Diagnosis of Hyperadrenocorticism in Dogs
Mark E. Peterson, DVM

A presumptive diagnosis of hyperadrenocorticism in dogs can be made from clinical signs, physical examination, routine laboratory tests, and diagnostic imaging findings, but the diagnosis must be confirmed by use of pituitary-adrenal function tests. Screening tests designed to diagnose hyperadrenocorticism include the corticotropin (adrenocorticotropic hormone; ACTH) stimulation test, low-dose dexamethasone suppression test, and the urinary cortisol:creatinine ratio. None of these screening tests are perfect, and all are capable of giving false-negative and false-positive test results. Because of the limitation of these diagnostic tests, screening for hyperadrenocorticism must be reserved for dogs in which the disease is strongly suspected on the basis of historical and clinical findings.

Once a diagnosis has been confirmed, the next step in the workup is to use one or more tests and procedures to distinguish pituitary-dependent from adrenal-dependent hyperadrenocorticism. Endocrine tests in this category include the high-dose dexamethasone suppression test and endogenous plasma ACTH measurements. Imaging techniques such as abdominal radiography, ultrasonography, computed tomography, and magnetic resonance imaging can also be extremely helpful in determining the cause.

KEYWORDS hyperadrenocorticism, Cushing’s syndrome, diagnosis, cortisol, ACTH, corticotropin, ACTH stimulation test, dexamethasone suppression test, urinary cortisol:creatinine ratio, ultrasonography, computed tomography, magnetic resonance imaging
more to do with the fact that detection of small tumors requires careful microdissection, experience, and special stains. In one study using immunocytochemical staining, more than 80% of dogs with pituitary-dependent hyperadrenocorticism were positive for pituitary adenomas.7

Most pituitary tumors in dogs with pituitary-dependent hyperadrenocorticism are microadenomas, defined as a tumor less than 10 mm diameter. Only about 10 to 15% of dogs have large corticotrophic adenomas (macroadenomas) at the time of diagnosis.7,8 These may compress the remaining pituitary gland and extend dorsally into the hypothalamus. However, they are generally slow growing and may not produce neurological signs. Although reported, corticotropic adenocarcinomas are rare.6

Adrenal-Dependent Hyperadrenocorticism
Cortisol-secreting adrenocortical tumors are responsible for approximately 15 to 20% of dogs with naturally occurring Cushing’s syndrome.1,4 The vast majority of adrenocortical tumors in these dogs are unilateral, but bilateral adrenal tumors do occur.9

Adrenocortical tumors may be benign or malignant, although it can be difficult histologically to distinguish between an adrenocortical adenoma and a carcinoma unless there is evidence of invasion or metastasis.3 In dogs, adrenocortical adenomas and carcinomas occur with approximately equal frequency.1,4 Adrenocortical adenomas are usually small, well-circumscribed tumors that do not metastasize and are not locally invasive. In contrast, adrenocortical carcinomas are usually large, locally invasive, hemorrhagic, and necrotic. Tumor calcification also occurs in over 50% of dogs with adrenal carcinoma.10,11 Carcinomas, especially of the right adrenal, frequently invade the phrenicoadominal vein and caudal vena cava and metastasize to the liver, lung, and kidney.2

Dogs with adrenocortical adenomas or carcinomas secrete cortisol autonomously, or independent of pituitary ACTH control. Through the negative-feedback effects of glucocorticoids on the pituitary gland, the excess cortisol secreted by the adrenal tumor chronically suppress endogenous ACTH secretion, resulting in atrophy of the contralateral (“normal”) adrenal cortex.2,3 This fact becomes extremely important to remember if the tumor is to be removed surgically, inasmuch as the dog will almost invariably develop hypoadrenocorticism postoperatively and will require temporary glucocorticoid supplementation. Because ACTH is not the primary stimulus for aldosterone secretion from the adrenal, however, the function of the zona glomerulosa will not be affected and mineralocorticoid supplementation will not be required.2,3

Iatrogenic Hyperadrenocorticism
Iatrogenic hyperadrenocorticism results in clinical signs and physical examination findings similar to those seen in the natural disease. Excessive or prolonged administration of corticosteroids causes iatrogenic hyperadrenocorticism. Because of the negative-feedback effects of glucocorticoids on the pituitary gland, endogenous ACTH production is suppressed, resulting in atrophy of the adrenal cortices.

Other Potential Causes of Hyperadrenocorticism
There are also a few reports of dogs with pituitary-dependent hyperadrenocorticism having concurrent adrenal tumors.12 Bilateral cortisol-secreting adrenal tumors have also been reported but appear to be extremely rare.9

In the ectopic ACTH syndrome, nonpituitary tumors synthesize and secrete ACTH, which, in turn, ultimately cause bilateral adrenocortical hyperplasia and hypercortisolemia. Ectopic ACTH production is not commonly recognized in the dog, but in humans a number of tumors (eg, oat cell carcinomas of the lung, pancreatic islet cell tumors, and carcinoid tumors) are capable of synthesizing and secreting excessive quantities of ACTH. Recently, ectopic ACTH secretion was described in two dogs with hyperadrenocorticism and abdominal neuroendocrine tumors.13,14

Signalment
Age, Breed, and Sex
Pituitary-dependent hyperadrenocorticism is usually a disease of the middle-aged to older dogs, with a median age of approximately 10 to 11 years.3,4 Dogs with adrenal-dependent hyperadrenocorticism tend to be slightly older, with a median age of 11 to 12 years.3,11

