

# Assessment of Buprenorphine, Carprofen, and Their Combination for Postoperative Analgesia in Olive Baboons (*Papio anubis*)

Sarah O Allison,<sup>1,\*</sup> Lisa C Halliday,<sup>3</sup> Jeffrey A French,<sup>4</sup> Dmitri D Novikov,<sup>2</sup> and Jeffrey D Fortman<sup>3</sup>

This study compared the efficacy of buprenorphine, carprofen, and a combination of the 2 analgesics in female baboons. Physiologic and behavioral parameters were assessed at baseline and postoperatively for 6 d by use of continuous noninvasive physiologic monitoring and twice-daily videotaping. Prior to surgery, all animals received a pre-emptive dose of either 0.01 mg/kg buprenorphine intramuscularly, 2.2 mg/kg carprofen intramuscularly, or a combination of 0.01 mg/kg buprenorphine and 2.2 mg/kg carprofen intramuscularly. All animals in the carprofen (n = 4) and buprenorphine+carprofen (n = 4) treatment groups appeared to have sufficient analgesia. Three of 4 animals in the buprenorphine group had adequate analgesia. The fourth animal had an elevated heart rate and spent less time standing during the postoperative period. In this study, the use of carprofen or a combination of carprofen plus buprenorphine provided more reliable postoperative analgesia than buprenorphine alone.

**Abbreviations:** NHP, nonhuman primate; NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase

Humane and ethical concerns as well as animal welfare regulations both stipulate the use of appropriate analgesics for procedures that may cause pain or distress to a laboratory animal. In particular, invasive surgical procedures such as laparotomy require the use of postoperative analgesia. The *Guide for the Care and Use of Laboratory Animals* recommends that personnel become familiar with the signs of pain in animals in order to provide analgesia when appropriate;<sup>24</sup> however, there is no scale that definitively assesses pain in all species of laboratory animals. In particular, there is a paucity of information regarding the assessment of postoperative pain in nonhuman primates (NHP).

The purpose of this study was to assess the postoperative analgesic efficacy of buprenorphine, carprofen, and a combination of the 2 drugs. Buprenorphine is a commonly used opioid for postoperative analgesia in NHP.<sup>15,19</sup> Carprofen is a nonsteroidal anti-inflammatory drug (NSAID) that is not typically used for postoperative analgesia in NHP, but it is used in dogs and rodents.<sup>9,18,29</sup> We also chose to assess the combination of buprenorphine and carprofen because multimodal analgesia generally is considered more effective than the use of a single analgesic.<sup>11</sup> We measured physiologic and behavioral parameters to compare analgesic efficacy in the 3 treatment groups.

Commonly used measures for the assessment of analgesia in animals include heart rate,<sup>1,5,9</sup> blood pressure,<sup>5,21</sup> blood cortisol level,<sup>1,13,22,30</sup> urinary cortisol level,<sup>13,30</sup> respiratory rate,<sup>1,9,22</sup> body temperature,<sup>1,21,22</sup> food intake,<sup>16,25,27</sup> and weight loss.<sup>16,25,28</sup> For this study, we used heart rate, skin temperature, activity, food consumption, body weight, and serum and urine cortisol levels to evaluate analgesic efficacy in baboons.

In addition to the assessment of physiologic parameters, behavioral pain scales have been used to assess postoperative

pain in animals including dogs,<sup>9,18</sup> cats,<sup>1,21</sup> farm animals,<sup>22</sup> and rodents.<sup>14,25,28,29</sup> Several studies that have focused on analgesio-metric and behavioral testing of NHP.<sup>2-5,7,20,25,32-34</sup> After a review of the literature, we had not found any reports that specifically addressed the assessment of postoperative pain in nonhuman primates. Because of a lack of a postoperative pain assessment scale in the literature for NHP, we developed an 8-parameter scale to evaluate pain in conscious female olive baboons (*Papio anubis*). The scale was used to determine whether a 3-d period of twice-daily analgesic administration was sufficient to control postoperative pain. We compared the degree of pain experienced postoperatively with the nonpainful baseline state. Furthermore, we compared the standard analgesic used at our facility, buprenorphine, with the parenteral form of carprofen and a combination of the 2 drugs.

## Materials and Methods

**Animals.** All activities were approved by the University of Illinois at Chicago Animal Care Committee. Baboons were housed in a facility approved by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the *Guide for the Care and Use of Laboratory Animals*,<sup>24</sup> Public Health Service Policy, and the Animal Welfare Act regulations. Twelve female olive baboons (*Papio anubis*) ranging in age from 8 to 18 y and weighing approximately 14 to 20 kg were used for these studies. Animals were housed singly in aluminum cages, each with a transparent Lexan (Streamwood Plastics, Streamwood, IL) panel to allow for videotaping. The cage provided 0.75 m<sup>2</sup> of floor space and 101.6 cm of vertical height. Animals were housed in visual and auditory contact with other baboons and were provided 20 biscuits of 15% Monkey Diet (8714, Harlan-Teklad, Madison, WI) daily and municipal tap water ad libitum. Rooms were maintained at 22 ± 2 °C and 30% to 70% relative humidity with 100% conditioned air at 10 to 12 room air changes hourly. Fluorescent lighting was provided on a 12:12-h light:dark cycle (lights on, 0600 to 1800).

