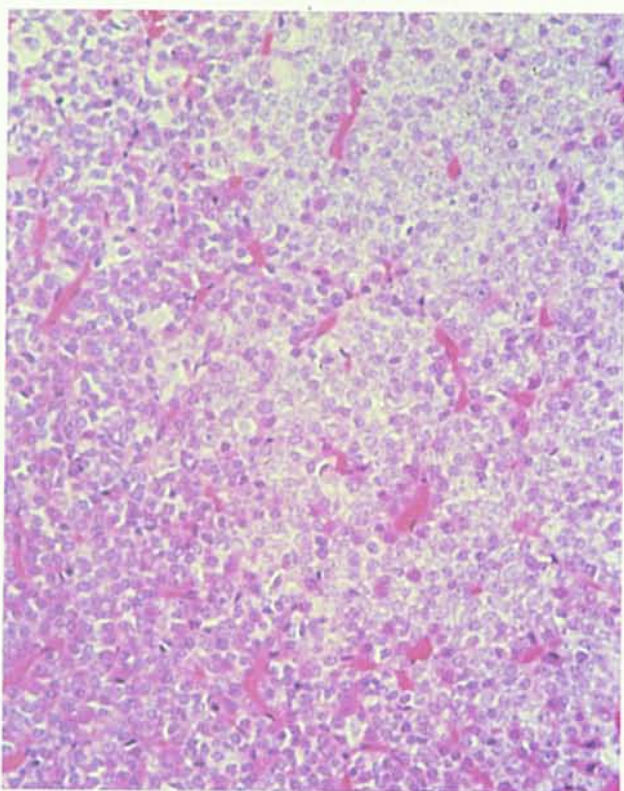


Fig. 4. Pars Distalis, Focal Hyperplasia - Higher magnification of Figure 3; cells in focus blend with adjacent tissue. (H&E, 200x)

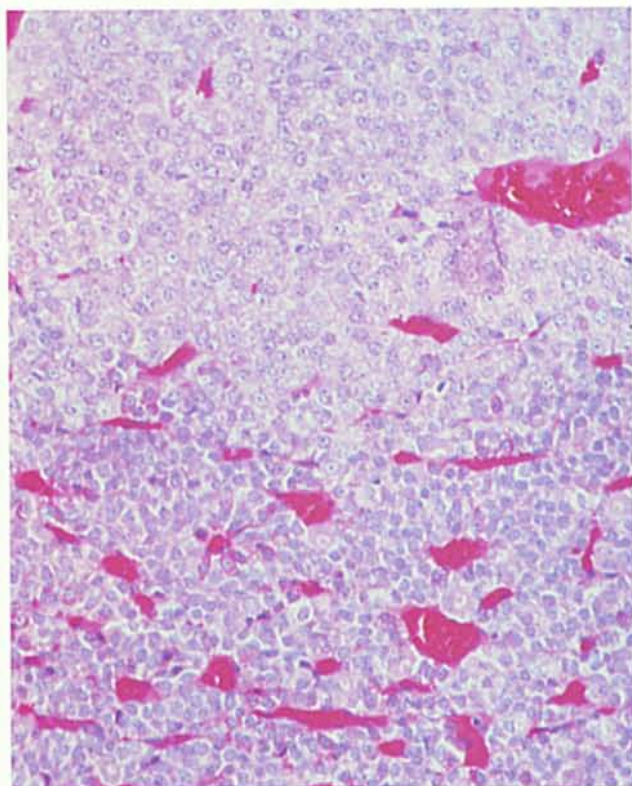


Fig. 5. Pars Distalis, Adenoma - Sharply demarcated proliferation of large cells with large vesicular nuclei. (H&E, 100x)

