

PROLIFERATIVE LESIONS OF THE ADRENAL GLANDS IN RATS

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INTRODUCTION

Although adrenal cortical degenerative lesions are common in aged rats, focal non-neoplastic proliferative lesions are less commonly reported within the cortical region (1-3). Furthermore, any attempts to separate literature reports of non-neoplastic proliferative changes of the adrenal cortex from neoplastic lesions is fraught with difficulty, owing to confusing terminology (4). Nodular proliferative changes have been described as arising spontaneously as well as secondary to reparative mechanisms (4). Their ultrastructural characteristics have been described in male Wistar rats (5). Experimentally induced hyperplasia is rarely reported (6-8). Serial-sections of glands have been recommended when studying the accurate prevalence of these often discrete changes (10).

Although spontaneously developing adrenal cortical tumors occur at a relatively low frequency in most rat stocks and strains (1-4), Osborne-Mendel rats (23) and one strain of Wistar rats (24) are exceptions (5,11). Laboratory rats commonly used currently in toxicity studies, such as the Sprague-Dawley and Fischer 344 rat, have a relatively low and sporadic incidence of spontaneously occurring adrenal cortical tumors (12). Hyperplastic and neoplastic changes in the rat adrenal

cortex are most commonly seen in females over 18 months of age (9,12).

Adrenal cortical neoplasms have been induced experimentally in rats with a wide variety of agents including estrogens (13), irradiation (14-16), certain chlorinated hydrocarbons (17-20) and benzenes (21). Many induced neoplasms have been reported to produce corticoids (22). Strandberg (12,26) provides an excellent review of the various agents that induce adrenal cortical adenomas and adenocarcinomas in the rat.

Adenomas appear to be far more prevalent than carcinomas; however, hormonally-active, transplantable cortical tumors have been described (25). Although some authors restrict the diagnosis of carcinoma to tumors with clear evidence of metastasis (3), direct invasion of the capsule is a more prevalent criterion (26).

In the rat adrenal gland, the medulla is centrally located and comprises about 10% of the weight of the gland (27). Grossly, the medulla is a gray, rounded structure sharply demarcated from the outer rim of golden brown cortex. While not essential for life, the medulla is a significant source of catecholamines, neuropeptides and other secretory products that have body-wide effects on homeostasis (28). In general, the adrenal medulla in the adult rat has been considered to have little proliferative activity (29). However, from the recent literature it is clear that various endogenous factors, such as strain, sex, age, and exogenous factors,

such as drugs and chemicals, can impact this situation resulting in augmented proliferative ability (27). Some authors feel that adrenal medullary proliferative lesions commonly occur in aged rats (30,31). Further, the adrenal gland has been ranked highest among the endocrine tissues with respect to susceptibility to drug/chemical induced lesions (i.e., hypertrophy or proliferation) (32).

Pheochromocytoma is a commonly observed tumor in aged rats, particularly in males (36-45). Tumors rarely occur before one year of age and the incidence increases thereafter (11,33,46-49). Reported incidences vary with strain (31,43-45,47,49-56), sex (41,43,44,49,51), age (31,33,50), diet (57-58), environmental conditions (37), and criteria for diagnosis (33,37,39,43-45,56,60-62). Tumors have been associated with the administration of growth hormone (61,63), thiouracil (64), estrogens (13), radiation (65,75), retinol acetate (66), neuroleptics (59), vitamin A analogues (59), nicotine (57), reserpine (67), zomepirac sodium (31), and 4-chloro-m-phenylenediamine (68) among others. These different agents generally can be grouped by common sites of action or mechanistic categories (e.g., hypothalamic-endocrine, autonomic nervous system modulated, calcium absorption associated and miscellaneous) (27,32).

Pheochromocytomas in rats are rarely detected clinically, but may be diagnosed at necropsy or, more frequently, microscopically. Grossly, the affected gland may appear enlarged or contain circumscribed tan or dark red nodules (46). Liver necrosis, chronic myocarditis, and nephrosclerosis are frequent findings in rats with complex pheochromocytomas (37).

Malignant pheochromocytomas are uncommon, but have been shown to invade the overlying capsule and to metastasize to lung, liver, and regional lymph nodes (11,33,69,70). Complex pheochromocytomas are rare (11). Actual incidence is difficult to determine as many of these lesions have been diagnosed as pheochromocytomas and ganglioneuromas. Reznik reported that tumors of the complex type are almost always associated with neoplastic pheochromocytes (71). Often these tumors have an intimate association with pheochromocytes with no distinct border between the neoplasms. Both cells are derived from fetal sympathoblasts and such findings may represent two differentiation stages of the same "stem cell" rather than two independently developing neoplastic cell types.

