Journal of Surgical Research **122,** 240–248 (2004)
doi:10.1016/j.jss.2004.05.016

Videoendoscopic Endotracheal Intubation in the Rat: A Comprehensive Rodent Model of Laparoscopic Surgery[1,2]

Joseph M. Fuentes, M.D., Eric J. Hanly, M.D., Sharon L. Bachman, M.D., Alexander R. Aurora, M.D., Michael R. Marohn, D.O., and Mark A. Talamini, M.D.[3]

Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted for publication March 15, 2004

Background. **Peritoneal absorption of CO_2 during abdominal insufflation in laparoscopy may disrupt the acid–base equilibrium and alter the physiological response to stress. Current nonventilated rodent models of laparoscopy do not manage the CO_2 load of pneumoperitoneum, but ventilated surgical rodent models are invasive (tracheotomy) and may independently induce the inflammatory response.**

Materials and methods. **A comprehensive rodent model of laparoscopy was developed. Rats were randomized to receive anesthesia alone, anesthesia plus CO_2 pneumoperitoneum, or anesthesia plus CO_2 pneumoperitoneum with videoendoscopic intubation and mechanical ventilation. Arterial blood-gas analysis was performed at baseline and after 30 min of intervention.**

Results. **Baseline pH, pCO_2, and HCO_3^- arterial blood gas parameters were normal for all rats. After 30 min, pCO_2 and pH changed slightly but remained normal among rats receiving anesthesia alone (pCO_2 = 46.5 ± 1.9; pH = 7.365 ± 0.009) whereas animals receiving anesthesia plus CO_2 pneumoperitoneum that were dependent on spontaneous respiration for ventilation developed significant hypercarbic acidosis (pCO_2 = 53.2 ± 1.9, $P < 0.05$; pH = 7.299 ± 0.011, $P < 0.001$). This acidosis was completely corrected with increased minute ventilation in intubated rats receiving mechanical ventilation (pCO_2 = 36.8 ± 1.5, $P < 0.001$; pH = 7.398 ± 0.011, $P < 0.001$).**

Conclusions. **CO_2 pneumoperitoneum induces significant hypercarbic acidosis in nonventilated rats. Noninvasive endotracheal intubation is feasible in the rat with videoendoscopic assistance. Our noninvasive rodent model of laparoscopic surgery controls for anesthesia- and capnoperitoneum-related acid–base changes and provides an environment in which the biological response to pneumoperitoneum can be studied precisely.**

***Key Words*: carbon dioxide; CO_2; laparoscopy; pneumoperitoneum; animal models; rats; acidosis; intubation; endotracheal intubation; minimally invasive surgical procedures.**

INTRODUCTION

As the principles of minimally invasive surgery have gained acceptance throughout the surgical community during the past two decades, laparoscopy has revolutionized the field of general surgery. Since the first laparoscopic cholecystectomy was performed in 1985, laparoscopic surgery has become preferred over open surgery for a variety of surgical conditions. The advantages of laparoscopic surgery over conventional surgery include decreased postoperative pain, decreased tissue trauma, shorter length of stay, faster recovery, more rapid return to oral intake, and less adhesion formation [1–5]. Although a great deal has been learned about the advantages of minimal access surgery, the tremendous potential this methodology has to offer is only beginning to be appreciated [6–9].

Many of the hemodynamic, immunological and hormonal effects of capnoperitoneum have been well characterized in both animal and human studies [10–19]. CO_2 pneumoperitoneum has been implicated in the release of cortisol, epinephrine, norepinephrine, renin, prostaglandins, and vasopressin, all leading to in-

[1] This work was supported by RO1-GM062899-02, National Institutes of Health, Bethesda, MD.

[2] All animal procedures were performed as part of a protocol reviewed and approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Protocol #RA01M445.

[3] To whom correspondence and reprint requests should be addressed at Department of Surgery, The Johns Hopkins Hospital, 600 North Wolfe Street, Blalock 665, Baltimore, MD 21287-4665. E-mail: talamini@jhmi.edu.

0022-4804/04 $30.00

creases in systemic vascular resistance [16, 18]. Regarding the immunological effects of laparoscopy, a number of studies from our group and others indicate that CO_2 pneumoperitoneum may alter the immunological profile in surgical patients [20–25]. The common denominator between the changes observed within these different physiological systems is the CO_2 pneumoperitoneum. To study the plethora of physiological differences between minimally invasive and conventional surgery, laparoscopy is becoming increasingly important in experimental animal models.

One of the main physiological perturbations that can occur during laparoscopic surgery is hypercarbic acidosis induced by the absorption of carbon dioxide during pneumoperitoneum. Although the direct depressive effects of acidemia on the myocardium may predominate over the indirect effects of hypercapnic stimulation of the autonomic nervous system [26], a number of studies have shown that hypercapnia has mixed effects on cardiac function [27–30]. This notion is consistent with studies in laparoscopy that show that CO_2 pneumoperitoneum leads to a reduction in stroke volume, tachycardia, and normal cardiac index with increased systemic and pulmonary vascular resistance. This is just one example. In fact, because of the pH sensitivity of many enzymatic functions, virtually every biological system is affected in some fashion by changes in systemic acid–base status. However, current animal studies do not always reflect the clinical conditions that are being "modeled." This is especially true in studies of laparoscopy in which animals are not mechanically ventilated.

In this work we address the magnitude of the effects of CO_2 pneumoperitoneum on acid–base status in anesthetized, nonventilated models of laparoscopic surgery. Furthermore, we have developed a comprehensive model for laparoscopic surgery in the rat that controls for CO_2 pneumoperitoneum-induced hypercapnic acidosis. We submit our experience with rodent models of laparoscopy anticipating that our recommendations will enable investigators to improve experimental planning for rodent models of laparoscopy.

MATERIALS AND METHODS

Effects of CO_2 Pneumoperitoneum on the Acid–base Equilibrium

In this article, 35 rats were randomized into three groups: 1) anesthesia alone (control, $n = 15$); 2) anesthesia plus carbon dioxide pneumoperitoneum ($n = 10$); and 3) anesthesia plus carbon dioxide pneumoperitoneum with videoendoscopic intubation and mechanical ventilation ($n = 10$). Baseline and 30-min arterial blood gas samples were collected from each animal through a femoral arterial line.