Any breed can develop hyperadrenocorticism but poodles, dachshunds, and small terriers, for example, the Yorkshire terrier, Jack Russell terrier, and Staffordshire bull terrier, appear more at risk at developing pituitary-dependent hyperadrenocorticism. Adrenocortical tumors occur more frequently in larger breeds with about 50% of dogs weighing greater than 20 kg.3,11

There is no appreciable difference in sex distribution in pituitary-dependent hyperadrenocorticism; however, female dogs are more likely to develop adrenal tumors than males. In one survey, between 60 and 65% of dogs with functional adrenocortical tumors were female.3,11

History, Clinical Signs, and Physical Examination
Hyperadrenocorticism has an insidious onset and is slowly progressive over many months or even years. Many owners consider the early signs as part of the normal aging process of their dog. In a few cases, clinical signs may be intermittent, with periods of remission and relapse, whereas in others there may be an apparent rapid onset and progression of clinical signs. Larger breeds of dogs and those with recent onset of disease, however, may only show a few characteristic signs rather than the classic array of clinical signs usually observed in smaller breeds. Recent corticosteroid administration (including eye, ear, and topical preparations) should be ascertained, to exclude iatrogenic hyperadrenocorticism.

The most common clinical signs associated with hyperadrenocorticism in dogs are polydipsia, polyuria, polyphagia, lethargy, abdominal enlargement or potbelly, panting, obesity, muscle weakness, and recurrent urinary tract infections. Dermatologic manifestations of hyperadrenocorticism commonly include truncal hair thinning or alopecia.1,4 On phys-
tical examination, the most commonly noted abnormalities include abdominal enlargement, hepatomegaly, panting, truncal obesity, bilaterally symmetric alopecia, comedones, pyoderma, and seborrhea. Hyperpigmentation, thin skin, bruising, and calcinosis cutis are less commonly recorded.

Routine Laboratory Findings

In dogs, the most common serum chemistry abnormality observed in association with hyperadrenocorticism include increased serum alkaline phosphatase activity (ALP), which is high in 85 to 90% of dogs.1-4 High serum alanine transferase activity (ALT), hypercholesterolemia, hyperglycemia, and low blood urea nitrogen are also common findings. The hemogram may reveal a mild erythrocytosis as well as a classic “stress leukogram” (ie, eosinopenia, lymphopenia, and mature leukocytosis).

The urine-specific gravity is usually less than 1.015 and is often hypostenuric (<1.008) provided water has not been withheld. Dogs with hyperadrenocorticism can usually concentrate their urine if water is deprived, but their concentrating ability is frequently reduced.15 Rarely, in some dogs with a pituitary macroadenoma, compression of the posterior lobe of the pituitary and suprasellar extension into the hypothalamus may cause disruption to antidiuretic hormone production and release resulting in signs of central diabetes insipidus. Finally, many dogs with hyperadrenocorticism have evidence of urinary tract infection without pyuria (positive culture), bacteriuria, and proteinuria resulting from glomerulonephritis.1-4,15,16

Pituitary-Adrenal Function Tests for Diagnosis of Hyperadrenocorticism

A presumptive diagnosis of hyperadrenocorticism can be made from clinical signs, physical examination, routine laboratory tests, and diagnostic imaging findings, but the diagnosis must be confirmed by hormonal assay.1-4 Screening tests are designed to diagnose hyperadrenocorticism, ie, to determine if the disease is present or not. Tests that fit into this category are the corticotropin (ACTH) stimulation test, low-dose dexamethasone suppression test, and the urinary cortisol:creatinine ratio. None of these tests are perfect, and all are capable of giving false-negative and false-positive test results.

When discussing the accuracy of these screening tests for hyperadrenocorticism, it is useful to consider the terms sensitivity and specificity. The sensitivity of a test result refers to the number of patients with the disease in question that also have test results diagnostic for that condition. If a test has a sensitivity of 0.8, for example, only 80% of dogs with the disease test positive for it, and the test misses the diagnosis (ie, gives a false-negative test result) in the other 20% of cases. The specificity of a test refers to the number of patients that do not have the disease but have test results diagnostic for that condition (false-positive test result). This concept becomes important when one understands that no screening test can be correct all of the time. In other words, sensitivity and specificity are never both 100% for any screening test.

When a dog suffering from hyperadrenocorticism shows a negative result with one of the screening tests, this should not be surprising inasmuch as no test will have 100% sensitivity. In such a dog, an alternative screening test should be used to help confirm the diagnosis if hyperadrenocorticism is still strongly suspected. It is extremely important to remember, however, that false-positive results are common in dogs suffering from nonadrenal disease.5 Because such false-positive test results occur for all of the commonly employed screening tests (ACTH stimulation, low-dose dexamethasone suppression, urinary cortisol:creatinine ratio), the definitive diagnosis of hyperadrenocorticism should never be made purely on the basis of results of one or more of these screening tests, especially in dogs without classic signs of hyperadrenocorticism or in dogs with known nonadrenal disease.