Ganglioneuroma is a neoplasm of neuronal elements composed of well-developed ganglion cells in a fibrous neural stroma (72). Grossly, ganglioneuromas of the rat adrenal medulla can vary greatly in size. Large lesions can displace virtually all of the adrenal, both the cortex and medulla. It is not unusual to find smaller lesions confined to the medulla without extension into

the cortex. As a rule, ganglioneuromas are unilateral.

The incidence of ganglioneuroma is extremely low (11, 33, 73); i.e., 28 were diagnosed in a retrospective study of the adrenal medulla of 60,048 rats (71). The chromaffin and neuronal cells of the adrenal medulla have a common origin (71). They differentiate from primitive sympathogonia of neuroectodermal crest origin. In keeping with their neural origin, the chromaffin cells of the mature adrenal medulla are innervated by cholinergic preganglionic sympathetic nerve fibers (74). The normal rat adrenal medulla consists almost entirely of chromaffin cells. Very few neurons are present (71).

Neuroblastoma of the adrenal medulla of rats appears to be a very rare tumor, as only two reports have been found in the literature (11,65). The tumors are mostly microscopic and display features and patterns consistent with a neural origin.

MORPHOLOGY

FOCAL ADRENAL CORTICAL HYPERPLASIA (Figures 1, 2)

Focal adrenal cortical hyperplasia of the rat occurs as single or multiple well-demarcated lesions, most commonly found in the zona fasciculata and zona reticularis. Foci maintain architectural relationships and produce minor or no compression of the adjacent parenchyma. If compression exists, it is regional around the circumference of the lesion. The cells may closely resemble those of the surrounding cortex, be tinctorially distinct and smaller in size, or display vacuolated cytoplasm. Highly vacuolated cells may be more numerous in the inner region of the foci. Mitotic activity typically is low and cellular atypia is not present. These foci may be associated with areas of telangiectasia, hemorrhage and thrombosis, all common degenerative changes in aged rat adrenal cortices.

ADRENAL CORTICAL ADENOMA (Figure 3)

Benign cortical tumors (adenomas) are of varying size, ranging from microscopic nodules to large tumors that cause distortion of the cortex and medulla. The tumors are usually well-defined nodular proliferations that arise from cells of the zona fasciculata and zona reticularis. Around the periphery, there is definite compression due to the expansile growth. Cells may be of a single or mixed population. Some tumors are comprised of large cells with vacuolated cytoplasm, while others are composed of small cells with a dense eosinophilic cytoplasm. The large cell variant must be differentiated from a simple focus of cortical cell

hypertrophy. Hypertrophic foci can cause compression due to cytomegaly, but otherwise do not show proliferative activity. Those tumors having a pure population are usually well-differentiated and may attain some cordal architecture within the tumor. Some of the large tumors may have areas of necrosis, hemorrhage and blood-filled cavities. These hemorrhagic and cystic vascular lesions should not be confused with cystic degeneration, a cortical lesion containing blood-filled cavities. These degenerative cavities also may cause compression due to the expansion of the blood-filled vascular channels.

ADRENAL CORTICAL CARCINOMA (Figure 4)

Tumors classified as adrenal cortical carcinomas are usually large with significant distortion of the cortex. The cells have varying degrees of differentiation ranging from well-differentiated cells consistent with normal cortical cells of the zona fasciculata to poorly differentiated or anaplastic epithelial cells with pleomorphic and vesicular nuclei. In some tumors, the cells are arranged in lobular and trabecular patterns. Mitotic figures are frequent. Areas of necrosis, hemorrhage and an angiomatous reaction may occur within the tumors. A significant feature for classification is evidence of invasion of the capsule, adjacent blood or lymphatic vessels or distant parenchyma. Metastasis is common and the usual sites of spread are to regional lymph nodes and the lungs.

ADRENAL MEDULLARY HYPERPLASIA (Figure 5)

Proliferative changes such as hypertrophy and hyperplasia generally are not discernible grossly, but may be evident by increased organ weights. Hyperplasia of the adrenal medulla can be either nodular or diffuse. Nodular, or focal, hyperplasia refers to collections of medullary cells that are distinguishable from the surrounding medullary tissue by cellular and/or tinctorial differences. Cells in hyperplastic areas may show higher nuclear:cytoplasmic ratios, nuclear pleomorphism and/or basophilia. Hyperplastic foci can be found anywhere within the medulla, but frequently are located at the corticomedullary junction. Hyperplastic areas do not compress adjacent normal tissue.

Diffuse hyperplasia of the medulla is defined by several criteria. Overall, there is an increased number of medullary cells in the absence of nodular formations. Cellular elements may be arranged in nests or cords. Mitotic activity is not a prominent feature. Compression of cortical tissue at the margins of the medulla is not found, although the cortex may be somewhat thinned.