Received: 26 Oct 2006. Revision requested: 8 Dec 2006. Accepted: 8 Dec 2006.

<sup>1</sup>Division of Animal Resources, <sup>2</sup>Illinois Statistics Office, University of Illinois at Urbana-Champaign, Champaign, IL; <sup>3</sup>University of Illinois at Chicago, Biologic Resources Laboratory, Chicago, IL; <sup>4</sup>University of Nebraska at Omaha, Omaha, NE.

\*Corresponding author. Email: allison3@uiuc.edu

Animals were provided with toys and manipulanda that were placed directly in the cage, and speakers in the animal rooms provided auditory enrichment. Animals were given polyvinyl chloride tubes on top of the cages for foraging Prima-Treats (Bio-Serv, Frenchtown, NJ). Foraging items were provided twice daily immediately prior to the videotaping sessions. All animals were tuberculosis-free as determined by quarterly skin testing and were healthy as assessed by regular physical examination, complete blood count, serum chemistry analysis, and fecal flotation.

**Baseline studies.** Each animal served as its own control. The baseline analgesic study was performed either before or 2 mo after surgery. Each animal was in the same analgesic treatment group for the baseline and postoperative periods. Baseline studies commenced on the first day of analgesic treatment (day 1) through day 6 of the study. During days 1 through 3, animals received analgesic treatment; during days 4 through 6, animals did not receive analgesic treatment.

**Postoperative studies.** All baboons underwent surgery as specified in other Animal Care Committee-approved protocols; no surgeries were conducted solely for the purposes of the present experiment. All animals were fasted the evening (1600) before surgery. On the morning of surgery, animals were sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Phoenix Pharmaceuticals, St Joseph, MO), and a presurgical body weight was obtained. At this time, a serum cortisol sample was obtained from the femoral vein. Depending on the treatment group assignment, each baboon received either an intramuscular injection of buprenorphine (0.01 mg/kg; Phoenix Pharmaceuticals), an intramuscular injection of carprofen (2.2 mg/kg; Pfizer Animal Health, New York, NY), or a combined intramuscular injection of 0.01 mg/kg buprenorphine and 2.2 mg/kg carprofen. After analgesic premedication, animals received an intravenous injection of propofol (3 to 5 mg/kg to effect; Baxter Healthcare, Irvine, CA) into the cephalic vein for induction of anesthesia. Animals were intubated and received 1% to 3% isoflurane (Baxter Healthcare) in 100% oxygen for maintenance of anesthesia. Each baboon underwent laparotomy for cesarean section, oocyte aspiration, or endometrectomy. In all procedures, a 10-cm midline abdominal incision was made through the skin, fascia, linea alba, and abdominal musculature. The uterus, ovaries, and oviducts were isolated and manipulated according to the type of surgical procedure required. The procedures lasted from 1 to 2 h and were performed by staff veterinarians who were experienced in performing surgical procedures on baboons. Postoperative monitoring commenced on the day of surgery (day 0) and continued through day 6. Animals received analgesic treatment from day 0 through day 3. Animals did not receive analgesic treatment from day 4 through day 6.

**Analgesic treatments.** Animals were assigned randomly to 1 of 3 analgesic treatment groups. The first treatment group ( $n = 4$ ) received an intramuscular injection of buprenorphine (0.01 mg/kg). The second treatment group ( $n = 4$ ) received an intramuscular injection of carprofen (2.2 mg/kg). The third treatment group ( $n = 4$ ) received a combination of 0.01 mg/kg buprenorphine and 2.2 mg/kg carprofen intramuscularly. All analgesics were administered preoperatively, at 1600 postoperatively, and for 3 additional days after surgery. Analgesics were administered twice daily, once at the beginning of the workday (0600) and again at the end of the workday (1600).

**Collection of radiotelemetry data.** Animals were placed in primate jackets (Lomir Biomedical, Malone, NY) that contained a pouch to hold the noninvasive radiotelemetry devices. Animals were acclimated to the primate jackets for a minimum of

24 h. The radiotelemetric devices were an activity monitor (IM Systems, Baltimore, MD) and a transmitter (Mini-Logger Series 2000, Mini-mitter, Bend, OR) set to measure heart rate and body temperature at 1-min intervals. Data were collected on days 1 through 6 for both the baseline and postoperative studies.

**Collection of body weight and food consumption data.** During the baseline study, food consumption was evaluated daily in the morning from day 1 through day 6. Body weights were not measured during the baseline study. During the postoperative study, food consumption was evaluated by counting biscuits; biscuits were counted daily in the morning, beginning on day 0 and continuing through day 7. Body-weight data were collected on days 0 and 7.

**Collection of serum cortisol data.** Serum cortisol was measured only during the postoperative study. Blood was collected on days 0 and 7 from the femoral vein of sedated baboons; all animals were sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Phoenix Pharmaceuticals). The blood was centrifuged to separate the serum, which was stored at  $-80^{\circ}\text{C}$  until assayed. Sampling occurred between 0900 and 1200 to minimize variation due to circadian patterns of hormone excretion. Commercially available solid-phase coated tube radioimmunoassay kits (ICN; Costa Mesa, CA) were used to measure the concentration of serum cortisol. All radioimmunoassays were conducted without sample extraction or chromatography. Each sample was assayed in duplicate, and all samples for each plasma endocrine measure were assayed in a single run. The intra-assay coefficient of variation was 2.9%.

