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Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider

Patricia V Turner,[1,*] Thea Brabb,[2] Cynthia Pekow,[3] and Mary Ann Vasbinder[4]

Administration of substances to laboratory animals requires careful consideration and planning to optimize delivery of the agent to the animal while minimizing potential adverse experiences from the procedure. For all species, many different routes are available for administration of substances. The research team and IACUC members should be aware of reasons for selecting specific routes and of training and competency necessary for personnel to use these routes effectively. Once a route is selected, issues such as volume of administration, site of delivery, pH of the substance, and other factors must be considered to refine the technique. Inadequate training or inattention to detail during this aspect of a study may result in unintentional adverse effects on experimental animals and confounded results.

Administration of substances to laboratory animals is often a critical component of experimental design. Administered substances may include: infectious disease agents; various therapeutics, such as vaccinations, antimicrobials, pharmacologic agents, anesthetics, and analgesics; chemical test agents; radiocontrast agents; electrolytes and other fluids; and nutritive support. Because substances may be administered repeatedly to the same animal or to multiple animals on the same study, the dosing methodology is an important consideration when planning an experiment and during protocol review by animal care and use committees and represents an essential opportunity for refining treatment of research subjects. Specific considerations for delivery of substances to animals are numerous and include factors such as absorption, distribution, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration; duration of treatment; pH, stability, homogeneity, and osmolality of the substance to be administered; selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation, including considerations for sterility if the substance is being administered parenterally; and dosing apparatus and animal restraint necessary for specific routes of delivery. In addition, research teams should be aware of potential adverse effects related to substance administration to avoid confounding effects with other aspects of study design and to permit accurate interpretation of research findings.

Although understanding the basic pharmacology of any administered therapeutic or chemical agent is important for experimental planning, it is beyond the scope of this article to review principles of pharmacokinetics and pharmacodynamics, and readers are referred to several excellent texts dealing with these subjects.[22,102,106] This article is the first of a 2-part review of substance delivery to laboratory animals and summarizes recommended practices for various routes of administration to a range of species and factors to consider during experimental planning. The second part of this review examines dosing equipment and apparatus needed for substance delivery, considerations for selecting vehicles, and solute preparation and handling.[134]

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[1]Department of Pathobiology, University of Guelph, Guelph, Canada; [2]Department of Comparative Medicine, School of Medicine, University of Washington and [3]Research and Development, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; [4]Platform, Technology and Sciences, Quality and Risk Management,Glaxo Smith Kline, Research Triangle Park, North Carolina.
[*]Corresponding author. Email: pvturner@uoguelph.ca

Routes of Administration

Selection of a route. Substances are administered to laboratory animals by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral [through the digestive tract] or parenteral [outside the digestive tract]) effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally administered chemicals and therapeutics. Parenteral routes also circumvent some of the unpredictability associated with enteral absorptive processes. Furthermore, regulatory requirements may influence the selection of a particular route, depending on the purpose of the study (for example, nonclinical safety testing, in which the route of delivery to animals should closely resemble the projected route of administration to humans).[37,38]

A substance may be given into the mouth (orally) or delivered directly into the stomach (gastric gavage); delivered into a blood vessel (intravenous); delivered onto, into, under, or across the skin or into a muscle (epicutaneous, intradermal, subcutaneous, transdermal, and intramuscular administration, respectively); instilled onto or into the eye (transcorneal or intraocular, respectively); into the brain (intracerebral) or the space surrounding the dura mater or that surrounding the distal spinal cord (epidural and intrathecal, respectively); administered into the peritoneal cavity (intraperitoneal), directly into the marrow cavity (intraosseous); sprayed into the nose for absorption across the nasal mucous membranes or into the lungs (intranasal) or delivered into the lungs by direct tracheal instillation (intratracheal) or inhalation; or administered by a range of less common routes using other body orifices, surgical

exposures, and species-specific anatomic features (for examples, see references 16, 41, 60, 64, 73, 91, and 127).

In laboratory species, many of the commonly used methods of delivery require restraint, sedation, or general anesthesia. The use of such manipulations should be considered when selecting the administration route to refine procedures so that they are less invasive or aversive to the animals. In addition, each route has advantages and disadvantages that should be considered depending on the final effect to be achieved, and ultimately the route selected will markedly affect the pharmacokinetics of the substance. This pharmacokinetic effect of route of administration is exemplified by naloxone, a potent opioid antagonist. Given intravenously, naloxone rapidly reverses opioid-induced central nervous system depression,[28] but when given enterally, the drug can be used to treat opioid-induced bowel stasis without antagonism of the analgesic effects of systemically administered opioids.[52] Another consideration regarding once-daily administration of substances to animals is their chronobiology or circadian rhythm. Depending on the aims and objectives of the experiment, the timing of substance administration may need to be considered carefully, for example, to administer a therapeutic when an animal's system is most or least metabolically active to induce or minimize toxicity.[119]

Enteral administration. Administration of substances directly into the mouth, admixed in diet or other foodstuffs, or by orogastric or nasogastric gavage is common in laboratory animal medicine and research. Per rectum administration of substances by enema or suppository is less common in animals than in humans. The oral route is economical, convenient, relatively safe, and some animals can be trained to cooperate voluntarily, depending on the compound being administered (Figure 1 A through C). Although voluntary consumption of the material being administered is ideal, this dosing technique may not be reliable in all animals or dose groups or for long-term studies, because of individual preferences for flavors, palatability issues, and changes in behavior over time. For substances being tested for safety, oral dosing mimics the most commonly used mode of administration of substances to humans. When placing substances directly into the mouth, it is important to ensure that tablets or gelatin capsules containing test material are placed far back in the mouth and that the animal swallows, to ensure receipt of the full dose. The number and size of capsules or tablets administered should be proportional to the size of the animal being dosed, to minimize regurgitation. Gavage (esophageal or gastric) is often used in research settings, instead of mixing substances in water or food, to ensure precise and accurate dosing of animals (Figure 1 D).

Selection of appropriate tubing size for orogastric or nasogastric gavage is important to minimize discomfort while optimizing delivery of substances. Nasogastric tubes are used commonly in rabbits for enteral nutrition and in nonhuman primates for dose administration and typically comprise 3- to 8-French soft rubber pediatric feeding tubes.[18,104] Tubing is measured from the external nares to the last rib and marked. To minimize discomfort, a small amount of xylocaine jelly can be placed on the end of the tubing or a drop of 0.5% proparicaine hydrochloride ophthalmic solution is placed directly in the nares prior to introducing the tubing into the ventromedial meatus (Figure 2).