PHEOCHROMOCYTOMA (Figure 6)

This tumor is a discrete nodular mass of medullary cells which may be limited to the medulla or extend into the cortex. The tumor is delineated and distinguished by its altered architecture and/or cytological characteristics. When large, the tumor may severely compress the surrounding parenchyma causing cortical atrophy. Tumor cells usually have a decreased cytoplasmic volume, but can be larger than normal cells, show increased cytoplasmic basophilia, and be arranged in variable-sized aggregates (islets) and/or trabeculae. Nuclei are generally larger, hyperchromatic, and the mitotic index is variable. The content of chromaffin granules in tumors is variable and generally no significant quantities of catecholamines are demonstrable by histochemistry. Small quantities of neurosecretory granules may be detected by electron microscopy.

MALIGNANT PHEOCHROMOCYTOMA

(Figures 7-9)

Numerous histologic features have been used to distinguish malignant pheochromocytoma from its benign counterparts. Some of these features include the following: increased nuclear:cytoplasmic ratio, cellular anaplasia, nuclear pleomorphism, increased mitotic index, hemorrhage, necrosis, larger size, increased vascularity, invasion and distant metastasis. However, the diagnosis of malignancy cannot be made strictly on the basis of cytologic characteristics; metastasis or capsular invasion must be identified.

COMPLEX PHEOCHROMOCYTOMA

(Figures 10, 11)

Complex pheochromocytomas are usually solitary masses within the medulla. The tumor consists of morphological components of both the pheochromocytoma and the ganglioneuroma. Thus, the tumor is composed of a mixture of pheochromocytes, ganglion cells, Schwann cells, and neurofibrils.

GANGLIONEUROMAS (Figure 12)

Ganglioneuromas are composed of ganglion cells and supporting neural stroma. They often are found together with pheochromocytomas. Ganglion cells have the typical appearance of large neurons. The cells are multipolar and their nuclei are large and pale with prominent nucleoli. The peripheral cytoplasm stains positively for Nissl substance. The supporting stroma is made up of Schwann's cells, capsular cells or satellite cells. These stromal cells have little cytoplasm and may have elongated nuclei arranged in parallel bundles

resembling the palisade arrangements of schwannomas.

NEUROBLASTOMA

The cells generally are small, round and unipolar, stain deeply and have elongated nuclei. Cellular proliferations are usually present in a palisading pattern around sinusoids and form rosettes resulting in a typical "neural" pattern. Tumors are confined to the adrenal medulla, as metastasis is not reported.

DISCUSSION

Although the classification of cortical proliferative lesions might be argued by experts, the distinction between hyperplasia and neoplasia in the rat adrenal medulla is far more controversial (47,69,76,77,96). Some authors consider the two processes to be indistinguishable and designate all medullary proliferative lesions as neoplasms (47). Most authors, however, agree that prominent compression of the normal surrounding tissue at the edge of the lesion and alteration of the normal architecture are characteristics of neoplastic growth (69,76,78). Accordingly, the criteria outlined above rely primarily on the absence of compression of surrounding tissue to separate hyperplasia from neoplasia.

The cell of origin in medullary nodular lesions, hyperplasias or neoplasias, is not clear in the rat. The adult rat adrenal medulla contains three types of cells, as well as scattered neurons (78). The cells can be characterized as epinephrine, norepinephrine, or small granule-containing cells (SGC cells) by the predominant type of membrane-bound secretory granules they contain (48). Granules are distinguishable ultrastructurally after fixation in glutaraldehyde (48). Epinephrine cells are the most numerous, containing moderately electron-dense granules (79). Norepinephrine cells are less common and contain highly electron-dense granules (79). SGC cells are the least common cell type, comprising less than 4% of the medullary cell population (80). Their granules are less than half the size of those in the other secretory cell types and are of variable electron density (80). Recent work suggests that many adrenal medullary nodules in aging Long-Evans rats are composed of SGC cells while diffuse hyperplasias, thought to precede the development of nodules, contain epinephrine and norepinephrine-type cells (36,80). There is still much to be done to clarify the origin and fate of rat adrenal medullary hyperplasias.

It is generally accepted by pathologists that the spectrum of change for proliferative adrenal medullary lesions represents a continuum and any classification

system is necessarily based on arbitrary distinctions (11,37,43,44,56,61,65,76,96). Consequently, statistical analyses should consider these lesions individually as well as a group (81).

