Lesions of the Adrenal Cortex

Anne Marie McNicol, BSc, MD, FRCPGlas, FRCPath

Context.—In surgical pathology practice adrenal cortical tumors are rare. However, in autopsy series adrenal cortical nodules are found frequently. These are now being identified more commonly in life when the abdomen is scanned for other disease. It is important to differentiate between benign and malignant lesions as adrenal cortical carcinoma is an aggressive tumor. Molecular genetic investigations are providing new information on both pathogenesis of adrenal tumors and basic adrenal development and physiology.

Objective.—To provide an overview of current knowledge on adrenal cortical development and structure that informs our understanding of genetic diseases of the adrenal cortex and adrenal cortical tumors.

Data Sources.—Literature review using PubMed via the Endnote bibliography tool.

Conclusions.—The understanding of basic developmental and physiologic processes permits a better understanding of diseases of the adrenal cortex. The information coming from investigation of the molecular pathology of adrenal cortical tumors is beginning to provide additional tests for the assessment of malignant potential in diagnosis but the mainstay remains traditional histologic analysis.

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Recent advances have significantly informed our understanding of the structure and function of the normal adrenal cortex and of the diseases that affect it. This review highlights some of the findings in relation to the human gland and their relevance to nonneoplastic disease. It also discusses the pathogenesis and diagnosis of adrenal cortical tumors.

NORMAL ADRENAL CORTEX

Structure and Function

The normal adult adrenal gland weighs 4 g at surgical excision or in cases of sudden death. At hospital autopsy the average is 6 g, reflecting the stimulation by adrenocorticotropic hormone (ACTH) in the stress of terminal illness. It is divided into head, body, and tail with alae extending laterally. The medulla comprises approximately 10% of the total weight and is present in the head and body and focally in the alae. The cortex comprises 3 zones with characteristic histologic features. The zona glomerulosa (ZG) is composed of small angular cells with a high nuclear-cytoplasmic ratio, dispersed focally under the capsule. It synthesizes the main mineralocorticoid, aldosterone. The major part is the zona fasciculata (ZF) with large clear lipid-laden cells arranged in columns from the capsule or ZG to the inner zona reticularis (ZR). It is now thought to be the major source of glucocorticoids (cortisol in the human gland). The ZR comprises eosinophilic (compact) cells with little lipid storage arranged in cords around vascular sinusoids. This zone appears to be the source of androgens. All steroids are derived from cholesterol and the enzymes involved in synthesis are 4 of the members of the cytochrome P-450 family, mainly with hydroxylase activities, and a 3β-hydroxysteroid dehydrogenase. These are distributed between the smooth endoplasmic reticulum and the mitochondria and precursors move between these loci during steroid synthesis. The first stage is the transport of cholesterol to the inner mitochondrial membrane by a key protein, steroid acute response protein.1 Cortisol production is mainly controlled by the hypothalamic-pituitary-adrenal axis, by the actions of ACTH. Aldosterone secretion is under the control of the renin-angiotensin system. The control of androgen secretion is still poorly understood. Adrenocorticotropic hormone can have an effect, but other factors are also involved as levels may rise without any change in ACTH (eg, at adrenarche). Other signaling molecules that may play a role in steroidogenesis include insulin-like growth factors (IGFs), IGF-1 and IGF-2,2 vasopressin, adrenomedullin,3 transforming growth factor β, and activin A.4 Catecholamines may also be involved.5

There is a complex vascular supply that may help regulate growth and function by altering blood flow to various compartments. This may be controlled by local release of neurotransmitters from nerve fibers within the cortical plexus.6–8 Interestingly, ACTH appears to be important in the development and maintenance of adrenal vasculature, possibly by regulating the secretion of vascular endothelial growth factor by the endocrine cells.9