Except when given in the diet or admixed with food, oral administration of substances typically requires some form of restraint. In many species, including rodents and nonhuman primates, restraint can be the greatest adverse effect of a procedure.[25,78,135] Habituation or positive reinforcement training to restraint may reduce the stress associated with the procedure.[1,107,120] In addition, the administration of large volumes of substances by orogastric or nasogastric gavage may cause stress due to gastric distension in species that are unable to vomit, such as rodents.[21] Therefore, using the smallest volume possible is recommended for the oral route of administration, optimally 5 mL/kg for all species (Table 1). When rats underwent gavage at this volume, no difference was noted between the stress induced by gavage compared with that induced by restraint alone.[135] When large volumes must be administered by gavage, a slower delivery rate may be better tolerated by animals.

Limitations of oral dosage may include a slower onset of action compared with parenteral delivery, a potentially significant first-pass effect by the liver for those substances metabolized through this route with reduced efficacy, lack of absorption of substances due to chemical polarity or interference with absorption by ingesta, poor compliance with voluntary consumption because of poor palatability or local irritation, lack of systemic absorption from the digestive tract, degradation of substances by digestive enzymes and acid, and inability to use this route in animals that are unconscious or have clinically significant diarrhea or emesis.[11] Oral gavage requires moderate technical skill and confidence. Research personnel should have training and practice prior to study initiation to minimize adverse events associated with the technique and to ensure that it is performed accurately, rapidly, and humanely in experimental animals.

Intravenous administration. The intravenous route of delivery is the most efficient means of delivering substances to animals because it bypasses the need for solute absorption. With this method, substances are administered as a bolus or infusion directly into blood vessels on either an acute or chronic basis (Figure 3). Precision electronic infusion pumps equipped with alarms to indicate flow interruptions and microdrop infusion sets are used to ensure accurate chronic intravenous delivery of many substances; however, less expensive precision and spring-operated disposable pumps have become available for this purpose in recent years and may represent a more economical alternative for experimental intravenous substance delivery, depending on the nature of the material to be administered and the duration of treatment.[2,32,117]

Although fluids and parenteral nutrition typically are infused on a continuous basis over several hours or days, the decision to administer other substances by the intravenous route often depends on the pharmacokinetics of the substance, as well as the maximum tolerated dose, the time interval over which delivery is required (referred to as dosing intensity), and the need to minimize variations in peak and trough blood levels in the substance being administered. The actual technique involves aseptic preparation of skin for percutaneous venous injection or surgical exposure of blood vessels for substance administration. Intentional intraarterial administration of substances should be avoided routinely and used only for specific experimental conditions, because of the potential for severe complications with this route, including blindness, cerebrovascular stroke, permanent motor deficits, and limb gangrene.[75,114,116,142] Suggested sites and volumes for intravenous injection and infusion of substances are given in Table 1.

Researchers designing experiments requiring single or repeated intravenous treatments should consider technique refinements that may enhance animal comfort, including the use of the smallest needle or catheter size possible to minimize injection trauma, butterfly needles for single injections to minimize perivascular trauma, indwelling catheters and vascular access ports for animal comfort and locomotor freedom, topical

Figure 1. (A) Rat voluntarily consuming nutritional supplement from a syringe. Photo courtesy of Colette Wheler. (B) Macaque voluntarily drinking medication from a syringe. Photo courtesy of Andrew Winterborn. (C) Pig voluntarily accepting medication when administered in a marshmallow. (D) Oral gavage of fish. Photo courtesy of Gerald Johnson.

Figure 2. Chronic nasogastric catheter placement in a rabbit for enteral nutrition. Photo courtesy of Colette Wheler.

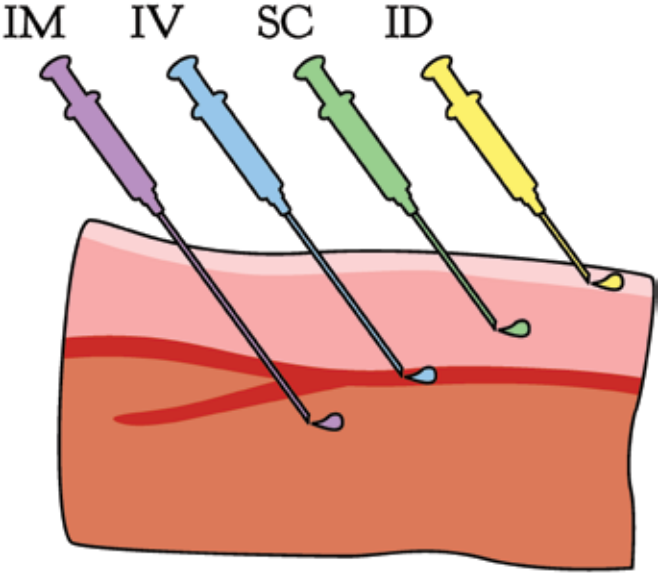

Figure 3. Different routes of skin administration of substances. Depicted are intramuscular (IM), intravenous (IV), subcutaneous (SC), and intradermal (ID) routes. Illustration courtesy of Gianni Chiappetta.

anesthetic creams and ointments prior to needle placement to minimize injection pain, and external pump packs to minimize the restriction of animal movement associated with tethering. Excellent recent reviews of techniques, equipment, and refinements for using catheters and vascular access ports in animals have been published.[16,53,89,124,125,128] A more detailed discussion of dosing equipment for intravenous delivery can be found in the companion article to the current work.[134]

Intraosseous administration of substances, particularly crystalloid fluids, is used in human pediatric medicine and emergency avian and rabbit medicine as an alternative for the intravenous route in hypovolemic patients with inaccessible

Table 1. Recommended volumes and sites of Administration of substances to laboratory animals

Route	Species	Optimal volume (range)	Site(s)	References
Gavage				
	All	5 mL/kg (to 20 mL/kg)	Mammals: intragastric	21, 82, 134
			Fish: esophageal[a]	127
	Fish	2 g/kg (gel capsules)		16
Intravenous				
	All	Up to 5 mL/kg (bolus)	Rodents: tail or saphenous vein Rabbits: ear or cephalic vein Larger species: jugular, cephalic, femoral, or saphenous vein	82
			Fish: caudal vein or artery[a,b]	16
		2 mL/kg hourly (to 4 mL/kg/h) continuous infusion)[c]		82, 89
Subcutaneous				
	Mammals	Maximum of 5 mL/kg per site	Intrascapular, neck, shoulder, flank	82
	Fish	1 mL/kg	Midline and just anterior to dorsal fin	53
Intradermal				
	All	0.05-0.1 mL per site	Skin	82
Intramuscular				
	All	Maximum of 0.05 mL/kg per site (rodents, rabbits, small nonhuman primates, fish)	Mammals: triceps, quadriceps, dorsal lumbar, semimembranosus, semitendinosus muscles	82
			Fish: base of dorsal fin or between dorsal fin and lateral line	16
Epidural				
	Mammals	0.15–0.2 mL/kg[d] (6 mL total volume in patients up to 35 kg)		47, 73, 138
Intraperitoneal				
	All	Maximum of 10 mL/kg	See text	82
Intranasal				
	Rodents	Minimum of 35µL per animal[e] (50 µL)		82, 121
	Dog, cats, nonhuman primates, rabbits	200 to 500 µL per animal		82

