When Normal Is Abnormal: Keys to Laboratory Diagnosis of Hidden Endocrine Disease

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Although veterinary clinicians commonly rely on panels of laboratory tests with individual results flagged when abnormal, care should be taken in interpreting normal test results as well. There are several examples of this in evaluating patients with endocrine disease. The finding of a normal leukogram (absence of a stress leukogram) can be indicative of adrenal insufficiency in dogs, and this disorder can be especially elusive when there are no overt indicators of mineralocorticoid deficiency. Cats with hyperthyroidism can have normal serum thyroid hormone concentrations, normal hematocrits, and normal serum concentrations of creatinine despite the presence of disease that affects these parameters. A normal serum phosphorus concentration, in the face of azotemia, isoosmolarity, and hypertension can point a clinician toward a diagnosis of primary hyperaldosteronism rather than primary renal disease. A normal serum parathyroid hormone concentration in the face of hypercalcemia is inappropriate and can indicate the presence of primary hyperparathyroidism. Similarly, hypoglycemia accompanied by a normal serum insulin concentration can be found in cases of hyperinsulinism. These normal findings in abnormal patients, and their mechanisms, are reviewed.

When laboratory markers mesh with clinical history and physical examination findings, the diagnosis of endocrine disease is often straightforward. Because of the complex nature of endocrine disorders, however, diagnosis depends on an understanding of the interplay between the endocrine system and other body systems. Serum biochemistry and hematology panels are widely available from veterinary diagnostic laboratories and are used extensively by veterinary clinicians. Typically, results of these panels of tests are reported with indicators flagging the results that are outside of the normal reference range for easy identification of abnormal results. There is a tendency on the part of clinicians to focus on abnormal results when interpreting panels of tests, but overlooking normal results can sometimes cause a diagnosis to be missed. This review article will present clinical situations in which normal test results can provide important clues to diagnosis of endocrine disease, illustrating the importance of interpreting all available clinical test results—not just those flagged by the diagnostic laboratory as being outside the normal range.

Normal Leukogram: A Clue to Adrenal Insufficiency

Adrenal insufficiency (hypoadrenocorticism; Addison’s disease) is a well-described condition in which destruction of the adrenal cortex, usually immune-mediated, results in a deficiency in both cortisol and aldosterone secretion.1-3 Classically, dogs with adrenal insufficiency have clinical findings referable to deficiencies in both hormones. Mineralocorticoid deficiency is the cause of an adrenal crisis, and affected dogs can be presented with hypovolemic shock, bradycardia, and circulatory collapse accompanied by hyperkalemia and hyponatremia. Diagnosis in these cases is readily accomplished by reviewing the clinical history and signalment, by documenting electrolyte abnormalities, and by demonstrating low (usually undetectable) serum concentrations of cortisol that do not increase appropriately in response to exogenously administered adrenocorticotropic hormone (ACTH).

Hyperkalemia, hyponatremia, and a low ratio of Na+/K+ are not found in all dogs with adrenal insufficiency, and there are a variety of other disorders that are associated with these electrolyte derangements, including gastrointestinal, urinary, cardiovascular, and respiratory disorders.4 A subtype of adrenal insufficiency in which dogs are deficient in cortisol but not aldosterone has been described.5-8 This condition is often referred to as “atypical” hypoadrenocorticism. It should be noted that isolated glucocorticoid deficiency with adequate mineralocorticoid secretion has not been demonstrated definitively in dogs by documentation of normal
aldosterone concentrations combined with abnormal or absent cortisol secretion in case series published in the veterinary literature. Rather, the presence of adequate aldosterone secretion has been assumed based on normal serum electrolyte results. For the purpose of this discussion, however, the “atypical” and “classic” forms of adrenal insufficiency will be considered as distinct entities.

Even the classic form of adrenal insufficiency can present a diagnostic challenge, but diagnosing the atypical disease can be more difficult. In a study comparing dogs with classic hypoadrenocorticism to those with the glucocorticoid-deficient form of the disease, investigators reported that dogs with atypical hypoadrenocorticism were an average of 2.6 years older at the time of diagnosis than the dogs in the mineralocorticoid-deficient group.9 There were no significant differences in clinical signs, although ataxia or collapse were not reported in any of the dogs with isolated glucocorticoid deficiency. Gastrointestinal signs were common in both groups, probably because both groups had glucocorticoid deficiency. Mechanisms of gastrointestinal (and other) signs in dogs with adrenal insufficiency have not been clearly elucidated in published studies, but the signs can be explained by considering the general physiologic roles of glucocorticoids. Cortisol-associated mechanisms are involved in maintenance of blood pressure, vascular volume, vascular tone, vascular permeability, and endothelial integrity, making it understandable how clinical signs of adrenal insufficiency could occur.10

