BIOCHEMISTRIES: WHAT THEY DO AND DON'T DO

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INTRODUCTION

One of the most striking developments in avian medicine over the last two decades has been the use of biochemistry analysis to detect disease and to monitor a patient’s response to treatment. It has lifted avian medicine from “some antibiotics in the drinking water” to the sophisticated field it is today. Biochemistry analysis allows a clinician to focus his/her diagnostic efforts to achieve an accurate diagnosis, a more appropriate treatment, and then follow a case through to a successful conclusion.

But biochemistry analysis is a tool; like any other tool it can be used or misused. This paper outlines the principles of biochemistry analysis, and then takes the clinician through its use in diagnosing disease of each organ system. The advantages, disadvantages and pitfalls of each biochemistry will be outlined, and biochemistries of limited or no use to the avian clinician will be identified.

PRINCIPLES OF BIOCHEMISTRY ANALYSIS

Clinical biochemistry involves the measurement of specific groups of chemicals within the body, and the interpretation of the results obtained. These chemicals include:

a. Metabolites - those chemicals that are produced as the end-products of various metabolic processes within the body;
b. Tissue enzymes, which catalyse chemical reactions within the body without being altered themselves;
c. Electrolytes including sodium, potassium and chloride;
d. Minerals such as calcium, phosphorous and magnesium; and
e. Bile acids, produced in the liver from cholesterol and used in the emulsification of dietary fats.

The levels of these chemicals in the blood can be influenced by either physiological or pathological processes. Physiological variations can be due to age, sex, body fat to muscle ratio, nutritional status, reproductive status and species. However, pathological processes, including cellular damage or abnormal function of an organ system (or systems), often produce significant changes in blood levels.

These changes in blood levels enable the clinician to detect illness when it is not readily detectable by a physical examination. Because birds are so limited in their ability to express clinical signs of illness and because their anatomy often precludes a comprehensive physical examination comparable to that which can be done on a mammal, hematlogy and biochemistry analysis become vital tools in reaching a diagnosis.

However, there is sometimes a trend to utilize biochemistry tests instead of, rather than as well as, a detailed history and thorough physical examination. When added to a lack of understanding of the differences between avian and mammalian physiology (and therefore differences in significant biochemistries), the result can be inappropriate testing and incorrect diagnoses.

Clinicians treating birds therefore need to be aware of just what biochemistry can do – and can’t do – for them. A sound understanding of avian biochemistry and its use in practice will provide the clinician with an invaluable tool for diagnosing disease and monitoring response to treatment.

There are three major causes of abnormal clinical biochemistries:

a. normal variation between species and individuals;
b. artifacts; and

NORMAL VARIATION BETWEEN SPECIES AND INDIVIDUALS

There are over 9,000 species of birds, with major differences in anatomy, physiology, form and function. Some are carnivorous, some are nectivorous, some are granivorous, some are omnivorous. To expect that they would all conform to a relatively narrow range of biochemical ‘normals’ is optimistic, to put it mildly! Some of these differences include:

a. raptors have a normal uric acid approximately double that of psittacines;
b. raptors are also able to maintain normal levels of glucose in the face of starvation much longer than other species, because of their different rate of gluconeogenesis; and
c. ratites, perhaps because they walk rather than fly, have much higher normal levels of Creatine Kinase (CK) than most other species;

Many other species differences exist, and the clinician is well advised to – wherever possible – utilise established normal ranges for the species they are dealing with.

Other variations arise between individuals of the same species. These variations occur because of differences in age, sex, diet, husbandry, etc. For this reason some clinicians recommend establishing a set of normal values for individual birds during annual health examinations, and then using these values as a comparison should the bird become ill.

ARTIFACTS

When interpreting a biochemistry analysis, care must be taken to distinguish between abnormal results due to disease, and abnormal results due to other factors.
These other factors, referred to here as artifacts, can occur for a variety of reasons, including:

- a. physiological changes
- b. previous therapy
- c. the clinical condition of the patient
- d. the collection method
- e. storage and transport of the sample

**Physiological Changes**

Stress due to transport and handling of the patient can lead to a release of endogenous corticosteroids, resulting in changes in the hemogram and in blood glucose.