The physicochemical properties of the substance to be administered will markedly affect the volumes that are tolerated. For example, lower volumes than those listed in this table may need to be used for highly viscous or irritating substances.
[a]Sedation or light anesthesia may be needed for larger species.
[b]Renal first-pass effect is possible when injecting by using this route.
[c]Rates considerably lower than 2 mL/kg hourly may result in catheter patency issues in rodents.
[d]Larger volumes may result in more rostral spinal effects. Intrathecal injection volumes and doses are typically 50% of those used for epidural delivery.
[e]In mice, volumes less than 35 µL have been reported to be distributed primarily to the upper respiratory tract, whereas a 50-µL volume was predominantly deposited in the lower respiratory tract.

or collapsed veins.[31,80,129] The medullary cavity contains noncollapsing venous sinuses that directly enter into the central venous circulation and substances administered intraosseously are generally detectable immediately after administration. The technique is difficult to perform without advanced training and is potentially invasive, with considerable risk for postprocedural osteomyelitis, fat embolization, iatrogenic fracture and growth plate injury, and pain. Intraosseous administration typically is conducted in fully anesthetized animals.

Substances administered intravenously or intraosseously must be delivered aseptically and should be sterile; free of particulates that may induce foreign body emboli; and minimally irritating to vascular endothelia, to prevent vasculitis and thrombosis, and to erythrocytes, to minimize lysis. Certain oily substances, such as cremaphor, and various alcohols, surfactants, and other vehicles and excipients may induce hemolysis when introduced intravenously; these substances should be avoided, whenever possible, or first evaluated in vitro for safety.[4,79,90] The intravenous route of substance delivery, although efficient, can be risky in animals, and persons conducting this technique require training and practice to ensure competency. Careful control of hemostasis must be instituted when the catheter or needle is removed, to minimize blood loss and painful hematoma formation. When fluids or infusions are administered chronically, animals should be monitored closely for signs of fluid overload and pulmonary edema, such as dyspnea and cyanosis.[77] Chronically implanted catheters and vascular access ports require regular cleaning and maintenance to ensure patency and prevent infection.

Administration to skin and muscle. Some substances can be administered directly to the skin surface (epicutaneous administration) for a topical affect. The extent of absorption of materials through the skin and into the systemic circulation (that is, percutaneous or transdermal delivery) depends on: the surface area over which the substance is applied; the concentration of the substance administered; the lipid solubility of the material or vehicle; whether the skin surface is intact; the skin thickness at the site of application; the length of time that the material is in contact with the skin surface; and the degree of skin hydration and surface occlusion, in that covered and well-hydrated skin absorbs substances faster than does uncovered or dry skin.[87] For fish, specialized chambers can be constructed to expose the skin or gills specifically to test substances.[16,53] When administering substances topically to the skin of mammals, overlying hair is clipped to minimize matting and maximize contact with the material to be applied, and the skin surface is cleaned prior to application. Absorption of substances across the epidermis occurs through paracellular and transcellular mechanisms into the stratum corneum, to the stratum spinosum, and then to the basal layers of the skin and later, the dermis, as well as into the subcutaneous space through hair follicles and accessory glands.[42,93]

Caution must be exercised to avoid applying caustic or irritating material directly onto the skin, and some substances may induce local sensitization reactions. Consideration should be given to the potential for systemic toxicity when administering substances topically, particularly if the site is readily accessible for grooming.[46] Application of thin layers of cream or ointment to the skin at more frequent intervals may be more efficacious with less potential for systemic toxicity than is less frequent application of thicker layers.

Transdermal or percutaneous delivery represents a similar route of administration except that materials are applied to the skin surface deliberately, usually by means of a patch, for absorption across the epithelial barrier into the systemic circulation. Typically, this method produces very constant blood levels of the substance being administered. Percutaneous delivery is an attractive alternative to other parenteral routes, avoiding the need for repeated animal restraint, painful injections, and sharps hazards. In addition, materials can readily be removed from the skin surface if dosing needs to be interrupted or if adverse effects are noted. Transdermal delivery of substances may be acute or chronic, and current techniques for delivering substances by this route have been reviewed recently.[7,45,100] The skin is prepared as for topical delivery. When a transdermal delivery system will be used, the agent and delivery system (for example, patch) must be applied in advance of when the desired effect needs to occur, based on the pharmacokinetics of substance absorption. The product should be applied in such a way to protect it from ingestion and contamination, and the signs of toxicity after inadvertent ingestion by the animal should be known. Commercially available human transdermal products can be difficult to use in animals because of the much larger doses of substances impregnated into products intended for adult human use. Cutting transdermal patches to scale-down the dose being administered is not recommended; however, covering a portion of the patch to limit substance administration may be used. Animals should be observed closely for toxicity, and as for topical delivery methods, skin sensitization may occur over time with transdermal product use.[84] Animals must be prevented from removing and ingesting patches.

Nonirritating substances may be given subcutaneously, which represents a rapid, inexpensive, and simple method of parenteral substance administration (Figure 3). Substances administered subcutaneously often are absorbed at a slower rate compared with other parenteral routes, providing a sustained effect. The exact mechanism of absorption is unknown but is thought to be due to uptake of macromolecules within the subcutis by small capillaries underling the skin, with minimal lymphatic absorption.[56] Substances delivered subcutaneously can be aqueous or oily fluids, depots of oily materials for slow absorption, solid pellets, or injected into suitably sized osmotic minipumps or other implantable pumps, which subsequently are surgically inserted into a subcutaneous pocket. Because the subcutaneous space is largely a virtual space, it can be an excellent site for large volume fluid delivery in small or dehydrated animals, avoiding technical difficulties and problems sometimes seen with direct intravenous administration, such as fluid overload and pulmonary edema, because excess subcutaneous fluid is excreted rapidly by the kidneys. Compared with intravenous delivery, the subcutaneous route is a simple one to master; however, training and competency of personnel should be monitored to ensure that substances are delivered accurately and that inadvertent intravenous injection is avoided. Careful consideration should be given to using an appropriately sized needle, and humane and aseptic periinjection techniques. The skin overlying the site selected for injection should be loose to minimize discomfort, and the needle should be inserted at a shallow angle to minimize damage to underlying tissues. Passing a small-gauge needle through a thick rubber stopper to fill an attached syringe prior to injection may dull the needle point, enhancing injection discomfort. Contaminated substances injected subcutaneously typically will result in abscess formation. Recommended volumes and locations for subcutaneous injections are presented in Table 1. Inadvertent subcutaneous administration is a common complication of intradermal injections, and small, sharp needles are required for success with intradermal delivery.[82]