Animal Care

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing approximately 250–350 g were housed in plastic cages where standard chow and water were available *ad libitum*. Animals were acclimatized to their environment for 3-5 days after arrival and were then fasted for 16 h prior to procedures. The animal housing environment maintained a 12-h light/dark cycle, a temperature of 72°F, and a humidity ranging between 30% and 70%. After completion of the experiment, all rats were euthanized via anesthetic overdose. All animal procedures were performed on a protocol approved by Johns Hopkins Medical Institutions Animal Care and Use Committee (IACUC; in this case, Protocol #RA01M445).

Videoendoscopy-Assisted Endotracheal Intubation

Before intubation, rats were preanesthetized rapidly in a 15 × 15-inch glass jar using vaporized isoflurane ("IsoFlo" Abbot Laboratories, North Chicago, IL). Spontaneously breathing anesthetized animals were placed supine on a 20 × 15 cm Styrofoam board and restrained with adhesive tape (Fig. 1). Anesthesia was maintained by delivering 3% of vaporized isoflurane thru a nose cone. With the neck extended, oppositional retraction of the superior and inferior incisor teeth was achieved using 1-cm "teeth loops" made from 3-O silk suture that were fixed to the Styrofoam board with needles (Fig. 2A). Use of this simple combination of Styrofoam, suture, and needles affords an inexpensive, completely adjustable rodent retraction system (useful for much more than jaw retraction). Inferior and lateral retraction of the tongue was achieved using a penetrating towel clamp. A rigid 30-degree 3-mm endoscope (generously donated by Karl Storz Endoscopy, Germany) was used to visualize the epiglottis, larynx, and vocal cords viewed on the monitor (Fig. 2B). Using its previously filed, blunted and bent metal insertion needle as a stylet, a 14-gauge intravenous catheter was inserted through the mouth in parallel with the scope. The catheter was carefully advanced between the vocal cords and into the trachea (Fig. 2C). The stylet was then removed and the catheter was secured to the snout with tape. The catheter was connected to a Harvard Rodent ventilator model 683, and during procedures requiring CO_2 pneumoperitoneum, ventilated with a min ventilation of approximately 850/ml/kg/min (approximately 250 ml/min for a 250–350 g rat).

Arterial Line Insertion

The right groin of the rat was shaved and prepped with 70% isopropyl alcohol. The lower extremities were taped in extension, and a 1-cm groin incision was made over the palpated pulse of the femoral artery (Fig. 3A). Dissection was conducted down to the muscular layer until femoral neurovascular complex was identified. A 1-cm segment of the femoral artery was bluntly dissected free from the vein, nerve, and associated connective tissue. A 3-O silk ligature was used to ligate the distal end of the isolated portion of artery. This suture was then used to apply gentle retraction along the axis of the extremity to straighten the vessel in preparation for catheterization. A second 3-O silk ligature was "looped" around the proximal portion of the isolated artery and a single throw "air knot" was made but not cinched. A small lightweight forceps was used to "tag" this second ligature and was draped over the animal such that the weight of the clamp produces slight upward traction on the vessel, thus collapsing the opposing vessel wall and preventing hemorrhage when the arteriotomy was performed. Castroviejo microscissors were used to make a small arteriotomy (approximately 30% of the circumference perpendicular to the axis of the vessel) between the distal ligation and the proximal loop (Fig. 3B). A mini catheter introducer made from a 25-gauge needle with the tip bent backward on itself was used to lift up the top arterial wall, and the beveled end of an arterial catheter made from polyethylene tubing with an outer diameter of 0.965 mm and an internal diameter of 0.58 mm flushed with heparinized saline was gently introduced approximately 1 cm into the artery (Fig. 3C). The "air knot" of the proximal ligature was then slid down over the catheterized portion of the vessel and was tied, thus securing the catheter in the vessel.

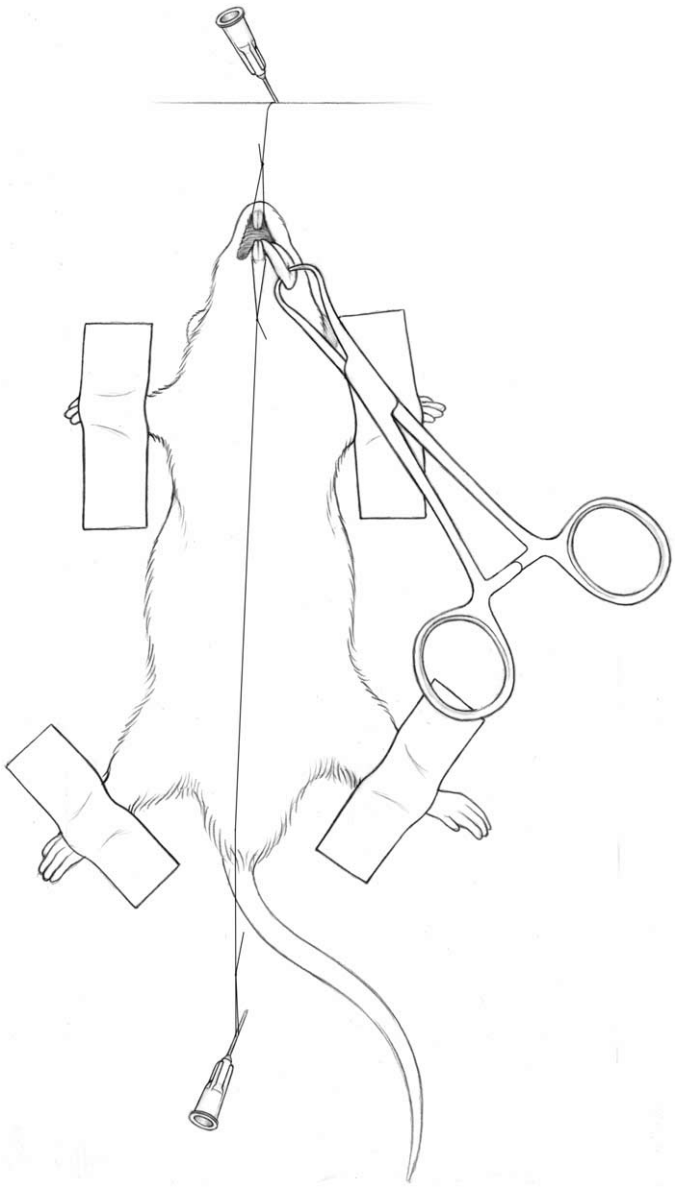

FIG. 1. Basic set up for videoendoscopic intubation. Spontaneously breathing rats anesthetized with volatile isoflurane delivered through a nose cone (not shown) are placed supine on a Styrofoam board. Oppositional retraction of the superior and inferior incisor teeth is achieved using 3-O silk suture loops. The tongue is retracted inferiorly and laterally using a penetrating towel clamp.

