

# NWL NEWS

The Newsletter of NationWide Laboratories

January 2007

## Welcome to the NationWide Laboratories quarterly newsletter.

In our first Newsletter, an overview of hyperadrenocorticism (HAC) in dogs was presented with details of the two most common diagnostic tests (ACTH stimulation test and Low dose dexamethasone suppression test) in current use with the pros and cons listed for each one. In this issue we continue with additional endocrine tests that are used in the diagnosis or screening for HAC. When to apply each test is also explained.

## Refresher...

### Hyperadrenocorticism (HAC)

#### Endocrine tests continued

### Urinary corticoid:creatinine ratio (UCCR):

This is best used in the exclusion of the diagnosis of HAC. The reference ratio for normal dogs is  $<10 \times 10^{-6}$ . The ratio is increased in dogs with HAC but also in many dogs with non-adrenal illness and therefore this test has a low specificity but a high sensitivity. Values within the reference range make a diagnosis of HAC highly unlikely. The test cannot differentiate between pituitary dependent (PD-) and adrenal dependent (AD-) HAC unless the ratio is  $>100 \times 10^{-6}$  when it is then very likely that the dog has PD-HAC. It has little value in monitoring medical treatment of HAC.

#### Test principles and method:

Cortisol and its metabolites (corticoids) are excreted in urine. By measuring the urine corticoids in the morning, it reflects cortisol release over several hours

and accounts for fluctuations in plasma cortisol concentrations. Urine corticoid concentration increases with increased plasma cortisol concentration. Any differences in the urine concentration are corrected by relating the urine corticoid concentration to the urine creatinine concentration. This is much simpler and as valuable as performing the 24 hour urinary corticoid excretion test which is highly labour intensive. Urine corticoid concentrations are not always directly reflective of plasma cortisol concentration as they can also be affected by other factors such as hepatic disease, drug administration.

#### Method:

Owner collects urine samples over three days, in the morning. Single samples are not suitable. These can be analysed separately or pooled to make analysis cheaper.

#### Calculate the UCCR:

Urine cortisol concentration (umol/l)/urine creatinine concentration (umol/l).

### Additional tests for Atypical HAC:

Atypical HAC is defined as a case of HAC that has classical clinical signs and changes on haematology and biochemistry but equivocal results on the usual endocrine tests performed in diagnosis – ACTH stimulation test and low dexamethasone suppression

test. Addition tests can be performed if repetition of the standard diagnostic tests still gives equivocal results and the clinical signs persist. Additional tests include:

- 1) **UCCR** ( see above)
- 2) **ACTH stimulation of 17-hydroxyprogesterone (17-OHP):** In atypical HAC there may be some derangement of the steroid production pathway and some of the precursors of cortisol, such as 17-OHP may be increased. Atypical HAC can affect PD and AD-HAC cases.

The method is identical to the standard ACTH stimulation test and 17-OHP can be measured on the same samples.

	Pre ACTH – 17-OHP nmol/l	Post ACTH – 17-OHP nmol/l
Normal	<3.0 – 4.0	1.0 – 8.0 (<10.0)
Classical and atypical HAC	3.0 – 10.0	>8.0 (6.5 – 38)

There is a degree of overlap between normal and affected animals and controversy over the cut off levels. A few normal animals have post ACTH 17-OHP values as high as 17.0 and some with “stressful” diseases can be as high as 38.0.

## Drug Monitoring...

### Anticonvulsants

### Phenobarbitone:

#### Introduction:

This is the drug of choice in the treatment of canine primary epilepsy. It is very cheap and has a proven efficacy. Its exact mechanism of action is unknown but some effects are mediated mainly via the gamma-aminobutyric acid (GABA) membrane receptor. Thus the net result is hyperpolarisation of the neuronal cell membrane which renders the cell more resistant to the effects of excitatory neurotransmitters.

#### Pharmacokinetic profile:

high oral bioavailability (86-96%) and long elimination  $T_{1/2}$  that ranges between 42-89 hours. After oral

administration, rapid absorption occurs within two hours and peak serum concentration occurs at 4-8 hours. As a potent inducer of hepatic enzymes it speeds up its own rate of clearance over time necessitating often an increase in dose.

#### Side effects: transient:

lethargy, sedation, polydipsia, polyphagia, behavioural. These usually resolve 1-2 weeks after initiation of treatment.

#### More serious side effects:

- 1) **hepatotoxicity:** This is not easy to diagnose since the result of treatment increases AP in almost all cases, but in cases of hepatotoxicity 83% showed concurrent increase in ALT that was disproportionately high when compared with the increase in AP. 67% were hypoalbuminaemia and 44% had raised fasting bile acids (Daryl-Hart et al. 1991).
- 2) Occasionally idiosyncratic bone marrow reaction with neutropenia, thrombocytopenia and anaemia. May or may not reverse on cessation of treatment.