Intramuscular administration of substances is a common parenteral route in large animals and humans but often is avoided in smaller species because of the reduced muscle mass. Generally, intramuscular injections result in uniform and rapid absorption of substances, because of the rich vascular supply (Figure 3). Smaller volumes are administered intramuscularly than for subcutaneous delivery (Table 1). The intramuscular technique requires more skill than does subcutaneous injection and should be conducted only by well-trained personnel. Intramuscular injection of irritating substances or inadvertent injection of nerves may result in paresis, paralysis, muscle necrosis, and localized muscle sloughing.[103] Repeated injections may result in muscle inflammation and necrosis.[30] Other considerations and cautions for using the intramuscular route for substance delivery are similar to the subcutaneous route.

Epidural and intrathecal administration. For rapid effects of substances on cerebrospinal tissues or meninges, substances can be administered into the epidural or subarachnoid (intrathecal) space of the spinal cord (Figure 4, Table 1). This technique avoids absorptive problems otherwise presented by the blood–brain barrier. The route is used commonly to induce spinal anesthesia or to introduce contrast media for visualizing vertebral bodies or the spinal cord of large animal species. The technique requires animals to be sedated heavily and given a local anesthetic block over the spinal needle insertion site; alternatively animals can undergo general anesthesia prior to implementation.[23,138] Aseptic preparation of the skin overlying the injection site and use of sterile technique for needle insertion are critical for success and animal recovery. The exact location of needle insertion and volume of injectate will vary between species and for intrathecal compared with epidural administration, and several factors contribute to procedural success (see reference 138 for review). Epidural fat, lipophilicity of the substance being administered, leakage of injectate through intervertebral spaces, and pronounced meningovertebral ligaments all will limit or alter the spread of material being introduced by epidural or intrathecal routes.[58] This limitation may be problematic, in that increased quantities of substances may need to be administered for effect, with the possibility of spill-over into systemic circulation, resulting in adverse effects, such as profound respiratory depression requiring prolonged ventilation. Visualization of cerebrospinal fluid after spinal needle insertion confirms intrathecal placement of the needle. If this fluid is noted when attempting an epidural injection, the needle should be withdrawn and repositioned, or the dose of the substance administered should be reduced, because the kinetics of substance absorption from epidural compared with intrathecal delivery can be markedly different.[138]

Intrathecal or epidural administration of substances requires considerable technical skill and in-depth knowledge of anesthesia, analgesia, and spinal cord and vertebral column anatomy. These techniques should be performed only by well-trained personnel. Adverse events associated with epidural administration of substances to small animals include prolonged time for hair regrowth over the injection site, pruritus, urinary retention, nausea, vomiting, and prolonged and severe respiratory depression.[132]

Intraperitoneal administration. Injection of substances into the peritoneal cavity is a common technique in laboratory rodents but rarely is used in larger mammals and humans. Intraperitoneal injection is used for small species for which intravenous access is challenging and it can be used to administer large volumes of fluid safely (Table 1) or as a repository site for surgical implantation of a preloaded osmotic minipump. Absorption of material delivered intraperitoneally is typically much slower

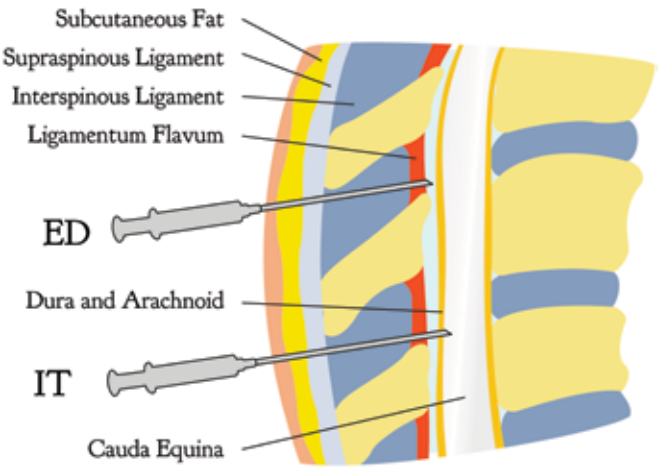

Figure 4. Epidural (ED) compared with intrathecal (IT) injections in the distal lumbar spine. Illustration courtesy of Gianni Chiappetta.

than for intravenous injection. Although intraperitoneal delivery is considered a parenteral route of administration, the pharmacokinetics of substances administered intraperitoneally are more similar to those seen after oral administration, because the primary route of absorption is into the mesenteric vessels, which drain into the portal vein and pass through the liver.[74] Therefore substances administered intraperitoneally may undergo hepatic metabolism before reaching the systemic circulation. In addition, a small amount of intraperitoneal injectate may pass directly across the diaphragm through small lacunae and into the thoracic lymph.[3]

In mammals, intraperitoneal administration typically is conducted in conscious animals by using firm manual restraint, with the head and body tipped downward to move viscera away from the surface of the ventral abdomen. Injections in rodents are made in the lower right abdominal quadrant away from the midline to avoid inadvertent injection into the urinary bladder or cecum.[26] The syringe plunger may be withdrawn prior to injection, specifically looking for urine, blood, or digesta in the needle hub; if these fluids are seen, the needle should be withdrawn, replaced, and repositioned prior to injection. The most common mistake is to puncture the skin at too acute an angle, resulting in subcutaneous rather than intraperitoneal administration. For intraperitoneal injections in fish, the animals are restrained on their side on a flat surface, and the needle should enter along the midline, just anterior to the pelvic fins. Larger fish may require sedation or light anesthesia for appropriate restraint.[16]

Materials injected intraperitoneally should be sterile, isotonic, and nonirritating. Irritating substances injected intraperitoneally may induce painful ileus and peritonitis in rodents, with subsequent adhesions.[43] This drawback is typified by the effects of undiluted chloral hydrate when administered intraperitoneally in rats.[36] Injections of identical doses of chloral hydrate in less concentrated solutions may avoid peritoneal irritation,[137] and this technique may be used for other potentially irritating substances. Although technically a simple procedure to perform, training and competency of personnel should be monitored to ensure that substances are delivered accurately and that inadvertent intracecal or intracystic injections are avoided.