Basal Serum or Plasma Cortisol Concentration

Basal cortisol concentrations, when used alone, are not reliable in the diagnosis of hyperadrenocorticism in dogs. Because of the episodic nature of cortisol secretion in dogs, basal cortisol concentrations fluctuate throughout the day, resulting in a high degree of overlap of values in normal dogs, dogs with nonadrenal illness, and dogs with hyperadrenocorticism.1-4,17,18 The sensitivity of basal cortisol determinations in the diagnosis of hyperadrenocorticism is only about 0.5 (approximately 50% of dogs with the disease have high random cortical concentrations), whereas the specificity can be extremely low in dogs with severe nonadrenal illness. This severely limits the usefulness of basal serum cortisol concentrations in the diagnosis of hyperadrenocorticism. Plasma or serum cortisol values are only useful after dynamic stimulation with ACTH or suppression after dexamethasone.

Corticotropin (ACTH) Stimulation Test

The ACTH stimulation test is commonly used as a screening test for hyperadrenocorticism in dogs. The basis for this test is that dogs with pituitary-dependent hyperadrenocorticism or cortisol-secreting adrenal tumors, because of their increased adrenocortical mass, have the capacity to secrete excessive amounts of cortisol. Advantages of this test include convenience, since it is a simple and quick test to perform. In addition, the ACTH stimulation test is the best screening test for distinguishing dogs with spontaneous from those with iatrogenic hyperadrenocorticism and provides valuable baseline information for monitoring mitotane or trilostane treatment.1-4

The preferred method for ACTH stimulation testing in dogs is to determine serum cortisol concentrations before and 1 hour after the intravenous or intramuscular injection of cosyntropin (Cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga, CA), administered at a dosage of at least 5 μg/kg (Table 1).3,4,19,20 This 5 μg/kg dosage will result in maximum stimulation of the adrenocortical reserve, the most important criteria for any ACTH stimulation protocol. Following reconstitution, the cosyntropin solution appears to be stable and bioactive for at least 4 weeks when refrigerated and for 6 months when frozen.19,21

If cosyntropin is not available, the ACTH stimulation test can also be performed by determining the serum cortisol
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concentration before and after the intramuscular injection of 2.2 U/kg of ACTH gel (Table 1).^{1,2} Acthar Gel (80 U/mL; Questcor Pharmaceuticals, Union City, CA) is available but is very expensive; if Acthar Gel is administered, the post-ACTH serum cortisol sample is collected at 2 hours. Alternatively, compounded forms of ACTH (usually 40 U/mL) can be purchased from several veterinary pharmacies. It should be noted, however, that the bioavailability and reproducibility of all of these compounded formulations have yet to be carefully evaluated. A recent study in dogs evaluated four compounded ACTH preparations and compared their cortisol fully evaluated. A recent study in dogs evaluated four compounded forms of ACTH (usually 40 U/mL) can be purchased from several veterinary pharmacies. It should be noted, however, that the bioavailability and reproducibility of all of these compounded formulations have yet to be carefully evaluated. A recent study in dogs evaluated four compounded ACTH preparations and compared their cortisol responses to that of cosyntropin.^{22} The data of that study showed that injection of the four compounded forms of ACTH increased serum cortisol concentrations to a similar magnitude as cosyntropin in samples collected 30 and 60 minutes after ACTH administration. However, serum cortisol concentrations at 90 and 120 minutes post-ACTH varied considerably, depending on the preparation of ACTH injected, with two compounded forms of ACTH producing much lower serum cortisol concentrations. Based on such variability in cortisol responses between compounded forms of ACTH, these investigators recommended determining serum cortisol concentrations at both 1 and 2 hours after ACTH administration when using a compounded preparation.^{22} Overall, the determination of a third cortisol concentration would likely offset any presumed cost-saving derived from using a compounded ACTH product. In addition, because the potential for lot-to-lot variability in compounded ACTH formulations has not been evaluated, one should consider assessing the activity of each new vial by performing an ACTH stimulation test on a normal dog.

In normal dogs, administration of a supraphysiological dose of ACTH, either cosyntropin or ACTH gel, produces a rise in serum cortisol to values usually greater than 10 μg/dL (>300 nmol/L). In contrast, dogs with hyperadrenocorticism tend to have an exaggerated response to ACTH administration, with post-ACTH serum cortisol concentrations rising to greater than 20 μg/dL (>600 nmol/L). The ACTH stimulation test identifies over half of dogs with cortisol-secreting adrenocortical tumors and about 85% of dogs with pituitary-dependent hyperadrenocorticism.\(^{1,4,11,23,24}\)

Based on these results, the sensitivity of the ACTH stimulation test in diagnosing pituitary-dependent hyperadrenocorticism can be calculated to be 0.85, whereas the specificity is only 0.60 in dogs with adrenal tumors.\(^{1,4,11,23,24}\) The specificity of the ACTH stimulation test varies depending on the population being evaluated. Dogs with nonadrenal illness may have ACTH stimulation test results in the range diagnostic for hyperadrenocorticism; the specificity of the test in this group of dogs may be as low as 0.60.\(^{5,25}\) A definitive diagnosis should not be entirely based on results of an ACTH response test in a dog that is sick. Ideally, the dog should be allowed to recover from its nonadrenal illness before testing.

One advantage of using the ACTH stimulation test is that it is the only screening test that can identify dogs with iatrogenic hyperadrenocorticism. Dogs receiving chronic glucocorticoid therapy can develop all of the clinical features of naturally occurring hyperadrenocorticism; this can develop with injectable, oral, topical, or ophthalmologic steroid preparations. In dogs with clinical signs and routine laboratory findings consistent with Cushings syndrome (especially if any history of exogenous steroid treatment), the finding of a low-normal baseline serum cortisol concentration with little or no response to ACTH stimulation is diagnostic for iatrogenic hyperadrenocorticism.