Of diagnostic importance is cortisol’s role in countering stress. Stress, which is an expected part of severe or chronic illness, is typically accompanied by changes in the number and distribution of leukocytes in peripheral blood, with neutrophilia, lymphopenia, eosinopenia, and monocytosis being common components of the classic “stress leukogram” in the dog.10,11 Before the wide availability of cortisol assays, the most valuable clinicopathological test for adrenal insufficiency was arguably the leukogram. The “Thorn test” is the best known example. In a series of studies published between 1948 and 1955, Thorn and colleagues described the diagnosis of adrenal insufficiency based on neutrophil, lymphocyte, and eosinophil responses to exogenously administered ACTH.12,13 A modified version of Thorn’s test was evaluated for use in dogs with adrenal insufficiency, but the test is generally not used.14 Still, stress leukograms are usually absent in dogs with adrenal insufficiency, despite the stress of severe illness, and this normal finding, which might otherwise be overlooked, should alert the astute clinician to the possibility of glucocorticoid deficiency. Because the clinical signs of canine adrenal insufficiency can be obscure, variable, and vague, the disorder is often not suspected. This might be more of an issue in cases of atypical adrenal insufficiency. A sick dog lacking indicators of cortisol-induced changes in blood leukocytes should be suspected of having adrenal insufficiency, even in the face of normal Na+ and K+ concentrations in the serum. In such cases, measurement of a basal concentration of serum or plasma cortisol can be performed to help rule out adrenal insufficiency. Although basal cortisol concentrations have not been traditionally considered useful, a recent study showed a very high negative predictive value for the test.15 If a normal or high concentration of cortisol is found, hypoadrenocorticism is highly unlikely, but if a basal cortisol concentration of less than approximately 2 µg/dL is found, an ACTH stimulation test should be used to confirm the diagnosis.

Normal Test Results in Cats with Hyperthyroidism

When Is a Normal T4 Abnormal?

Hyperthyroidism is a common endocrine disease in middle-aged and older cats. In many cases the diagnosis is not particularly challenging, involving recognition of clinical signs and demonstration of a high serum concentration of total thyroxine (T4). “Occult hyperthyroidism” in the cat was first described in 1990 and is defined as the presence of thyrotoxicosis with a serum T4 value within the normal range.16 This phenomenon, which occurs in approximately 10% of cats with hyperthyroidism, has several possible explanations. It has been shown, for example, that T4 concentrations can fluctuate significantly in hyperthyroid cats, with some cats having T4 concentrations well within the normal range on some days and significantly elevated T4 concentrations on others.17 (Fig 1) Another explanation could be the effects of nonthyroidal illness on serum thyroid hormone concentrations. Peterson and Gamble reported that a large proportion of cats with nonthyroidal illness have low serum T4 concentrations in the absence of hyperthyroidism.18 In that study, serum T4 concentrations were reported to be below the normal range in 59% of cats with diabetes mellitus, in 54% of cats with liver disease, in 48% of cats with renal disease, and in 41% of cats with neoplasia, with low T4

![Figure 1. Serum T4 concentrations measured daily for 15 days in a cat with hyperthyroidism. Dashed lines represent the upper and lower ends of the reference interval (adapted from Peterson et al, J Vet Intern Med, 1987).17](image-url)
concentrations found in cats with a variety of other illnesses as well. Based on those findings it is reasonable to hypothesize that cats with hyperthyroidism and a concurrent nonthyroidal illness could experience a T4-lowering effect that could mask overt hyperthyroidism.

Nonthyroidal illness syndrome (NTIS) has also been referred to as “euthyroid sick syndrome” because sick patients with low thyroid hormone concentrations are not clinically hypothyroid. NTIS is the term used more commonly today, possibly because of controversy surrounding the debate over whether these patients can benefit from thyroid hormone supplementation.19,20 It is not known if NTIS is an adaptive response to the stress of illness, with a beneficial decrease in metabolism and tissue oxygen demand, or if it is pathologic in itself. Low T3 syndrome is much more common in sick human patients than a syndrome of low T4, with low T4 occurring in the most severe cases of NTIS and carrying a poorer prognosis.19 Patients with NTIS have thyrotrophin (TSH) concentrations that are below the normal range despite the presence of low T3.19,20 Recovery from illness results in increased TSH secretion from the pituitary gland, and TSH concentrations can remain elevated for a period of time after the recovery period. The low TSH associated with NTIS is likely due to decreased thyrotrophin-releasing hormone (TRH; thyroliberin) from specific paraventricular nucleus neurons. TRH gene expression was lost in patients who died from prolonged illnesses with associated NTIS.21