**Collection of urine cortisol data.** Urine samples were collected only during the postoperative study on days 0 through 7. The urine collection method was free-catch, with urine removed from the cage pan prior to the daily pan change. Urine was frozen at  $-80^{\circ}\text{C}$  until assayed. Sampling occurred between 0600 and 0900 to minimize variation due to circadian patterns of hormone excretion. A cortisol enzyme immunoassay was used to assess free cortisol, which is excreted in the urine of baboons.<sup>8,35</sup> All assays involved direct measurement of appropriately diluted urine samples, with no extraction or chromatography.<sup>10,13</sup>

Microtiter plates (Immuno MaxiSorp F96, Nunc, Rochester, NY) were coated with 50  $\mu\text{l}$  of antibody raised against a cortisol-bovine albumin antigen in rabbits and diluted in bicarbonate buffer. Coated plates were sealed, incubated for 1 to 2 d, and washed to remove antibody not covalently bonded to the plate well. Enzyme immunoassay buffer was added to each well, along those containing duplicate aliquots of reference standard (Sigma Chemical, St Louis, MO) and urine samples, diluted to appropriate concentrations in buffer. Steroid-horseradish peroxidase conjugate was added to the wells, and the plates were incubated for 2 h. After incubation, the plates were washed to separate unbound from bound hormone. Substrate solution (Sugen, San Francisco, CA) was added, and absorbance was measured at 410 nm (reference, 570 nm) with an automated plate reader (MR5000 Microtiter Plate Reader, Dynatech Laboratories, Chantilly, VA) when the absorbance in the day 0 baseline wells reached an optical density of 1.0. A 4-parameter sigmoid curve-fit function was used to calculate sample concentrations.<sup>30</sup> To control for variable fluid intake and output, each sample was assayed for creatinine concentrations by use of a modified Jaffé reaction.<sup>12</sup> Urinary cortisol concentrations were divided by creatinine concentrations, yielding an index of nanograms of cortisol per milligram creatinine.

**Development of behavioral scale.** To develop the behavioral scale, we randomly selected 5 female baboons that had not undergone a surgical procedure in the past 2 mo and acclimated them to the Lexan panel cage for a minimum of 24 h prior to

**Table 1.** Independent variables of female olive baboons in an analgesia study

Variable	Definition
Porb	Comparison of the postoperative condition with the baseline condition.
Porb treatment factor	Comparison of the three treatment groups in the postoperative condition with the baseline condition.
Day	Refers to day of the study; days 1 through six for the baseline study and days 0 to 7 for the postoperative study.
Time: radiotelemetric data	Daylight hours are 06:00 to 17:59, and nighttime hours are 18:00 to 05:59.
Time: behavioral data	Maximal analgesic benefit measured at 09:30 to 10:30, after the morning analgesic injection. Minimal analgesic measured at 14:30 to 15:30, prior to the afternoon analgesic injection.
Drug	Days 1 through 3 is the period of drug administration. Days 4 through 6 is the period of drug administration.
Treatment	Refers to 1 of the 3 drug treatment groups: buprenorphine, carprofen, or a combination of buprenorphine and carprofen.
Drug treatment factor	Days 1 through 3 (drug administered) and days 4 through 6 (drug not administered) evaluated for each drug treatment group.
Day (porb)	The comparison of the postoperative condition to the baseline condition in terms of day. Only evaluated for food consumption.

**Table 2.** Dependent variables of female olive baboons in an analgesia study

Variable	Type
Heart rate	Physiologic
Temperature	Physiologic
Activity	Physiologic
Food consumption	Physiologic
Body weight	Physiologic
Serum cortisol	Physiologic
Urine cortisol	Physiologic
Standing	Behavioral
Checking	Behavioral

Heart rate, temperature, and activity data were collected via radiotelemetry.

videotaping. These animals were videotaped for 31 min at a time during a 1- to 2-d period. Two authors (SA and LH) viewed the videotapes and noted the predominant behaviors of the animals during 1-min timed intervals. The behaviors expressed by the baboons were position in the cage (floor or perch), locomotion, posture (standing, sitting, or lying), checking (actively observing as determined by moving the head at least 45° in any direction), foraging, yawning, scratching, and grooming. Additional behaviors were noted as they occurred, including threatening postures, manipulating enrichment devices, and licking of cage bars or panels. Review of these tapes revealed that the behaviors to quantify were locomotion, foraging, posture, and checking.