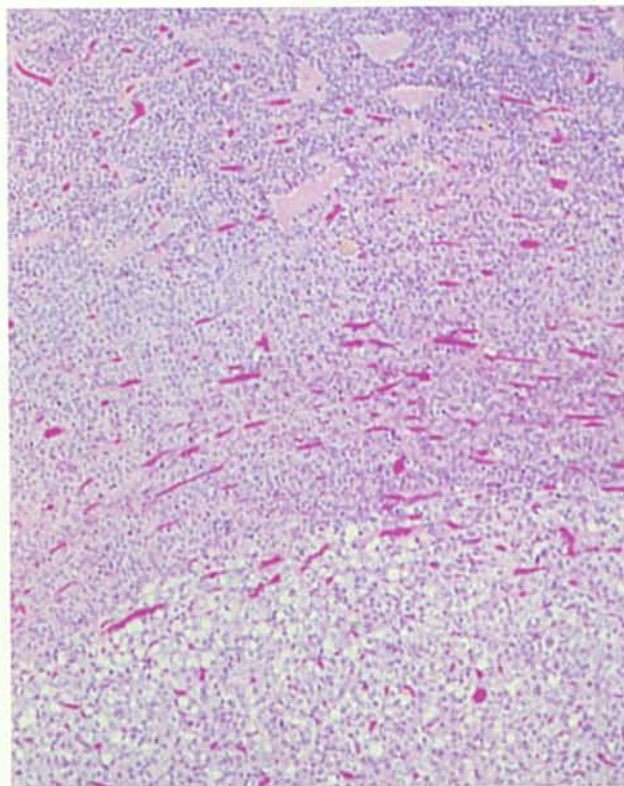


Fig. 6. Pars Distalis, Adenomas, - vacuolated and pseudofollicular patterns within one gland. (H&E, 40x)

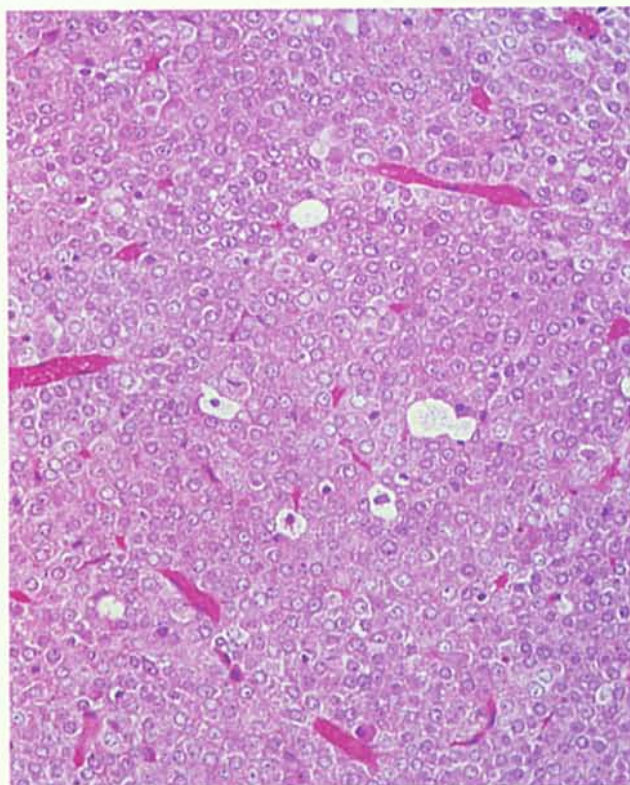


Fig. 7. Pars Distalis, Adenoma - diffuse sheets of uniform large eosinophilic cells. (H&E, 100x)