#### Monitoring:

This should be started after 2-3 weeks of treatment when a steady state of the phenobarbitone has been reached in the serum.

#### Single or paired samples?:

Single samples should be trough concentrations and are beneficial particularly if underdosing or overdosing may be expected. However with the  $T_{1/2}$  of phenobarbitone the time the sample is taken does not often matter and there is frequently only a small difference between peak and trough values.

Paired samples are beneficial in that the  $T_{1/2}$  in a particular individual can be calculated. This may be of use in cases where the apparent serum levels are within therapeutic range but there is ongoing seizure activity.

#### Therapeutic range:

15-40 ug/ml (Farnbach 1984). However better seizure control can be obtained if serum levels are maintained between 20-40 ug/ml. Dayrell-Hart et al. (1991) reported that serum concentration of phenobarbitone above 35 ug/ml had the highest correlation with hepatic dysfunction.

In combination with Potassium bromide (KBr) – similar serum levels required but 25 ug/ml for phenobarbitone and 150-200 mg/dl for KBr (Podell 1996, 1998).

#### Cats:

Therapeutic range: 10-30 ug/ml. Dosing is highly individualised. Can be used with KBr but there is individual variation as to tolerance of its use (Podell 1998).

# Allergy Testing...

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## Important considerations prior to serological testing:

- The age of patients at initial testing.
- The effects of recent or ongoing antipruritic therapy on serum IgE levels.

### Age:

Requests for the UK environmental screening tests or full environmental panels are not unusual for animals (usually dogs) less than 12 months of age. Our advice is to proceed with caution. Atopy is recognised in dogs less than a year old however, the sensitivity of serological tests may be reduced if these are carried out on young dogs, not least because these animals have not experienced a full calendar year of allergens. A screening test for juveniles may help to establish a diagnosis of atopy but we strongly recommend retesting when animals are more than 12 months old if unexpected negative results are obtained or for cases where immunotherapy is a consideration.

### Corticosteroids:

Many animals suspected of suffering from atopy or other hypersensitivity disorders rely from time to time on tailored courses of antipruritics, to alleviate severe clinical signs and improve "quality of life". Few thorough clinical trials have been conducted to evaluate the effects of antipruritic therapy on serum IgE levels. Short courses (three to six weeks) of oral corticosteroids at antipruritic doses did not significantly affect serum IgE levels in a study conducted by Miller, Scott et al (1992). **We recommend that, prior to serological testing; short term anti-inflammatory doses of oral corticosteroids (< 0.5 mg/kg) should be the goal.** The effects of long-term or injectable corticosteroids, especially depot steroid preparations, on serological testing have not been sufficiently evaluated.

### Antihistamines:

The pharmacological efficacy of antihistamines results from their blocking the effects of histamine, one of the potent inflammatory mediators released following mast cell degranulation during the type I hypersensitivity reactions. This class of antipruritics have no known effect on serum IgE levels. Immunomodulating drugs: Limited information is available regarding the effects of cyclosporin A and other immunomodulating drugs such as tacrolimus on serological testing. The suggested mode of action of these drugs is via inhibition of cytokine gene expression in T lymphocytes, thereby blocking the production and secretion of interleukins, and other cytokines involved in initiating inflammation following mast cell activation. A study conducted by Clarke et al (2002) on the effects of therapeutic doses of cyclosporin A on concentrations of flea allergen specific IgE found no significant differences at 5 and 8

weeks in treated dogs when compared to untreated controls. It seems likely that cyclosporin A therapy does not need to be suspended prior to serological testing, or could be stopped for just a few days (5-7 days) to rule out the possibility of any interference.

## References:

### Phenobarbitone:

- Dayrell-Hart B, Steinberg S, Van Winkle T, Farnbach G (1991) Hepatotoxicity of Phenobarbital in dogs: 18 cases (1985-1989) JAVMA 199, 1060-1066.
- Farnbach GC (1984) Serum concentrations and efficacy of phenytoin, phenobarbitone and primidone in canine epilepsy JAVMA 184, 1117.
- Podell M (1996) Seizures in dogs. Vet Clinics of N. Am.: Small animal practice 26 (4) 779-809.
- Podell M (1998) Antiepileptic drug monitoring. Clin. Tech Small An. Pract. 13 (3), 185-192.

### Allergy:

- Miller, WH et al: The influence of oral corticosteroids or declining allergen exposure on serologic allergy test results. Veterinary dermatology, Vol. 3, No. 6. 237-244, 1192.
- Clarke, K et al: The effects of cyclosporine A and oral prednisolone on flea allergen specific IgE and intradermal tests in experimentally sensitized laboratory beagles. Free communication.