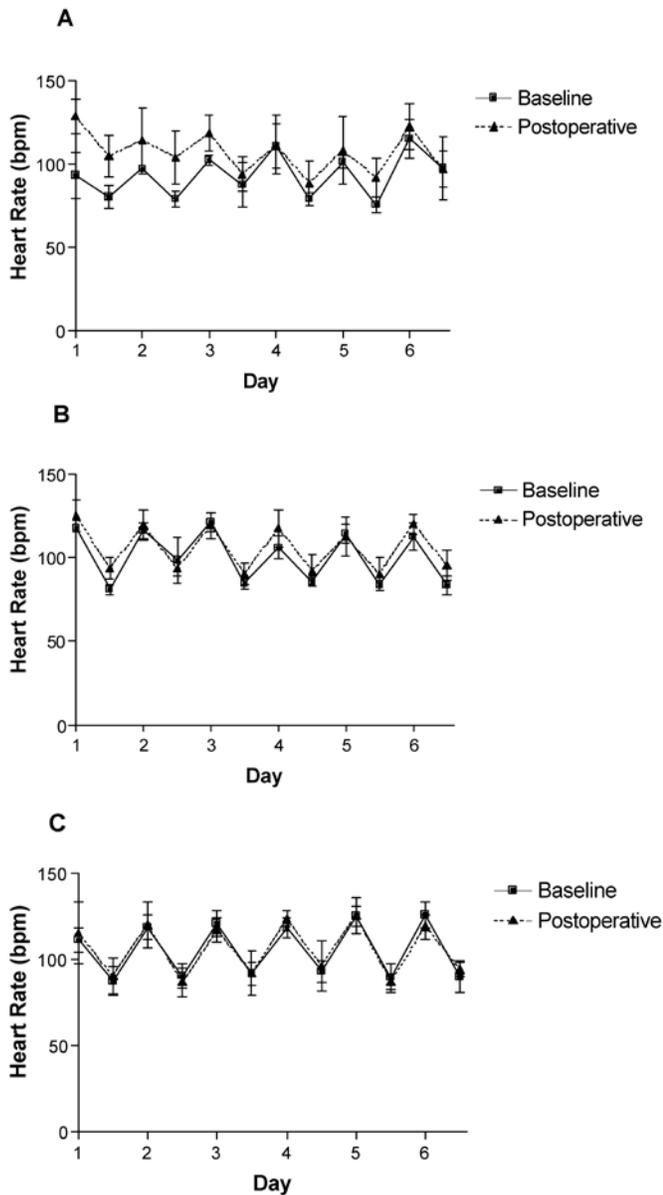
**Collection of behavioral data.** The behavioral parameters were assessed by videotaping each baboon for 31-min periods. One author (SA) viewed and evaluated the videotapes and was blinded to treatment group assignment. Animals were videotaped twice daily during the baseline and postoperative studies. The first videotaping session was approximately 3 to 4 h after the morning analgesic injection at the time considered to have maximal analgesic benefit (0930 to 1030). The second videotaping session occurred approximately 1 to 2 h prior to the afternoon analgesic injection at the time considered to have minimal analgesic benefit (1430 to 1530). These time periods were used to ascertain whether any behavioral differences

existed between the time of maximal analgesic benefit and minimal analgesic benefit. The percentage of time that an animal displayed a particular behavior was calculated by dividing the number of 1-min intervals in which the behavior was displayed by 31 (total number of intervals in each videotaping session). Of the behavioral parameters evaluated, only posture and checking were important for behavioral analysis.

**Statistical analysis of data.** Data manipulation and statistical analysis were performed using SAS software (version 9.1.3, SAS Institute, Cary, NC), which is commercially available from <http://www.sas.com/>. For each set of data, the statistical significance of the effects of the independent variables and their interactions on the outcome was evaluated by analysis of variance by use of the GLM and MIXED procedures in SAS. Results were presented in terms of P values for the F tests. The F tests used type III sums of squares, which accounted accurately for all factors in a model rather than the individual variables. Relative changes in the values of a dependent variable due to some effect were assessed by use of least squares estimates of the means of the effects. For significant factors with more than 2 possible values, pairwise comparisons of least squares means were made with Tukey-Kramer adjustments. The threshold for statistical significance was set at  $P = 0.1$ . Data are expressed graphically as mean  $\pm$  standard error of the mean. Table 1 describes the independent variables, and Table 2 describes the dependent variables.

## Results

**Radiotelemetric data.** Baseline and postoperative heart rate were increased significantly ( $P < 0.0001$ ) during daylight hours (Figure 1). The postoperative heart rate was increased in the buprenorphine group on days 1 through 3 ( $P = 0.0598$ ). Both baseline and postoperative temperatures were increased during daylight hours ( $P < 0.0001$ ). Postoperative temperature was increased on days 1 through 3 ( $P = 0.0352$ ; Figure 2). Baseline and postoperative activity were increased during daylight hours ( $P < 0.0001$ ). Activity was decreased in the postoperative condition as compared with the baseline condition ( $P = 0.0164$ ; data not shown). Both baseline ( $P = 0.0705$ ) and postoperative ( $P = 0.0809$ ) activity were increased during days 4 through 6. Baseline activity was increased during days 1 through 3 in the