Laparoscopic Techniques

The abdominal wall was shaved and prepped with 70% isopropyl alcohol. Insufflation was achieved using a laparoscopic insufflator (Olympus) connected to a simple 20-gauge angiocatheter acting as a Veress needle. A pneumoperitoneum of 4 mmHg was established with gas flow rates being limited by the diameter of the catheter to less than 1 liter/min. When performing pneumoperitoneum experiments that require intra-abdominal tissue manipulation, we connect the insufflator to the side port of the endoscope sheath. A 1-cm sub-xyphoid midline abdominal incision is made in the skin followed by a deep 5-mm abdominal wall and peritoneal incision. The Hasson technique is used to introduce a 4-mm endoscopic sheath (Storz) into the abdominal cavity, which is secured using a 3-O silk purse string stitch. Gas flow rates are maintained in the 1–2 liter/min range. A rigid 0-degree 3-mm endoscope (Storz) is connected to a fiberoptic light source and video camera (Olympus America Inc., Melville, NY) to visualize the peritoneal cavity on a 13-inch color video monitor (Olympus). We use six Kleinsasser endolaryngeal microsurgery instruments (Storz): two straight micro grasping forceps, one curved micro grasping forceps, one micro cupped jaw forceps, one miniature scissors, and one needle holder. All instruments have a working length of 20 cm and are introduced directly through small holes in the abdominal wall (without trochars) created by puncture with a 14-gauge angiocatheter. The holes are small enough to allow maintenance of the pneumoperitoneum.

Experimental Set-up Special Considerations

In our comprehensive model, the ideal gas for rodent laparoscopic surgery is carbon dioxide because of its properties of being odorless, colorless, stable, buffered in the blood, eliminated by the lungs, having a low risk of venous gas embolism, and being nonflammable/nonexplosive. Furthermore, CO_2 pneumoperitoneum has been shown to have beneficial immune-modulating effects [25, 31]. The amount of pneumostatic pressure that should be used to insufflate the rat abdomen warrants particular consideration. There is increasing evidence in the literature that maintaining the lowest possible pressure to create the CO_2 pneumoperitoneum is the best option, mainly because fewer deleterious hemodynamics changes occur at lower pressures [32–40]. In our experience, pressures of 3–4 mmHg are ideal for performing complex laparoscopic surgical tasks in the rat. Also of note is the importance of fluid support: we use a 10-ml/kg bolus of 0.9% NaCl administered subcutaneously in the scruff of the neck in the immediate post-operative period. Maintaining body temperature with a heating device such as a simple heat lamp or more complex forced air warming devices is important to prevent hypothermia. Hypothermia increases both morbility and mortality under critical conditions, and can have significant effects on gross and molecular physiology [41–46].

Description of Statistical Methods

The one-way analysis of variance test was used to detect general differences in arterial blood gas parameters between groups. To elucidate specific significances in these parameters between groups, multiple pairwise comparisons were performed using Tukey's test. All results were presented as the mean ± the standard error of the mean. Differences between groups were considered significant when $P < 0.05$. Analysis was performed using SigmaStat 3.0 (SPSS Inc., Chicago, IL) software.

RESULTS

The learning curve associated with videoendoscopy-assisted endotracheal intubation was not insignificant. After trying this procedure on 10–15 "practice" animals, the overall success rate for operators in our laboratory was between 80% and 90%. The "failures" were caused largely by the fact that animals with even relatively mild iatrogenic laryngeal trauma had significant respiratory difficulty postoperatively. Thus, because our laboratory also performs survival surgery using this model, we limit the intubation process to three attempts. Difficulties with intubation are primarily related to animal movement due to laryngeal stimulation in unparalyzed animals. For this reason, the concentration of vaporized isoflurane was in-