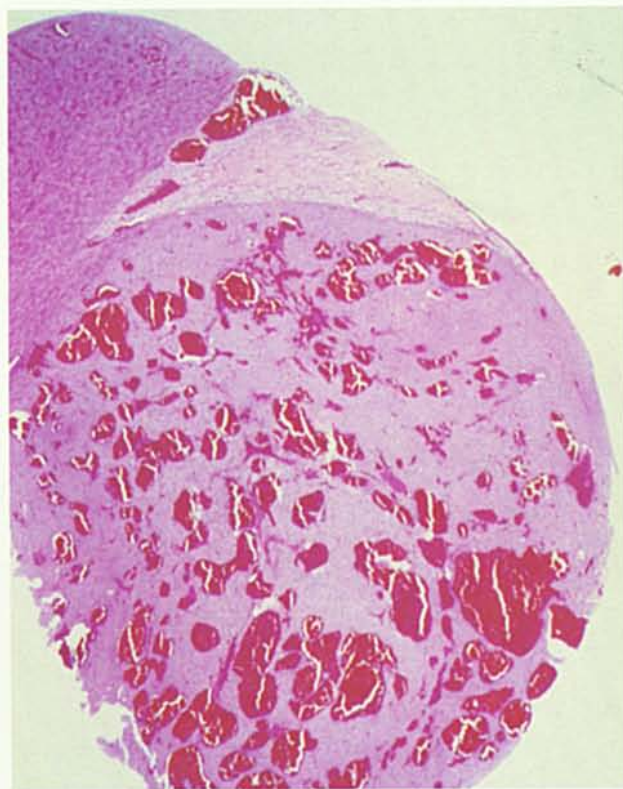


Fig. 8. Pars Distalis, Adenoma - Angiomatous or cavernous pattern. (H&E, 20x)

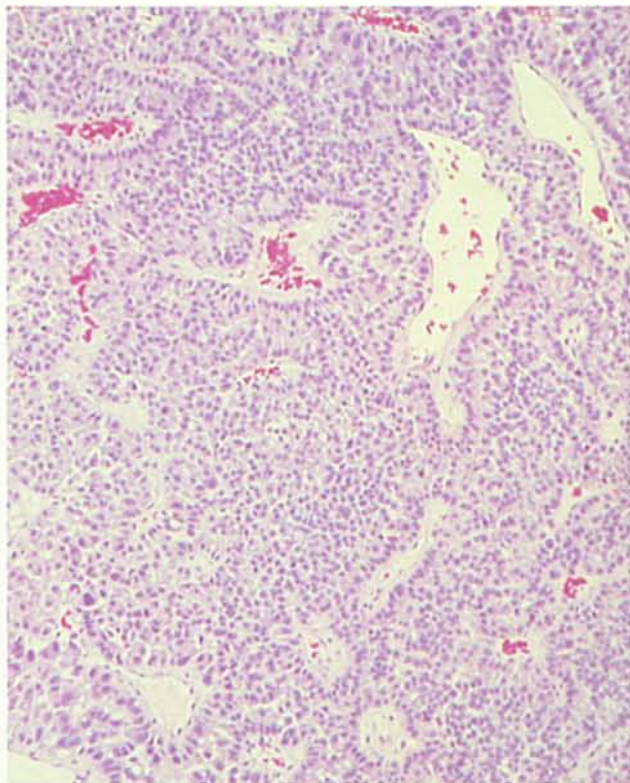


Fig. 9. Pars Distalis, Adenoma - Glandular pattern. (H&E, 40x)

