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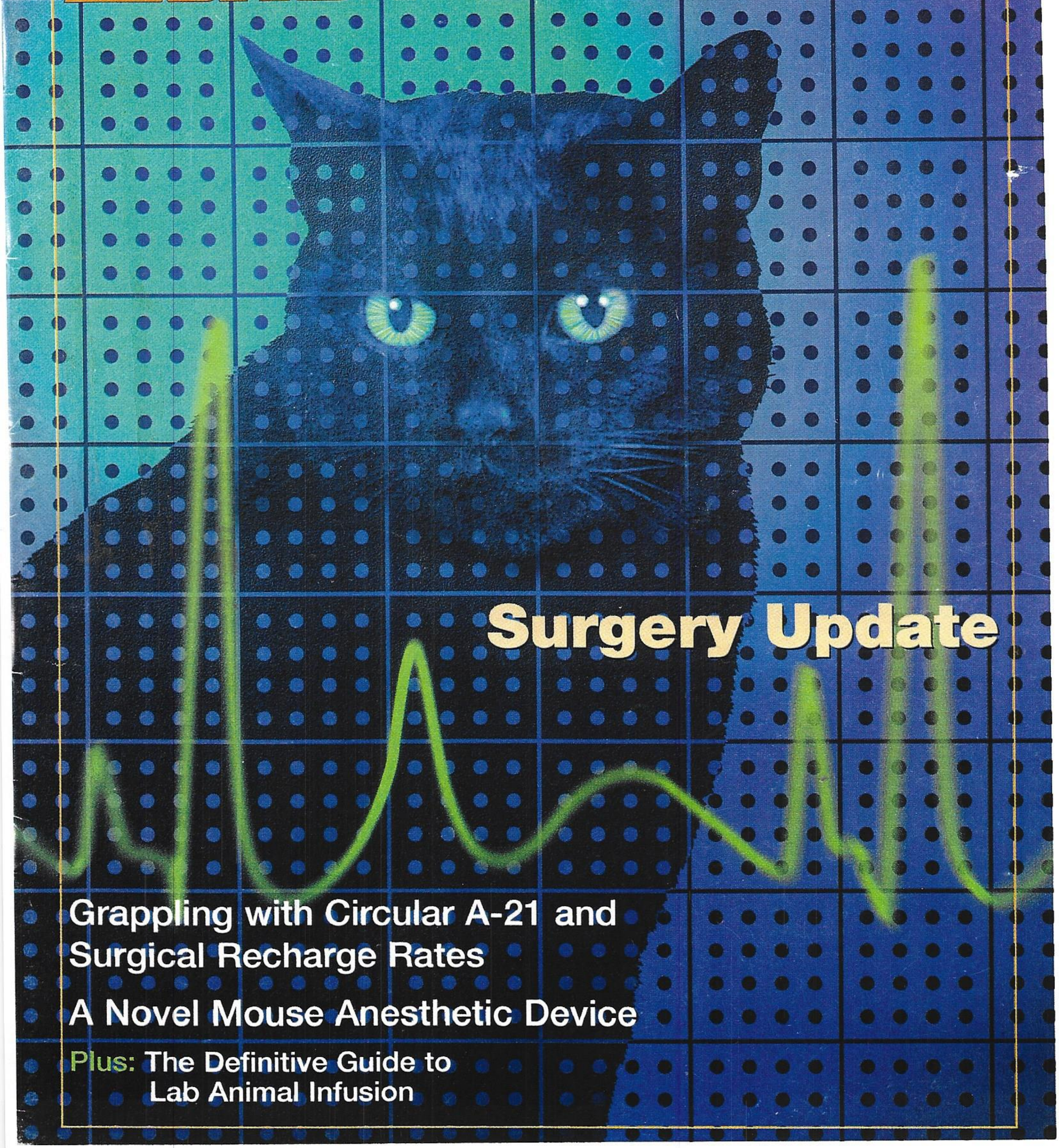

Surgery Update

Grappling with Circular A-21 and
Surgical Recharge Rates

A Novel Mouse Anesthetic Device

Plus: The Definitive Guide to
Lab Animal Infusion

A Nonrebreathing Anesthetic Delivery System for Mice

Dee Horne, LATG, Bryan Ogden, DVM, Jim Houts, BS, and Art Hall, DVM

The authors present a technique for constructing an inexpensive, reliable, and easy-to-use nonrebreathing anesthetic delivery system. The system meets the needs of both the animal care facility and investigators, and is convenient for use under laminar flow hoods.