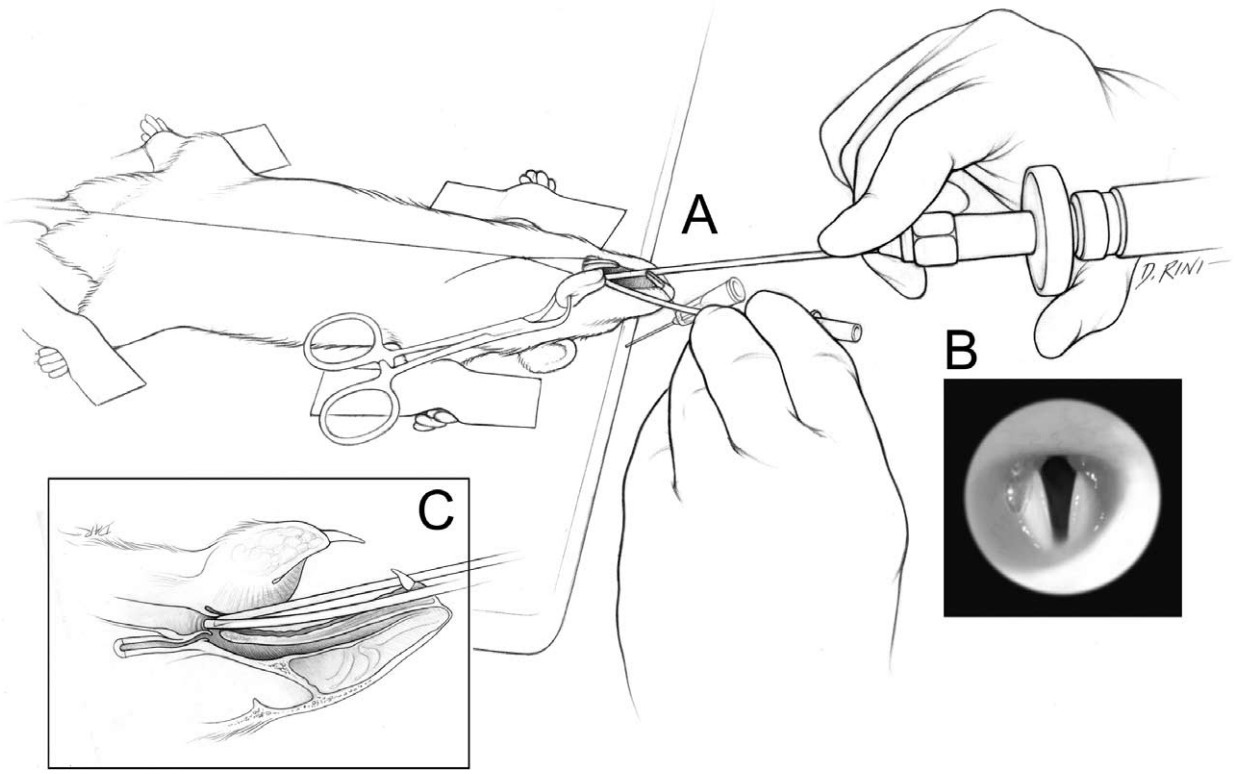

FIG. 2. Videoendoscopic intubation in the rat. With the rat ergonomically positioned, the operator easily introduces the endoscope and catheter simultaneously into the open jaws of the rat (A). A 3-mm rigid endoscope is used to visualize the epiglottis, larynx, and vocal cords on the monitor (B). A 14-gauge angiocatheter with its interior needle being used as a stylet is inserted into the trachea in parallel with the scope and threaded between the vocal cords under direct visualization (C).

creased (4–5%) briefly immediately prior to intubation. In this study, two failed intubation attempts occurred. These rats were replaced with new animals.

The learning curve associated with our method of arterial cannulation plateaus much more quickly, and after 10-15 "practice" animals, the operators in our laboratory achieve a near-100% success rate. All 35 femoral artery catheterization attempts were successful in this study. All attempts at creating a pneumoperitoneum in this study were successful as well.

Arterial blood gas parameters were measured in rats at baseline and after 30 min of anesthesia alone, anesthesia plus carbon dioxide pneumoperitoneum, or anesthesia plus carbon dioxide pneumoperitoneum with mechanical ventilation. Animal weight (mean = 317 g), intraabdominal insufflation pressure was 4 mmHg, and the total duration of all procedures (mean = 118 min) were comparable between groups. Baseline pH, pCO_2, and HCO_3^- arterial blood gas parameters were normal for all rats (Table 1). After 30 min of anesthesia alone pCO_2 increased slightly (from 42.6 ± 1.1 to 46.5 ± 1.9) and pH decreased slightly (from 7.399 ± 0.009 to 7.365 ± 0.009) thus reflecting the mild respiratory suppressive effects of isoflurane anesthesia. After 30 min of anesthesia and CO_2 pneumoperitoneum, rats dependent on spontaneous respiration for ventilation developed significant hypercarbic acidosis with a mean pCO_2 of 53.2 ± 1.9 ($P < 0.05$ *versus* anesthesia alone) and a mean pH of 7.299 ± 0.011 ($P < 0.001$ *versus* anesthesia alone). This acidosis was completely corrected with increased min ventilation in the intubated rats receiving mechanical ventilation (mean pCO_2 = 36.8 ± 1.5, mean pH = 7.398 ± 0.011, $P < 0.001$ for both *versus* spontaneous ventilation anesthesia + CO_2 pneumoperitoneum).

DISCUSSION

Laparoscopic surgery has become the gold standard for a variety of surgical indications [8], with laparoscopic cholecystectomy being the most widely and rapidly accepted throughout the world [47–51]. Other organs routinely being approached via the laparoscope include the adrenal glands [52], the esophagus for antireflux surgery [53–56], and the spleen [57, 58] to mention a few. Continued advancement of the field of laparoscopic surgery depends on the use of animal models. The rodent model of laparoscopy is being applied to investigations spanning the spectrum from clinical to basic science. Everything from enteric resection and anastomosis, gastric bypass, splenectomy, and nephrectomy [59] to surgically- and endotoxin-induced