Intranasal, intratracheal, and inhalational administration. In research settings, animals generally are sedated or anesthetized[47] for the intranasal and intratracheal routes of delivery, to minimize struggling and sneezing. Volumes administered intranasally are small compared with those of other routes (Ta-

ble 1), to minimize the potential for suffocation and death. The technique may not be useful in animals with signs of rhinitis or conjunctivitis. Intranasal delivery is readily taught and simple to perform in an anesthetized animal. Substances administered by this route should be nonirritating to minimize sneezing, posttreatment rhinitis, and epistaxis.

Intranasal techniques may be used for either local (for example, vaccinations or decongestant sprays) or systemic delivery of substances. The nasal mucosa lines the nasal cavity and is richly supplied with blood vessels, potentially resulting in rapid substance absorption and subsequent systemic effects, avoiding the hepatic first-pass effect seen with oral delivery. Blood drug levels of substances administered intranasally may approach those seen after intravenous administration, and small, lipophilic molecules are absorbed more rapidly by this route than are large molecular weight or highly polar substances.[54]

The lung has a large surface area, which is supplied by a dense capillary network, making absorption from this site rapid. Intrapulmonary delivery is the most common route by which substances are administered to fish. With this method, substances are dissolved in a static or flow-through aquatic system into which fish are placed. Material is absorbed rapidly across the gills, which are richly supplied with capillaries, resulting in systemic uptake. Because the entire fish is submerged in the tank, the dissolved substance should not be corrosive or irritating to minimize skin and ocular damage (Figure 5 A and B).

Intrapulmonary delivery to other species is accomplished by either intratracheal instillation or inhalation. Intratracheal instillation is an easier delivery method requiring less specialized equipment and knowledge; however, this route typically is not as effective as are inhalational techniques in ensuring even pulmonary exposure to a substance. Intratracheal instillation involves injecting small volumes of solutions directly into the trachea of anesthetized animals and results in rapid but localized and uneven distribution of material over a relatively small volume of the lung. Volumes administered by the intratracheal route must be small to avoid suffocation. Those performing the intratracheal technique should be competent at intubating the species being treated, or a surgical cutdown can be used to expose the trachea for direct injection.

Inhalational delivery typically uses vapors (for example, volatile anesthetic gases) or aerosols of nebulized particles in solution. Animals are conscious with this delivery method and are restrained with or without a specialized nose mask to optimize delivery. Substances administered by aerosol are deposited by gravitational sedimentation, inertial impaction, or diffusion. As a rule of thumb, larger particles are deposited in the airways by gravitational sedimentation and inertial impaction, whereas smaller particles make their way into distal alveolar spaces by diffusion. Particles less than 3 µm in diameter penetrate the alveoli, and those that are 3 to 5 µm in diameter distribute uniformly throughout the lung. Materials deposited in the oropharynx, proximal trachea, or airways will be transported up the trachea by the mucociliary apparatus, into the mouth, and swallowed with subsequent first pass-effect after absorption.[95] In addition, solvent and propellant effects must be taken into account, because evaporation may cause particles to change in size.

Inhalational administration is a highly complex technique requiring specialized equipment and knowledge, and it is beyond the scope of this article to discuss this methodology in further detail (for more information, see references 99, 113, and 140). Substances administered by this route should be nonirritating to minimize pharyngeal edema, bronchial spasm, anaphylaxis, peracute death, and chronic pulmonary fibrosis. Animals should be conditioned to restraint devices and nose masks prior to experimental initiation.

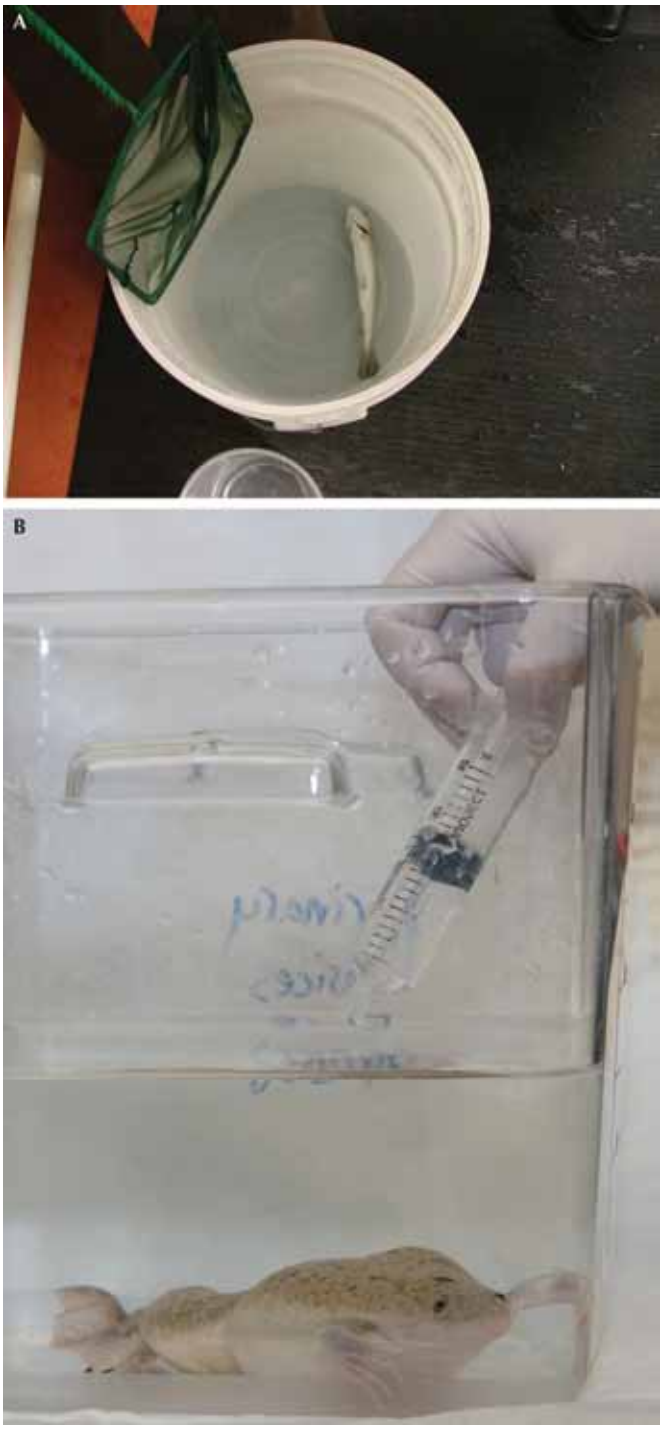

Figure 5. (A) Deep anesthesia of a fish with tricaine methane sulfonate (MS222), achieved by immersion in aqueous solution with the drug. Drug is taken up across the gills during respiration and, to a lesser extent, across the skin. Photo courtesy of Gerry Johnson. (B) Amphibians may be similarly dosed in aqueous chambers as in the *Xenopus laevis* depicted; however, substance uptake is solely through trancutaneous absortion.

