INTRODUCTION
Approximately 80 to 85% of cases of hyperadrenocorticism in both dogs and cats are due to pituitary dependent hyperadrenocorticism (PDH), with the remainder due to an adrenocortical tumor (AT). Cortisol is the most common secretory product of the adrenal gland in hyperadrenocorticism, but excessive production of other steroid hormones (sex hormones, mineralocorticoids) has been reported in dogs and other species.

PHYSIOLOGY OF THE ADRENAL CORTEX
Adrenal steroids are derived from cholesterol and all contain the cyclopentanoperhydrophenanthrene (CPPP) nucleus. Four cytochrome P450 enzymes present in the adrenal cortex (17α-hydroxylase, 3β-dehydrogenase, 21β-hydroxylase, and 11β-hydroxylase), catalyse formation of the different adrenal steroids. The adrenal cortex is composed of three layers, the zona glomerulosa (ZG), the zona fasciculata (ZF), and the zona reticularis (ZR). The ZG is the outermost layer of the adrenal gland and synthesizes and secretes aldosterone. The ZG is deficient in 17α-hydroxylase activity, so it is incapable of synthesizing cortisol or androgens. The ZF and ZR both synthesize sex hormones and cortisol. Most sex hormones secreted from the adrenal gland are androgens (dehydroepiandrosterone, androstenedione). Progesterone is synthesized in the adrenal gland as a precursor to androgens but in normal dogs very little progesterone is secreted into the circulation. Only small amounts of testosterone and estrogen are synthesized by the adrenal, however adrenal androgens serve as substrates for synthesis of estrogen and testosterone in peripheral tissues. Cortisol production from the adrenal gland is regulated by corticotrophin releasing hormone (CRH) released from the hypothalamus and ACTH from the pituitary gland. Regulation of adrenal androgen and estrogen secretion is poorly understood, but likely involves both ACTH and other non-ACTH pituitary factors.

CLINICAL SIGNS OF HYPERADRENOCORTICISM
In most cases of hyperadrenocorticism, secretion of abnormal quantities of glucocorticoids is believed to be the cause of the clinical syndrome (Cushing’s syndrome). Clinical signs include polydipsia, polyuria, polyphagia, abdominal enlargement, hepatomegaly, cutaneous changes (alopecia, cutaneous atrophy, calcinosis cutis, hyperpigmentation), muscle weakness, decreased exercise tolerance, excessive panting, truncal obesity, lethargy, weight gain, immunosuppression, insulin resistance, and decreased sexual function. More recently it has been recognized that increased circulating concentrations of progesterone or 17α-hydroxyprogesterone may cause clinical signs that are indistinguishable from those due to glucocorticoids. This is hypothesized to be due to the marked intrinsic glucocorticoid activity of progestagens. In rare cases of hyperadrenocorticism, increased concentrations of adrenal androgens, estrogens, and mineralocorticoids, may cause virilization, feminization, or hypertension respectively.

DIAGNOSIS OF HYPERADRENOCORTICISM
Diagnosis of Cushing’s syndrome is made by consideration of historical findings, physical examination, review of a laboratory minimum data base (CBC, serum biochemical profile, urinalysis) and performance of specific endocrine function tests (ACTH stimulation test, low dose dexamethasone suppression test, urine cortisol:creatinine ratio). Measurement of a baseline cortisol is of little value in evaluating the pituitary-adrenal axis. More recently evaluation of sex hormones before and following administration of ACTH has also been used to support the diagnosis. 

ACTH stimulation test: This is the most common test used in the diagnosis of HAC. It relies on the assumption that hyperplastic or neoplastic adrenals have abnormally large reserves of cortisol and that these animals therefore hyper-respond to maximal stimulation by ACTH. Most protocols recommend collection of a baseline sample, the administration of synthetic ACTH at a dose of 0.25 mg/dog IV or IM or 5 µg/kg IV. A second sample is collected one hour later. In order to interpret the ACTH stimulation test, normal values must be established for the individual laboratory. The ACTH stimulation test is abnormal in 85 - 90% of PDH cases and in 50% of ADH cases. The ACTH stimulation test does not distinguish between ADH and PDH. Because of the low sensitivity of this test, a diagnosis of HAC should not be excluded based on a normal ACTH stimulation. In situations in which sex hormones are measured the same protocol for the ACTH stimulation test is used.