Development and Growth

Extended reviews of this subject are available elsewhere.10,11 The adrenal cortex arises from the adrenogenital primordium that develops from the urogenital ridge.11 The Wilms tumor gene (WT1) and wingless-type mouse mammary tumor virus integration site family, member 4 (WNT4) play an early role. Important regulators of development include transcription factors such as steroidogenic factor 1 and a nuclear hormone receptor, dosage sensitive sex reversal–adrenal hypoplasia congenita gene on the X chromosome, gene 1 (DAX1).12 The inner...
Historically, this has been recognized, cells in cycle, as demonstrated by immunopositivity though the bulk of evidence supports the migration theory. This theory also fits with the observation of apoptosis in the inner zones. In the rat, more recent immunohistochemical studies have demonstrated an undifferentiated zone between the ZG and ZF that is proposed as a stem cell zone. This theory also fits with the observation of apoptosis in the inner zones. The zonal theory, in contrast, suggests that each zone proliferates to maintain itself. Although the bulk of evidence supports the migration theory, cells in cycle, as demonstrated by immunopositivity for Ki-67, can be seen in the inner zones suggesting that both mechanisms may coexist.

Hypophysectomy and exogenous glucocorticoids result in atrophy of ZF and ZR, implicating ACTH and related factors in control of growth. Adrenocorticotropic hormone induces hypertrophy of ZF and ZR in vivo followed by increased mitotic activity. However, ACTH is not directly mitogenic in vitro, so the in vivo effects may represent interaction with other factors. Peptides from the N-terminal region of the ACTH precursor, proopiomelanocortin, can cause hypertrophy and hyperplasia. In addition, ACTH stimulates the release of intra-adrenal growth factors including IGF-I and IGF-II, which have trophic and steroidogenic effects. Other factors thought to be involved include epidermal growth factor, basic fibroblast growth factor, and cytokines including interleukin 1, Angiotensin II, vasopressin, vasoactive intestinal peptide, and endothelin 1 may also be important, particularly with reference to the ZG. The actions of ACTH are mediated via immediate early genes Jun and Fos. Transforming growth factor β and activin may have inhibitory roles, the latter by increasing apoptosis.

ASPECTS OF GENETIC DISEASES OF THE ADRENAL CORTEX

Congenital adrenal hyperplasia is a group of autosomal recessive diseases affecting cortisol synthesis. In most forms, mutations or translocations of genes encoding the steroidogenic enzymes lead to inefficient steroidogenesis with decreased negative feedback to the pituitary. This results in increased secretion of ACTH, with adrenocortical hyperplasia. The glands have a characteristic cerebriform appearance. The cortex is lipid depleted, because all cholesterol stores are used for steroidogenesis in an attempt to achieve normal cortisol levels. Each enzyme defect is associated with a characteristic profile of steroid secretion and clinical findings. The most common form is 21-hydroxylase deficiency. In the salt-losing variant of this disease there is abnormal development of the adrenal medulla, with chromaffin cells extending neurites between cortical cells. This is in keeping with the proposed role for cortisol in the development and maintenance of the medulla. There is also a higher frequency of adrenal cortical tumors in patients with congenital adrenal hyperplasia than in the general population, suggesting that chronic stimulation by ACTH may have a role in tumorigenesis. Myelolipomas have also been reported. Congenital lipoid hyperplasia is a very rare cause of congenital adrenal hyperplasia and the histologic appearance of the gland differs from the other variants in that there is significant accumulation of lipid within the cells. Until recently, this was thought to be due to an abnormality in the side-chain cleavage enzyme that starts the process of steroidogenesis. It is now known to be associated with mutations in steroid acute response protein, thus preventing transport of cholesterol to the mitochondrion for steroid synthesis. The accumulation of cholesterol in the cytoplasm adequately explains the unusual histologic appearance.

Primary congenital hypoplasia shows an X-linked pattern of inheritance and is due to mutations or deletions in the DAX1 gene on Xp21, important in the development of steroidogenic tissues. The condition is often fatal and the adrenals are small and difficult to find at autopsy. Secondary hypoplasia may result from lack of ACTH, either as a genetic disease or secondary to acquired hypopituitarism. In isolated familial glucocorticoid insufficiency, glucocorticoid synthesis is impaired, whereas aldosterone is unaffected. This is explained in some cases by the detection of mutations in the ACTH receptor. The pathology is poorly documented.

