IMPORTANT CLINICAL ASPECTS OF AMPHIBIAN PHYSIOLOGY

Kevin Wright, DVM
University Animal Hospital
Mesa, AZ

This talk covers aspects of amphibian anatomy and physiology with immediate relevance to the veterinarian dealing with amphibians.

THERMAL HOMEOSTASIS

Amphibians are poikilothermic ectotherms. Their core body temperatures are determined by ambient environmental temperatures, and an amphibian thermoregulates by moving through the mosaic of environmental temperatures to find those appropriate for its current physiological needs. The preferred operating temperature zones (POTZ) vary tremendously between species, between geographic variants of the same species, and even between different physiological states of an individual (eg, full stomach, empty stomach, fighting infection) and its larval and adult life stages.

If an amphibian cannot maintain its body temperatures appropriately it will fail to thrive. Captive amphibians maintained at temperatures below their POTZs typically show inappetence, lethargy, abdominal bloating from decomposition of ingesta, poor growth rate, and infections associated with immunosuppression. If captive amphibians are maintained at temperatures above their POTZs, they show signs such as agitation, excessive movement, changes in skin color, inappetence, weight loss despite good appetite, seizures, infections associated with immunosuppression, and sudden death. An appropriately constructed vivarium should provide a mosaic of temperatures so that the inhabitants can thermoregulate. (The thermal mosaic should be integrated with other aspects of the environment, such as moisture, illumination, substrate, so that the amphibian is not forced to satisfy one physiological need at the expense of another.)

Amphibians will often use behavioral fever when fighting infection, moving to stay within temperatures at or exceeding the upper limit of their POTZs for longer periods of time than healthy amphibians would. Fever's potential impact on disease in amphibians must be accommodated in the treatment plan. Recent work with chytridiomycosis has demonstrated that fever alone can successfully eliminate this fatal fungal infection. Providing supplemental heat sources is recommended for the treatment of many infectious diseases.

Conversely, sometimes cooling is an appropriate choice for an amphibian showing signs of infectious disease. Since bacteria have a growth curve that is temperature-dependent, the doubling time of a pathogenic bacterium in an amphibian patient may be significantly delayed by cooling the amphibian. This inhibits the spread of bacteria while allowing antibiotics to disperse through the amphibian. The goal of treatment is for the antibiotic to reach tissue levels that achieve or exceed MIC for the pathogen before the pathogen overcomes the patient. Caution must be exercised before adding cooling to an amphibian's treatment plan since some pathogens, such as saprolegniasis, actually cause more significant lesions at cooler temperatures.

WATER HOMEOSTASIS

The two conditions commonly encountered consist of dehydration and fluid overload (ie, hydrocoelom). Dehydration is quickly recognized because the moist areas of the skin feel tacky rather than slick. The eyes may be sunken and the colors dark, and the "abdomen" may be slightly withdrawn. Amphibians lack a diaphragm so there is no separate division between the thorax and the abdomen; their organs sit in a single cavity called the coelom. Fluid overload generally results in distention of the coelom due to accumulation of a fluid with low specific gravity and protein, a condition called hydrocoelom. If there is also fluid in the subcutaneous space, a rare condition in amphibians, it is called anasarca or hydrops.

The majority of amphibians obtain very little water through drinking (ie, oral ingestion of water and subsequent absorption through the gastrointestinal tract). The majority of water uptake is through their skin. Several features facilitate water uptake through the skin: the epidermis is only a few layers thick and has few barriers to evaporative water loss; there is a highly vascularized dermis; the surface of the amphibian is coated with a slime layer that is hygroscopic; and there is a high surface area to volume ratio in many amphibians so water (and oxygen and other compounds) can diffuse across the skin in sufficient quantities to support metabolic activity. The ventral surface is an important route for the uptake of water from the environment, and in some frogs and toads there is a modified area of the ventral pelvic area as a "drink patch." The drink patch accounts for up to 80% of the water uptake of a frog or toad. Some amphibians that have a modified skin to prevent water loss, such as the waxy tree frog, do rely on drinking for a large portion of their metabolic needs. Because the skin is the site of water uptake, dehydration should be treated using baths of shallow water (or other rehydration fluids) or via intracoelomic injections rather than oral administration of fluids. Only in a few species, such as the waxy tree frog, will oral administration have much impact.