Factors to Consider for Substance Administration

There are a number of factors to consider to optimize substance delivery to animals and to minimize complications associated with delivery. Complications may arise from the method of delivery as well as those associated with volume of substance administered, rate of administration, temperature of substance, fasting state, and subject age. Checklists may be developed for use in experimental planning to ensure that all factors have been considered adequately; these factors should also be considered during ethical review of study protocols.[82]

Adverse effects associated with dosing route. Any method of substance administration has inherent potential side effects. For enteral administration, complications depend on the delivery method: force feeding, pilling, delivery in food, or gavage. Oral gavage can result in passive reflux if the stomach is overfilled, aspiration pneumonia, pharyngeal, esophageal, and gastric irritation or injury with stricture formation, esophageal and gastric rupture (Figure 6), and stress.[17,21,40,85] Even when small volumes are used, microaspiration has been suggested to occur in as many as one third of gavaged mice, resulting in detection of radiolabeled particles outside the gastrointestinal tract.[27] Highly viscous substances can affect both the risk of aspiration and the systemic stress response to the gavage procedure, and oily vehicles increase the likelihood of both.[21] Highly viscous substances are difficult to deliver through a small-diameter dosing needle or catheter and should be diluted, whenever possible, for ease of administration.

Habituation to restraint and gavage may reduce struggling and the risk of associated injuries. Without habituation, rats and mice have increased blood pressure and heart rate for as long as 1 h after gavage, as well as higher serum corticosterone levels.[5,17,51] Repeated periods of brief restraint in the week before experimental gavage reduce physiologic responses to the gavage procedure in rats.[135] Generally, rodents adjust quickly to repeated gavage, and corticosterone levels return to baseline after the second day of gavage in mice.[51] Heart rate and blood pressure return to normal by the third day of oral gavage in rats.[92] In addition, elevations in corticosterone levels in mice can be mitigated by dipping the gavage needle in a sucrose solution prior to gavage.[51] Adverse effects may also be reduced by using soft gavage tubing. In rats, changes in heart rate and blood pressure were reduced if soft gavage tubes (Teflon) were used in place of stainless steel dosing needles.[92,141] A potential drawback of soft tubing for oral gavage is the possibility that an animal may bite through the tube. Sedation prior to gavage is not necessarily a refinement and may interfere with pharmacokinetic measurement. Prolonged gastric retention occurred in rats that were anesthetized briefly with halothane for oral gavage.[85] Although not clinically significant in the cited study,[27] incomplete gastric retention of substances can result in variable rates of absorption and immune stimulation, thereby affecting study outcome.

Other methods of enteral administration include sprinkling or mixing the substance with food, food treats, or water, and introducing substances directly into the mouth by using a syringe or pill. When substances are mixed with food or water, dehydration, weight loss, and inefficient drug administration can occur if palatability is poor or if taste aversion develops.[5,82] In addition, gingivitis, tooth decay, and tooth overgrowth can result from diets high in sugar or after the addition of diet softeners, such as polyethylene glycol.[19,62,86,105,122,131] Finally, marked species-, breed-, strain-, and stock-associated variations in food and water consumption exist, so caution must

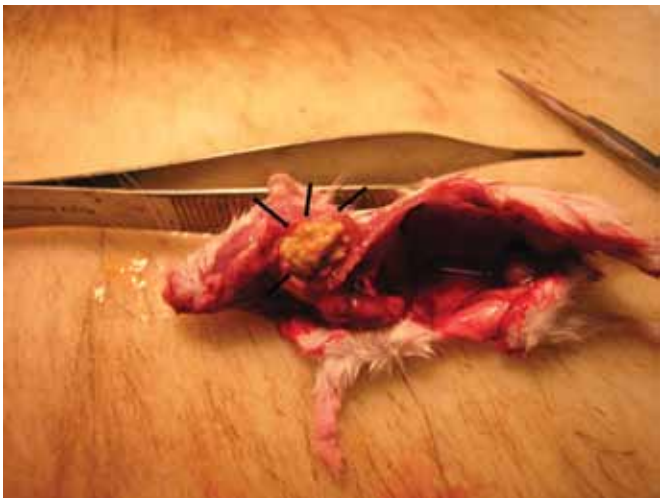

Figure 6. Inadvertent esophageal rupture (arrows) with food contamination and local cellulitis after oral gavage in a mouse. Photo courtesy of David Hobson.

be applied when using standard estimates of consumption of test substance in food or water, to avoid over- or under-dosing animals.[6,35,68,69,70,94] Obtaining accurate body weights prior to dosing and throughout the study is critical.

Syringe feeding or feeding by dropper requires training and timing with relation to natural feeding. This technique can be time-consuming, particularly with studies of more than 20 animals.[5] Not all animals readily acclimate to this method of delivery, and it may not be possible to use this method in studies in which strict accuracy of animal dosing is required. Palatability and taste aversion both can affect this type of delivery, similar to mixing substances into food or water. Absorption directly across the oral mucosa can affect substance pharmacokinetics, an effect that should be considered when oral routes of delivery are employed.[5]

With common parenteral routes of administration complications associated with substance administration include local irritation, pain, infection, and damage to the surrounding tissue depending on the species, method of restraint, route, volume, and substance administered. Generally, administering smaller volumes over multiple injection sites will minimize adverse reactions and can be used for subcutaneous, intramuscular, or intradermal delivery.[82,143] Some substances cause species-specific complications. For example, complete Freund adjuvant can result in pulmonary granulomata in rodents, irrespective of the site of administration.[97] The mechanism underlying this reaction is unknown.

Different routes of parenteral administration may be associated with specific inherent complications. Intramuscular injections can cause muscle necrosis or inflammation of the nerves, resulting in lameness and self-mutilation of the affected area. Pain, necrosis, and self-mutilation of the feet have been reported in response to intramuscular injection in rabbits, rodents, and other species.[14,39,63,118,123,136] With intranasal injections, aspiration pneumonia and suffocation can occur, depending on the volume and formulation of the compound administered.[48] Dosing can be inaccurate, because animals often sneeze in response to intranasal administration. Deep sedation or light anesthesia can be useful adjuncts to this procedure to ensure dosing accuracy.