ADRENAL CORTICAL HYPERFUNCTION

Response to Stress

Chronic stress causes increased output of ACTH and increased stimulation of the adrenal cortex. Adrenal weight increases with enlargement of ZF and ZR. This is probably a combination of hypertrophy, hyperplasia, and reduced apoptosis. Lipid depletion occurs in the ZF in a centrifugal manner. Degenerative changes may be seen in the outer ZF with cords of cells converted into tubular structures. Lipid reversion is characterized by reaccumulation of lipid, also in a centrifugal manner. These changes are often seen in hospital autopsies. Care should be taken not to misinterpret outer lipid depleted cells as hyperplastic ZG.

Chronic Hypersecretion of Hormones

Three classical clinical syndromes are associated with hypersecretion of adrenal cortical steroids: primary hyperaldosteronism (including Conn syndrome), Cushing syndrome (hypercortisolism), and adrenogenital syndrome (hypersecretion of sex steroids).

Primary Hyperaldosteronism.—Historically this has been thought to be a rare cause of hypertension, accounting for less than 1% of patients attending clinics although some would say it is more common, responsible for up to 10% of cases. High aldosterone levels are coupled with low renin and hypokalemia. About two thirds of patients have classical Conn syndrome with an adrenal adenoma. Carcinomas are extremely rare. The tumors are usually small, often less than 2 cm in diameter, and half weigh less than 4 g. Women are affected more commonly than men, with a peak incidence in the third to fifth decades. The cut surface has a golden-yellow color. Histologic examination shows various cell types. Most resemble ZF cells with only a minority of ZG morphology. Hybrid cells show mixed features, containing lipid but with a higher nuclear-cytoplasmic ratio than ZF cells. Compact cells are also found. It has been reported that tumors with ZG morphology respond to angiotensin, whereas those with ZF
The clinical features associated with Cushing syndrome may be a continuum, nodules developing in long-standing disease. The emergence of adrenal autonomy in occasional cases suggests that neoplastic transformation can occur on a background of hyperplasia.

Ectopic ACTH syndrome accounts for 15% of cases, about half caused by secretion of ACTH from a bronchial carcinoid or small cell lung carcinoma. Other tumors associated with the syndrome are thymic carcinoids, islet cell tumors of pancreas, medullary carcinoma of thyroid, and pheochromocytoma. The adenals show marked bilateral symmetrical enlargement weighing on average 15 g each and rarely contain nodules. Compact cells extend close to the capsule, mitotic figures may occasionally be found and pleomorphism is common. Metastases are often present in the gland in patients with bronchial carcinoma.

Fifteen percent to 20% of adults with Cushing syndrome have an adrenal tumor, equally divided between benign and malignant and most common in the fourth and fifth decades. In contrast, more than half of children with the disease have a tumor, and the majority are malignant. Females are affected 4 times as often as males at all ages. Coexistent virilization is more common in carcinomas. Because the high levels of cortisol suppress ACTH secretion from the pituitary, the ZF and ZR of the adjacent cortex and the contralateral gland are atrophic. The ZG may appear more prominent than in the normal gland, due to the relative loss of the other 2 zones.

A rare variant is macronodular hyperplasia without ACTH hypersecretion. The glands are markedly enlarged and distorted. The nodules are composed mainly of lipid-laden cells and the intervening cortex can be difficult to recognize but has been reported atrophic. Adrenocorticotrophic hormone levels are suppressed. It has now been shown that some of these cases are due to the aberrant or ectopic expression of receptors not normally present in the adrenal cortex and the stimulation of cortisol release is due to a peptide that does not usually play a role. A range of receptors have been identified including β-adrenergic and those for gastric inhibitory polypeptide, vasopressin, luteinizing hormone, and serotonin. In patients with gastric inhibitory polypeptide receptor expression, the hypersecretion of cortisol is in relation to intake of food. Occasional adenomas also express aberrant receptors.

