Medicine and Surgery of Amphibians

Edward J. Gentz

Abstract

Amphibians are most notably characterized by their glandular skin, which they shed regularly and ingest routinely. It is advisable to handle amphibians only with protective gloves to avoid damaging their skin. These animals absorb water readily across the skin as a means of maintaining hydration. They also easily absorb drugs and anesthetics that are applied directly to the skin. Investigators commonly utilize cutaneous respiration in amphibians and evaluate skin abnormalities via wet mount preparations, skin scrapes, and biopsy. The examination of blood samples can be useful in evaluating the status of ill amphibians, although the similarity in function of amphibian blood cell types and those of other species is largely unknown. If surgery is required, it is necessary to fast the animals before surgery, and to monitor their hydration. The wet environment required for amphibian surgery makes sterile technique challenging, and it is advisable to institute prophylactic antibiotic therapy before the procedure. The anesthetic of choice for amphibian surgery is tricaine methanesulfonate (MS-222). Postoperative recommendations include fluids, nutritional support if necessary, and analgesia. If euthanasia is required, MS-222 overdose or pentobarbital injection are the preferred methods.

Key Words: amphibians; analgesia; anesthesia; euthanasia; hematology; radiology; surgery

Clinical Anatomy and Physiology

The three orders of amphibians are Caudata, Anura, and Gymnophiona. The Caudata (salamanders and newts) have glandular skin and four legs, except for sirens, which have only two legs. The Caudata also may have external gills. The Anura (frogs and toads) have four legs, and the rear pair is larger than the front pair. Anuran external gills are absent. The glandular skin of frogs tends to be smooth whereas that of toads tends to be less smooth. The

Edward J. Gentz, M.S., D.V.M., DACZM, is Associate Veterinarian, Albuquerque Biological Park, Albuquerque, New Mexico.

Address correspondence and reprint requests to Dr. Edward J. Gentz, Albuquerque Biological Park, 903 Tenth Street SW, Albuquerque, New Mexico 87012 or email ngentz@cabq.gov.

Gymnophiona (caecilians) have no limbs and have no common use in the research setting. Some salamanders can regrow missing toes, limbs, and tails. Among anurans, tadpoles can regrow missing limbs, but this ability is lost in adults (Stebbins and Cohen 1995).

The amphibian heart has three chambers, which include two atria and a single ventricle. The highly developed lymphatic system has lymph hearts that beat independently of the cardiovascular system's heart (Wallace et al. 1991). Erythropoiesis is centered in the amphibian spleen and liver (Helmer and Whiteside 2005). In general, adult amphibians breathe through functional lungs, and larval forms through gills, although numerous neotenic forms utilize gills into adulthood. The amphibian lung is generally simple, has no partitioning or infolding, and usually lacks alveoli. All adult amphibians are carnivores, but many larval amphibians are herbivorous. Although the teeth of most amphibians are continually shed and replaced, bufonid toads lack teeth. The tongue in many amphibian species is essential for food handling.

The amphibian has a simple stomach and a straight intestinal tract that terminates in a cloaca. The amphibian mesonephric kidney produces urine that is less concentrated than the urine of higher species. Most aquatic amphibians excrete the majority of their nitrogenous waste as ammonia, whereas terrestrial species tend to excrete at least a portion of their nitrogenous waste as urea. A urinary bladder is present in all species examined to date (Mitchell et al. 1988; Stebbins and Cohen 1995).

Both male and female amphibians have paired gonads. Sexual dimorphism is uncommon, except in some frog species in which males are considerably smaller than females. Although a majority of salamanders utilize internal fertilization, anurans, in contrast, utilize external fertilization. Adrenal, thyroid, and pituitary glands function as in higher species. The amphibian brain resembles that of fish, with most brain function involved in vision, hearing, and olfaction. Larval amphibians and adult aquatic amphibians possess a lateral line system that is similar to fish (Mitchell et al. 1988).

Amphibians lack external ears. The external tympanic membrane is essential for sound transmission to the inner ear. In the amphibian eye, the iris is controlled by voluntary striated muscle rather than involuntary smooth muscle. For this reason, a pupillary light reflex is generally absent (Mitchell et al. 1988).

The most important organ system in the amphibian is

generally considered to be the skin. These animals shed their skin regularly and ingest it routinely. Amphibian skin is easily damaged by handling, therefore it is recommended that all personnel wear premoistened powder-free latex gloves whenever they handle these animals. Gloves can also protect the handler from irritating and toxic secretions produced by the dermal glands of many amphibians. Because cutaneous respiration exists to some degree in all members of the species, the skin of amphibians is also an effective route for topical administration of antibiotics as well as anesthetics (Helmer and Whiteside 2005). However, before administering a substance by this route, it may be necessary to dilute it (e.g., enrofloxacin) to avoid irritating the animal's skin.