Some dogs have classic clinical signs of hyperadrenocorticism and typical hematological and biochemical findings but have a normal cortisol response to both ACTH stimulation and low-dose dexamethasone suppression. These cases have been termed atypical hyperadrenocorticism.\(^{26}\) It has been suggested that cases of atypical hyperadrenocorticism may have a derangement of the steroid production pathway and that some of the precursors of cortisol, such as 17-hydroxyprogesterone, may be abnormally increased. Plasma 17-hydroxyprogesterone concentrations show an exaggerated response to ACTH stimulation in both typical and atypical hyperadrenocorticism.\(^{26,29}\) Both pituitary-dependent and adrenal-dependent atypical hyperadrenocorticism cases have been reported. (See the article by Greco in this journal entitled Diagnosis and Treatment of Sex Steroid Excess: The Other Cushings Syndrome for a more complete discussion of the diagnosis and treatment of this syndrome.\(^{30}\))

### Table 1 Protocols for Corticotropin (ACTH) Stimulation Test

<table>
<thead>
<tr>
<th>Protocol A (Cosyntropin; Cortrosyn, Amphastar Pharmaceuticals)</th>
<th>Protocol B (ACTH gel; Acthar gel, Questcor Pharmaceuticals)</th>
<th>Protocol C (compounded ACTH, from several veterinary pharmacies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Collect basal blood sample for determination of plasma or serum cortisol</td>
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</tr>
<tr>
<td>2. Reconstitute 250 μg cosyntropin (1 vial) in 5 ml of saline solution to make concentration of 50 μg/ml</td>
<td>2. Inject ACTH gel at dosage of 2.2 U/kg body weight, IM</td>
<td>2. Inject compounded ACTH gel at dosage of 2.2 U/kg body weight, IM</td>
</tr>
<tr>
<td>3. Inject cosyntropin at dosage of ≥5 μg/kg body weight</td>
<td>3. Collect single post-ACTH stimulated cortisol sample at 1 h</td>
<td>3. Collect two post-ACTH stimulated cortisol samples at 1 and 2 h</td>
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### Low-Dose Dexamethasone Suppression Test

The low-dose dexamethasone suppression test is considered by many to be the test of choice for the diagnosis of hyperadrenocorticism in dogs.\(^{31}\) Normally, glucocorticoids (eg, dexamethasone) feed back onto the pituitary gland, turning off or suppressing ACTH secretion. As circulating ACTH falls, cortisol secretion from the adrenal cortex is also diminished (ie, suppressed). This test takes advantage of the fact that, in dogs with hyperadrenocorticism, the pituitary-adre-
nal axis controlling ACTH and cortisol secretion is abnormally resistant to suppression by dexamethasone.1-4,18,32 Dexamethasone is used for the low-dose dexamethasone suppression test not only because it is a potent glucocorticoid, but also because it does not cross-react with the standard cortisol assays, allowing one to use serum (plasma) or urine measurements as an endpoint.1-4,18,32,33

Compared with the ACTH stimulation test, the low-dose dexamethasone suppression test is much more sensitive in confirming hyperadrenocorticism, since the results are diagnostic in the virtually all dogs with cortisol-secreting adrenal tumors and in 90 to 95% of dogs with pituitary-dependent hyperadrenocorticism.24 However, in contrast to the ACTH stimulation test, the low-dose dexamethasone suppression test is not helpful in the detection of iatrogenic hyperadrenocorticism.1-4 The test is also affected by more variables than the ACTH stimulation test, takes 8 hours to complete (or 3 days, if urine samples are collected; see below), and does not provide pretreatment information that may used in monitoring the effects of mitotane or trilostane therapy.1-4,32

Two similar protocols have been described for low-dose dexamethasone suppression testing in dogs, both of which yield similar patterns of cortisol suppression (Table 2).1-4,18,32,35 To perform this test, serum or plasma cortisol concentrations are determined before, 4 and 8 hours after the administration of the dexamethasone preparation. Either a solution of dexamethasone in polyethylene glycol (0.015 μg/kg, IV or IM) or dexamethasone sodium phosphate (0.01 μg/kg, IV) can be administered for the test with equivalent results.1,18,24,32,35 In general, the dexamethasone is best diluted in sterile saline to allow for the dog to be dosed accurately (eg, dilute 2 mg (2000 μg) of dexamethasone with 5 mL of saline to make a final concentration of 400 μg/mL). Interpretation of the results of a low-dose dexamethasone suppression test must be based on the laboratory’s normal range for the dose and preparation of dexamethasone administered.

If the low dose of dexamethasone fails to adequately suppress circulating cortisol concentrations in a dog with compatible clinical signs, this is consistent with a diagnosis of hyperadrenocorticism. While basal and 8-hour postdexamethasone samples are most important for interpretation of the test, one or more samples taken at intermediate times (eg, 4 hours) during the test period may also prove helpful. Approximately 30% of dogs with pituitary-dependent hyperadrenocorticism have serum cortisol suppression at 4 hours (<1 μg/dL or <30 nmol/L), with a rise in cortisol values by 8 hours after dexamethasone administration.1-4,32 This escape from suppression is diagnostic for pituitary-dependent hyperadrenocorticism, and further tests to determine the cause of hyperadrenocorticism are not necessary.1,4,18,32,35 Failure to show adequate suppression of serum or plasma cortisol at both 4 and 8 hours (ie, >1 μg/dL or >30 nmol/L) is diagnostic for hyperadrenocorticism but cannot aid in determining the cause of the hyperadrenocorticism.