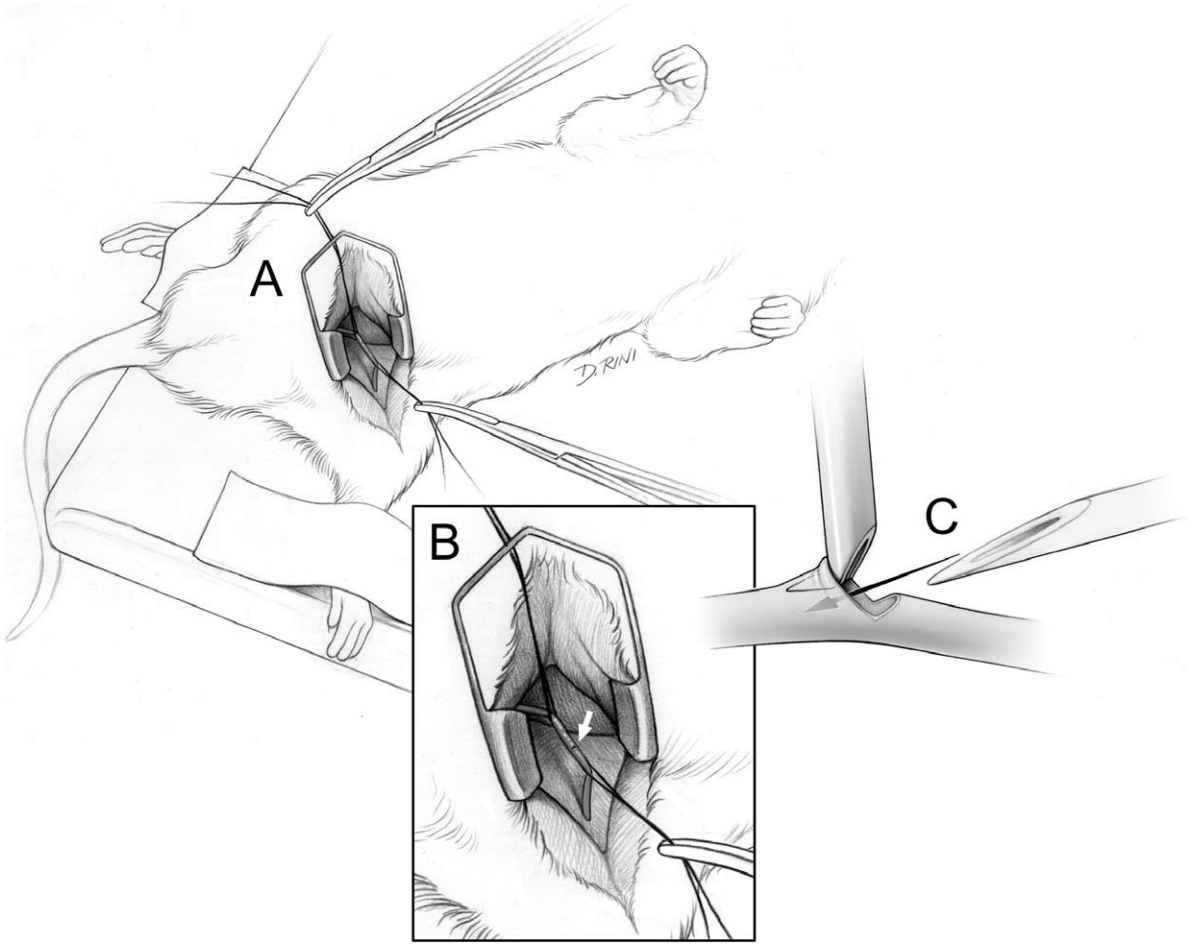

FIG. 3. Femoral arterial cannulation in the rat. The lower extremities are taped in extension, a groin incision is made over the femoral artery, and a 1-cm segment of artery is bluntly dissected free (A). Micro-scissors are used to make a small arteriotomy between the distal arterial ligation and the proximal loop (B). A mini catheter introducer is used to lift up the top arterial wall, and the beveled end of an arterial catheter is gently introduced into the artery (C).

models of sepsis is being performed in rats and mice applying the laparoscopic paradigm. Only a few publications describe the use of such models for specific minimal access surgery applications [60, 61].

We have developed an effective rodent model of minimally invasive abdominal surgery that we anticipate will serve as a guide for investigators in the surgical research community interested in studying the physiology, immunology, and outcomes unique to the laparoscopic approach. This comprehensive laparoscopic

TABLE 1

Arterial Blood Gas Parameters before and after Intervention Among Rats Receiving: Anesthesia Only, CO_2 Pneumoperitoneum, or CO_2 with Mechanical Ventilation

	Baseline			After 30 min		
	pH	pCO_2	HCO_3^-	pH	pCO_2	HCO_3^-
Anesthesia	7.399 ± 0.009	42.6 ± 1.1	25.7 ± 0.4	7.365 ± 0.009	46.5 ± 1.9	25.9 ± 0.9
CO_2 Pneumo	7.409 ± 0.006	39.3 ± 0.9	24.2 ± 0.4	7.299 ± 0.011*	53.2 ± 1.9†	25.5 ± 0.5
CO_2 Pneumo + Vent	7.419 ± 0.017	38.2 ± 2.5	23.9 ± 0.9	7.398 ± 0.011	36.8 ± 1.5	22.1 ± 0.7

Note. Values expressed as mean ± SEM ($n = 15$ for anesthesia group, $n = 10$ for CO_2 Pneumo and CO_2 Pneumo + Vent groups). Statistical analysis via one-way ANOVA (analysis of variance).

* $P < 0.001$ vs. both Anesthesia pH and CO_2 Pneumo + Vent pH.

† $P < 0.05$ vs. Anesthesia pCO_2 and $P < 0.001$ vs. CO_2 Pneumo + Vent pCO_2 after 30 min.

surgery rat model addresses important issues such as anesthesia- and pneumoperitoneum-induced acid–base effects, circulatory system access and fluid support, pneumoperitoneum insufflation pressure and gas, and choice of anesthetic agents.

Because of the ease of using anesthetic regimens that do not cause severe respiratory suppression in rodent experimentation, mechanical ventilation is not performed in the majority of animal surgical experiments. Although a number of minimally invasive techniques for gaining airway access in rodents have been described, tracheotomy is used most commonly. This method is excellent for nonsurvival experiments and for studies in which the addition of the tracheotomy would likely not directly affect the outcome. But because those investigating laparoscopy are concerned about the invasiveness of their surgery, these researchers usually choose to avoid tracheotomy. Furthermore, because many of the noninvasive means of intubation in rodents require specialized skill and/or equipment to employ, the majority of laparoscopy researchers elect not to mechanically ventilate their animals at all, but rather depend on anesthetic regimens believed to have minimal respiratory suppressive effects.

Our findings corroborate those of other investigators indicating that carbon dioxide pneumoperitoneum can have profound effects on acid–base equilibrium in nonventilated animals [30, 62, 63]. We found that after only 30 min of abdominal insufflation with CO_2, rats anesthetized with an agent touted as possessing only mild effects on respiratory drive at low doses (isoflurane) develop statistically and clinically significant accumulation of CO_2 (pCO_2 = 53.2) and acidosis (pH = 7.299). We have shown that adding endotracheal intubation with an appropriate min ventilation restores acid–base equilibrium completely (pCO_2 = 36.8, pH = 7.398). It is our concern that, because the enzymatic function of many proteins can be profoundly affected by changes in pH, models that allow the accumulation of CO_2 produce an environment that may influence outcomes and are thus not representative of the clinical scenarios that these models are intended to mimic. Mechanical ventilation should be considered in laparoscopy experimentation whenever it is possible that systemic acid–base balance could affect the results of the experiment.

In the current study we have demonstrated that endotracheal intubation can be performed in the rat by a single operator with videoendoscopic assistance. When animals are deeply anesthetized or sedated, this method consistently provides excellent exposure of the vocal cords, thus making the technique relatively easy to learn. A number of other methods for endotracheal intubation in rodents have been described in the literature. Rigid cannula intubation was first reported by Jaffe and Free in 1973 [64]. Pena and Cabrera reported improved visualization of the vocal cords by enlisting the aid of a surgical microscope [65], and Alpert *et al.* reported a simple means of direct laryngoscopy emphasizing the importance of a good light source [66]. Although rarely used today, Stark *et al.* described a method for blind intubation [67]. Weksler *et al.* reported intubation using an otoscope [68], and Linden *et al.* described the use of a modified laryngoscope to intubated rats [69]. Worthley *et al.* were the first to report use of a fiber-optic scope to aid in the intubation of rabbits [70]. Recently, Vergari *et al.* reported intubation in mice using arthroscopic equipment very similar to that used in our laboratory [71]. The fact that so many different methods for rodent intubation have been described probably indicates that there is no one "best" way. Our method of using our rigid 3-mm laparoscope as a laryngoscope works well and provides our laboratory the additional advantage of being cost-effective. By leveraging previously acquired videoendoscopic equipment, laparoscopic surgery researchers can improve the validity of their experiments at very low cost.