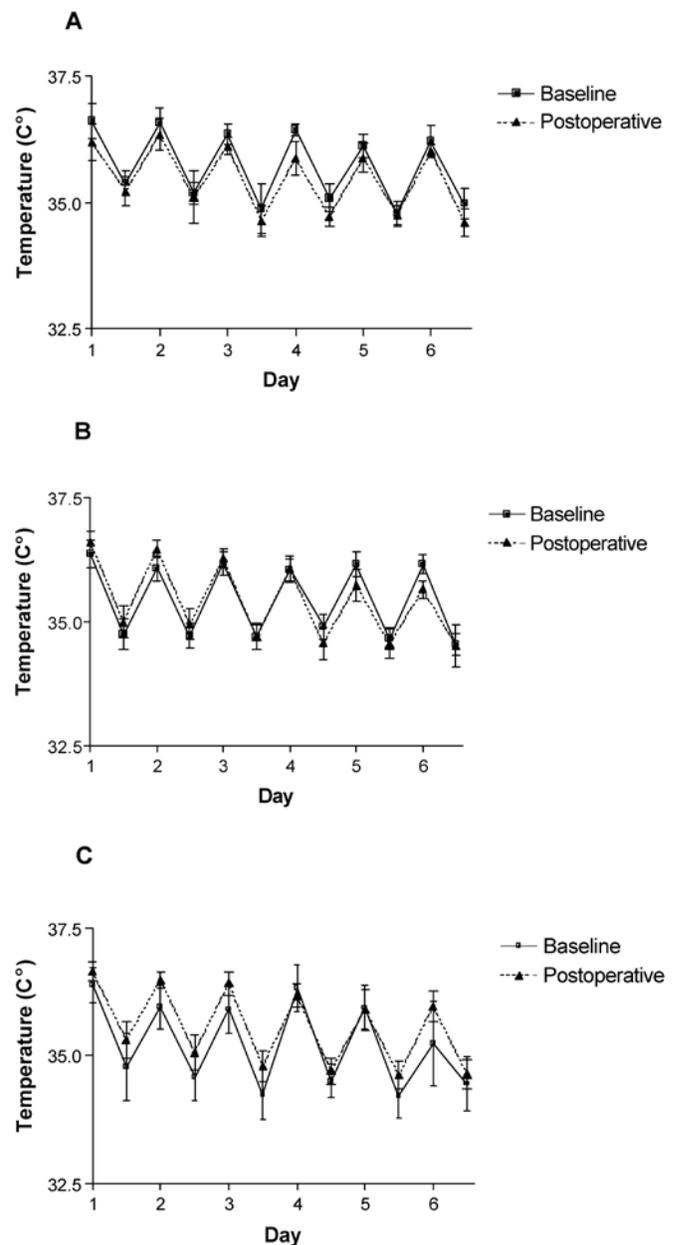
**Figure 1.** Heart rate (beats per minute, bpm) in (A) buprenorphine, (B) carprofen, and (C) buprenorphine+carprofen treatment groups of female olive baboons ( $n = 4$  per group) during postoperative and baseline conditions. Drugs were administered on days 1 through 3 but not on days 4 through 6. Whole numbers refer to daytime hours; half-numbers refer to nighttime hours. Data are presented as mean  $\pm$  standard error. Postoperative and baseline heart rates were increased during daylight hours ( $P < 0.0001$ ).

buprenorphine group as compared with the carprofen ( $P = 0.0763$ ) and buprenorphine+carprofen groups ( $P = 0.0026$ ). In addition, postoperative buprenorphine activity was decreased as compared with baseline buprenorphine activity ( $P = 0.0662$ ).

**Food consumption data.** Food consumption was decreased in the postoperative condition as compared with the baseline condition ( $P = 0.0893$ ; data not shown). Food consumption was decreased on postoperative day 1 as compared with postoperative days 2 through 7 ( $P < 0.0001$ ).

**Body weight data.** No significant loss in body weight occurred in the postoperative condition as compared with the presurgical body weight in all treatment groups (data not shown).

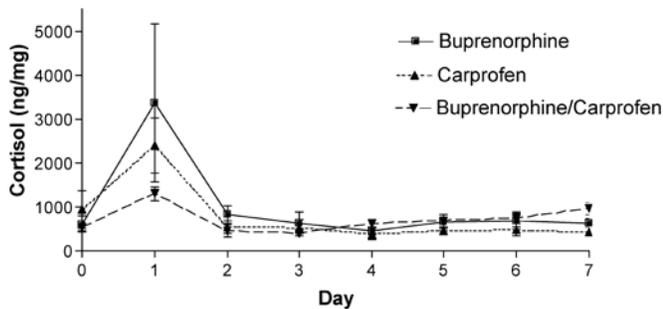
**Serum and urine cortisol data.** There were no statistically significant findings in the serum cortisol data, which ranged from 23.84 to 46.74  $\mu\text{g}/\text{dl}$  on day 0 and 18.0 to 44.94  $\mu\text{g}/\text{dl}$  on



**Figure 2.** Body temperature ( $^{\circ}\text{C}$ ) in (A) buprenorphine, (B) carprofen, and (C) buprenorphine+carprofen treatment groups of female olive baboons ( $n = 4$  per group) during postoperative and baseline conditions. Drugs were administered on days 1 through 3 but not on days 4 through 6. Whole numbers refer to daytime hours; half-numbers refer to nighttime hours. Data are presented as mean  $\pm$  standard error. Postoperative and baseline body temperature were increased during daylight hours ( $P < 0.0001$ ). [1]

day 7. There was a statistically significant ( $P = 0.0223$ ) effect of day, with urine cortisol significantly increased on day 1 as compared with days 0 and 2 through 7 (Figure 3).