Lipemia, while occasionally seen in diseases of the liver and reproductive system, can also occur naturally in the reproductively active female. Regardless of the cause, lipemia can cause false elevations in bile acids, protein, calcium, phosphorous, and uric acid. It may also falsely decrease amylase. Post-prandial lipemia is uncommon in pet birds, so fasting will not help; the clinician needs to check with the laboratory if the sample submitted was lipemic before interpreting these biochemistries.

**Previous Therapy**

Before interpreting biochemistries, the clinician should consider if any treatment given prior to the sample collection could have had an effect on the results. Therapy given by another veterinarian or in an attempt to stabilize a crashing patient can have marked effects. Parenteral fluids can dilute biochemistries; exogenous corticosteroids can markedly elevate AST, CK and LDH; intramuscular injections, particularly of irritant drugs, can do the same.

**The Clinical Condition of the Patient**

Trauma, starvation and dehydration can all have marked effects on biochemistries, and need to be considered when interpreting results. Trauma can cause elevations in AST and CK, and possibly glucose; Starvation can lower glucose, and also elevate AST and CK if protein catabolism has begun; dehydration can elevate uric acid.

**The Collection Method**

Ideally sample collection should be performed in such a manner that it has minimal impact on the patient while providing an artifact-free sample suitable for analysis. This usually requires venipuncture performed on a minimally stressed patient. Inexperienced clinicians may need to consider gaseous anaesthesia in order to collect a good sample without the bird struggling.

Venipuncture should be performed on a large vein eg the jugular, using a needle that is large enough to minimise hemolysis while been small enough to minimise trauma to the blood vessel wall. Hemolysis can cause elevations in bile acids, LDH, CK, ALP, potassium, and phosphorous. Glucose and albumin may be decreased. Calcium may be elevated or decreased, according to the methodology used.

Toenail clipping should be discouraged. Not only is it unduly painful, but crush artifacts and contamination with uric acid and bacteria from droppings on the perch can cause elevations in uric acid and decreased glucose if testing is delayed long enough for bacterial growth to occur in the sample.

**Storage and Transport of the Sample**

Plasma is collected from centrifuged, anti-coagulated blood before clotting occurs; therefore it contains clotting factors and an anti-coagulant. Serum, on the other hand, is collected after clotting has occurred - therefore it has no clotting factors, but no anticoagulant. Because serum is in contact with hemolyzing red blood cells for a longer time than plasma, there are more artifactual changes. Serum is lower in total protein, albumin, and perhaps calcium; it is higher in CK, calcium, magnesium, and phosphorous. It is therefore recommended that plasma be used instead of serum.

Blood collected for biochemistry analysis should be placed immediately into a lithium heparin tube. Ideally, miniature tubes as used in medical pediatrics should be used. The sample should be gently rolled or rocked – clotting must be avoided, but hemolysis must be as well. If the analysis is to be performed in-house, it should be processed immediately. If a delay is likely, or if the sample is to be shipped to an outside laboratory, the sample should be centrifuged and the plasma harvested. Sending whole blood to an outside blood can result in decreased glucose (as cell metabolism continues) and hemolysis.

EDTA tubes are unsuitable for biochemistry analysis, but can be used for hematology, lead analysis and fibrinogen determination.

**BIOCHEMISTRY ANALYSIS BY ORGAN SYSTEM**

Although no part of the body can be evaluated separately from the rest of the body, it is sometimes convenient to discuss clinical signs, laboratory testing and treatment by organ system; this paper continues that modus operandi. However, it needs to be emphasised that the patient as a whole needs to be considered, and that no one organ system can be evaluated without reference to the rest of the body.

**The Liver**

The detection of liver disease through biochemistry is complicated by the fact that there are no specific 'liver enzymes' that can be evaluated conclusively in each and every case. Liver disease can be broadly classified into three conditions: hepatocellular rupture; decreased hepatic function; and cholestasis. These conditions can occur either separately or concurrently.