Amphibians are poikilothermic. When they are kept either above or below their preferred optimal temperature zone (POTZ[1]), these animals may exhibit signs of anorexia and immunosuppression. Additionally, individuals kept above their POTZ may become agitated and exhibit abnormal color changes in the skin, while those kept below their POTZ may become lethargic and bloated, and show poor growth. Most amphibian species also absorb orally administered fluids poorly. For this reason, soaking, subcutaneous, or intracoelomic administration of fluids is significantly more effective than oral administration (Helmer and Whiteside 2005).

Diagnostic Tests

The skin is one of the most important amphibian organs, therefore it is imperative to investigate any skin abnormality promptly and thoroughly. It is advisable to use wet-mount preparations, which are made by direct touch of the skin with a microscope slide and are less traumatic than skin scrapes. However, if neither procedure yields a diagnosis, it is necessary to consider performing a biopsy. Ascites in amphibians can result from cardiovascular, hepatic, or renal disease, therefore it is important to submit excess coelomic fluid collected via celiocentesis for fluid analysis, cytology, and bacterial and fungal culture. It is advisable to examine fecal samples routinely by both direct smear and flotation techniques. In cases in which fecal collection is difficult, an alternative method is to obtain samples via cloacal wash. Stomach content samples obtained via gastric wash can be informative in cases of bloat and other gastrointestinal disorders. Tracheal wash is indicated in cases of suspected pneumonia (Whittaker and Wright 2001).

Radiology is useful for investigating cases of skeletal, respiratory, and gastrointestinal disease. It is possible to take most amphibian radiographs without chemically restraining the animal but instead, by placing it in a resealable plastic bag that contains a small amount of water (Stetter

2001). With mammography and dental radiography units, it is possible to produce amphibian radiographs with greater detail and quality than with standard radiography machines. However, mammography and dental radiography film can be used successfully with traditional radiography machines. A viable alternative is to use digital radiography units, if they are available, because they are capable of producing high-quality images. As with mammalian or avian radiology techniques, two views (lateral and ventrodorsal) are recommended. Contrast studies are indicated for suspected cases of gastrointestinal obstruction (Hadfield and Whitaker 2005).

It is advisable to consider hematology in the diagnostic evaluation of any ill amphibian. In anurans, accessible venipuncture sites include the heart, ventral abdominal vein, femoral vein, and lingual vein. In salamanders, the preferred venipuncture site is the ventral tail vein. It is generally considered safe to take a blood volume of 1% of a healthy amphibian's body weight. In the case of ill amphibians, blood collection should be limited to half of that amount (Wright 2001a). Lithium heparin is the anticoagulant of choice. Gentle pressure applied with a cotton ball to the venipuncture site after the needle is removed will assist hemostasis (http://www.nwhc.usgs.gov/publications/ amphibian_research_procedures/blood_samples.jsp). Amphibian erythrocytes are nucleated and are the largest blood cell among the vertebrates. It may be possible to perform complete blood counts in amphibians in the same manner as described in the literature for birds and reptiles (Wright 2001a). The ratio of leukocytes to erythrocytes in healthy amphibian blood ranges from 1:20 to 1:70 (Wright 2001a). Few studies have attempted to correlate leukogram changes in amphibians with specific disease processes. Early publications related to amphibian hematology described amphibian leukocytes based on their staining characteristics, as in other species (Jerrett and Mays 1973; Rouf 1969); however, the amount and degree of similarity in function of these cells between species remain largely unknown. Often red and white blood cell morphology is more informative than absolute cell counts. Viral inclusions, hemoparasites, and phagocytized bacteria are particularly informative. Many anurans possess paired dorsal lymph sacs, which constitute a convenient site for obtaining diagnostic samples of lymph fluid (Wright 2001a).

Preoperative Considerations

To prevent intraoperative regurgitation, it is necessary to establish a fasting period for small amphibians before surgery for a minimum of 4 hours, and larger insectivorous amphibians for 48 hours. For amphibians on a diet of whole vertebrate prey, the fast before surgery should continue for 1 week.

Adequate presurgical hydration is essential for a successful surgical outcome, therefore soaking the amphibian patient in water for 1 hour before surgery is recommended

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[1]Abbreviations used in the article: MS-222, tricaine methanesulfonate; POTZ, preferred optimal temperature zone.