In the current study, femoral arterial catheters were placed to allow measurement of arterial blood-gas parameters. In our model in general, the placement of a femoral arterial line also facilitates blood sampling at different time points during kinetic studies and measurement of hemodynamic parameters. Our experience in using this model in survival experiments has shown that when the femoral nerve is uninjured during the arterial dissection, rats have a fully functional limb post-operatively with no evidence of cyanosis. Therefore, investigators using this technique do not need to be overly concerned about distal limb ischemia when ligating the rat femoral artery. For studies that necessitate repeated intravenous drug delivery or blood sampling over a number of weeks, implantable catheters with subcutaneous access ports are a good option. Numerous investigators have reported on the use of these devices, and some have used them for experiments lasting over a month [72, 73]. In our experience, placement of a femoral arterial catheter in a 250- to 350-g rat takes between 3 and 5 min among individuals who have mastered the technique during the course of 10–14 attempts (5–7 rats). A few technical points discovered through our experience with this simple but delicate procedure may be of interest to the investigator who plans to use this model: 1) We have found that dipping the tip of the catheter in mineral oil eases the insertion of the catheter a great deal. 2) Applying gentle steady tension on the vessel by retracting (along the axis of the limb) the distally ligated artery using its 3-0 silk suture facilitates a simple and atraumatic catheter tip insertion. 3) The use of a homemade "catheter introducer" (described earlier) placed through the arteriotomy greatly facilitates the opening of the vessel lu-

men. 4) Minimizing the length of vessel dissection to 1 cm or less prevents the artery from being a "moving target" as the connective tissue stabilizes the vessel.

We limited blood-gas analysis to that of the arterial blood in both spontaneously breathing and mechanically ventilated rats undergoing CO_2 pneumoperitoneum. Kuntz *et al.* studied in rats the pH in three different compartments (arterial blood, subcutaneous fat tissue, and intra-abdominal) during CO_2 pneumoperitoneum in spontaneously breathing animals only, and found that pressures above 6 mmHg had dramatic pH changes in all compartments, while pressures below this level had minimal changes in all three compartments [74]. Future experiments should evaluate such parameters in the portal system where one might expect an even greater degree of acidemia from absorbed CO_2 that persists to some degree even after the systemic arterial pH has been corrected via mechanical ventilation.

In summary, we have demonstrated that noninvasive endotracheal intubation is feasible and beneficial in the rat with videoendoscopic assistance. Furthermore, our study confirms that CO_2 pneumoperitoneum induces significant hypercarbic acidosis in non-ventilated rats, but that mechanical ventilation can completely compensate for this accumulation of CO_2. Our comprehensive rodent model of laparoscopic surgery addresses circulatory system access and fluid support, pneumoperitoneum insufflation pressure and gas, choice of anesthetic agent, and animal thermoregulation. Furthermore, our model controls for anesthesia- and pneumoperitoneum-related acid–base changes and provides an environment in which the biological response to pneumoperitoneum can be studied precisely.

ACKNOWLEDGMENTS

We thank Karl Storz Endoscopy, Germany, who provided laparoscopic instruments. Special thanks to David Rini, Associate Professor, Art as Applied to Medicine, Johns Hopkins University, who created all illustrations for this paper.

REFERENCES

1. Barkun, J. S., Wexler, M. J., Hinchey, E. J., *et al.* Laparoscopic versus open inguinal herniorrhaphy: preliminary results of a randomized controlled trial. *Surgery* **118:** 703, 1995.
2. Buanes, T., and Mjaland, O. Complications in laparoscopic and open cholecystectomy: a prospective comparative trial. *Surg. Laparosc Endosc.* **6:** 266, 1996.
3. Glaser, F., Sannwald, G. A., Buhr, H. J., *et al.* General stress response to conventional and laparoscopic cholecystectomy. *Ann. Surg.* **221:** 372, 1995.
4. Jatzko, G. R., Lisborg, P. H., Pertl, A. M., *et al.* Multivariante comparison of complications after laparoscopic cholecystectomy and open cholecystectomy. *Ann Surg.* **221:** 381, 1995.
5. Tanovic, H., and Mesihovic, R. Differences in the postoperative course and treatment in patients after laparoscopic and standard cholecystectomy. *Med. Arh.* **57:** 219, 2003.
6. Vierra, M. Minimally invasive surgery. *Annu. Rev. Med.* **46:** 147, 1995.
7. Hunter, J. G. Minimally invasive surgery: the next frontier. *World Surg.* **23:** 422, 1999.
8. Himal, H. S. Minimally invasive (laparoscopic) surgery; The future of general surgery. *Surg. Endosc.* **16:** 1647, 2002.
9. Fuchs, K. H. Minimally invasive surgery. *Endoscopy* **34:** 154, 2002.
10. Ivankovich, A. D., Miletich, D. J., Albrecht, R. F., Heyman, H. J., and Bonnet, R. F. Cardiovascular effects of intraperitoneal insufflation with carbon dioxide and nitrous oxide in the dog. *Anesthesiology* **42:** 281, 1975.
11. Motew, M., Ivankovich, A. D., Bieniarz, G., Albrecht, R. F., Zahed, B., and Scommegna, A. Cardiovascular effects and acid–base and blood gas changes during laparoscopy. *Am. J. Obstet. Gynecol.* **113:** 1002, 1973.
12. Constantino, G. N., Vincent, G. J., Mukalian, G. G., and Kleifoth, W. L. Laparoscopic cholecystectomy in pregnancy. *J. Laproendosc. Surg.* **4:** 161, 1994.
13. Alexander, G. D., and Brown, E. M. Physiologic alterations during pelvic laparoscopy. *Am. J. Obstet. Gynecol.* **105:** 1078, 1969.
14. El-Minawi M. J., Wahbi, O., El-Bagouri E. S., Sharawi, M., El-Mallah S. Y. Physiologic changes during CO2 and N2O pneumoperitoneum in diagnostic laparoscopy. *J. Reprod. Med.* **26:** 338, 1981.
15. Hein, H. A., Joshi, G. P., Ramsay, M. A., *et al.* Hemodynamic changes during laparoscopic cholecystectomy in patients with severe cardiac disease. *J. Clin. Anesth.* **9:** 261, 1997.
16. Joris, J. L., Chinche, J. D., Canivet, J. L. M., *et al.* Hemodynamic changes induced by laparoscopy and their endocrine correlates: effects of clonidine. *J. Am. Coll. Cardiol.* **32:** 1389, 1998.
17. Leighton, T. A., Liu, S. Y., and Bongard, F. S. Comparative cardiopulmonary effects of carbon dioxide versus helium pneumoperitoneum. *Surgery* **113:** 527, 1993.
18. O'Leary, E., Hubbard, K., Tormey, W., and Cunningham, A. J. Laparoscopic cholecystectomy: hemodynamic and neuroendocrine responses after pneumoperitoneum and changes in position. *Br J Anaesth.* **76:** 640, 1996.
19. Wahba, R. V. M., Beique, F., and Kleiman, S. J. Cardiopulmonary function and laparoscopic cholecystectomy. *Can. J. Anaesth.* **42:** 51, 1995.
20. Sheen-Chen, S. M., Chen, H. S., Eng, H. L., Chen, W. J., and Jawan, B. Systemic immune response after laparoscopic and open cholecystectomy. *World J. Surg.* **26:** 1418, 2002.
21. Gutt, C. N., Heinz, P., Kaps, W., and Paolucci, V. The phagocytosis activity during conventional and laparoscopic operations in the rat; A preliminary study. *Surg. Endosc.* **11:** 899, 1997.
22. Rotstein, O. D. Peritoneal host defense: modulation by carbon dioxide insufflation. *Surg. Infect.* **2:** 163, 2001.
23. Mendoza-Sagaon, M., Gitzelmann, C. A., Herreman-Suquet, K., et al. Immune response: effects of operative stress in a pediatric model. *J. Pediatr. Surg.* **33:** 388, 1998.
24. Gitzelmann, C. A., Mendoza-Sagaon, M., Talamini, M. A., *et al.* Cell-mediated immune response is better preserved by laparoscopy than laparotomy. *Surgery* **127:** 65, 2000.
25. Are, C., Talamini, M. A., Murata, K., and De Maio, A. Carbon dioxide pneumoperitoneum alters acute phase response induced by lipopolysaccharide. *Surg. Endosc.* **16:** 1464, 2002.
26. Ho, H. S., Saunders, C. J., Gunther, R. A., and Wolfe, B. M. Effects of hemodynamics during laparoscopy: CO2 absorption or intra-abdominal pressure? *J. Surg. Res.* **59:** 497, 1995.