Low-dose dexamethasone suppression test: This test is probably a better screening test for HAC since it is more sensitive (95%) than the ACTH stimulation test. It does not however, allow detection of iatrogenic Cushings. This test relies on the fact that the administration of exogenous glucocorticoids should suppress the production of ACTH from the normal pituitary and therefore the production of cortisol from the normal adrenal. This suppression persists in the normal dog for 24 - 48 hours. Since dexamethasone is not detected by the assay for cortisol, this suppression
can be measured after administration of exogenous dexamethasone. Adrenal tumors function independently of ACTH and a hyperplastic or neoplastic pituitary gland is relatively resistant to negative feedback from circulating steroids, so in either cause of hyperadrenocorticism no suppression occurs. The test requires a blood sample for the measurement of a baseline cortisol, followed by the administration of dexamethasone sodium phosphate IV at a dose of 0.01 mg/kg. The patient is then left undisturbed in a cage and a second blood sample is collected 8 hours later. In normal dogs the second sample will show suppression of the cortisol concentration to less than 1.5 µg/ml (Purdue lab). Dogs with HAC will not suppress. Additional information may be obtained by measuring a 4-hour cortisol concentration. If suppression occurs at 4 hours but "escape" occurs at 8 hours this is diagnostic for PDH and further differentiation testing is unnecessary.

**Which test should be performed first?** In general if HAC is suspected and there is no history of exogenous corticosteroid administration, I recommend starting with the LDDS. If this test is abnormal or borderline I would perform an ACTH stimulation test to confirm the diagnosis and obtain a baseline for monitoring response to treatment. If hyperadrenocorticism is not confirmed by the LDDS test, an ACTH stimulation test should be performed if clinical suspicion for the disease is high. Testing should be repeated in 1-3 months if testing is negative but no other cause of the clinical signs is identified. In dogs with obvious clinical signs of hyperadrenocorticism and persistent normal cortisol testing, measurement of a sex hormone profile should be considered (see below). In animals with concurrent disease or any history of exogenous steroids, the ACTH stimulation test is the test of choice for initial testing.

**Urinary cortisol:creatinine ratio:** The urinary cortisol:creatinine ratio is a useful screening test for HAC. This test is performed on a voided morning urine sample so is a very convenient initial screening test. The normal range in our lab is < 20. This test is extremely sensitive for HAC but is not very specific. Many other diseases other than hyperadrenocorticism increase the cortisol:creatinine ratio. It is however useful in some circumstances in order to rule out HAC as a differential diagnosis. A dog with a normal cortisol:creatinine ratio is unlikely to have hyperadrenocorticism.

**Sex hormone panel:** Recent studies at our institution and others have demonstrated that dogs with hyperadrenocorticism also secrete excessive quantities of other adrenal steroids, in particular progesterone, 17-hydroxy-progesterone, androstenedione and DHEA. Measurement of a sex hormone panel (University Tennessee Endocrinology Laboratory) before and after administration of ACTH may be useful in the diagnosis of early or atypical hyperadrenocorticism. Sex hormone testing may also be useful in diagnosis of atypical hyperadrenocorticism caused by sex hormone secreting adrenal tumors (see later). In these cases cortisol secretion may be normal or sub-normal.

**ATYPICAL HYPERADRENOCORTICISM**

This term has been used to describe cases of hyperadrenocorticism (both PDH and ADH) in which clinical signs and response to treatment are consistent with a diagnosis of hyperadrenocorticism, but the standard screening tests (ACTH stimulation test, low dose dexamethasone suppression test) are normal. Note that in these cases, cortisol secretion is normal rather than suppressed as in the sex hormone secreting adrenal tumors. Initial studies suggested that sex hormone profiles might be a more sensitive screening test compared with cortisol alone for diagnosis of hyperadrenocorticism. More recent studies suggest that the sensitivity might not be as high as initially suspected. Specificity of sex hormones in diagnosis of hyperadrenocorticism is also not yet well documented. At this time it is recommended that sex hormone screening is not utilized for routine diagnosis of hyperadrenocorticism. A sex hormone panel before and after ACTH stimulation should be considered in dogs that have clinical signs and clinical laboratory evidence of hyperadrenocorticism, no evidence of other cause for their clinical signs, but normal or borderline cortisol testing. Sex hormone measurement should also be considered in dogs with clinical signs of hyperadrenocorticism and suppressed cortisol concentrations after ACTH stimulation if treatment with exogenous steroids or mitotane can be ruled out.

**DIFFERENTIATION OF PDH FROM ADRENAL TUMORS**

Clinical and routine laboratory findings are not useful in distinguishing PDH from functional adrenal tumors. The ACTH stimulation test and cortisol:creatinine ratio are also unable to differentiate adrenal tumors from PDH. Endogenous ACTH concentrations, and endocrine function tests such as the high and low dose dexamethasone suppression tests, are the most useful endocrine tests for distinguishing AT from PDH.