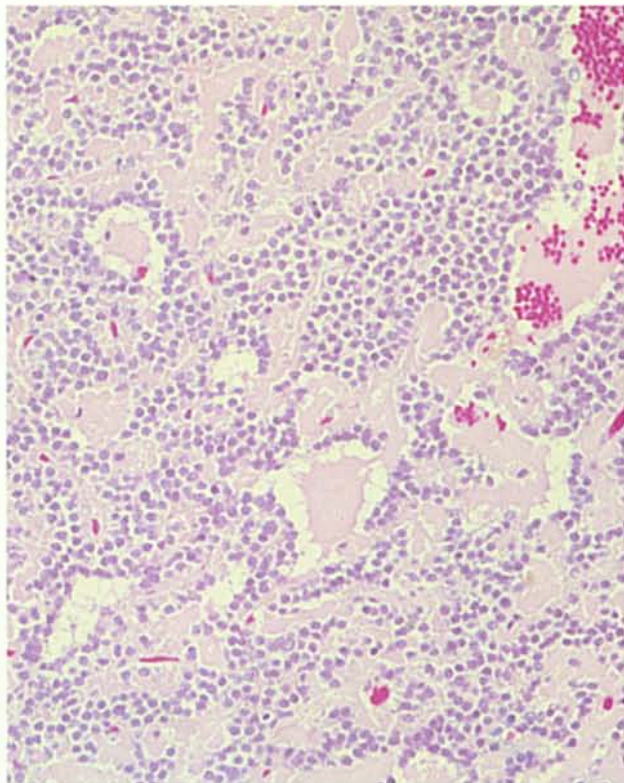


Fig. 10. Pars Distalis, Adenoma - Pseudofollicular pattern. (H&E, 40x)

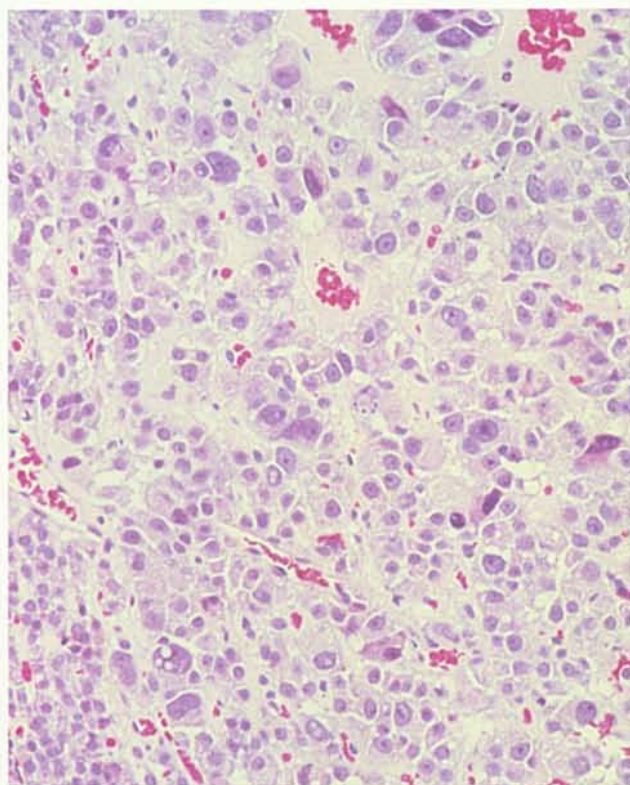


Fig. 11. Pars Distalis, Adenoma - Area of pleomorphism, giant cells, and increased mitotic index. (H&E, 200x)

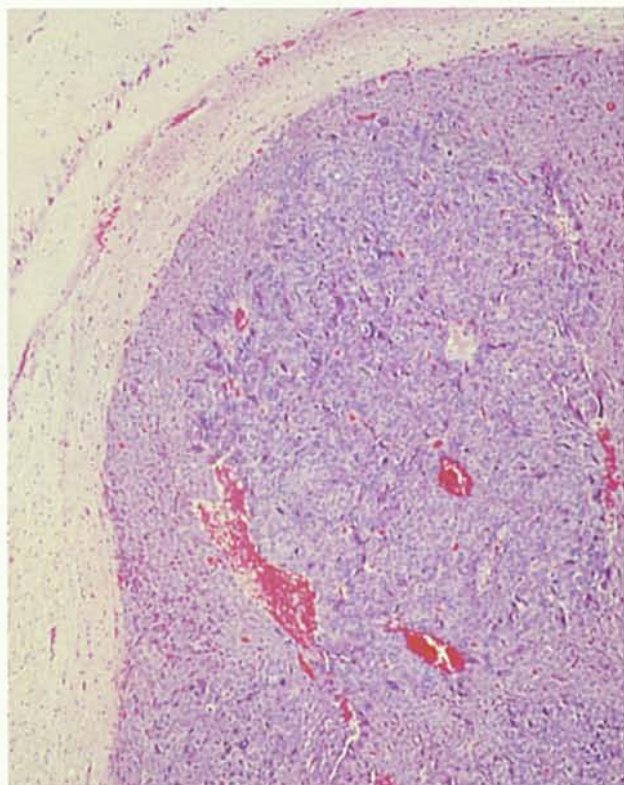


Fig. 12. Pars Distalis, Adenoma - Expansive growth with prominent compression of brain parenchyma. (H&E, 40x)