Due to unresolved issues of pathogenesis, cell of origin, and relevance to the human counterpart, some differences of opinion exist concerning the most appropriate terminology to be used in the diagnosis of proliferative medullary lesions (35). Citing published reports indicating dissimilarities in clinical function and histochemical features of tumors of the adrenal medulla of the rat and human (48,65,80,83,85), some investigators have suggested the use of adrenal medullary tumor in rats and pheochromocytoma in humans as distinct diagnoses for tumors arising in the adrenal medulla of these different species (31,33). Others prefer the diagnosis of pheochromocytoma for both species because of the similarities in function, morphology, and clinical context between rat adrenal medullary nodules and human pheochromocytomas (27,83). This polarity of opinion concerning the most appropriate terminology for diagnosis of medullary proliferative lesions in the rat has not been resolved. Appropriately sensitive methods, e.g., fluorescence methods employing formaldehyde or glyoxylic acid, may identify hormone, hormone precursors, or other granule constituents (84,85). Until further evidence is provided, the commonly used term, pheochromocytoma, is recommended for the diagnosis of these tumors of the adrenal medulla.

The diagnosis of malignant pheochromocytoma can only be made with certainty if there is evidence of capsular invasion and/or metastasis. Metastasis occurs most frequently to the lung, liver, and regional lymph nodes with a small percentage of tumors. The low reported incidence of metastases of this tumor is supported by transplantation studies indicating pheochromocytomas possess a low grade of malignancy (85). Human pathologists generally agree that metastasis is the only reliable indicator of malignancy in man (84,86-88). Some authors have argued for the use of similar diagnostic criteria for rodent lesions (31).

It is important to distinguish between true capsular and/or periadrenal tissue invasion and artifact resulting from rough handling during necropsy and/or tissue trimming. Also, the plane of section should be considered, as tangential cuts close to the hilus can result in apparent intracortical and subcapsular medullary cells. Also, capillaries of the adrenal gland are of the fenestrated closed type (78). Occasionally, medullary cells, associated with a lesion which would otherwise be classified as focal hyperplasia, are observed within the lumen of a vessel. This phenomenon should not be confused with true vascular invasion by neoplastic cells.

Complex pheochromocytomas often are observed in conjunction with proliferative lesions of the pituitary,

thyroid C-cells and/or pancreatic islet cells (22). This apparent relationship bears some similarities to Mixed Endocrine Neoplasm Syndrome of humans (89). Manger reported that hypophysectomy eliminated proliferative medullary lesions in NEDH rats (90).

Processes by which proliferative medullary lesions arise is unclear (37,67). They may arise as the result of neoplastic transformation and dedifferentiation of pheochromocytes or possibly by the proliferation of small granule-containing cells (77, 91-94). Recently neurotrophic factors have been shown to influence differentiation of medullary cells (95).

Accepting a common progenitor cell for the pheochromocyte and the ganglion cell, it is possible to speculate that pheochromocytomas and ganglioneuromas follow the same developmental steps (33,71,73). The frequent finding in complex neoplasms of ganglioneuromas with associated pheochromocytomas supports this view. Some authors refer to such lesions as "mixed tumors" or "ganglioneuroma-pheochromocytomas." Others do not accept such terminology and consider ganglioneuromas and pheochromocytomas as distinct entities sometimes occurring together (73). In man, neoplasms have been described with a mixture of neuroblast- and pheochromocyte-derived cells (33). Histogenesis of this interesting tumor requires further detailed investigations.

RECOMMENDED NOMENCLATURE AND DIAGNOSTIC CRITERIA FOR THE ADRENAL GLAND

Focal Cortical Hyperplasia

1. Single or multiple, well-demarcated foci.
2. Composed of tinctorially-distinct cells.
3. Little or no compression of adjacent parenchyma.
4. Low mitotic activity or atypia.

Cortical Adenoma

1. Discrete nodules of single or mixed cytological character.
2. Prominent compression of adjacent parenchyma.
3. Vary tinctorially and architecturally from normal parenchyma.
4. Solid, degenerative, or less commonly, glandular.

Cortical Carcinoma

1. Large, compressive, expansile mass.
2. Considerable cytological variability and anaplasia.
3. Frequent mitoses.
4. Direct invasion or distant metastases.

Focal/Nodular Medullary Hyperplasia

1. Single or multiple aggregates of cells with a high nuclear:cytoplasmic ratio.
2. Increased cytoplasmic basophilia.
3. No prominent compression of adjacent tissue.
4. Located anywhere within the medulla but frequently at the corticomedullary junction.

Diffuse Medullary Hyperplasia

1. Expand the medulla due to absolute increase in number of cells but without formation of nodules.
2. Cells are arranged in nests or cords which are wider than seen in normal medulla.
3. Mitotic figures are rare.

Pheochromocytoma

1. Discrete nodular mass of medullary cells usually limited to the medulla but may distort the cortex. Prominent compression of surrounding tissue.
2. Altered architecture with cells arranged in islets and/or trabeculae.
3. Altered cytologic characteristics with cells generally smaller in size and increased in cytoplasmic basophilia.
4. Mitotic rate is variable.

Malignant Pheochromocytoma

1. Diagnosis of malignancy cannot be made on cytologic characteristics alone.
2. Malignant if there is invasion of the capsule and/or metastasis.