The sensitivity of the low-dose dexamethasone suppression test is excellent, approximately 0.90 to 0.95 in dogs with pituitary-dependent hyperadrenocorticism and 1.0 in dogs with an adrenal tumor.1-4,24,33,35 Thus, only 5 to 10% of dogs with the pituitary-dependent form of the disorder show “normal” cortisol suppression with this protocol. The specificity of the low-dose dexamethasone suppression test, however, can be low, especially when measured in a population of sick dogs.1-5 In fact, the specificity of the low-dose dexamethasone suppression test is considerable lower than that of the ACTH stimulation test. Because of the low specificity of the low-dose dexamethasone suppression test, diagnosis of hyperadrenocorticism should never be based on results of a low-dose dexamethasone suppression test alone, especially in a dog with nonadrenal disease. It is best to delay testing for hyperadrenocorticism until the dog has recovered from the concurrent illness.

### Table 2 Protocols for Low-Dose Dexamethasone Suppression Test

<table>
<thead>
<tr>
<th>Protocol</th>
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<tbody>
<tr>
<td><strong>Protocol A (Plasma or serum; dexamethasone solution in polyethylene glycol)</strong></td>
<td></td>
</tr>
<tr>
<td>1. Collect basal blood sample for determination of plasma or serum cortisol</td>
<td></td>
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<tr>
<td>2. Administer dexamethasone solution at dosage of 0.015 μg/kg, IV or IM</td>
<td></td>
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<tr>
<td>3. Collect additional serum or plasma cortisol samples at 4 and 8 h</td>
<td></td>
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<tr>
<td><strong>Protocol B (Plasma or serum; dexamethasone sodium phosphate)</strong></td>
<td></td>
</tr>
<tr>
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<td>2. Administer dexamethasone sodium phosphate at dosage of 0.01 μg/kg, IV or IM</td>
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<tr>
<td><strong>Protocol C (Urine cortisol:creatinine ratios; oral dexamethasone)</strong></td>
<td></td>
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<tr>
<td>1. Owners collect urine from their dogs on two consecutive mornings (0800 h) for determination of baseline cortisol:creatinine ratios</td>
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<tr>
<td>2. Immediately after collection of the second urine sample, owners administer dexamethasone at the dosage of 0.01 mg/kg, PO</td>
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<tr>
<td>3. Owners walk dog at 1200 and 1400 h to ensure bladder emptying</td>
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<tr>
<td>4. Owners collect a third urine sample for measurement of cortisol:creatinine ratio at 1600 h (8 h after oral administration of dexamethasone)</td>
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### Urinary Cortisol:Creatinine Ratio

Calculation of a cortisol:creatinine ratio by use of cortisol and creatinine concentrations measured in a single urine sample is a simple and valuable screening test for hyperadrenocorticism in dogs.35-38 The main advantages of the urine cortisol:creatinine ratio over the low-dose dexamethasone suppression and ACTH stimulation tests include the test’s convenience and high sensitivity.

Cortisol and its metabolites are normally excreted into the urine, with urine cortisol excretion rising with increased adrenal secretion of the hormone. By measuring cortisol in morning urine, an integration of cortisol secretion over a period of about 8 hours is achieved, thereby adjusting for the wide and rapid fluctuations in circulating cortisol concentrations. Because creatinine excretion is relatively constant when kidney function is stable, dividing the urine cortisol by the creatinine concentration negates the effect of urine volume (and therefore the degree...
of urinary concentration) in interpreting the urine cortisol concentration. To this end, the urine cortisol:creatinine ratio is determined by dividing the urine cortisol concentration (in μmol/L) by the urine creatinine concentration (in μmol/L). In most laboratories, the reference range for the ratio is generally less than 15 to 20 (there are no units associated with the cortisol:creatinine ratio).

To perform this test, the owner is instructed to collect morning urine samples at the same time of day (eg, 0700 to 0800 hours) on two to three consecutive days. On the preceeding evening, the dog should have its last walk at the identical times (eg, 2300 hours). No special precautions are needed for the urine collection itself. However, after collection, each of the urine samples should be kept refrigerated until the owner is able to bring the specimens to the veterinary hospital. This home-collection protocol avoids the "stress" of a car ride or visit to the veterinary clinic for sample collection, both of which may cause slight elevations in the ratio in dogs without evidence of hyperadrenocorticism.

After submission of the dog's morning urine samples to the laboratory for determination of cortisol and creatinine concentrations, the veterinarian should average the results of the two to three urine cortisol:creatinine ratios. The mean urine cortisol:creatinine ratio clearly differentiates between clinically normal dogs and dogs with hyperadrenocorticism (ie, it is a sensitive diagnostic test). A high mean cortisol:creatinine ratio will be found in most dogs with hyperadrenocorticism, with a reported sensitivity ranging from 0.85 to 0.99. Unfortunately, the cortisol:creatinine ratio is also high (false-positive) in many dogs with nonadrenal illness. It has been demonstrated that 79 and 76% of dogs with moderate to severe nonadrenal disease had a high ratio (false-positive test result), consistent with Cushing's syndrome. Therefore, while this simple test appears highly sensitive in detecting hyperadrenocorticism in dogs, it lacks specificity and commonly produces false-positive results in dogs with severe nonadrenal illness.