**Behavioral data.** Standing was significantly ( $P = 0.0179$ ) decreased in the postoperative condition as compared to the baseline condition (Figure 4). Baseline standing was increased during the time of maximal analgesic benefit ( $P < 0.0023$ ). Postoperative standing was decreased in the buprenorphine group on day 4 as compared with days 2 ( $P = 0.0244$ ) and 3 ( $P = 0.0036$ ). Checking was significantly ( $P = 0.0960$ ) decreased in the postoperative condition as compared with the baseline condition (Figure 5). Postoperative checking was increased on days 1 through 3 as compared with days 4 through 6 ( $P = 0.0209$ ).



**Figure 3.** Urine cortisol levels (ng/mg creatinine) in female olive baboons receiving buprenorphine, carprofen, and buprenorphine+carprofen. Baboons monitored the day of surgery (day 0) through day 7 demonstrated significant ( $P = 0.0223$ ) increases in urine cortisol on day 1 as compared with days 0 and 2 through 7.

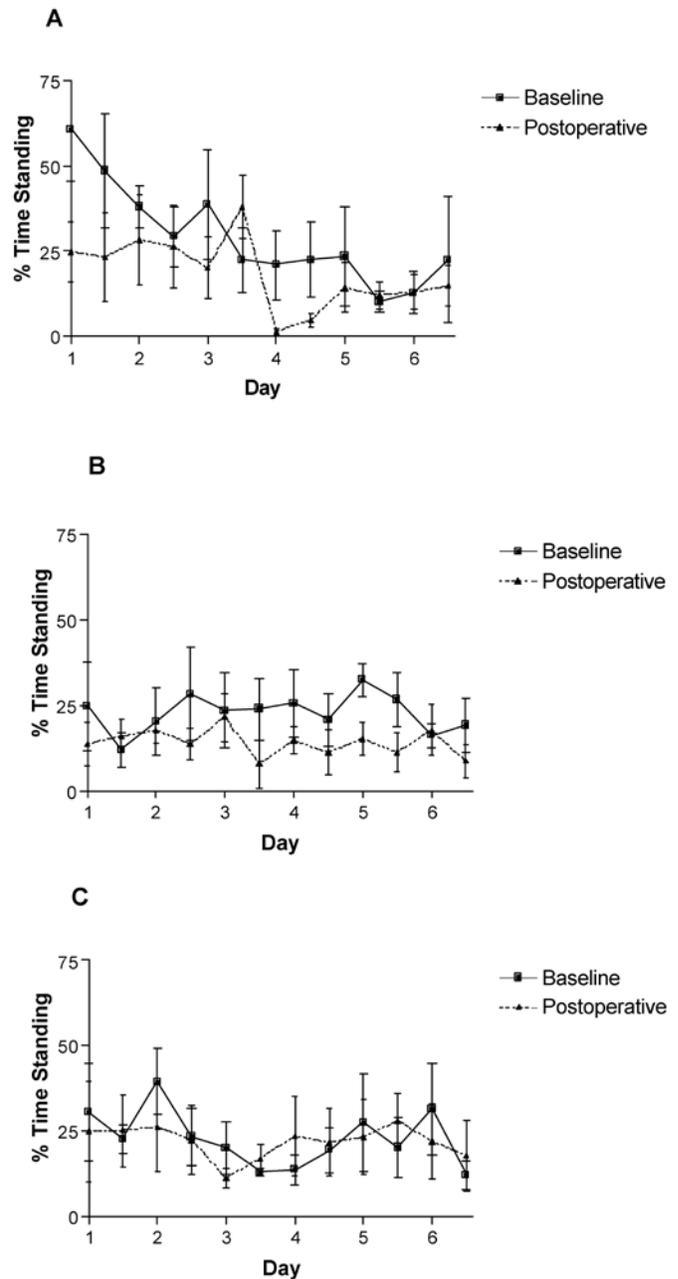
## Discussion

In this study, we chose to assess buprenorphine because it is routinely used to provide postoperative analgesia to NHP in our facility. This drug has a long duration of action (6 to 12 h) and typically is administered by twice-daily intramuscular injections. Buprenorphine is a controlled drug, requiring a license from the Drug Enforcement Agency to obtain and administer this compound. It is a Schedule 3-controlled drug, which requires less stringent recordkeeping as compared with that for more potent Schedule 2 opioids such as fentanyl and morphine.

Buprenorphine is an opioid analgesic that is a partial mu-receptor agonist and a kappa-receptor antagonist.<sup>31</sup> Mu opioid receptors are responsible for analgesia, sedation, euphoria, and respiratory depression.<sup>31</sup> Buprenorphine is considered a suitable analgesic agent for NHP because it has a long duration of action compared with that of other opioids.<sup>15</sup> Other effects include decreased blood pressure, decreased heart rate, and decreased food intake.<sup>16,26,27</sup> Because buprenorphine is a partial agonist, there is a potential ceiling effect to its opioid agonist effects at higher doses.<sup>27,31</sup> The ceiling effect may be a concern at dose rates much higher than the dose (0.01 mg/kg) we evaluated in this study.