Complex Pheochromocytoma

1. Generally discrete medullary mass.
2. Composed of neoplastic pheochromocytes and ganglion cells interspersed among nerve fibers and Schwann cells.

Ganglioneuroma

1. Large ganglion cells scattered within neural stroma.
2. Palisading stromal cells with elongated nuclei and scant cytoplasm.
3. Often with ipsilateral pheochromocytomas.

Neuroblastoma

1. Cells with elongated nuclei and thin cytoplasm.
2. Often form parasinusoidal pallisades or rosettes.
3. Absence of ganglion cells.

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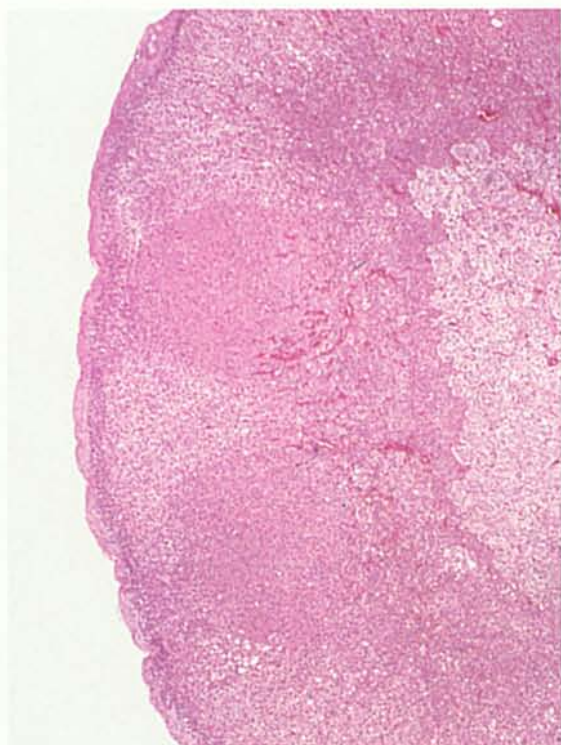


Fig. 1 – Low magnification of multicentric nodular foci of cortical hyperplasia in an aged rat.

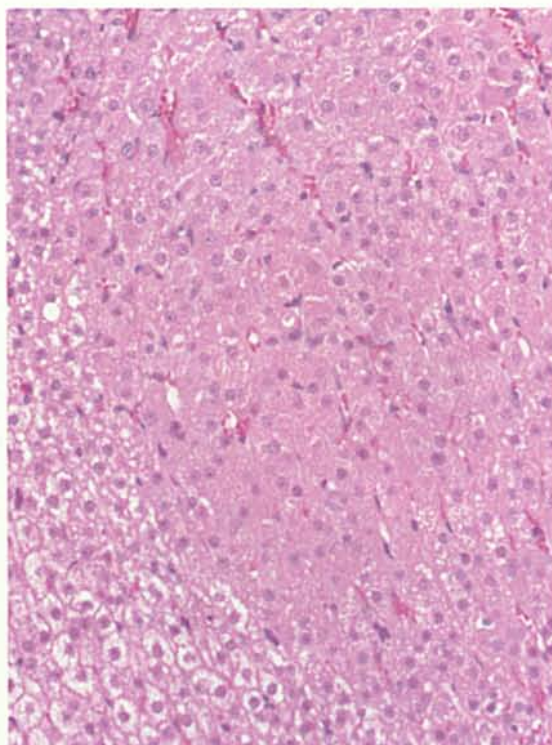


Fig. 2 – Well-demarcated focus of tinctorally-distinct cells with negligible mitotic activity or compression of parenchyma adjacent to focal hyperplasia in an aged rat.

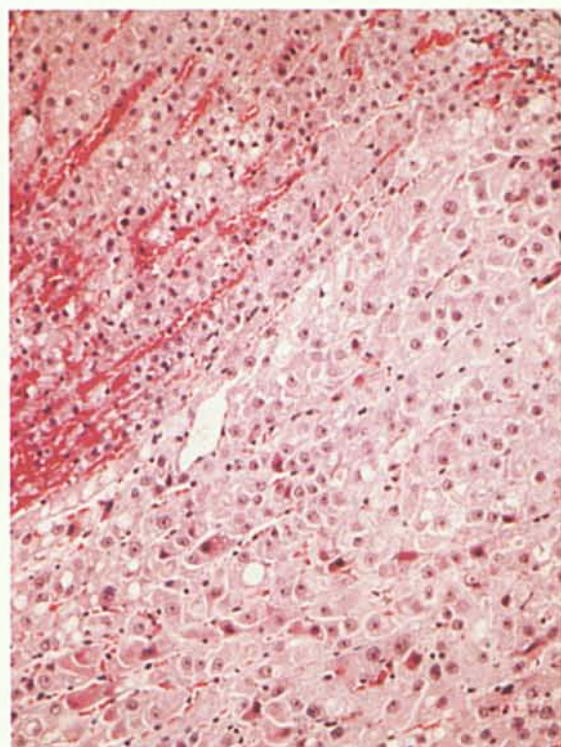


Fig. 3 – Polymorphic cytology in a solid, nodular cortical adenoma, with distinct compression and congestion in adjacent adrenal parenchyma of an aged rat.