Overall, because of the high sensitivity and low specificity of the urinary cortisol:creatinine ratio, it is recommended that this test be used primarily for its negative-predictive value. In other words, if the results of cortisol:creatinine remain within reference range limits, the presence of hyperadrenocorticism is highly unlikely. On the other hand, if a high cortisol:creatinine ratio is found, especially in a dog with concurrent disease (eg, diabetes mellitus), we recommend this positive result for Cushing's syndrome be confirmed with use of a low-dose dexamethasone suppression or ACTH stimulation test.

The urine cortisol:creatinine ratio cannot be used to reliably differentiate pituitary-dependent from adrenal-dependent hyperadrenocorticism. However, the finding of very high urine cortisol:creatinine ratios (>100) make it more likely that the dog is suffering from pituitary-dependent hyperadrenocorticism.

**Urinary Cortisol:Creatinine Ratio (Low-Dose Dexamethasone Suppression Test)**

Although the low-dose dexamethasone suppression test is typically performed by measuring serum or plasma cortisol concentrations before and after dexamethasone injection, measurement of urinary cortisol:creatinine ratios in samples collected before and after administration of a low dose of dexamethasone can also be proposed. With this protocol, the owner collects urine from the dog on two consecutive mornings at 0800 hours for the measurement of baseline cortisol:creatinine ratios. Immediately after collection of the second urine sample, the owner administers dexamethasone at the dosage of 0.01 mg/kg PO. After walking the dog at 1200 and 1400 hours to ensure bladder emptying, the owner collects a third urine sample for measurement of a cortisol:creatinine ratio from their dog at 1600 hours (8 hours after the oral administration of dexamethasone).

Preliminary evidence suggests that a 50% suppression in the mean urine cortisol:creatinine ratio (and a decrease in the ratio to <10) would be consistent with a normal response. Dogs with hyperadrenocorticism would be expected to fail to show adequate suppression of the urine cortisol:creatinine 8 hours following oral dexamethasone administration.

**Tests to Determine the Cause of Hyperadrenocorticism**

Once a diagnosis of hyperadrenocorticism has been confirmed, the next step in the workup is to use one or more tests and procedures to distinguish pituitary-dependent from adrenal-dependent hyperadrenocorticism. The ability to differentiate between dogs with pituitary-dependent hyperadrenocorticism from those with functional adrenocortical tumors has important implications in providing the most effective method of management for the disease. An accurate test is therefore required to determine the cause of the dog's hyperadrenocorticism.

Endocrine tests in this category include the high-dose dexamethasone suppression test and endogenous plasma ACTH measurements. Imaging techniques such as abdominal radiography, ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) can also be extremely helpful in determining the cause. In addition, it is only possible to detect metastatic lesion from an adrenal carcinoma by use of these imaging techniques, in the absence of adrenal biopsy and histopathology.

**High-Dose Dexamethasone Suppression Test**

Even though the ability of glucocorticoids, such as dexamethasone, to suppress pituitary ACTH secretion in dogs with pituitary-dependent hyperadrenocorticism is abnormal and is resistant to suppression with low doses of dexamethasone, much higher doses of dexamethasone will usually overcome this resistance to negative-feedback inhibition. Therefore, most dogs with pituitary-dependent hyperadrenocorticism demonstrate suppression of serum cortisol levels following administration of a high dose of dexamethasone. In contrast, because pituitary ACTH secretion has already been chronically suppressed in dogs with cortisol-secreting adrenal tumors, administration of dexamethasone, no matter how high the dose, will fail to suppress serum cortisol concentrations.
Table 3 Protocols for High-Dose Dexamethasone Suppression Test

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<td>1. Owners collect urine from their dogs on two consecutive mornings for determination of baseline cortisol:creatinine ratios</td>
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<tr>
<td>2. Owners then administer three oral doses of dexamethasone to their dog at the dosage of 0.1 mg/kg every 8 h (eg, 0800, 1600, and 2400 h)</td>
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<tr>
<td>3. Owners collect post-dexamethasone urinary cortisol:creatinine ratio the next morning (eg, 0800 h)</td>
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</table>

The high-dose dexamethasone suppression test is performed in a manner similar to that of the low-dose suppression test with respect to sample collection timing. The recommended protocols for the high-dose dexamethasone suppression test is to collect samples before and 8 hours after administration of dexamethasone (0.1 to 1.0 mg/kg, IV or IM). Either a solution of dexamethasone in polyethylene glycol or dexamethasone sodium phosphate can be administered for the test with equivalent results. Demonstration of cortisol suppression to concentrations less than 1.5 μg/dL (40 nmol/L) is generally considered diagnostic for pituitary-dependent hyperadrenocorticism and excludes an adrenal tumor. Interpretation of the results of the high-dose dexamethasone suppression test should be based on the laboratory’s reference ranges for the dose and of dexamethasone administered.

