HERP BIOCHEMISTRY RESULTS – WHAT DO THEY MEAN AND WHY DO I NEED THEM

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It should always be remembered that the results obtained from a sample will always be affected by the quality of that sample. The method of collection, storage, shipping and analysis must always be of the highest in order to get the best results. Blood samples are usually collected into heparin, as EDTA can cause haemolysis of red blood cells of most reptiles.

BLOOD VOLUME

The total amount of blood in a reptile varies from 5-8% of body weight (50-80mls/Kg) and 10% can safely be removed (5-8ml/Kg) although this does depend upon the health status of the animal. Sites that can be sampled include the jugular, brachial vein, ventral/dorsal coccygeal vein (sinus) orbital sinus and trimmed toenails. Other sites include the heart (snakes) and supravertebral sinus.

Care must be taken not to dilute the sample with lymph.

ENZYMOLOGY & BASIS OF BIOCHEMISTRY

INTERPRETATION

The measurement of Serum Alkaline Phosphatase as a diagnostic aid was introduced in 1927 (by King and Armstrong) and based on the success of this basic diagnostic tool, clinical enzyomology has gone from strength to strength. However, it is important to remember that there are many factors that will influence the results other than the patient and how the sample is taken and handled. (The same is true for the other analytes that are measured.)

1) The kinetics of the method used to measure the enzymes.
2) the isoenzyme being measured (eg LDH has 5 isoenzymes).
3) temperature effects, all enzymes have an optimal temperature range.
4) the presence of lipoaemia. Ph effects, as all enzymes have an optimal Ph range.

No single parameter should ever be thought of as diagnostic by itself. The aim is to be able to get a screen of a number of parameters which then by consideration of their values and comparison of groups; a suspected diagnosis should be reached. It may be necessary to repeat certain parameters over a period of time.

Interpretation of results using “ranges of normal values.” Although in the literature there appears to be many incidences of normal ranges for reptiles being published, indeed normal ranges are also included here, they are not highly specific as they are not quoted for each of the 7500 odd species but used as a catchall for reptiles (or an order). For domesticated mammals (dog, cat, horse etc) this would be considered as totally unacceptable but yet we insist on doing this for reptiles and to a lesser extent for birds. It has been suggested that it may be more beneficial in reptiles to monitor the changes of biochemical parameters in an individual than to rely on absolute values.

UREA

Urea does not indicate renal function in reptiles. The production of urea is low and variable. Normal range 0-16.7mmol/l (0-46mg/dl). High levels may be indicative of dehydration.

CALCIUM & PHOSPHORUS

Calcium blood levels are dependent upon renal function, dietary calcium, hormonal activity (especially reproductive, Calcitonin, Parathyroid, Vitamin D₃ ). Calcium can be either protein bound or non-protein bound, of the non-bound calcium, ionised (active calcium) accounts for about 40-50%. The protein bound calcium will be affected by the total plasma proteins (albumin) and if this is reduced, then the measured bound calcium will also be reduced. In mammals there is a formula to work out the adjusted calcium by using the measured calcium and subtracting the albumin value plus adding a correction constant (3.5). In reptiles, such a calculation has not been verified and the correction constant has not been worked out.

In ovulating females the higher levels of circulating egg proteins will also increase the measured bound calcium levels, to a falsely elevated level. Ionised calcium levels are a better indication of the true calcium activity.

Calcium & Phosphorus ratios are considered very important in reptile medicine. Elevation of the calcium but decrease of the phosphorus may indicate a primary hyperparathyroidism; while an elevation of phosphorus and decrease in calcium (reverse Ca:P) is suggestive of renal disease; whereas if there is not a reverse ratio but still a reduced calcium and elevated phosphorus then either nutritional secondary hyperparathyroidism or renal secondary hyperparathyroidism is suspected. However, with both nutritional secondary hyperparathyroidism or renal secondary hyperparathyroidism, it is also possible that the calcium may be normal or even elevated.

Phosphorus elevation can be associated with renal function, diet, and/or anorexia.
PROTEIN
Protein is an indication of the health of the reptile and if it is feeding. Normal range of 32-66 g/L (3.2-6.6g/dl). Protein, if low, may indicate malnutrition, malabsorption, maldigestion, intestinal parasites, protein loosing enteropathy, blood loss, hepatic disease, or renal disease. High protein levels may indicate dehydration, or inflammatory disease.
Albumin-normal range 10-35 g/L (1-3.5g/dl). This will fall if there is prolonged anorexia, protein loosing enteropathies, some neuropathies or chronic liver disease. It will become elevated when dehydration occurs, providing it was depressed before that.

GLUCOSE
Glucose is an energy analyte and is particularly important in the immediate post hibernation phase. Normal range 2.5-5.5 mmol/l (45-99mg/dl). Glucose when low may indicate malnutrition, post hibernation anorexia, hepatopathies, septacemia, and endocrinopathies. High levels can indicate a short period of fast or a recent meal.

LIVER ENZYMES
AST (Aspartate aminotransferase) 5-130 U/L (5-130IU/L) is considered a normal range. Elevation suggests tissue damage which may include muscles, liver, heart. Interpretation really requires assessment of the CK, LDH values as well. This parameter can also sometimes be elevated in cases of renal disease.
CK values are indicative of muscle damage and are used for trying to show that the elevation of the AST/ LDH and GGT are not solely associated with muscle damage.
LDH is also indicative of muscle damage and liver damage.
GGT (gamma glutamyltransferase) is thought to be more liver specific.

ELECTROLYTES
Sodium if high indicates dehydration.
Potassium will become high if there is severe acidosis whereas with diarrhoea, it may becomes low.
Chloride will be high if there is dehydration or renal disease.

LIPIDS
Assessment of Lipids is important as abnormalities can lead to artherosclerosis, hepatic lipodosis and hypothyroidism. Accumulation of fat in the liver will lead to a Fatty Liver Syndrome. However, fatty livers can also be associated with egg laying. Here cholesterol will be elevated or the blood sample is found to be lipaemic. Cholesterol deposits will depend with species and the diet.

BILE ACIDS
Bile acids are synthesised from cholesterol and the cholesterol esters. Bile acid concentration is a reflection of the liver's ability to clear the bile salts. The bile acid levels suggest the function of the livers ability to perform extraction, conjugation and excretion of bile acids. It is thus an indication of the total function of the liver, it is liver specific.
In the fasted state there is still an enterohepatic cycle and thus, even an unhealthy liver will eventually extract the bile acids from the blood over a period of time which could give the appearance of "normal" bile acid levels. Normal range is up to 60 umol/l. However the bile acids do different between various groups.
Reptiles (as well as birds and amphibians) lack bilirubin reductase and therefore biliverdin is the main end product of haem catabolism. As biliverdin is excreted into urine it will not build up in the body and cause jaundice like that seen in mammals were bilirubin is the main end product of haem catabolism.

FURTHER READING