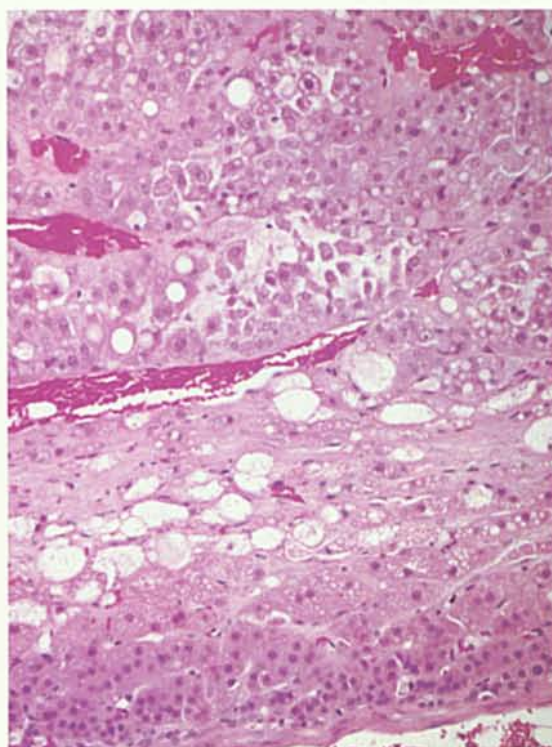


Fig. 4 – Adrenal cortical carcinoma with considerable anaplasia, degeneration, cytological variability, but lower than usual number of mitoses, in an aged rat.

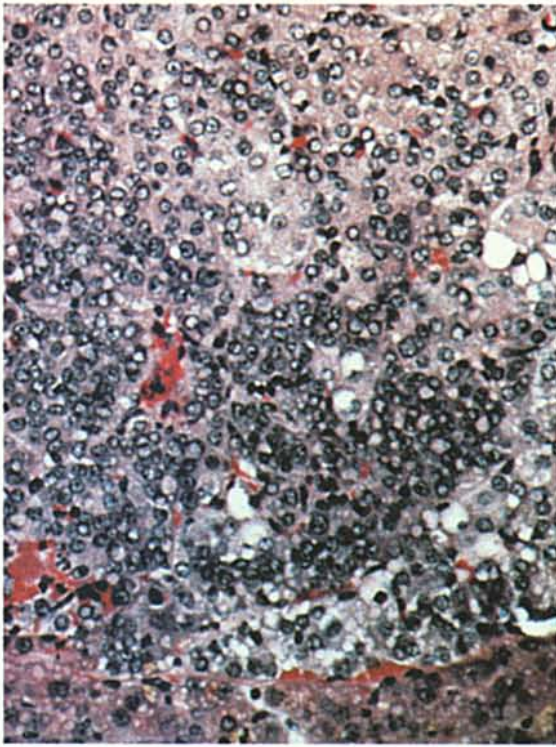


Fig. 5 – Basophilic, poorly demarcated hyperplastic focus of adrenal medullary cells at the typical corticomedullary junction; note high nuclear:cytoplasmic ratio, suggesting greater cell density in this aged rat.

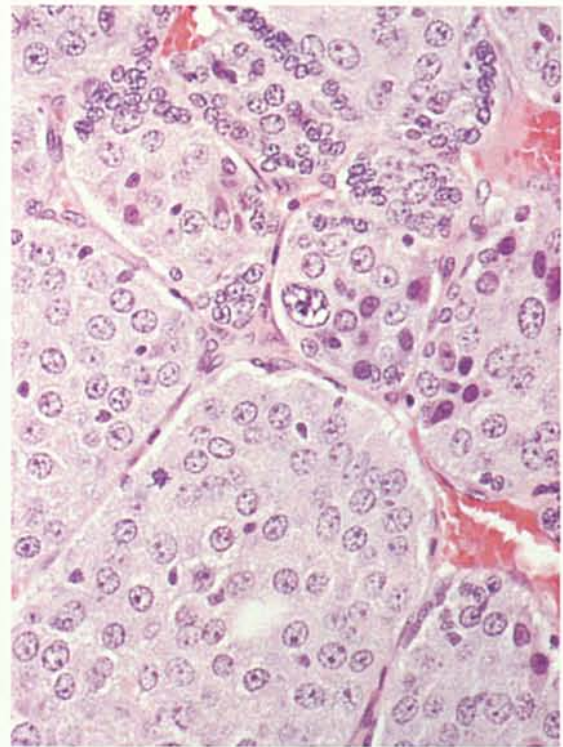


Fig. 6 – Well-differentiated islets or nodules of benign adrenal medullary pheochromocytoma separated by fine fibrovascular tissue; note variable nuclear:cytoplasmic ratios, but negligible mitotic activity.