Suppression of plasma or serum cortisol concentrations after administration of high-dose dexamethasone, regardless of the high dose given, indicates pituitary-dependent hyperadrenocorticism. Unfortunately, lack of cortisol suppression is not always diagnostic for an adrenal tumor, because anywhere from 15 to 50% of dogs with pituitary-dependent hyperadrenocorticism fail to demonstrate cortisol suppression, depending on the dose of dexamethasone used. Dogs given higher doses of dexamethasone tend to suppress more consistently than those receiving the lower “high dose” of dexamethasone for the test. In addition, the larger the pituitary tumor, the less likely the dog is to suppress with dexamethasone, no matter how high the dose.

In dogs with confirmed hyperadrenocorticism that fail to demonstrate cortisol suppression during high-dose dexamethasone suppression testing, there is approximately a 50:50 chance that it has pituitary-dependent disease versus an adrenal tumor. In these dogs, additional tests are required to determine the cause of hyperadrenocorticism (see below).

### Urinary Cortisol: Creatinine Ratios and the High-Dose Dexamethasone Suppression Test

The protocol for performing this high-dose dexamethasone suppression test, with monitoring of the percent suppression of urine cortisol:creatinine ratios, is as follows. Owners collect urine from their dogs on two consecutive mornings (eg, at 0700 to 0800 hours) for determination of baseline cortisol:creatinine ratios. The owners then administer three oral doses of dexamethasone to their dog at the dosage of 0.1 mg/kg every 8 hours. In other words, immediately after collection of the second basal urine sample, the first dexamethasone dose is administered; the second and third doses are administered in the afternoon and evening of the same day, respectively. The third urine sample is collected 8 hours after administration of the final dexamethasone dose, which would be the next morning. Thus, for this test, urine is collected on three consecutive mornings, and the three dexamethasone doses are all administered on the second day (Table 3).

The finding that the urinary cortisol:creatinine ratio after dexamethasone administration is suppressed by more than 50% of the average basal cortisol:creatinine ratio is diagnostic for pituitary-dependent hyperadrenocorticism. If suppression is less than 50%, no discrimination is possible, as is the case for the standard high-dose dexamethasone suppression test described above. Similar to the standard test, approximately 30% of dogs with pituitary-dependent hyperadrenocorticism fail to suppress with the urinary high-dose dexamethasone suppression test. In these dogs, additional testing is required to determine the cause of hyperadrenocorticism.

It is important to remember that the baseline urinary cortisol:creatinine ratio is very sensitive in diagnosis of hyperadrenocorticism but its specificity is quite poor. Therefore, a diagnosis of Cushing’s syndrome must be supported by typical clinical signs and routine laboratory abnormalities and is best confirmed by use of a second screening test (ie, ACTH stimulation or low-dose dexamethasone suppression test).

### Combined Dexamethasone Suppression/ACTH Stimulation Test

This test combines a differentiation test (high-dose dexamethasone suppression test) with a screening test (ACTH stimulation test). The combined test cannot be recommended inasmuch as the shortened version of the high-dose dexamethasone suppression test is less reliable than the standard test described above. In addition, administration of dexamethasone just 4 to 6 hours before ACTH stimulation can influence the cortisol response to the ACTH. Overall, there is more variability in the ACTH-stimulated cortisol response with the combined dexamethasone suppression/ACTH stimulation test compared with an isolated ACTH stimulation test.
Plasma Endogenous ACTH Concentration

Measurement of basal endogenous ACTH concentrations is of no value in the diagnosis of hyperadrenocorticism because dogs with the disease commonly have values within reference range limits. On the other hand, measurement of plasma ACTH concentrations is an extremely reliable means of determining the cause of hyperadrenocorticism once the diagnosis has been confirmed. There is relatively little overlap in ACTH concentrations between dogs with pituitary tumors and those with adrenal tumors, making this a valuable discriminatory test.1-4,17,18,32,49

Endogenous ACTH concentrations are normal to high in dogs with pituitary-dependent hyperadrenocorticism (eg, >40 pg/mL or >8.8 pmol/L), whereas ACTH concentrations are usually low or undetectable (eg, <20 pg/mL or <4.4 pmol/L) in dogs with adrenal tumors or with iatrogenic hyperadrenocorticism. Unfortunately, about 20% of dogs with hyperadrenocorticism will have random plasma ACTH concentrations in the “gray zone” (ie, too low for pituitary-dependent disease but too high to be classified as adrenal-dependent disease). One option in such dogs with nondiagnostic plasma ACTH values is to repeat the plasma ACTH measurement, either collecting another basal sample or by using stimulation testing with corticotropin-releasing factor or vasopressin (see below). Alternatively, one could use one of the other differentiation tests (eg, high-dose dexamethasone suppression, imaging studies) to determine the cause of the dog’s hyperadrenocorticism.

Samples for accurate endogenous ACTH concentration determination can be difficult to collect and are somewhat costly to perform. Samples must be collected in heparin or EDTA tubes and centrifuged immediately; the plasma is then placed into plastic or polypropylene tubes (ACTH will stick to glass) and immediately frozen until the assay is performed.30 Plasma samples should be sent on dry ice by overnight delivery service to the nearest laboratory. If such conditions are not feasible, one can add aprotinin, a protease inhibitor, to the EDTA tube as a preservative for ACTH; in such cases, the sample can be shipped cold via refrigeration packs and freezing is not necessary. Nevertheless, while this test is reliable in determining the cause of hyperadrenocorticism, its routine use may not be feasible for small animal practitioners primarily because of the difficulty and cost of shipping the sample to the laboratory.