NSAIDs also are used to provide postoperative analgesia. Both their beneficial and detrimental effects are achieved due to blockage of prostaglandin synthases cyclooxygenase 1 and 2 (COX1 and -2).<sup>6,17</sup> COX1 enzymes produce beneficial physiologic effects including maintenance of renal blood flow and protection of the gastrointestinal mucosa.<sup>6,17,18</sup> COX2 enzymes produce undesirable inflammatory changes, including hyperalgesia, chemotaxis of inflammatory mediators, and alterations in capillary permeability.<sup>6,17</sup> Certain NSAIDs such as aspirin are nonselective, thereby halting the actions of both COX1 and COX2 enzymes.<sup>6</sup> The disadvantage of nonselectivity is that the beneficial effects of COX1 enzymes are blocked, leading to side effects such as gastrointestinal tract ulceration.<sup>6,17</sup> NSAIDs such as carprofen are part of a new generation of selective COX2 inhibitors that block the inflammatory events while preserving some beneficial COX1 activity.<sup>6,17,18</sup> COX2-selective NSAIDs are more desirable because they inhibit the formation of the COX2 prostaglandins that are responsible for the clinical signs associated with inflammation while sparing the homeostatic effects of COX1 prostaglandins.<sup>6</sup>

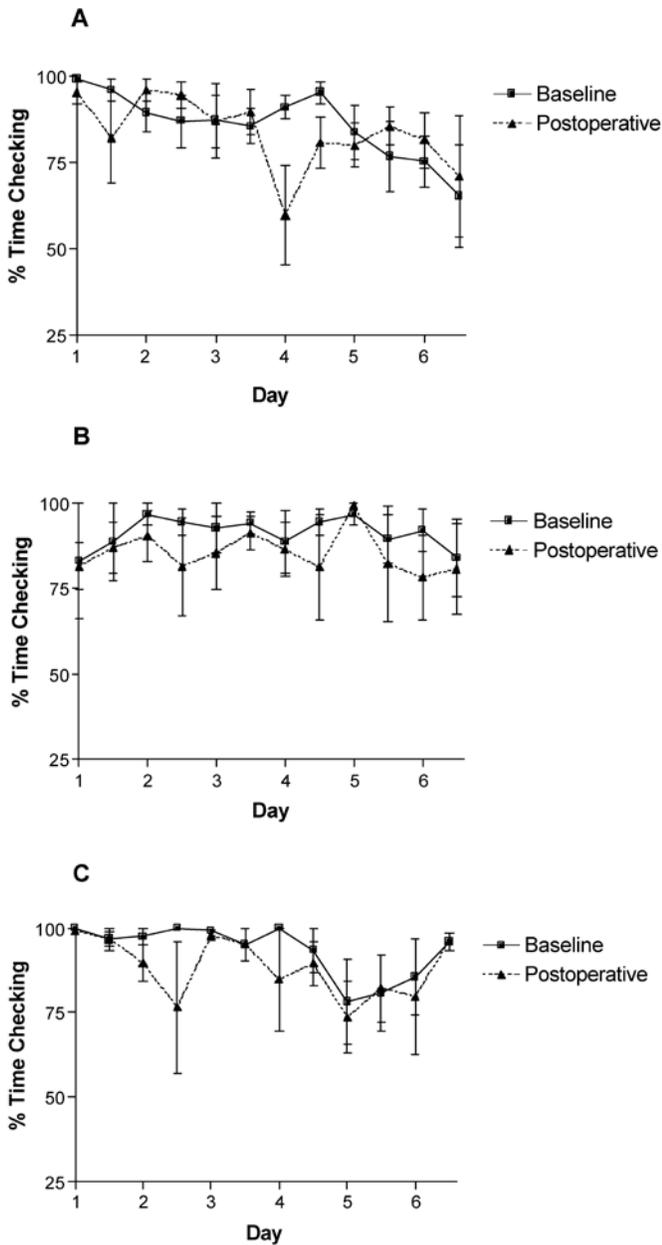
Similar to buprenorphine, carprofen has a long duration of action (as long as 12 h) and is administered by intramuscular injection twice daily for postoperative analgesia. A review of the literature suggested that carprofen is not used routinely to provide postoperative analgesia to NHP, although other



**Figure 4.** Standing in (A) buprenorphine, (B) carprofen, and (C) buprenorphine+carprofen treatment groups of female olive baboons ( $n = 4$  per group) during postoperative and baseline conditions. Drugs were administered on days 1 through 3 but not on days 4 through 6. Whole numbers refer to period of maximal analgesia; half-numbers refer to period of minimal analgesia. Data are presented as mean  $\pm$  standard error. Standing was increased during the baseline condition as compared with the postoperative condition ( $P = 0.0179$ ). Postoperative standing in the buprenorphine group was increased on days 2 ( $P = 0.0244$ ) and 3 ( $P = 0.0036$ ) as compared with day 4.