27. Van den Bos G. C., Drake, A. J., and Noble, M. I. The effect of carbon dioxide upon myocardial contractile performance, blood flow, and oxygen consumption. *J. Physiol. (Lond.)* **287:** 149, 1979.

28. Kelman, G. R., Swapp, G. H., Smith, I., Benzie, R. J., and Gordon, N. L. Cardiac output and arterial blood gas tension during laparoscopy. *Br. J. Anaesth.* **44:** 1155, 1972.

29. Rassmussen, J. P., Dauchot, P.J., DePalma, R.G., *et al.* Cardiac function and hypercarbia. *Arch. Surg.* **113:** 1196, 1978.

30. Price, H. L. Effects of carbon dioxide on the cardiovascular system. *Anesthesiology* **21:** 652, 1960.

31. Hanly, E. J., Mendoza-Sagaon, M., Murata, K., Hardacre, J. M., De Maio A., and Talamini, M. A. CO2 pneumoperitoneum modifies the inflammatory response to sepsis. *Ann. Surg.* **237:** 343, 2003.

32. Berguer, R., Cornelius, T., and Dalton, M. The optimum pneumoperitoneum pressure for laparoscopic surgery in the rat model. *Surg. Endosc.* **11:** 915, 1997.

33. Dalton, M., Hildreth, J., Matsuoka, T., and Berguer, R. Determination of cardiorespiratory function and the optimum anesthetic regimen during laparoscopic surgery in the rat model. *Surg. Endosc.* **10:** 297, 1996.

34. Sefr, R., Puszkailer, K., Frana, J., and Penka, I. Effect of carbon dioxide pneumoperitoneum on selected parameters of the acid–base equilibrium in laparoscopic cholecystectomy. *Rozhl. Chir.* **80:** 206, 2001.

35. Kantorova, I., Svoboda, P., and Ochmann, J. Does insufflation of the abdomen in laparoscopic surgery affect acid–base and ventilatory parameters? *Rozhl. Chir.* **78:** 332, 1999.

36. Wakizaka, Y., Sano, S., Koike, Y., Nakanishi, Y., and Uchino, J. Changes of arterial CO_2($PaCO_2$) and urine output by carbon dioxide insufflation of the peritoneal cavity during laparoscopic choleystectomy. *Nippon Geka Gakkai Zasshi.* **95:** 336, 1994.

37. Mynbaev, O. A., Molinas, C. R., Adamyan, L. V., Vanacker, B., and Koninckx, P. R. Pathogensis of CO(2) pneumoperitoneum–induced metabolic hypoxemia in a rabbit model. *J. Am. Assoc. Gynecol. Laparosc.* **9:** 306, 2002.

38. Dexter, S. P. L., Vucevic, M., Gibson, J., and McMahon, M. J. Hemodynamic consequences of high-and low-pressure capnoperitoneum during laparoscopic cholecystectomy. *Surg. Endosc.* **13:** 376, 1999.

39. Shuto, K., Kitano, S., Yoshida, T., Bandoh, T., and Mitarai, Y., Kobayashi, M. Hemodynamic and arterial blood gas change during carbon dioxide and helium pneumoperitoneum in pigs. *Surg. Endosc.* **9:** 1173, 1995.

40. Schmandra, T. C., Kim, Z.-G., and Gutt, C. N. Effects of insufflation gas and intraabdominal pressure on portal venous flow during pneumperitoneum in the rat. *Surg. Endosc.* **405**, 2001.

41. Sessler, D. I., and Akca, O. Nonpharmacological prevention of surgical wound infections. *Clin. Infect. Dis.* **35:** 1397, 2002.

42. Kurz, A., Kurz, M., Poeschl, G., Faryniak, B., Redl, G., and Hackl, W. Forced air warming maintains intraoperative normothermia better than circulating water mattresses. *Anesth. Analg.* **77:** 89, 1993.

43. Kurz, A., Sessler, D. I., Narzt, E., *et al.* Postoperative hemodynamic and thermoregulatory consequences of intraoperative core hypothermia. *J. Clin. Anesth.* **7:** 359, 1995.