Intraperitoneal delivery represents a theoretically easy method of introducing material into rodents, but the associated accuracy can be questionable. In one study in rats, 19.6% of in-

traperitoneal injections conducted by competent staff resulted in the material being injected in the gastrointestinal tract, subcutaneously, retroperitoneally, or into the urinary bladder.[67] In addition, the true prevalence of associated complications likely is underestimated, given that many animals are not necropsied after injection. Potential complications include infection, pain, local irritation and chemical peritonitis, formation of fibrous tissue and adhesions within the abdominal cavity, perforation of an abdominal organ, hemorrhage, and respiratory distress or discomfort from administration of too large a volume. Repeated administration can result in a cumulative irritant effect and needle-induced damage.[82]

Complications associated with intravenous delivery methods are more readily apparent than after intraperiotenal delivery. Asepsis is critical, as intravenous administration of contaminated material can result in bacteremia and septicemia (Figures 7 and 8). Extravascular delivery of compounds that are irritating may result in local soft tissue damage, infection, pain, and tissue sloughing. In all species, injection of compounds that contain particulate material or are of low pH that precipitate when mixed with blood can result in vascular occlusion, emboli, and thrombosis of local and distant capillary beds such as those found in the ears, tail, toes, or lungs.[8,50,61,81,98,126] Substances also may induce hemolysis, coagulation, or anaphylaxis when administered intravenously, and these complications may vary depending on the species and the nature of the material being administered. For example, the vehicle Tween 80 causes anaphylaxis when administered intravenously to dogs but not rodents.[44,130] For studies involving multiple venipunctures and injections, those evaluating histologic sections should remember that pulmonary microthrombi or foreign-body granulomas related to shedding of catheter materials or hair fragments are not uncommon histologic findings in chronic infusion studies (Figure 9 A and B).[24,34,81,98]

For continuous infusion studies, the nature of the catheter material may affect irritation at the site of catheterization, and this consideration is important when catheters will be in place long term (see Table 2 of reference 134).[134] In long-term continuous infusion studies, the local concentration of the substance in the cannulated vessel can be higher close to the catheter insertion site for a longer period of time when low flow rates are used. In combination with the mild local inflammation that is typically associated with the implanted catheter, this higher concentration may result in phlebitis and vascular thrombosis.[49] Even substances that do not induce phlebitis when given as a rapid intravenous bolus may cause irritation when given by continuous infusion, because of the background inflammation in catheterized vessels.[49]

Considerations for administration volumes. The volume of solution that can be given varies with species, strain, route, frequency of administration, speed of administration, and composition of the solution. For example, gavage administration of large volumes (20 mL/kg or more) of oil-based formulations is associated with greater toxicity than are aqueous-based formulations.[21] Large volumes (10 mL/kg or more) administered by oral gavage can result in absorption changes associated with rapid shunting of the compounds to the duodenum[88,133,144] or aspiration pneumonia associated with passive reflux of the material into the esophagus.[21] Large volumes given subcutaneously, intramuscularly, and intradermally can result in pain, necrosis, and changes in absorption as well as leakage from the site of injection. Volume of administration also influences the absorption of substances given intraperitoneally,[9,20,33] and larger volumes can result in pain and respiratory distress.[82]

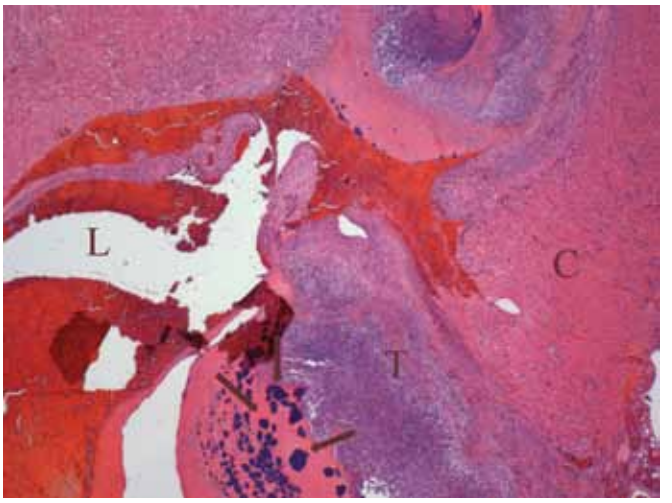

Figure 7. Photomicrograph of atrial thrombosis with secondary bacterial infection and myocarditis in a rat with a chronic indwelling jugular vein catheter. A large septic thrombus (T; bacterial colonies indicated by arrows) is firmly adherent to the endocardium and there is significant infiltration of the myocardium (C) with neutrophils. The thrombus has not entirely occluded the atrium, as a small lumen (L) is present. Hematoxylin and eosin stain; magnification, ×40.

Figure 8. Photomicrograph demonstrating multifocal suppurative encephalitis with perivascular neutrophilic cuffing (arrows) after inadvertent contamination of an indwelling jugular vein catheter in a rat. Hematoxylin and eosin stain; magnification, ×40. Photo courtesy of Leah Schutt.

The volume of substances given intravenously should be calculated carefully, because large volumes can result in immediate distress, pulmonary and cardiac abnormalities, and death. The maximum volume of substances that can be administered depends on dosing rate, in which smaller total volumes should be given for bolus administration (over 1 min or less) than for slow infusion (5 to 10 min) or chronic (continuous) infusion. In rats, large volumes (40 mL/kg or greater) of fluid given as a slow infusion (1.0 mL/min) induce clinical signs of distress, including tachypnea and porphyrin pigment staining, as well histologic evidence of pulmonary changes.[81] Large volumes of substances given by bolus administration caused increased central venous pressure, hemodilution, acid–base disturbances, and diuresis. [30, 6,82]

Generally, best practices for intravenous substance delivery suggest that the blood volume should not acutely be increased