Primary nodular adrenal cortical disease is a rare familial condition of children and young adults. Patients have typical features of Cushing syndrome but osteopenia is more severe. Both glands usually consist of multiple small brown to black nodules and the combined weights range from 4 to 21 g. The intervening cortex may be difficult to identify but comprises small regular cells with clear cytoplasm consistent with functional suppression. Plasma ACTH levels are low consistent with adrenal autonomy. In some patients this forms part of the Carney complex, with myxomas, spotty skin pigmentation, schwannomas, and tumors of the pituitary, testis, and thyroid, frequently caused by mutations in the PRKARIA gene, which encodes the 1α regulatory subunit of protein kinase A.

ADRENAL CORTICAL TUMORS—GENERAL FEATURES

Adrenal Cortical Tumors—General Features

Adrenal cortical tumors are not uncommon at autopsy, with lesions reported in up to 54% of unselected cases.

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Figure 1. Gross appearance of adrenal gland from a patient with Cushing syndrome showing macronodular cortical hyperplasia. The contralateral gland had a similar appearance.

Figure 2. Gross appearance of an adrenal adenoma from a patient with Cushing syndrome. The atrophic cortex caused by inhibition of adrenocorticotrophic hormone is seen on the left.

Figure 3. Adrenal adenoma showing characteristic alveolar architecture and lipid-laden cells (hematoxylin-eosin, original magnification ×100).

Figure 4. Gross appearance of adrenal carcinoma showing invasion of the adjacent fat (arrow).

Figure 5. Adrenal carcinoma showing diffuse architecture, compact cells, and mitotic activity (hematoxylin-eosin, original magnification ×400).

Figure 6. Adrenal carcinoma showing focal immunopositivity for inhibin-α (original magnification ×200).

Larger lesions are usually defined as adenomas, but in many cases there are small, multiple, bilateral nodules. They are more common with increasing age and in those with hypertension or diabetes mellitus. The size ranges from microscopic to several centimeters. The cut surface is yellow with focal brown areas. They are usually circumscribed but not encapsulated. Most comprise ZF-like cells, although compact cells may predominate. Their pathogen-
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Histologic Features to Be Assessed to Determine Malignant Potential

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<th>Feature</th>
<th>Scoring</th>
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<td>Diffuse architecture</td>
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<td>Clear cells &lt;25% of total</td>
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<tr>
<td>Significant nuclear pleomorphism</td>
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<td>Confluent necrosis</td>
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<td>Mitotic count ≥ 6 per 50 high-power fields</td>
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<td>Atypical mitoses</td>
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<td>Capsular invasion</td>
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<td>Sinusoidal invasion</td>
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Functioning tumors comprise between 24% and 74% of cases. Cushing syndrome is most common, often accompanied by androgen excess (mixed Cushing syndrome). Virilization may occur alone; feminizing tumors are rare. Other symptoms include abdominal or loin pain, abdominal fullness, and fever. Most respond poorly to treatment. Complete surgical excision is the mainstay of cure but may not be possible. The tumor is extremely resistant to chemotherapy, which may be explained in part by the expression of P-glycoprotein and glutathione S-transferases, which play roles in various types of drug resistance. Mitotane (o,p′-dichlorodiphenyldichloroethane, a derivative of dichlorodiphenyltrichloroethane) has a nonspecific adrenolytic effect and may be of use in controlling the disease.

Most carcinomas weigh more than 100 g but small tumors have behaved in a malignant fashion. Grossly, they may appear encapsulated or may be obviously adherent to or infiltrating surrounding structures (Figure 4). Lobulation is common with fibrous tissue separating tumor nodules. The cut surface is fleshy, with variable coloration, ranging from pink-brown to yellow. Hemorrhage and necrosis are common and there may be cystic change. In occasional cases, there is gross evidence of vascular invasion.