The amphibian kidney cannot concentrate urine above the osmolality of plasma. One of the main methods of water conservation is the ability to tolerate a wide fluctuation in the osmolality and composition of its plasma. This physiological adaptation can confound the interpretation of plasma biochemistry from a single point in time, or a single parameter without assessing the rest of the biochemistries (enough to assess plasma osmolality). The kidney does not have a loop of Henle, so loop diuretics have no impact on amphibians. Many veterinarians contact me after having tried furosemide to relieve hydrocoelom (ascites). It does not work because the part of the kidney this impacts is not present in amphibians!
Aquatic amphibians are under a different water flux than terrestrial species as they are immersed in a hypotonic environment. Aquatic amphibians are adapted to excreting excess water while conserving plasma solutes. Water is continually being absorbed through the skins and gills, if present. If the excretory function of the kidneys fails, or the cutaneous exchange functions fail, the plasma will rapidly dilute with absorbed water while ions and other solutes are lost. This is problematic for it is difficult to initiate any corrective action (ie, re-establishing "normal" plasma osmolality via parenteral dextrose/electrolyte solutions or colloid solutions) that does not exacerbate the problem. Volume overload through expansion of the blood (plasma) volume eventually places undue stress on the heart and quickly incapacitates the amphibian.

Aquatic amphibians generally excrete ammonia as their main nitrogenous waste and this is excreted not only through the kidney but also by the skin (and gills if present). Since ammonia is so toxic, this is not an option for amphibians that are not surrounded by water at all times. Terrestrial amphibians excrete urea which is less toxic than ammonia and can be stored inside the bladder without consequence. When the amphibian has access to water, it will void its urea-laden urine. Some species can actually switch back and forth between ammonia as the main nitrogenous waste and the production of urea depending on the availability of water. A few species of anurans (eg, waxy tree frogs) produce uric acid. Some terrestrial amphibians can tolerate a loss of body water up to 40% of their bodyweight, and can maintain constant plasma solute concentration until the reservoir of water in the bladder is expended. Under conditions of restricted water, amphibians cease to produce urine as conservation of body water takes precedence over excretion of nitrogenous wastes. Thus dehydrated amphibians are often suffering from varying levels of ammonia or even urea intoxication following dehydration.

The amphibian skin is highly permeable. Topical administration of many medications is effective.

The typical fluids used to rehydrate mammals are hypertonic to amphibians, which have plasma osmolalities in the neighborhood of 200 to 250 mOsm. Amphibian Ringer’s solution is designed to be within this range, and is made by adding 6.6 g NaCl, 0.15 g KCl, and 0.2 g NaHCO$_3$ to 1 liter of distilled water. It may be stored for up to 7 days, longer if the solution is able to be autoclaved and stored in sterile containers. I am unaware of a commercial source for this as a prepackaged product but the chemicals may be purchased through chemical supply companies. An alternative formulation consists of 6.5 g NaCl, 0.42 g KCl, and 0.25 g CaCl$_2$ to 1 liter of distilled water. (http://www.msu.edu/user/eisthen/lab/methods/anatomy/recipes/ringers.html) Up to 10 g of glucose may be added to the solution too, but this reduces the shelf life of unsterilized solution to 24 hrs.