(Wright 2001c). For the procedure, it is advisable to submerge the amphibian surgical patient at least partially in anesthetic-free water. The wet environment required for amphibian surgery makes sterile surgery a challenge, and these procedures are generally considered to be clean-contaminated. Sterile clear plastic drapes can be helpful, but adhesive drapes should never be used on amphibian skin. It is essential to institute prophylactic antibiotic therapy before surgery (Wright 2001c).

Surgical scrubs that contain soaps, detergents, isopropyl alcohol, or iodine products are contraindicated in amphibians. The recommended presurgical disinfectant is 0.75% chlorhexidine solution. It is necessary for sterile gauze soaked in the chlorhexidine to have direct contact with the surgical site for at least 10 minutes before surgery, and then for personnel to rinse the site with sterile saline. Absorbable suture such as polyglactin (Vicryl, Ethicon, Somerville, NJ), polydioxanone (PDS, Ethicon, Somerville, NJ), and polyglycolic acid (Dexon, United States Surgical, Norwalk, CT) are appropriate for internal use including muscle. Non-absorbable monofilament suture such as nylon should be used for skin closure; 3-0 or 4-0 suture is generally adequate. Taper needles are preferable to cutting needles, and it is usually possible to remove sutures after 14 days (Brown 1995).

Anesthesia and Sedation

In almost all instances, the anesthetic of choice for amphibian surgery is tricaine methanesulfonate (MS-222[1]; Argent Chemical Laboratories, Redmond, WA), which is an isomer of benzocaine. Most larval amphibians can be successfully anesthetized in a MS-222 solution of 0.2 g/L; however, adults generally require the higher concentration of 1.0 g/L. Induction times are typically ≤ 30 minutes using these techniques. It is important to buffer MS-222 solutions by adding sodium bicarbonate (baking soda) to the water to achieve a pH range between 7.0 and 7.4. When using MS-222 in amphibian patients, a light plane of anesthesia is indicated by the loss of the righting reflex and the corneal reflex. A deep plane of anesthesia is indicated when the withdrawal reflex to deep pain is lost. Once anesthesia is successfully induced, it is essential to transfer the amphibian patient to fresh water. If the amphibian patient begins to recover before the completion of the surgical procedure, it is advisable to deepen anesthesia by returning the animal to a 50% dilution of the induction solution (Downes 1995). Injectable ketamine at a dose of approximately 70 to 100 mg/kg delivered intramuscularly is an acceptable alternative to MS-222, but recovery can be prolonged up to 12 or even 18 hours.

Clove oil (eugenol) has been found to be an effective anesthetic in some amphibian species; however, there is the potential for the undesirable side effect of gastric prolapse to occur (Lafortune et al. 2001). Other techniques for amphibian anesthesia that were described in the older literature

(e.g., ether, acepromazine, or phencyclidine) are no longer appropriate, and investigators and instructors should not use these outdated techniques. Hypothermia is also unacceptable as a sedation technique for painful procedures.

Inhalant anesthetics such as isoflurane and sevoflurane may be delivered by conventional methods or bubbled directly into the water. These latter techniques, however, make scavenging of waste gas difficult. It is possible to perform minor procedures using no more than 1.0 mg/kg total dose of 2% lidocaine as a local anesthetic. Anesthetic monitoring is recommended during amphibian surgery, as in any other species. In some amphibian patients, it is possible to visualize the heartbeat directly; the use of a Doppler probe is also effective. An additional procedure for monitoring is to attach pulse oximeters to the extremities of the amphibian during surgery (Wright 2001b).

It is essential to monitor the amphibian patient during anesthetic induction to prevent drowning. If the anesthetic level becomes too deep, it is imperative to rinse the patient with clean well-oxygenated water until the animal recovers. During MS-222 anesthesia, gular respiration will slow or even cease; however, the heart rate is rarely affected. It is advisable to bubble 100% oxygen into the water during the surgical procedure to assist the amphibian patient's cutaneous respiration. Large amphibians may be intubated and ventilated for prolonged procedures. If the heart rate of the amphibian patient drops 20% or greater from baseline, it is necessary to remove the animal from the anesthetic solution and to recover the patient (Downes 1995).

Common Surgical Procedures and Techniques

Skin incisions in amphibian patients are best made with a number 15 or number 11 scalpel blade. Everting suture patterns are recommended for skin closure, and interrupted patterns are preferred over continuous patterns. Alternatively, it is possible to use cyanoacrylate tissue adhesives for skin closure in some instances.