Hepatocellular rupture releases intracellular enzymes which then reach elevated levels in the blood. These so-called 'leakage enzymes' include:

- a. Aspartate Aminotransferase (AST) – formerly known as Serum Glutamic-Oxaloacetic Transaminase (SGOT). This cytosolic enzyme is found in many tissues in the body, but the highest
concentrations are found in skeletal muscle and liver. Significant elevations usually represent either muscular or hepatocellular damage. AST therefore must be interpreted alongside Creatine Kinase (CK – released from damaged muscle) to distinguish between the two. In general, an elevated AST with a normal CK indicates hepatocellular rupture. However, CK has a much shorter half-life than AST; a single-point muscle injury (eg an injection) 4-7 hours before sample collection could duplicate this biochemistry pattern. Although AST is considered to be the most useful liver enzyme, it cannot be considered in isolation as an indicator of liver disease.

b. Glutamate Dehydrogenase (GLDH), a mitochondrial enzyme, is the most specific enzyme for the detection of liver disease, but its sensitivity is low – because it is bound to mitochondria, extensive and severe liver damage is required before elevations are detectable.

c. Lactate Dehydrogenase (LDH) is not specific to any tissue; its main advantage lies with a half-life shorter than CK – persistent elevations in the presence of normal CK is strongly suggestive of liver disease.

d. Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) are not considered useful in detecting liver disease in birds. ALT in birds is very non-specific for the liver, and normal levels have been shown in cases with severe liver damage. ALP elevations are more commonly associated with bone disease in birds.

Decreased liver function can occur with any number of liver diseases – not all of which involve hepatocellular rupture. Chronic cirrhosis, amyloidosis and hepatic lipidosis can all be having an adverse effect on liver function without causing any cellular damage. In these cases a “liver function test” is necessary to detect the problem. Bile acids serve this purpose well. Produced in the liver, they are excreted in bile into the small intestine where they act to emulsify fat. Most of the bile acids are then resorbed in the small intestine, enter the portal system and are taken up by the liver to be recycled. Elevated levels occur when there is impairment of the liver’s ability to remove bile acids from the portal circulation. A 2-4-fold increase in bile acids indicates a significant decrease in liver function. It needs to be noted though that a severely dysfunctional liver (eg end-stage cirrhosis) may not be able to produce normal levels of bile acids, leading to low to normal results. Total protein, especially albumin, may also be decreased with decreased liver function.

Cholestasis occurs when the biliary system is partially or totally obstructed. This can be seen with biliary neoplasia, pancreatic disease, or diffuse swelling of the entire liver. Gamma Glutamyl Transferase (GGT) is an enzyme found in the cell membranes of the bile ducts. Elevations can be seen in cholestatic disease (eg bile duct carcinoma) but it is considered to be a relatively insensitive test for liver disease in psittacines. Bilirubin is not produced in birds – they utilize biliverdin instead. There are no commercial assays for biliverdin.

Interpreting patterns of elevations of these liver enzymes can be an art form. It should be obvious by now that a detailed history and a thorough physical examination is necessary to ensure the clinician is aware of events that could change the levels of some enzymes. Has the bird lost a lot of weight quickly? Was it difficult to catch and restrain? Has it had a recent injection? Has it been subjected to any physical trauma, including feather picking? Knowledge or detection of these and other events is essential to correctly interpret a ‘liver panel’ (Table 1).

Table 1. Avian Liver Panel

<table>
<thead>
<tr>
<th>AST</th>
<th>CK</th>
<th>GLDH</th>
<th>LDH</th>
<th>Bile Acids</th>
<th>GGT</th>
<th>Possible Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>Liver disease is unlikely – most likely this reflects muscle damage</td>
</tr>
<tr>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++ -</td>
<td>-</td>
<td>Liver disease</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>Decreased liver function</td>
</tr>
<tr>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++ -</td>
<td>-</td>
<td>Severe liver disease</td>
</tr>
<tr>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Possible liver disease, or single point muscle damage 4-7 hours previously</td>
</tr>
<tr>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>Muscle damage</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>Hemolysis of sample</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++ -</td>
<td>-</td>
<td>Decreased liver function without cellular damage</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal liver?</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>Cholestasis – bile duct carcinoma?</td>
</tr>
</tbody>
</table>

Interpretation of possible patterns in liver biochemistry panels (+ = elevated levels; - = normal levels)
The Kidney
The end product of protein metabolism in birds is uric acid. It is produced in the liver, enters the circulation, and is then secreted by renal tubules (>90%) or filtered in the glomerulus (< 10%). Significant loss of renal tubules will therefore see elevations of uric acid. Dehydration is less likely to cause hyperuricemia because glomerular filtration is relatively unimportant.