ENERGY METABOLISM

Amphibians use aerobic pathways for most activity and switch to anaerobic glycolysis for heavy activity. During anaerobic activity, the muscles fatigue rapidly due to the buildup of lactate due to an increase in the level of free hydrogen ions in the muscle. These hydrogen ions in turn create acidified inorganic phosphorus ions, such as H$_2$PO$_4^-$, which are known to be a proximate cause of fatigue in amphibians. Many amphibians may struggle a few minutes before collapsing. An amphibian oxidizes lactate at a rate about nine times slower than an equivalent-sized mammal so it may take hours to recover from a fatiguing event. Critical patients may need elevated levels of atmospheric oxygen before and after handling.

The role of lactate in acid-base metabolism in amphibians has been poorly investigated. The pH of amphibian blood can vary widely in normal specimens. Since we do not understand the actual way that endogenous lactates are completely eliminated in amphibians or even have a full understanding of the physiology of metabolic acidosis or alkalosis, it seems prudent to avoid adding sodium lactate (found in lactated Ringer’s solution) or using other lactated buffer solutions as fluid therapy for amphibians. Amphibian Ringer’s solution, described previously, is one of the most effective fluids for managing amphibians. If that is not available, 0.5 to 0.6% saline solution (ie, 5 to 6 g table salt per liter of water) is approximately isotonic for amphibians.

CALCIUM METABOLISM

Calcium metabolism is not well understood in amphibians although it is an important regulatory ion involved in many metabolic pathways throughout the body including activity of muscles and nerves. Calcium is absorbed from the environment either across the skin or through the gastrointestinal tract, and is excreted through urine, feces, and skin. In addition to bone and the endolympathic sacs, the skin can be a significant storage site of calcium with up to 30% of the total body calcium stored in the skin of some anurans.

There are at least four different hormones involved in calcium homeostasis. Vitamin D has only been proven to be involved in frogs and toads, although my clinical impression suggests it is important for caecilians and salamanders, too. The roles of parathormone and calcitonin have only been elucidated in frogs, toads, and salamanders, too. The roles of parathormone and calcitonin have only been elucidated in frogs, toads, and salamanders, too. Prolactin plays a role in salamanders but has not been documented in other taxa.

Vitamin D$_3$ appears to be important for normal calcification of the amphibian skeleton based on my long-term experiences with breeding and raising amphibians. Amphibians raised without access to vitamin D$_3$ develop a metabolic bone disease, often first noticed as a bowing of the mandibles, pathologic fractures of the long bones, and weak pelvic girdle. Amphibians given too much vitamin D$_3$, as sometimes
happens by feeding too many live goldfish, develop extraskeletal calcification. Vitamin D₃ has been documented to elevate plasma calcium levels in frogs and increase calcium uptake in tadpoles.

Calcitonin is the hypocalcemic hormone, lowering plasma calcium by increasing its deposition onto the bones, into the endolymphatic sacs, or in structures within the skin. The functional structure of amphibian calcitonin varies widely between species as the calcitonin derived from one species of frog did not work in a different species of frog. More importantly, the commercially available forms of calcitonin are derived from bovine or piscine sources. These non-amphibian calcitonins confound results of laboratory investigations and ultimately any work needs to be done with same-species derived calcitonin in order for the data to be consistent.

Parathormone is the hypercalcemic hormone, raising blood calcium by extracting calcium from the stored depots such as the mineralized skeleton, endolymphatic sacs, or skin. Prolactin induces hypercalcemia in adult salamanders.

Hypocalcemia is a serious metabolic derangement, and can affect the striated and smooth musculature of the amphibian. Tetany may develop in hypocalcemic striated muscle while smooth muscle dysfunction may result in lack of peristalsis and associated gastrointestinal bloating. The permeability of the skin to calcium allows treatment of hypocalcemia via baths of calcium ion solutions such as calcium gluconate. Daily baths in 2% to 5% calcium gluconate and vitamin D₃ (2 IU per 10–100 ml of water) are clinically effective in many species, but should be used as a supplementary aid and not as the single course of corrective efforts. Injectable calcium should be given intracoelomically for amphibians in tetany and may need to be administered weekly in addition to the calcium bath.

Hypercalcemia has not been a clinically apparent syndrome.