There are several factors that negatively influence TRH production in patients with NTIS. Diminished calorie intake causes decreased paraventricular nucleus TRH, and this is thought to occur through decreases in leptin.22 Arcuate nucleus neurons that contain a-melanocyte-stimulating hormone stimulate TRH production, and this mechanism is enhanced by leptin. Neuropeptide Y and agouti-related protein, arcuate nucleus proteins that generally stimulate appetite and are increased in fasting, also suppress TRH gene expression.23 This effect is inhibited by leptin and supported by ghrelin, the well-described orexigenic hormone. Sepsis and trauma are also implicated in decreased TRH expression of NTIS. The mechanism is believed to involve increased deiodinase activity in the tanycytes that line the third ventricle and extend to the hypothalamus.24 These cells produce more T3 (by deiodination of T4) during sepsis and trauma, thereby suppressing TRH production. Inflammatory cytokines also contribute to NTIS by suppression of pituitary TSH release. Interleukin-6 possesses the best-known and most potent TSH-suppressing effect.20

The role of deiodinases in NTIS is controversial. It has generally been thought that decreased deiodinase activity in illness is responsible for the decreased conversion of T4 to T3, resulting in a low T3 state. This idea has been challenged in recent years, and it is currently not known if low T3 in NTIS is a cause or effect of decreased deiodinase activity.25 NTIS may also be related to the decrease in thyroid hormone-binding proteins that occurs as part of the “acute phase response” in illness.26

How should a veterinary clinician approach a cat with a normal serum T4 concentration in which hyperthyroidism is suspected clinically? Because T4 can fluctuate, a diagnosis may be reached by simply repeating a T4 measurement on a different day. In cases of NTIS, however, thyroid hormone concentrations may remain suppressed. Free thyroxine (fT4) measurement has been advocated for use in this situation, but it is important to realize that a significant percentage of cats with NTIS have increased concentrations of fT4 that are not associated with thyrotoxicosis.27 For this reason it is unwise to depend on fT4 alone for diagnosis of hyperthyroidism in the cat, and it is unclear whether currently available fT4 assays are more sensitive for feline hyperthyroidism than total T4 assays. T4 and fT4 tests may have some value when used in combination, but care should be taken in interpreting these combined results. The finding of a high-normal T4 and a high fT4 in a cat in which hyperthyroidism is suspected supports the diagnosis, but not strongly. In sick cats with NTIS and a high serum fT4 concentration, T4 can be expected to be low because almost 50% of cats with NTIS have serum T4 concentrations below the normal range, and the result is in the low half of the normal range or lower in more than 80% of these cats.27 Of 14 cats with NTIS and high serum concentrations of fT4, 11 had serum T4 concentrations that were either below the normal range (N = 8) or in the lower half of the normal range (N = 3). The other 3 cats had serum T4 concentrations in the upper half of the normal range (T4 > 3.3 nmol/L).27 Based on this report, the combination of a high fT4 and a high-normal T4 has poor specificity for hyperthyroidism in the cat. Other tests that can be used to diagnose occult hyperthyroidism in cats include the T3 suppression test,28 the TRH stimulation test,29 and thyroid scintigraphy.30 These tests all have advantages and disadvantages. The T3 suppression test is reportedly very accurate, but time-consuming. The TRH stimulation test is associated with adverse effects that, although transient and not life-threatening, can be severe. Thyroid scintigraphy requires the use of radioisotopes and the availability of nuclear medicine facilities.

Is a Normal Hematocrit Really Normal?

The results of a complete blood count (CBC) from a 14-year-old cat with weight loss and polyuria are presented in Table 1. When interpreting CBC results from sick, older cats, a low hematocrit is expected. A low hematocrit could be from anemia of chronic disease and associated with any type of illness,31 but anemia of chronic renal failure would be a top differential in an older cat with polyuria and weight loss.32 In the present example, rather than simply considering the hematocrit normal, the astute clinician should question why it is normal. The cat in this case also had a normal serum concentration of T4; a diagnosis of hyperthyroidism was eventually made by T3 suppression testing, and the best clue to the diagnosis on screening blood tests may well have been the normal hematocrit.
Mild erythrocytosis occurs in approximately half of cats with hyperthyroidism. Observations of this phenomenon in human patients were published in 1939. Several factors may contribute to the increase in red blood cell (RBC) mass seen in cats with this disease. Generalized volume depletion could raise the hematocrit by hemoconcentration, but this should be accompanied by a concomitant increase in plasma total solids or albumin concentrations, and this has not been reported as a result of thyrotoxicosis in cats.