Microchips are often used to identify research amphibians. In anurans, a microchip may be placed subcutaneously; however, in salamanders, which lack subcutaneous space, the microchip should be placed intracoelomically through a small paramedian incision (Wright 2001c). It is also possible to identify individual amphibians by utilizing a toe-clipping technique, although this technique is recommended only for adult anurans (http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp).

Lightweight intramedullary pins may be used to repair simple long-bone fractures. Compound or comminuted fractures are often best treated with amputation (which may well regenerate in salamanders). When amputating the hind limb of an anuran, it is necessary to remove the entire femur at its articulation to the pelvis. It may be necessary to manually assist these animals with shedding. For forelimb amputations, it is important to preserve a stump because male

anurans without forelimbs may be unable to successfully reproduce. It is necessary to consider euthanasia in cases of mandibular fractures, which carry a grave prognosis (Wright 2001c).

Laparoscopy is a tool that can be used to determine the gender of sexually monomorphic amphibians. Organ biopsies may also be obtained via laparoscopy. A paramedian approach is the preferred method for visualizing the heart, liver, bladder, and gastrointestinal tract, whereas a lateral approach is preferred for gonads, kidneys, and adrenals. Exploratory celiotomy may be required to investigate cases of anasarca (ascites) or coelomic masses, among other indications. A paramedian incision is recommended to avoid transecting the ventral abdominal vein. It is advisable to close the incision in two layers (Wright 2001c).

Endoscopy can be used to remove gastrointestinal foreign bodies; however, if this method is not successful, it is necessary to proceed to gastrotomy. The stomach should be stabilized with stay sutures before incision. Care is required to avoid contaminating the coelomic cavity with spilled gastric contents. Cystotomy to remove cystic calculi is less commonly required. In larger amphibians specimens, it is necessary to close gastrotomy and cystotomy incisions using a double layer technique. In smaller amphibians, a single layer closure may be adequate. Gonadectomy is a common amphibian surgery in a research setting. Surrounding mesenteric blood vessels can be prominent and can require ligation. Gonadectomized amphibians tend to become obese after surgery. Cloacal prolapse is a common problem in anurans more than salamanders. It is often not possible to replace such a prolapse without first shrinking the hyperemic tissue with either a hyperosmotic saline or a sugar solution. It is then necessary to coat the prolapsed tissue with a water-soluble lubricant before replacing it back through the cloacal opening using a cotton-tipped applicator. It may also be necessary to use a pericloacal pursestring suture to prevent the prolapse from recurring. If enucleation is required in an amphibian patient, it is important to avoid damaging the membrane that separates the eye from the oral cavity. Second intention healing is often preferred in such cases (Wright 2001c).

Ovariectomy is a common method used in various frog species for obtaining oocytes for embryological studies. This method makes it possible to excise a portion of egg mass from a donor animal without ligating any blood vessels, although complete ovariectomy does require ligation of the surrounding blood vessels. Small surgical clips work well for this purpose. Testicular biopsy is also a commonly used method in embryological and reproductive studies (Wright 2000).

Postoperative Care

If the amphibian patient is still in a MS-222 solution at the conclusion of surgery, it is essential to transfer the animal to a warm, anesthetic-free bath and to rinse it copiously with fresh, well-oxygenated water. It is helpful to administer subcutaneous or intracoelomic postoperative fluids, especially after a gastrotomy. Two easily formulated solutions for use in amphibian patients are (1) one part of saline (0.9% NaCl) mixed with two parts of 5% dextrose, and (2) seven parts of saline mixed with one part of sterile water. An appropriate dose of either solution is 25 mL/kg of body weight (Wright 2006). An important adjunct to maintain hydration and restore the health of ill amphibians is to soak the animals in balanced electrolyte solutions. Two such formulations are (1) amphibian Ringer's solution (6.6 g NaCl, 0.15 g $CaCl_2$, 0.15 g KCl, and 0.2 g $NaHCO_3$ per liter of dechlorinated water), and (2) Holtfreter's solution (3.46 g NaCl, 0.1 g $CaCl_2$, 0.05 g KCl, and 0.2 g $NaHCO_3$ per liter of dechlorinated water).

It may be necessary to assist the amphibian patient with feeding for a brief period after any surgical procedure. Suitable choices for nutritional support in amphibians that are not self-feeding include the following: A/D Prescription Diet (Hill's Pet Nutrition, Topeka, KS); and a slurry made of ReptoMin pellets (Tetra, Blacksburg, VA) (Hadfield and Whittaker 2005). After gastrotomy, it is essential to withhold feeding for 1 week and to offer only small meals for the next month.