The architecture is less ordered than in adenomas (Figure 5). Trabecular and diffuse patterns of growth are seen and alveolar arrangement is uncommon. Compact cells often predominate. Nuclear pleomorphism is common, sometimes with multinucleated giant cells. Mitotic activity is usually seen, often with atypical forms. Oncocytic variants have been described. Broad fibrous bands are present in many cases and confluent necrosis is common. Both sinusoids and veins may be invaded and capsular invasion can be seen. Both local invasion and distant metastasis define malignancy.

Diagnosis of Malignant Potential and Prognostic Markers

As indicated previously, the diagnosis of carcinoma is easy in many cases. However, the risk of malignant potential must be assessed in all adrenal cortical tumors, even intra-adrenal lesions. This is best done by an overview of clinical, biochemical, and histologic findings and multifactorial analysis. Features to be assessed have been identified by examining differences between tumors with known benign and malignant outcome. Virilizing, feminizing, or large nonfunctional tumors are more usually carcinoma. Malignant tumors are usually heavier, and extensive necrosis, broad fibrous bands, and capsular, venous, and sinusoidal invasion are all more common in carcinoma. The overall architecture is more usually trabecular or diffuse and the proportion of clear cells lower. Nuclear pleomorphism, high mitotic activity, and the presence of atypical mitoses are important. These latter features should be assessed in the areas showing most marked change. A number of protocols for diagnosis have been published. In some, there is a combination of clinical, biochemical, and morphologic features that have been given a numerical weighting. The sum of the scores in a specific case defines the tumor as adenoma, of uncertain malignant potential, or carcinoma.

However, the pathologist may not have all the appropriate information to apply these approaches and may be limited to a histologic assessment. Weiss assessed 9 features (Table) and the presence of any 3 of these indicated...
malignant potential. This system is widely used by pathologists. It was validated in a more recent study with a specificity of 96% and sensitivity of 100% and there was good correlation of the overall score ($r = 0.94$). However, there was poorer correlation on some of the individual features including nuclear pleomorphism and vascular invasion and the group proposed omitting these features and incorporating the others into a weighted numerical score. All of these systems have value, but they may not always give the same diagnosis in an individual case. In difficult cases all clinical and histologic information should be taken into account. In a few cases a diagnosis of indeterminate or borderline tumor may have to be made.

There are a few additional investigations emerging. The Ki-67 (MIB-1) index is higher in carcinomas with levels of more than 4% to 5% seen only in malignant lesions. A low Ki-67 index does not define behavior as many carcinomas have levels below this threshold. Adrenal carcinoma is associated with overexpression of IGF-2, which can be detected by immunohistochemistry. Abnormal expression of p53 protein and p53 mutations are present in most carcinomas and rarely in adenomas, so again immunoactivity is supportive of a malignant diagnosis.

High proliferative activity is associated with more aggressive behavior, tumors with a mitotic rate greater than 20 per 50 high-power fields or a Ki-67 index of greater than 3% showing a shorter disease-free interval. However, there appears to be no correlation with overall survival.

**Immunohistochemistry**

Occasionally adrenal cortical carcinoma may have to be distinguished from hepatocellular carcinoma, renal cell carcinoma, or pheochromocytoma. Antibody D11 has been reported as useful in identifying adrenal cortical tumors as have immunoreactivity for inhibin a (Figure 6) and Melan A clone A103. Immunopositivity for SF-1 and DAX-1 has been reported but is not yet widely used in diagnostic practice. Immunopositivity for cytokeratins is weak or absent and they are negative for epithelial membrane antigen. Renal cell carcinoma is usually positive for both cytokeratins and epithelial membrane antigen. Hepatocellular carcinoma may be positive for α-fetoprotein, α1-antitrypsin, and carcinoembryonic antigen. Adrenal cortical carcinoma can show positive staining for general neuroendocrine markers including synaptophysin, so chromogranin A is the only marker that will positively discriminate between adrenal cortical carcinoma and pheochromocytoma.