At first glance it would appear that uric acid offers a sensitive and specific test for renal disease. There are, however, several confounding factors. Firstly, species differences; carnivorous birds have higher normal uric acid levels than granivorous birds. Secondly, age; juvenile birds may have lower levels than adults. Thirdly, although significant elevations usually indicate renal disease, normal levels do not mean the kidneys are normal; mild increases could indicate early renal disease or dehydration (or both). There must be severe renal damage before uric acid levels begin to rise.

Because of this relative insensitivity of uric acid in detecting renal disease, levels are best interpreted alongside a determination of the bird’s water intake and loss, and a physical examination. To distinguish renal disease from dehydration, the patient’s hematocrit, total protein and blood urea nitrogen (BUN) should be evaluated concurrently. Dehydration can lead to decreased glomerular filtration rates (GFR), in turn leading to elevated levels of BUN; this same decrease in GFR can lead to elevations of uric acid without primary renal disease being present. It is therefore prudent, in cases of an elevated uric acid level, to rehydrate the patient over 2-3 days before definitively diagnosing renal disease. Persistent hyperuricemia after fluid therapy, and with hematocrit, total protein and BUN returning to normal, confirms a diagnosis of renal disease.

Creatinine is generally accepted as being of little or no value in evaluating renal function in birds. Phosphorous elevations are usually not seen in birds with renal disease.

The Reproductive System
Clinical biochemistries can tell the clinician little about the male reproductive tract; they can however, reveal something about the activity of the female reproductive tract. Estrogen, produced by developing follicles, induces the production of calcium binding protein and vitellogenesis in the liver. The serum may appear lipemic. Radiographic evidence of hepatomegaly and increased long bone density can confirm reproductive activity. It should be noted though, that normal calcium and protein does not reflect a lack of reproductive activity.

The Gastrointestinal System
Gastrointestinal disease typically only gives non-specific results with clinical biochemistry. Elevations of CK, AST and LDH are not uncommon, and are not specific to the intestinal tract. Electrolytes may give more information: Sodium may be elevated with excessive water loss through vomiting or diarrhoea; Chloride may be elevated with vomiting or regurgitation; and Potassium may be decreased with vomiting/diarrhoea and elevated with dehydration. There are many other possible causes of electrolyte disturbance, and our understanding of avian electrolyte balance is still in the very early stages.

Amylase and lipase have been proposed as useful parameters in the detection of pancreatic disease. There is still considerable discussion of the incidence of pancreatic disease and the specificity of these enzymes. Significant elevations of these enzymes, when accompanied by clinical signs of gastrointestinal dysfunction (vomiting, ileus, diarrhoea, abdominal pain) should lead the clinician to consider pancreatic disease as a differential diagnosis. However, normal levels do not preclude a diagnosis of pancreatic disease, nor do abnormal levels confirm such a diagnosis.

Blood Glucose
Glucose is an essential energy source for nearly every cell in the body. Blood levels are governed by its intake, absorption, the interactions of hormones controlling carbohydrate metabolism (insulin, glucagon, and somatostatin), the body’s metabolism, its ability to store glucose, and its excretion. As disorders of glucose metabolism involve so many organ systems, it is treated here as a separate entity.

Hyperglycemia (>500mg/dL / 27.5 mmol/L) may be a normal physiological process eg in juvenile birds. However, elevated levels are usually related to increased production or release (eg stress) or failure of tissues to take it up out of the blood (diabetes mellitus). Iatrogenic hyperglycemia occurs when corticosteroids are administered, or intravenous dextrose is given. Female reproductive disease may also elevate blood glucose, but this may be an indirect result due to inflammation affecting the endocrine pancreas.

Hypoglycemia (< 150mg/dL / 8.25 mmol/L ) may result from poor handling of blood samples ie it may be artifactual, rather than factual. However, it is seen in cases with decreased intake (starvation, anorexia), increased usage (septicemia, neoplasia, multi-organ failure) or decreased production (liver disease).

CONCLUSION
Clinical biochemistry is a valuable diagnostic tool in avian medicine. But, like all diagnostic tools, it has its limitations. Clinicians need to be aware of the range of possible reasons for abnormal results, and not just assume that disease is the only cause. They also need to be aware that biochemistry is only an aid to a diagnosis, and that a detailed history and physical exam are essential elements in reaching an accurate diagnosis.

Recommended Reading