Antibiotic therapy is routinely recommended after any surgical procedure. The recommended duration of therapy is 2 weeks after an amputation, 3 weeks after fracture repair, and up to 6 weeks for open fractures (Wright 2001c). Some antibacterials commonly used in amphibians include amikacin (5 mg/kg IM, SC, or ICe, q48h), ceftazadime (20 mg/kg IM q72h), and enrofloxacin (5-10 mg/kg IM, SC, or PO q24h) (Hadfield and Whitaker 2005). Currently there is a poor understanding of analgesia in amphibians, but some of the analgesics that are recommended for amphibian patients include the following: buprenorphine (38 mg/kg SC), butorphanol (0.2-0.4 mg/kg IM), fentanyl (0.5 mg/kg SC), meperidine (49 mg/kg SC), morphine (38-42 mg/kg SC), and nalorphine (122 mg/kg SC) (Machin 1999). A current "Compendium of Drugs and Compounds Used in Amphibians" (Smith 2007) appears elsewhere in this issue.

Humane Euthanasia

Prolonged immersion in a MS-222 solution (10 g/L) is an effective and stress-free form of amphibian euthanasia. Also effective is pentobarbital (100 mg/kg) administered by intracardiac, intracoelomic, or subcutaneous lymph sac injection. It is important to note that pithing and traumatic blows to the head are not recommended methods of euthanasia. Moreover, the following techniques are also not acceptable for euthanasia of amphibians: carbon dioxide, decapitation, electrocution, exsanguination, freezing, and hyperthermia (Wright 2001b).

Conclusion

Amphibians require special care in captivity. All personnel who handle these animals must be careful to avoid damaging their characteristically glandular skin. It is not appropriate to handle amphibians with bare hands. Not only can drugs be absorbed when applied directly to the skin, but also oxygen, which is an important ancillary form of respiration, can diffuse directly across the skin. Surgery in amphibians is uncomplicated; however, sterility is problematic. For that reason, postsurgical antibiotic therapy is generally indicated. When required, it is essential to administer euthanasia in a humane manner.

References

Brown CS. 1995. Rear leg amputation and subsequent adaptive behavior during reintroduction of a green treefrog, *Hyla cinerea*. Bull Assoc Reptil Amphib Vet 5:6-7.

Downes H. 1995. Tricaine anesthesia in amphibia. Bull Assoc Reptil Amphib Vet 5:11-16.

Hadfield CA, Whitaker BR. 2005. Amphibian emergency medicine and care. Sem Av Exot Pet Med 14:79-89.

Helmer PJ, Whiteside DP. 2005. Amphibian anatomy and physiology. In: O'Malley B, ed. Clinical Anatomy and Physiology of Exotic Species. Edinburgh: Elsevier Saunders. p 3-14.

Jerrett DP, Mays CE. 1973. Comparative hematology of the hellbender, *Cryptobranchus alleganiensis*, in Missouri. Copeia 1973:331-337.

Lafortune M, Mitchell MA, Smith JA. 2001. Evaluation of medetomidine, clove oil, and propofol for anesthesia of leopard frogs, *Rana pipiens*. J Herp Med Surg 11:13-18.

Machin KL. 1999. Amphibian pain and analgesia. J Zoo Wildl Med 30:2-10.

Mitchell LG, Mutchmor JA, Dolphin WD, eds. 1988. Amphibians. In: Zoology. Menlo Park: Benjamin-Cummings. p 727-748.

Rouf MA. 1969. Hematology of the leopard frog, *Rana pipiens*. Copeia 1969:682-687.

Smith SA. 2007. Compendium of drugs and compounds used in amphibians. ILAR J 48:297-300.

Stebbins RC, Cohen NW. 1995. A Natural History of Amphibians. Princeton: Princeton University Press.

Stetter MD. 2001. Diagnostic imaging of amphibians. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger. p 253-272.

Wallace RA, Sanders GP, Ferl RJ. 1991. The chordates. In: Wallace RA, Sanders GP, Ferl RJ, eds. Biology: The Science of Life. New York: Harper Collins. p 718-751.

Whitaker BR, Wright KM. 2001. Clinical techniques. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger. p 89-110.

Wright KM. 2000. Surgery of amphibians. Vet Clin North Am Exot Anim Pract 3:753-758.

Wright KM. 2001a. Amphibian hematology. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger. p 129-146.

Wright KM. 2001b. Restraint techniques and euthanasia. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger. p 111-122.

Wright KM. 2001c. Surgical techniques. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger. p 273-283.

Wright KM. 2006. Overview of amphibian medicine. In: Mader DR, ed. Reptile Medicine and Surgery, 2nd ed. St. Louis: Saunders Elsevier. p 941-971.