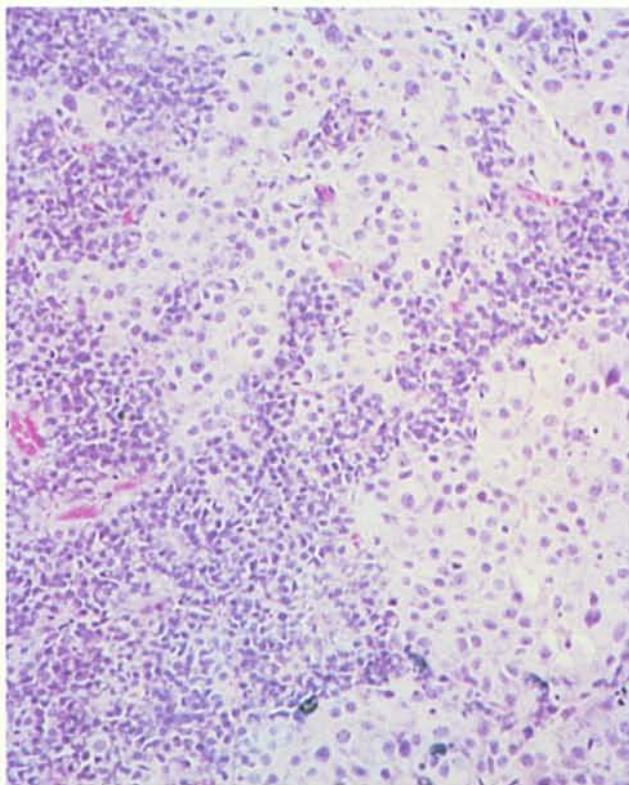


Fig. 13. Pars Intermedia, Adenoma - Extension into the pars distalis. Note the presence of nuclear pleomorphism. (H&E, 100x)

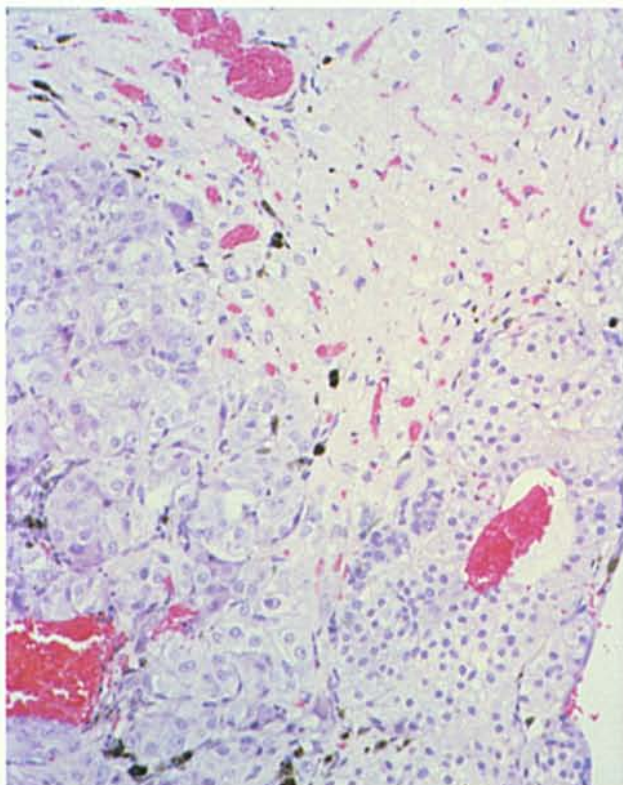


Fig. 14. Pars Intermedia, Adenoma - Tumor cells have extended into the pars nervosa. (H&E, 40x)