**Low and High dose dexamethasone suppression tests:** These tests are the most commonly used tests to differentiate pituitary dependent hyperadrenocorticism (PDH) from adrenal dependent hyperadrenocorticism (ADH). The tests rely on the assumption that adrenal tumors are more resistant to the feedback effects of dexamethasone than are hyperplastic or neoplastic pituitary glands. The protocol for the high dose test is the same
as the low-dose test except that the dose of dexamethasone is 10 x higher (0.1 mg/kg) and that a 4 hour sample is usually not collected. It is important that dexamethasone SP is used in this test since dexamethasone in polyethylene glycol can cause CNS signs at this high dose. Suppression is defined as a 4 or 8 hour plasma cortisol concentration that is < 50% of the baseline sample. One hundred percent of adrenal tumors will fail to suppress while 75 - 80% of pituitary dependent cases will show suppression. Therefore suppression after the low or high dose dexamethasone test is diagnostic for PDH. Lack of suppression however, is not diagnostic for ADH and additional testing is required. If greater than 50% suppression is seen at 4 or 8 hours on a LDDS, a HDDS test is unnecessary.

**Endogenous ACTH concentration:** An understanding of the pathophysiology of HAC indicates that it should be possible to distinguish PDH from ADH based on the circulating ACTH concentration. In PDH, circulating plasma ACTH concentrations should be high while in ADH they should be low. Endogenous ACTH is susceptible to rapid enzymatic degradation in vitro so handling recommendations from the laboratory should be carefully followed. Different laboratories may have different reference ranges. In our laboratory ACTH concentrations >15 pg/ml are consistent with a diagnosis of PDH. ACTH concentrations <10 pg/ml are suggestive of ADH however some dogs with PDH will also have low values probably due to episodic ACTH secretion. The result of the endogenous ACTH should therefore never be relied on alone to confirm a diagnosis of adrenal tumor.

Additional tests that may be useful to differentiate PDH from ADH include abdominal radiography, abdominal ultrasound, and CT scans of the brain and abdomen. Fifty to sixty percent of dogs with adrenal neoplasia have a radiographically visible mineralized mass on abdominal radiographs. In those AT in which mineralization is not evident, the neoplasm is usually identified by abdominal ultrasound. Computed tomography (CT) may also be used to identify adrenal and pituitary masses. Results of the various differentiation tests can be difficult to interpret, particularly in the presence of bilateral adrenal neoplasia, concurrent AT and PDH, or concurrent pheochromocytoma.

Preliminary evidence from recent studies at our institution suggests that adrenocortical carcinomas secrete larger amounts of certain sex hormones in response to ACTH than do benign malignant tumors and dogs with PDH, although there is significant variability between dogs. In the future measurement of sex hormone profiles may be useful in identification of malignant adrenal tumors.

Occasionally a functional or non-functional adrenal tumor is identified as an incidental finding during imaging studies for evaluation of other related or unrelated problems. We have shown in recent studies that some of these apparently non-functional tumors have abnormalities of sex hormone secretion. Whether these tests will be clinically useful in evaluation of the incidentally discovered adrenal tumor is still unclear.

**NON CORTISOL SECRETING ADRENOCORTICAL TUMORS**

In some adrenocortical tumors, cortisol concentrations are normal or suppressed and the tumor secretes excessive quantities of other adrenal steroids. Increased production of adrenal steroids other than cortisol may be due to deficiencies of one or more enzymes involved in normal steroidogenic pathways. Deficiency of these enzymes causes accumulation of precursor steroids proximal to the blocked step, with shunting of precursors into other metabolic pathways. Increases in enzyme activity can also play a role in steroid hypersecretion. In humans, adrenal carcinomas are usually inefficient in conversion of cholesterol to cortisol and production of cortisol precursors is disproportionately high. In contrast adrenal adenomas exhibit very efficient steroidogenesis and production of precursors may be low or normal in relation to cortisol production. Whether this is also true in dogs is unknown, however all reported cases to date of adrenal tumors in which the predominant secretory product is a steroid other than cortisol have been carcinomas.

In dogs with sex hormone secreting adrenocortical carcinomas, clinical signs are consistent with hyperadrenocorticism, and adrenal tumors are identified by imaging studies, however endocrine function tests (ACTH stimulation, low dose dexamethasone suppression tests) do not demonstrate hypercortisolemia. Hormones that may be increased in different combinations included 17-hydroxyprogesterone, progesterone, estradiol, testosterone, and androstenedione. In dogs with clinical signs of HAC but normal or low cortisol concentrations, concentrations of other adrenal steroids should be measured. It is unclear whether sex hormone secreting adrenocortical tumors in dogs and cats are a distinct clinical syndrome or are one end of the spectrum of functional adrenocortical tumors. A dog with a deoxycorticosterone secreting adrenocortical carcinoma that had hypokalemia, metabolic acidosis, and mild hypertension has also been reported. This dog also had no evidence of hypercortisolemia.

**REFERENCES**
Frank LA, Schmeitzel LP, Oliver JW. Steroidogenic response of adrenal tissues after administration of ACTH to dogs with hypercortisolemia. JAVMA 2001;218:214-216