## Answers to Case Question 1...

### CLINICAL DETAILS:

**Case 1** - 15 year old DSH MN cat. History: Progressive weight loss, polyphagia, tachycardia (>240 bpm).

### Diagnostic tests:

Haematology	within normal limits
Biochemistry	<b>ALP 126 iu/l (0-55)</b> <b>ALT 112 iu/l (30-60)</b> Bilirubin 1.0 umol/l (0-4) <b>Total protein 83 g/l (55-78)</b> Albumin 34 g/l (26-40) <b>Globulin 49 g/l (19-48)</b> Alb: Glob ratio 0.69: 1 (0.53-1.36) <b>Urea 9.8 mmol/l (3.5-8.0)</b> Creatinine 102 mmol/l (40-180) Calcium 2.39 mmol/l (2-2.8) <b>Phosphorus 1.7 mmol/l (0.81-1.61 &gt; 2 years)</b> Glucose 4.2 mmol/l (4.3 - 6.6 Fasting) <b>Sodium 160 mmol/l (141-155)</b> Potassium 4.5 mmol/l (3.5 - 5.5)

### Differential diagnoses:

Raised liver enzymes	Hyperthyroidism, hepatic pathology (inflammatory, neoplastic)
Raised urea, normal creatinine	Probable prerenal increase e.g. reduced renal perfusion
Hyperphosphataemia	Sample quality (delayed separation, haemolysis), hyperthyroidism, renal disease (see above)

### Notes for interpretation:

Test	Origin	Increases: common causes
AP	Hepatocytes and bone; short half-life for liver isoenzyme in the cat (<8hr)	Hyperthyroidism*, diabetes mellitus and hepatic pathology
ALT	Mainly from hepatocytes	Endocrine disease* and hepatic pathology (inflammatory, neoplastic, toxic, traumatic)
Globulins	Synthesised by the liver (except immunoglobulins)	Dehydration, inflammation, neoplasia (lymphoma, myeloma)
Urea	Synthesised in liver; renal excretion	Prerenal (hypovolaemia, cardiac insufficiency, intestinal haemorrhage), renal or post renal (obstruction, trauma)
Phosphorus	Concentration determined by renal clearance, intestinal absorption and age	Decreased GFR, urinary trauma, hyperthyroidism, hypoparathyroidism, spurious (haemolysis, delayed sample separation)
Sodium	Interpreted in the light of total body water (hypo-, normo- or hypervolaemia)	Dehydration, restricted H <sub>2</sub> O intake, salt poisoning, hyperaldosteronism

\*Concurrent hepatic pathology should be considered in hyperthyroid cats if mild or modest increases in T<sub>4</sub> concentrations are accompanied by marked increases in liver enzymes.

### What further tests would be advised?

- Total T<sub>4</sub>
- A proportion of hyperthyroid cats have normal T<sub>4</sub> levels (>30nmol/l) particularly when there is significant concurrent non-thyroidal illness. In these cases FT<sub>4</sub> by equilibrium dialysis is useful for further assessment.
- Assessment of hydration status and urine SG

## DIAGNOSIS

**Hyperthyroidism confirmed with a T<sub>4</sub> of 86**

## Case Question...

### CLINICAL DETAILS:

**Case 2** - 8 year old male neutered Yorkshire Terrier presents with polydipsia, polyuria, thinning of the hoar coat, pot bellied appearance and lethargy.

### Diagnostic tests:

- Haemogram - Neutrophilia, lymphopenia, monocytosis
- Biochemistry - Increased AP, ALT, cholesterol, glucose
- Urinalysis - USG 1.013, +protein, +blood, WBCs 0-5, RBCs 0-5

### Differentials:

HAC was top of the list but other differentials included D. Mellitus, primary hepatopathy.

### Further Diagnostic tests:

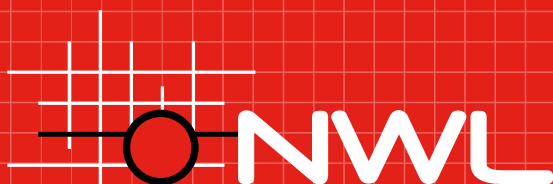
Low dose dexamethasone suppression test - results:

Endocrinology	Result	Reference interval
Cortisol basal (nmol/l)	123	28-250
Cortisol 3 hour post dex. (nmol/l)	40	<40
Cortisol 8 hour post dex. (nmol/l)	89	<40

### QUESTIONS:

How do you interpret these results?  
Is this the test you would have chosen if HAC was suspected?  
Are further test required?

Answer: next issue



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## Coming up next issue...

- HAC in the cat
- Drug monitoring: KBr
- Infectious diseases
- Answers to case quiz