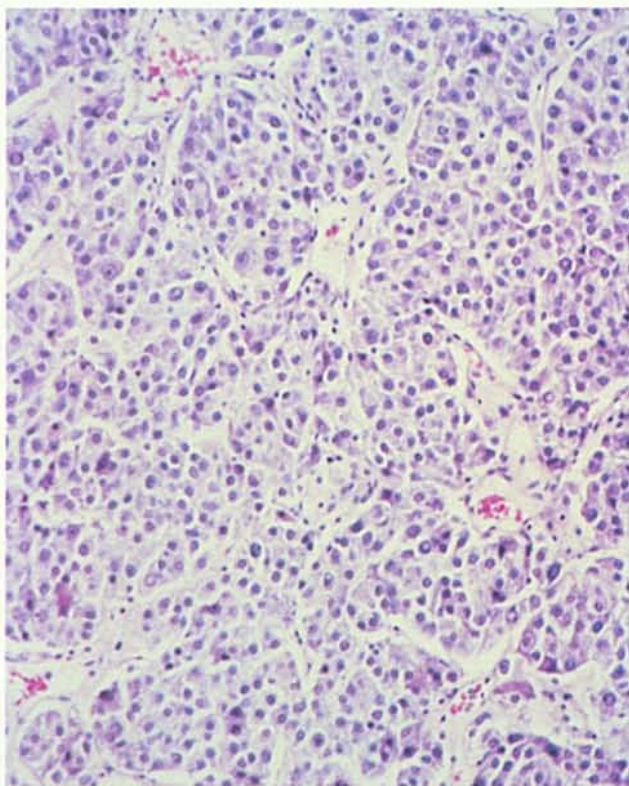


Fig. 15. Pars Intermedia, Adenoma - Islands of tumor cells are separated by thin strands of connective tissue. Slight nuclear pleomorphism is present. (H&E, 100x)

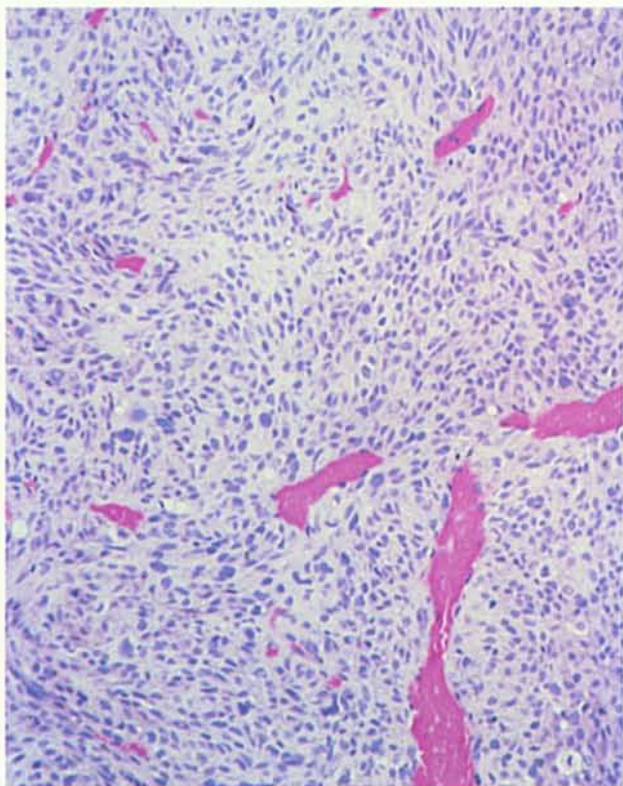


Fig. 16. Pars Intermedia, Adenoma - Note ill-defined whorls, nuclear pleomorphism and prominent vascular spaces. (H&E, 100x)