The mechanism of erythrocytosis in hyperthyroidism is probably mediated, at least in part, by erythropoietin. Normocytic, normochromic anemia is common in human patients with hypothyroidism, and is associated with decreased erythropoietin concentrations. Conversely, human patients with hyperthyroidism and increased RBC mass have increased serum concentrations of erythropoietin, and thyroid hormone causes enhanced erythropoiesis in hypoperfused rat kidneys experimentally. Increased cellular oxygen requirement is well established as the main physiologic stimulus for erythropoietin production, and the increased metabolic rate accompanying hyperthyroidism clearly exerts an increased oxygen demand on metabolically active tissues. There may, however, be other mechanisms at play. Early work by Meineke and Crafts showed that dinotrophenol, although causing more of an increase in oxygen demand, stimulates erythropoiesis to less of an extent than does thyroid hormone. There is also an increase in erythropoiesis in response to D-T3, similar to that seen with L-T3, even though the D isomer is not metabolically active and does not increase tissue oxygen consumption. This suggests some other thyroid-hormone receptor-mediated mechanism contributes to the increased RBC mass seen in hyperthyroidism.

Although studies of thyroid hormone effects on erythropoiesis were conducted decades ago, reports of mechanisms of erythrocytosis in cats with hyperthyroidism are lacking.

### Normal Serum Creatinine in Hyperthyroid Cats

Of the components of a typical serum chemistry panel, creatinine concentration is the best indicator of glomerular filtration rate. Still, the test is insensitive at best. A rise in serum creatinine concentration is not observed until more than 75% of renal function has been lost. Accurate assessment of renal function is of critical importance in the management of cats with hyperthyroidism. Reportedly, between 17% and 49% of cats develop overt renal insufficiency after treatment for hyperthyroidism. A deleterious effect of thyrotoxicosis on the kidney has not been demonstrated conclusively in the cat, but it is likely that many cats with hyperthyroidism also have underlying renal disease, and that normal renal autoregulation in these patients is lost; in this case, the increased cardiac output and decreased peripheral resistance observed in hyperthyroidism could lead to an increase in glomerular filtration rate, falsely lowering the concentration of creatinine in the serum of a cat with concurrent renal and thyroid disease. In light of this probability, findings of normal serum creatinine in cats suspected of having hyperthyroidism should not be taken lightly. A normal serum creatinine concentration, especially in a cat with hyperthyroidism, does not indicate the absence of renal pathology. For this reason careful assessment of renal function before and after treatment of feline hyperthyroidism is recommended.

### If This Is Renal Azotemia, Why Is The Phosphorus Normal?

#### Case Example

A 13-year-old, castrated male domestic shorthair cat was presented for evaluation of acute onset of weakness and lethargy. The cat was kept as an indoor pet. All vaccinations were current. The diet consisted of a mixture of free-choice adult dry maintenance food and a small amount of canned food (a variety of flavors) daily. The owner reported the cat’s appetite had been mostly normal, but the owner thought the cat may have been drinking more than usual.

On physical examination, the cat was depressed and weak. The body condition score was 3/9. There was pronounced cervical ventroflexion. The cat was mildly dehydrated, with a normal body temperature. The heart rate was 155 bpm, and there was a grade 2/6 systolic murmur, with a point of maximum intensity heard at the left sternal border. Abdominal palpation revealed a large urinary bladder, but no obvious abnormalities. There was no palpable goiter. The cat was depressed but mentally appropriate. All cranial nerve reflexes were normal. Proprioception was normal. All spinal reflexes were intact but were subjectively diminished. Causes of weakness were considered, including neurologic, metabolic, cardiopulmonary, and musculoskeletal diseases. The initial diagnostic plan included urinalysis, serum chemistry profile, CBC, total T4, thoracic radiographs, and systolic blood pressure measurement.

The urine was pale and clear with a trace of protein, and was negative for glucose, bilirubin, blood cells, and ketones. There were no casts, cells, or crystals seen in the urine sediment. The specific gravity of the urine was 1.016. All findings on the CBC were normal. Abnormalities on the serum chem-
tions despite 8 of the 11 cats being azotemic.