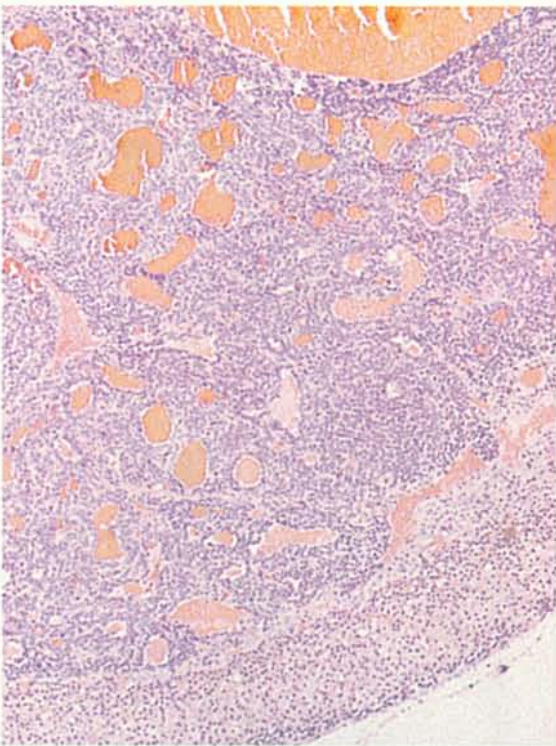


Fig. 7 – Large, telangiectatic, medullary, malignant pheochromocytoma causing considerable cortical compression in an aged rat.

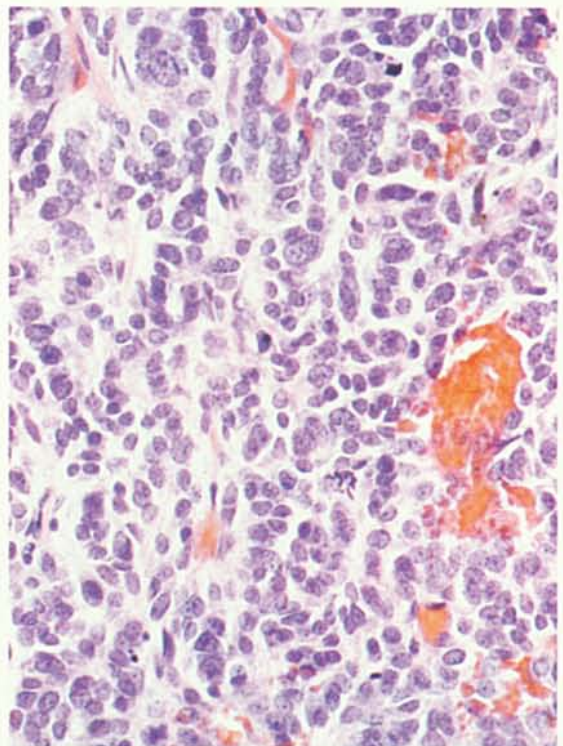


Fig. 8 – Malignant pheochromocytoma with marked cytologic variability and negligible glandular uniformity, occasional focal hemorrhage or hypremia but low mitotic activity in this aged rat.

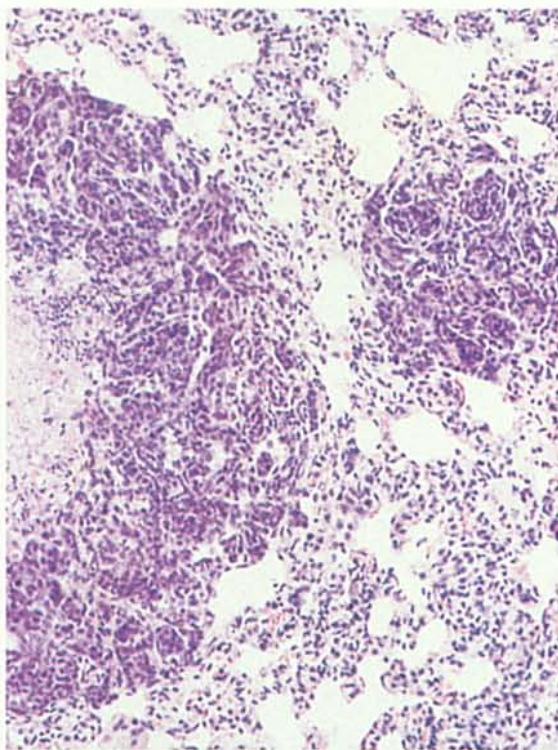


Fig. 9 – Pulmonary metastases of a malignant pheochromocytoma in an aged rat; metastasis or invasion is key to diagnosis of malignancy.