44. Buhre, W., and Rossaint, R. Perioperative management and monitoring in anesthesia. *Lancet* **362:** 1839, 2003.

45. Hynson, J. M., and Sessler, D. I. Intraoperative warming therapies: a comparison of three devices. *J. Clin. Anesth.* **4:** 194, 1992.

46. Kurz, A., Sessler, D. I., and Lenhardt, R. Perioperative normothermia to reduce the incidence of surgical wound infection and shorten hospitalization. Study of wound infection and temperature group. *N. Engl. J. Med.* **334:** 1209, 1996.

47. Cappuccino, H., Cargill, S., and Nguyen, T. Laparoscopic cholecystectomy: 563 cases at a community teaching hospital and a review of 12,201 cases in the literature. Monmouth medical center laparoscopic cholecystectomy group. *Surg. Laparosc. Endosc.* **4:** 213, 1994.

48. Hasaniah, W. F., Ghada, I., Sabah, A. H., Sulaiman, A. H., Jamal, M. J., and Derar, S. A. Laparoscopic cholecystectomy in 2750 cases in a teaching hospital in Kuwait. *Med. Princ. Pract.* **11:** 176, 2002.

49. Lichten, J. B., Reid, J. J., Zahalsky, M. P., and Friedman, R. L. Laparoscopic cholecystectomy in the new millennium. *Surg. Endosc.* **15:** 867, 2001.

50. Patel, S. C., and Bhatt, J. R. Laparoscopic cholecystectomy at the Aga Khan Hospital, Nairobi. *East Afr. Med. J.* **77:** 194, 2000.

51. Paredes, J. P., Carrillo, A., and Ramirez, J. A. Laparoscopic cholecystectomy in Spain: multicentric study of 2432 patients. *Rev. Esp. Enferm. Dig.* **85:** 19, 1994.

52. Smith, C. D., Weber, C. J., and Amerson, J. R. Laparoscopic adrenalectomy: new gold standard. *World J. Surg.* **23:** 389, 1999.

53. Oleynikov, D., and Oelschlager, B. New alternatives in the management of gastroesophageal reflux disease. *Am. J. Surg.* **186:** 106, 2003.

54. Pitcher, D. E., Curet, M. J., Martin, D. T., Vogt, D. M., Mason, J., and Zucker, K. A. Successful laparoscopic repair of paraesophageal hernia. *Arch. Surg.* **130:** 590, 1995.

55. Dunnington, G. L., and DeMeester, T. R. Outcome effect of adherence to operative principles of Nissen fundoplication by multiple surgeons: the department of Veterans affairs gastroesophageal reflux disease study group. *Am. J. Surg.* **166:** 654, 1993.

56. Bammer, T., Hinder, R. A., Klaus, A., and Klingler, P. J. Five to eight year outcome of the first laparoscopic Nissen fundoplications. *J Gastroinstest Surg.* **5:** 42, 2001.

57. Katkhounda, N., and Mavor, E. Laparoscopic splenectomy. *Surg. Clin. North Am.* **80:** 1285, 2000.

58. Rescorla, F. J., Engum, S. A., West, K. W., Tres Scherer L. R. 3rd, Rouse, T.M., and Grosfeld, J. L. Laparoscopic splenectomy has become the gold standard in children. *Am. Surg.* **68:** 297, 2002.

59. Giuffrida, M. C., Marquet, R. L., Kazemier, G., *et al.* Laparoscopic splenectomy and nephrectomy in a rat model; description of a new technique. *Surg. Endosc.* **11:** 491, 1997.

60. Gutt, C. N., Riemer, V., Brier, C., Berguer, R., and Paolucci, V. Standardized technique of laparoscopic surgery in rat. *Dig. Surg.* **15:** 135, 1998.

61. Allendorf, J. D. F., Bessler, M., and Whelan, R. L. A. Murine model of laparoscopic-assisted intervention. *Surg. Endosc.* **11:** 622, 1997.

62. Clary, E. M., Bruch, S. M., Lau, C. L., *et al.* Effects of pneumoperitoneum on hemodynamic and systemic immunologic responses to peritonitis in pigs. *J. Surg. Res.* **108:** 32, 2002.

63. Brown, R. H., Walters, D. M., Greenberg, R. S., and Mitzner, W. A. method of endotracheal intubation and pulmonary functional assessment for repeated studies in mice. *J Appl Physiol.* **87:** 2362, 1999.

64. Jaffe, R., and Free, M. A simple endotracheal intubation technic for inhalation anesthesia of the rat. *Lab. Anim. Sci.* **23:** 266, 1973.

65. Pena, H., and Cabrera, C. Improved endotracheal intubation technique in the rat. *Lab. Anim. Sci.* **30:** 712, 1980.

66. Alpert, M., Goldstein, D., and Triner, L. Technique of endotracheal intubation in rats. *Lab. Anim. Sci.* **32:** 78, 1982.

67. Stark, R. A., Nahrwold, M. L., and Cohen, P. J. Blind oral tracheal intubation of rats. *J. Appl. Physiol.* **51:** 1355, 1981.

68. Weksler, B., Ng, B., Lenert, J., and Burt, M. A. Simplified method for endotracheal intubation in the rat. *J. Appl. Physiol.* **76:** 1823, 1994.

69. Linden, R. D., Shields, C. B., Zhang, Y. P., Edmonds, H. L., and Hunt, M. A. A laryngoscope designed for intubation of the rat. *Cont. Topics Lab. Anim. Sci.* **39:** 40, 2000.

70. Worthley, S. G., Roque, M., Helft, G., Soundararajan, K., Siddiqui, M., and Reis, E. I. Rapid oral endotracheal intubation with a fibre-optic scope in rabbits: a simple and reliable technique. *Lab. Anim.* **34:** 199, 2000.

71. Vergari, A., Polito, A., Musumeci, M., Palazzesi, S., and Marano, G. Video-assisted orotracheal intubation in mice. *Lab. Anim.* **37:** 204, 2003.

72. De Wit M., Raabe, A., Tuinmann, G., and Hossfeld, D. K. Implantable device for intravenous drug delivery in the rat. *Lab. Anim.* **35:** 321, 2000.

73. Thrivikraman, K. V., Huot, R. L., and Plotsky, P. M. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. *Brain Res. Protoc.* **10:** 84, 2002.

74. Kuntz, C., Wunsch, A., Bodeker, C., *et al.* Effect of pressure and gas type on intraabdominal, subcutaneous, and blood pH in laparoscopy. *Surg. Endosc.* **14:** 367, 2000.