Corticotropin-Releasing Hormone (CRH) and Vasopressin Stimulation Testing Using Plasma ACTH Concentrations

Dogs with pituitary-dependent hyperadrenocorticism typically retain their responsiveness to CRH and vasopressin and have plasma ACTH concentrations that rise above reference range limits.18,51,52 In contrast, pituitary ACTH secretion is chronically suppressed in dogs with functional cortisol-secreting adrenal tumors; therefore, these dogs have low-normal or low basal ACTH concentrations that do not respond to stimulation with either CRH or vasopressin. Therefore, stimulation with either CRH or vasopressin can be used to help determine the cause of hyperadrenocorticism. However, major disadvantages, which limit the use of these testing protocols in veterinary practice, include the expense, lack of availability of CRH and vasopressin, and the special sample handling required for plasma ACTH assays.30

Diagnostic Imaging

Abdominal radiography, abdominal ultrasonography, and computed tomography or magnetic resonance imaging (of either the abdominal or the brain) can be used to differentiate between pituitary-dependent hyperadrenocorticism and adrenal-dependent hyperadrenocorticism. Diagnostic imaging of the abdomen can also be used in many cases to distinguish between benign and malignant tumors of the adrenal cortex.

Radiography

On radiographic examination, changes in dogs with hyperadrenocorticism may include hepatomegaly (most consistent radiographic finding), pendulous abdomen, calcification cutis, bronchial mineralization, osteopenia, and dystrophic mineralization of the renal pelvis and abdominal aorta.1-4 Although these radiographic findings may increase the suspicion for hyperadrenocorticism, their presence would not aid in confirmation or differentiation of the disease.

Abdominal radiography can be helpful in differentiation of the cause of hyperadrenocorticism if an adrenal mass is found. Mineralization in the region of an adrenal gland strongly suggests the possibility of an adrenal tumor.1-4,10,53 Although the presence of mineralization cannot be used to distinguish benign from malignant tumors, most adrenal tumors with extensive calcification are carcinomas.

Abdominal Ultrasonography

Ultrasonography is much more useful than radiography in distinguishing dogs with pituitary-dependent from those with adrenal-dependent hyperadrenocorticism. Ultrasonography is much less time consuming than the 8-hour high-dose dexamethasone suppression test. In addition, abdominal ultrasonography allows differentiation in a much higher percentage of cases than with measurement of the endogenous ACTH concentration or high-dose dexamethasone suppression testing. Because of these reasons, as well as widespread use of abdominal ultrasonography in companion animal practice, this technique has become the test of choice for determining the cause of hyperadrenocorticism in dogs.

With abdominal ultrasonography, small or noncalcified unilateral adrenal tumors can generally be readily detected, and bilateral adrenal enlargement can be visualized in dogs with pituitary-dependent hyperadrenocorticism. Ultrasonography may, in addition, detect the presence of liver metastasis or invasion of the vena cava from an adrenal carcinoma. The contralateral adrenal gland would be expected to be small in dogs with unilateral cortisol-secreting tumor due to the fact that pituitary ACTH secretion has been chronically suppressed leading to contralateral adrenal atrophy. However, the small contralateral adrenal gland may not always be visible at the time of ultrasound examination.

One may sometimes have difficulty in visualizing the adrenal glands because they are normally quite small and lie in a cranial retroperitoneal position. Development of higher resolution ultrasound machines diminishes this problem, but operator experience is of critical benefit when performing adrenal evaluation by ultrasound.
Despite the great usefulness of abdominal ultrasonography in evaluating dogs with hyperadrenocorticism, the technique does have limitations. Abdominal ultrasonography cannot be used as a screening test for hyperadrenocorticism; it can only be used to help determine the cause once a diagnosis has been established by use of the pituitary-adrenal function tests outlined above. There is great overlap in adrenal gland length and diameter between clinically normal dogs, dogs with pituitary-dependent hyperadrenocorticism, and dogs with nonadrenal disease; thus, ultrasound cannot always distinguish between the groups.

In addition, it is extremely important to remember that the finding of an adrenal mass on ultrasonography is not diagnostic or synonymous with a cortisol-secreting tumor leading to hyperadrenocorticism. Ultrasonography cannot differentiate a functional cortisol-secreting adrenocortical tumor from a nonfunctional tumor, a pheochromocytoma, a metastatic lesion to the adrenal, or a granuloma.

**Computed Tomography and Magnetic Resonance Imaging**

Use of either CT or MRI is extremely accurate and reliable methods to image either the adrenals or the pituitary glands. Both CT and MRI require general anesthesia (up to 2 hours) and an experienced radiologist to interpret the study. In general, the study recommended (CT versus MRI) should simply be whichever is least expensive or most readily available.

With either technique, bilateral adrenal enlargement can be readily differentiated from a unilateral adrenal tumor. With adrenocortical carcinoma, the location of the primary adrenal tumor and its metastasis can be identified in most dogs.

Use of CT or MRI is most helpful in the diagnosis of pituitary tumors, as well as to confirm the macrotumor syndrome. CT scanning will allow visualization of large pituitary tumors, but not smaller ones. MRI provides superior soft-tissue contrast as compared with CT and is also more accurate for visualization of smaller pituitary tumors.

**References**

Diagnosis of hyperadrenocorticism in dogs