The authors are affiliated with Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Rd., Portland, OR 97201-3098. Please send reprint requests to Horne at the above address.

Our facility, like many others, has experienced a great increase in the use of valuable and possibly irreplaceable transgenic and knockout mice. Many procedures involving these animals require the use of anesthesia.

While most investigators are accustomed to using ether, methoxyflurane, or injectable anesthetics in mice for a variety of procedures, these options are associated with a number of problems, including: the flammability of ether; waste gas scavenging problems (including toxicity to humans handling methoxyflurane)[1,2]; variability in dosing requirements; prolonged recovery times of injectable anesthetics; adverse cardiopulmonary effects; hypothermia; and unacceptable mortality rates. We decided to convert from these types of anesthesia to isoflurane to achieve better control of the depth of anesthesia, quicker recovery time, and a lower mortality rate[3].

We tried a number of commercially available and homemade nonrebreathing circuits and face masks for use with inhalant anesthetics[4-8]. Investigators had several objections: they were too cumbersome, did not seal properly around the animal's face, or did not allow easy access to the animal's facial area. We decided to develop a standardized device using a "tube-within-a-tube" model that would be both easy to use and safe for administering inhalant anesthesia to mice.

Tube-Within-a-Tube Model

See Table 1 for materials, and Fig. 1 for diagram.

- Cut bubble tubing to a length of 19 cm;
- Cut one end so that a flared orifice is created for the face mask [10 mm outer diameter (o.d.)];
- Attach the nonflared end to a double-sided "male" adapter (19 mm o.d. male x 6 mm o.d.);
- Drill a hole at a 45% angle into the 19 mm adapter;
- Pass a 3 mm o.d. feed tube (approximately 40 cm long) through the hole;
- Pass the feed tube through the adapter into the lumen of the bubble tubing until it reaches 2 cm from the distal, flared end;
- Cut a 1/4" length of Tygon tubing [3 mm inner diameter (i.d.) x 4 mm o.d.], glue it as a cuff over the feed tube, and pull it into the drilled hole of the adapter to anchor the feed tube in place;
- Attach a standard 15 mm male adapter to the proximal end of the feed tube. This connector facilitates hook-up to the anesthesia device.

TABLE 1. Materials.

Parts	Company/Product
Face mask & tube	Bubble tubing (part #8889-224054) 50 foot rolls Oxford Labware, St. Louis, MO
Feed tube	Adult nasal canulae (part #1600) Salter Labs, Arvin, CA
15 mm adapter	3 mm x 15 mm male endotracheal tube adapter (part #00-309-3) Anesthesia Associates, San Marcos, CA
19 mm adapter with 45 degree angle hole pre-drilled	(part #96-0534) VASCO, Bend, OR
Mouse anesthetic tubes	VASCO, Bend, OR
Omnicon f/air™	A.M. Bickford, Inc. Wales Center, NY

One may shorten the overall length of the tube to accommodate special devices used during procedures such as stereotactic surgery. Another option is to cut the bubble tubing at the site of its maximum diameter for use in rat anesthesia. A local

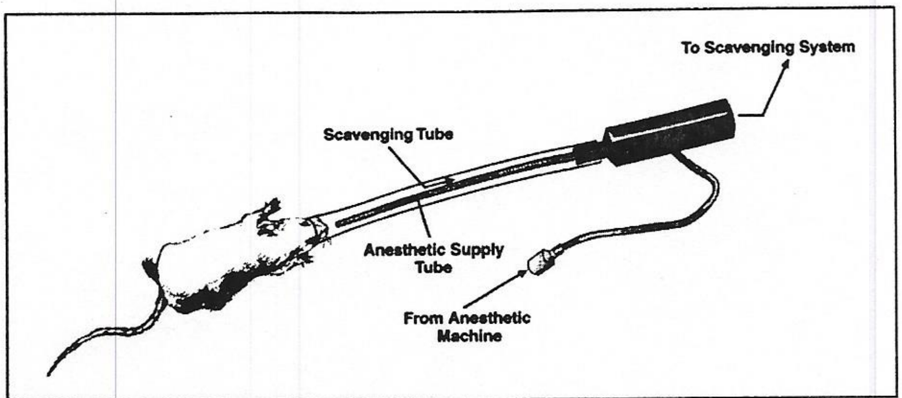

FIGURE 1. Nonrebreathing mouse anesthetic tube.

anesthetic supply company now makes these tubes for us (Veterinary Anesthesia Systems Company, Bend, OR). There are no patent restrictions.