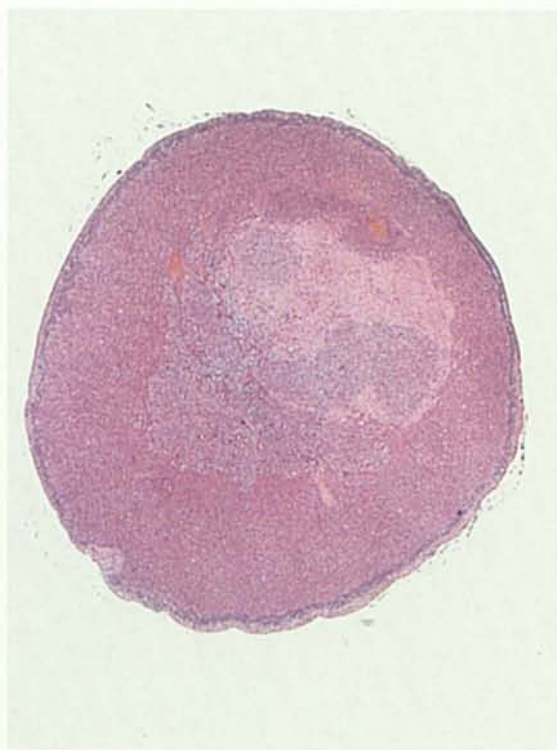


Fig. 10 – Adrenal medullary complex pheochromocytoma with basophilic cellular elements and a pale eosinophilic zone of apparent low cellularity in an aged rat. Shown on higher magnification in Fig. 11.

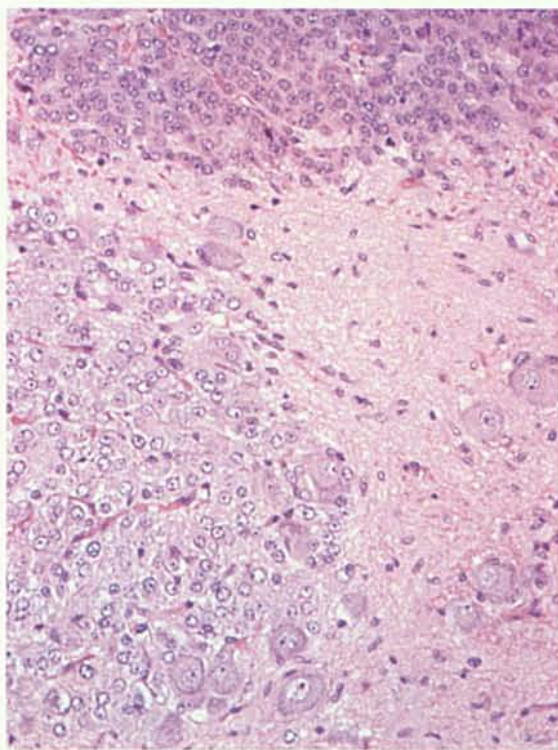


Fig. 11 – Complex pheochromocytoma comprised of neoplastic pheochromocytes and ganglion cells, surrounded by nerve fibers, and Schwann cells within the adrenal medulla of an aged rat.

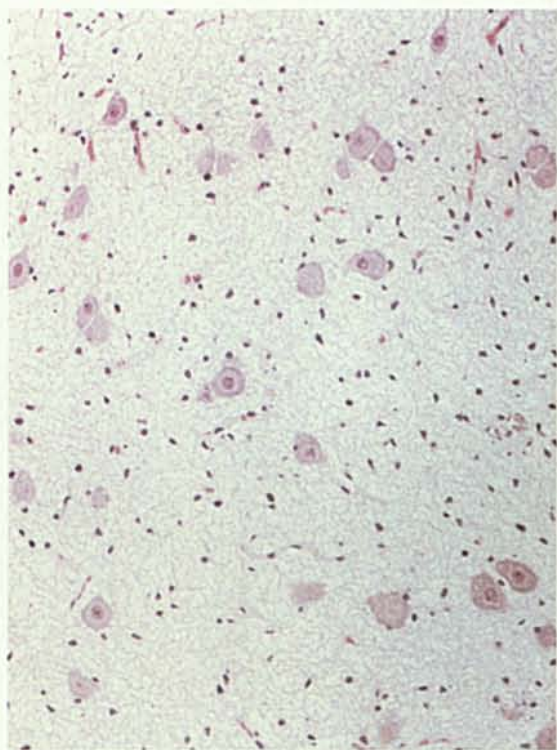


Fig. 12 – Ganglioneuroma from the adrenal gland of an aged rat, with large ganglion cells scattered among an abundant stroma of neural elements.