**Molecular Pathogenesis of Adrenal Cortical Tumors**

Clonality studies based on X chromosome inactivation have demonstrated that carcinomas are monoclonal but that adenomas may be monoclonal or polyclonal. Comparative genomic hybridization, loss of heterozygosity, and interphase cytogenetics have been used to examine changes in individual chromosomes and some conflicting data have emerged. Chromosomal changes have been reported in between 28% and 51% of adenomas. There is evidence to suggest accumulation of changes in tumor progression. Losses have been found on chromosomes 1p, 17p, 22p, 22q, and 11q and gains on 5, 12, 19, and X. Loss of heterozygosity or allelic imbalance have been demonstrated at 11q13 (90%), 17p13 (85%), and 2p16 (92%) in carcinomas. Changes in chromosomes 3, 9, and X may be early events.

A number of oncogenes and tumor suppressor genes have been investigated. Adrenal cortical carcinoma is one of the tumors seen in Li-Fraumeni syndrome, associated with germline mutations in the p53 gene. The majority of sporadic adrenal cortical carcinomas also show abnormal p53 expression and/or p53 mutations, whereas few adenomas do. An unusual inherited mutation in the p53 gene is thought to account for the high numbers of childhood adrenal carcinomas in Brazil and is also found in a proportion of the adult cases. Conflicting data exist on the ras family of oncogenes. Although 2 studies have shown no involvement, others report 12.5% of tumors with mutations in N-ras but none in Ki-ras or Ha-ras and 46% of cases with mutations in Ki-ras, but none in Ha-ras. Expression of c-myc protein may vary with tumor type, but it does not seem to be involved in neoplastic transformation.

Somatic mutation of the menin gene is rare in adrenal cortical tumors. Familial tumors also occur in Beckwith-Wiedemann syndrome, associated with dysregulation of a group of growth controlling genes on 11p15 including paternal disomy of the IGF2 gene. Rearrangement at this locus and overexpression of IGF-2 has been reported in the majority of sporadic cases. Other growth factor interactions that may be involved are transforming growth factor α and epidermal growth factor receptor, IGF-1, its binding proteins and receptors, and the activins and inhibins. Mutations in the ACTH receptor are found in a subset of adrenal cortical tumors but are probably not of major importance in pathogenesis.

Immortalization of cells by the action of the protein/RNA telomerase complex, not normally expressed in differentiated cells, may also play a role in tumorigenesis. Published data to date on expression in adrenal cortical tumors are equivocal. The role of apoptosis is unclear. A microarray study using 10000 genes has confirmed IGF2 as important in carcinoma and has identified new candidate genes including fibroblast growth factor receptor 1, osteopontin, and 11β-hydroxylase (CYP11B1). A further investigation examined cancer-related genes and adrenal cortex–related genes, including steroidogenic enzymes, cyclic adenosine monophosphate (cAMP) signaling components, and the IGF2 system. On the basis of the analysis of a combination of 8 genes from the IGF2 cluster and 14 from the adrenal cluster, the predictive value for malignancy was similar to that of the Weiss histologic score. The adrenal cluster was more highly expressed in adenomas and the IGF2 cluster in carcinomas. In addition, using expression profiles of 14 genes, it was possible to separate recurring from nonrecurring tumors in a group of 13 carcinomas. Correlation of molecular markers with outcome suggest that loss of heterozygosity at 17p13 and overexpression of IGF2 are associated with shorter disease-free survival and 17p13 loss of heterozygosity is independently associated with recurrence. These data require validation.

**Other Tumors**

Adrenal oncocytomas resemble similar lesions at other sites and are characterized by large eosinophilic cells (Figure 7), due to mitochondrial accumulation. Although
originally described as nonfunctioning and benign, hormone-secreting\textsuperscript{22,25} and malignant\textsuperscript{25} variants have been reported. Myelolipomas (Figure 8) comprise a mixture of mature adipose tissue and hemopoietic tissue. Their histogenesis has not been clear, but the recent demonstration of clonality in both elements suggests that they are neoplastic.\textsuperscript{126} Focal myelolipomatous change may be seen in other adrenal cortical tumors and in cortical hyperplasia.

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