In addition to causing renal potassium loss, aldosterone causes renal calcium loss and a resultant decrease in plasma Ca^{2+}. Hypocalcemia results in parathyroid hormone secretion—a physiological response directed at restoring calcium homeostasis. One of the classic effects of parathyroid hormone is renal phosphorus loss, and this explains the presence of normal or low serum phosphorus in syndromes of aldosterone excess.

Normal Parathyroid Hormone in Hyperparathyroidism

Primary hyperparathyroidism (PHP) is the third most common cause of ionized hypercalcemia in dogs, occurring in 13% of canine hypercalcemic patients according to a recent retrospective study. Humoral hypercalcemia of malignancy occurred in 58% of the dogs of that study and was the most common cause of ionized hypercalcemia. In the cat, PHP appears to be less common. In a retrospective study of total hypercalcemia in 71 cats, PHP was diagnosed in only 4 (6%). In both species, PHP is usually caused by an autonomously hyperfunctioning adenoma of one or more parathyroid glands. The resulting high concentration of parathyroid hormone (PTH) causes hypercalcemia, and it is hypercalcemia that is responsible for clinical signs when they are present. Diagnosis can be confirmed by demonstration of a high serum concentration of PTH in the face of hypercalcemia. This is one of the most striking examples, however, of cases in which normal is abnormal in patients with endocrine disease. In a study of 185 dogs with PHP, serum concentrations of PTH were within the normal range in 135 (73%). Reports of serum PTH results in cats with PHP are less extensive, and the prevalence of normal serum concentrations of PTH in these patients is not known. Based on personal clinical observations, however, normal serum PTH concentrations in cats with PHP are common.

PHP illustrates clearly the differences in interpretation of normal test results in normal animals versus those with disease. Although a PTH concentration within the reference range may be normal for a healthy animal, it is inappropriate for an animal with hypercalcemia. The effect of hypercalcemia should be to suppress PTH secretion rapidly and effectively. Therefore, the finding of anything other than a low concentration of PTH in an animal with hypercalcemia is indicative of dysregulated PTH secretion.

When Glucose Is Low, Insulin Should Be Too

Very similar to the situation with normal PTH in animals with PHP is the finding of normal serum concentrations of insulin in an animal with an insulin-secreting neoplasm of the pancreatic beta cell. One of the main stimuli for beta cells to secrete insulin is hyperglycemia. Conversely, hypoglycemia should be accompanied by very low pancreatic insulin secretion. A normal insulin concentration in the face of hypoglycemia can only occur when insulin secretion is independent of regulation by blood glucose concentrations. Beta cell tu-
mors, referred to as “insulinomas,” are uncommon in dogs and even less common in cats. These tumors cause chronic hypoglycemia by secreting insulin unregulated by concentrations of glucose in the extracellular fluid.

In the most recent retrospective study of dogs with insulinoma, serum insulin concentrations were within the normal reference range, despite hypoglycemia, in 3 of 8 dogs. Because of the large proportion of dogs with insulinomas and normal serum insulin concentrations, the ratio of insulin: glucose is often used in diagnosis. In such cases, hypoglycemia must be documented at the time a serum sample is collected for insulin measurement. Simple ratios of insulin: glucose and amended ratios have both been proposed, but neither has particularly good diagnostic accuracy. Diagnosis of insulinoma, therefore, depends on a combination of clinical signs and clinical test results. In a patient in which insulinoma is suspected, abdominal ultrasound often is used to detect a pancreatic nodule consistent with a beta cell tumor. The disease is confirmed by surgical exploration of the pancreas and histopathology of the tumor. Before the advent of insulin assays, the indication for pancreatic surgery in the investigation of hypoglycemia was based on “Whipple’s Triad.” Named for Dr. Allen Whipple, a surgeon at Columbia University who pioneered the field of pancreatic surgery in the 1930s, Whipple’s Triad consists of 1) signs of hypoglycemia, 2) alleviation of clinical signs on exogenous administration of glucose, and even less common in cats. These tumors cause chronic hypoglycemia by secreting insulin unregulated by concentrations of glucose in the extracellular fluid.

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Summary

Clinicians are trained to identify problems and to generate differential diagnosis lists for those problems. As a result, clinicians are often drawn to test results that are flagged as abnormal. Test results that are not flagged are often ignored. In physiological systems in which positive and negative feedback mechanisms maintain homeostasis, normal is a relative term. Clinical examples in which seemingly normal test results should alert the clinician to the possible presence of an endocrine disease have been reviewed herein. It is important to pay attention to all test results, and to consider them not just in relation to a reference interval, but in relation to each other, and in view of the patient as a whole.

References