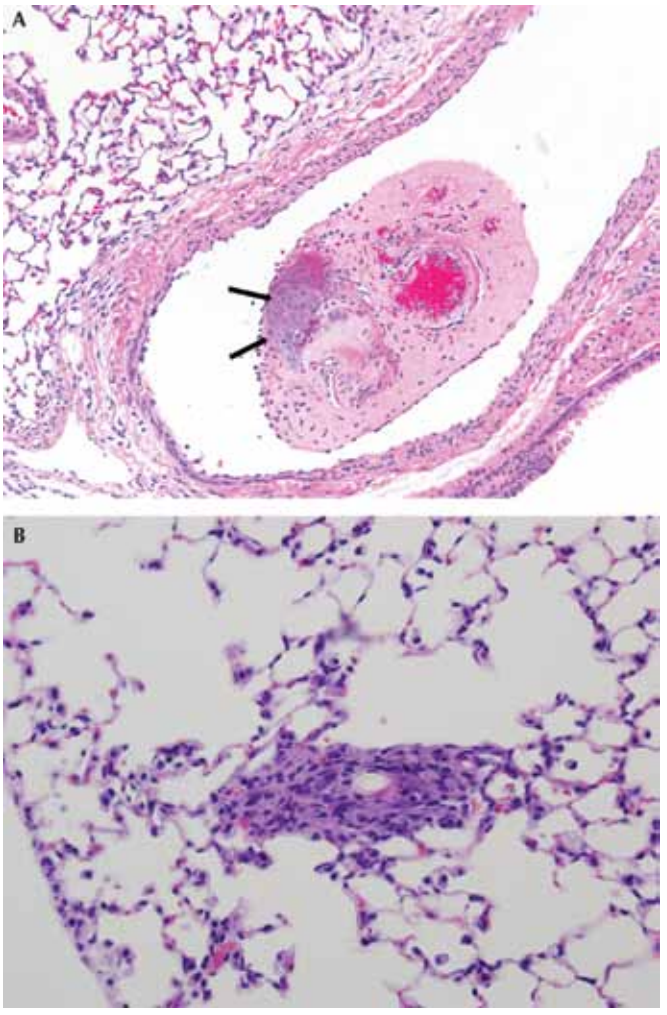

Figure 9. Photomicrographs demonstrating incidental findings. (A) Raft of epithelial cells (arrows) forming microthrombus within a pulmonary vessel of a chronically infused rat. Hematoxylin and eosin stain; magnification, ×200. Photo courtesy of Igor Mikaelian. (B) Hair shaft embedded centrally within pulmonary microthrombus of rat receiving a bolus IV injection. Hematoxylin and eosin stain; magnification, ×400. Photo courtesy of Heather Workman.

more than 4%.[82] Special practices may require larger volumes, such as in hydrodynamic gene delivery, in which volumes ranging from 25% to more than 100% of the circulating blood volume are administered to deliver genes to the liver.[111] In rodents, volumes of 80 to 100 mL/kg body weight typically are injected into the tail vein over 5 s, resulting in expression levels of reporter genes in approximately 40% of the hepatocytes without the use of viral vectors or other carriers (for review, see reference 111). Similar methods attempted in other species have been less successful.[111] Hydrodynamic gene delivery results in swelling of the liver and outflow obstruction, which is believed to be critical to the gene delivery. Although rodents generally survive, this delivery method has considerable side effects. Blood pressure and heart rate drop dramatically (in mice, from 500 to 200 beats per minute), and cardiac electrical abnormalities are observed.[110,145] Other approaches have been used to minimize some of these generalized effects and include inferior vena cava delivery and regional methods, such as delivery directly to particular lobes of the liver or isolated muscle groups.[57,111,112]

With slow or chronic infusion, the composition of the compound, the type of excipient [134] and the age, size, and sex of the recipient can induce potential complications.[134] For example, a chronic infusion rate of 2 to 3 mL/kg/h usually is well tolerated in rats; however, increased fetal toxicity has been reported in pregnant rats at infusion rates exceeding 1 mL/kg/h.[49] In general, chronic infusion results in decreased water consumption and weight loss. Higher rates of slow or chronic infusion (greater than 1 to 1.5 times the total circulating blood volume per 24 h) can result in hemodilution and diuresis.[49]

Considerations for substance temperature during administration. Substances given at or near body temperature will have fewer side effects in animals. The administration of large volumes of cold substances (below body temperature) intraperitoneally or intravenously can induce distress and hypothermia.[96,115] Local absorption rate can be influenced by the temperature of substances administered intraperitoneally.[13,55]

The effect of feeding and fasting prior to substance delivery. The timing of dosing related to the diurnal rhythm of various species may affect absorption and toxicity in animals and introduce unwanted variability, regardless of the route of administration.[12,66] For example, the activity of several hypnotic drugs including ketamine, pentobarbital, propofol, midazolam, and ethanol was tested at different times of the photoperiod, and longer periods of sleeping and anesthesia were observed when drugs were administered in the early active phase (early in the dark phase) as compared with during the early inactive period (early light phase).[109] Aminoglycosides have greater toxicity when administered during the resting period than during the active period.[10]

In addition, fasting and water deprivation affect the absorption of many substances.[29,65,108,139] Duration of fasting will vary with the species involved, in that gastric emptying times vary considerably across species, with mice and rats having significantly shorter gastric emptying times than those of larger animals.[139]

Considerations for subject age when administering substances. Neonatal animals that receive experimental manipulations may undergo maternal rejection. Important techniques to minimize rejection and cannibalism include wearing gloves to mask hand odors, handling all young in a litter, and ensuring that pups are rewarmed before returning them to their dams.[71] Neonatal stress associated with maternal separation for experimental purposes can profoundly affect behavioral indices later in life.[72] However, when care is given to use volumes appropriate for the size, species, and route, neonatal animals can be dosed at early time points. Because they are undergoing rapid growth, establishing accurate body weights prior to administering substances is critical. In rodents, oral gavage can be administered as early as postnatal day 1, although waiting until postnatal day 4 is more common.[83] In neonates, esophageal tissues are very thin, and care must be taken to use appropriately sized equipment and correct technique. Typically for oral gavage of rodent pups, a 30-gauge needle attached to size 10 polyethylene tubing is used. The end is lubricated, and a small amount (up to 10%) of food coloring may be added to the material to be gavaged (typically 0.05 mL for mouse pups) to permit immediate visualization of the substance within the stomach (through the body wall) after administration.[101] Intravenous injections can be started at postnatal day 3 for rodents, and the external jugular and superficial temporal veins are readily accessible sites.[59] Intraperitoneal and subcutaneous injections can be given early, although careful attention to volume is required and intraperitoneal injections are more difficult due to limited space in the abdomen. Administration of irritating substances or large volumes that result in discomfort may influence the outcome of

the study significantly, in that either of these properties could affect the nursing behavior of the pups, causing them to go off feed.[15] In many species, dams lick and consume the feces and urine of their litters, and consideration should be given to the effects on the dam of any test substance or its metabolites that are excreted by the pups.

Conclusion

The administration of substances to animals is a key component of many scientific projects. There are many factors that must be considered by the research team, veterinarian, institutional animal caregivers, and animal ethics committee members to ensure that studies involving experimental administration of substances to animals are planned and conducted appropriately. Careful attention to detail and consideration of the route of administration will contribute to experimental refinement and minimize adverse effects on animals.

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