Delivering and Scavenging Isoflurane

We use a table-top anesthetic device, consisting of a standard isoflurane vaporizer and an oxygen delivery system, to deliver the isoflurane. An extension tube (6 mm x 6 ft or any length) with a 15 mm "female" connector is attached to the nonrebreathing tube with a 15 mm male adapter to the 3 mm feed tube.

The scavenging system consists of a 6 ft x 19 mm tube attached to the evacuation side of the nonrebreathing apparatus for collection of waste anesthetic gases into an activated charcoal filter (f/air$^{t/m}$). Excess gas flow forces waste gas down the outer tube for collection in the f/air$^{t/m}$ by passive scavenging. If active or negative pressure scavenging is preferred, interfacing or pressure balance is necessary[9].

Anesthetizing Mice

Method One: Physical Restraint

- Physically restrain the mouse by holding the scruff of the neck and base of the tail;
- Hold the nose of the mouse into the opening of the flared end of the nonrebreathing apparatus;
- Set the anesthetic machine to deliver 0.5-1 liter of oxygen per minute and 4% isoflurane. The mouse will be relaxed enough to release physical restraint within 15-30 seconds;
- The mouse is in a surgical plane of anesthesia within 1 minute. At this time, reduce the isoflurane to 2.5% and adjust as necessary.

To keep the tube from moving, it can be taped to the work surface with a 3" length of tape. Similarly, the operator may hold the mouse in place by lightly taping its feet to the work surface for long procedures. For short procedures, this is not usually necessary. The mouse will stay in position with its nose in the tube unless the operator removes it.

Method Two: Induction Box

Another option is to use an induction box before placing the mouse in the anesthetic tube:

- Attach an f/air$^{t/m}$ to the induction box for passive collection of waste gas;
- Attach the anesthetic supply to a "Y" connector and tubing with stop valve controls for delivery to both the induction box and nonrebreathing tube separately or simultaneously (Fig. 2).

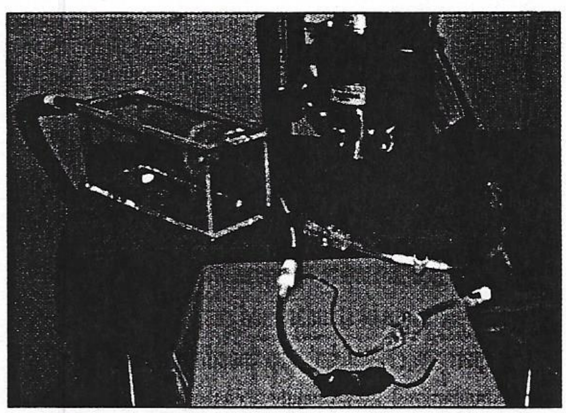

FIGURE 2. Portable anesthetic machine with nonrebreathing tube and induction chamber.

We have had the work area and the operator monitored for exposure to escaped gas during typical anesthetic procedures. The level of waste gas was within the allowable level for operator exposure. The only time that gas levels registered over the acceptable level was when the lid to the induction box was opened to remove the mice. We are currently working on methods of scavenging waste anesthetic gases from the induction box when it is open.

Conclusion

We have found the use of this all-in-one mask and nonrebreathing anesthetic delivery system to be safe, reliable, easy to use, and inexpensive. We have safely anesthetized over 1,000 mice at our facility for durations of a few minutes to several hours, and have easily trained personnel to use the system. An added benefit is that the mouse anesthetic tube used with a table-top anesthetic machine is portable and easily sanitized, using gas or cold sterilants. Steam sterilization can also be used, but it does turn the tubing opaque. In addition, the anesthetic machine can be as far away from the work surface as is reasonably desired. This is especially useful in a transgenic mouse facility when anesthetizing mice under a laminar flow hood. The only item that needs to be placed in the hood is the mouse anesthetic tube, f/air$^{t/m}$ canister, and a small portion of connecting tubing that runs from the mouse anesthetic tube to the anesthesia machine. The anesthetic machine can even be outside of the procedure room when used with extension tubing.

Currently, we maintain several portable anesthetic machines with precision isoflurane vaporizers, induction boxes, and mouse anesthetic tubes for use by our investigators in a variety of settings and procedures throughout our institution.

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