NSAIDs such as flunixin meglumine and acetaminophen are used frequently.<sup>15</sup> Unlike buprenorphine, carprofen is not a controlled drug, which eliminates the need for a Drug Enforcement Agency license. We also studied a buprenorphine+carprofen treatment group because the recommended approach to multimodal analgesia is to use drugs that control pain by means of 2 different pathways.<sup>11</sup>

In this study, baseline and postoperative heart rate were increased during daylight hours (Figure 1); this result was expected because baboons are diurnal animals. The postoperative



**Figure 5.** Checking in (A) buprenorphine, (B) carprofen, and (C) buprenorphine+carprofen treatment groups of female olive baboons ( $n = 4$  per group) during postoperative and baseline conditions. Drugs were administered on days 1 through 3 but not on days 4 through 6. Whole numbers refer to period of maximal analgesia; half-numbers refer to period of minimal analgesia. Data are presented as mean  $\pm$  standard error. Checking was increased during the baseline condition as compared with the postoperative condition ( $P = 0.096$ ). Postoperative checking was increased during days 1 through 3 as compared with days 4 through 6 ( $P = 0.0209$ ).

heart rate increased during days 1 through 3 for the buprenorphine group. Buprenorphine is an opioid drug that depresses heart rate.<sup>31</sup> Such an elevation in heart rate is unexpected in animals receiving sufficient analgesia because heart rate and blood pressure are reported to increase in response to painful stimuli.<sup>5,21</sup> Review of the raw data revealed that 1 animal in the buprenorphine group had an elevated heart rate throughout the postoperative study. The increased postoperative heart rate in the presence of buprenorphine likely is indicative of pain in this animal. Because the sample size was small, this animal influenced the results, making it appear that buprenorphine an-

algnesia was ineffective for the entire group. The other 3 animals in the buprenorphine group had postoperative heart rates that were not significantly different from their baseline heart rates.

The increase in the baseline and postoperative body temperatures during daylight hours (Figure 2) was another expected result in diurnal animals. The postoperative temperature increase during days 1 through 3 for all treatment groups likely was due to inflammation associated with healing of the surgical site.

Activity was decreased overall in the postoperative condition as compared with the baseline condition. In particular, postoperative buprenorphine activity was decreased as compared with baseline buprenorphine activity. The increased baseline activity in each of these circumstances is expected because the animals are not recovering from the effects of anesthesia and surgery. Both baseline and postoperative activity levels were increased during the daylight hours. Along with heart rate and body temperature, daytime activity is expected to be increased in diurnal animals.

Food consumption is an important parameter used to evaluate pain and general well-being in animals.<sup>16,26,27</sup> In the present study, food consumption was significantly decreased on postoperative day 1 as compared with postoperative days 2 through 7. Most likely, animals on postoperative day 1 had decreased food consumption at least in part due to the lingering effects of anesthesia and surgery. The animals' appetites increased coincident with the diminishing acute effects of anesthesia and surgery across the subsequent postoperative days. Animals in all of the treatment groups maintained normal body condition and did not lose weight during the postoperative period.

Cortisol has been used to assess stress in animals, with increased cortisol levels indicating stress.<sup>13,30</sup> Urine cortisol demonstrated a statistically significant increase on postoperative day 1 as compared with all other postoperative days (Figure 3). This increase in urine cortisol likely is due to the physiologic stress of anesthesia and surgery. As the animals recovered from these stressors, urine cortisol decreased to baseline (cortisol value from day 0) during subsequent postoperative days. These results demonstrate that animals experienced more stress on postoperative day 1 as compared with postoperative days 2 through 7. This finding is not unexpected, because there is an inevitable degree of stress associated with a surgical procedure. Unlike the urine cortisol data, serum cortisol data were not statistically significant. This difference was due to the fact that serum cortisol was only sampled twice, on postoperative days 0 and 7, whereas urine cortisol was sampled daily. If serum cortisol had been measured every day, an increase in serum cortisol may have been apparent on postoperative day 1, corresponding to the increase in urine cortisol on postoperative day 1.

The behavioral data demonstrated that standing was decreased in the postoperative condition as compared with the baseline condition (Figure 4). The animals may have been reluctant to stand postoperatively because this position stretches the abdominal incision, causing discomfort. The decrease in postoperative standing on day 4 for the buprenorphine treatment group as compared with days 2 and 3 was clinically significant. When the buprenorphine treatment was stopped on day 4, it appeared that animals were uncomfortable, and standing behavior decreased. However, this finding must be interpreted cautiously, because a review of the raw data revealed that the same animal with an elevated heart rate also had a marked decrease in standing behavior on day 4. The decrease in standing behavior in conjunction with an elevated heart rate further suggests that this single animal is the reason buprenorphine did not appear to provide adequate postoperative analgesia.

This animal might have benefited from the carprofen or the buprenorphine+carprofen treatment. Because the sample size was small, this animal influenced the results, making it appear that buprenorphine analgesia was ineffective for the entire group. As was seen with the postoperative heart rate data, 3 of 4 animals in the buprenorphine group had congruent postoperative standing data. It appears that buprenorphine was an effective analgesic for these 3 animals.

The decreased checking seen in the postoperative condition as compared with the baseline condition (Figure 5) was expected because animals are less alert after anesthesia and surgery. Postoperative checking was increased during days 1 through 3 as compared with days 4 through 6, suggesting that animals were more comfortable during drug treatment. Extending postoperative analgesic treatment beyond 3 d may have been beneficial; however, all other findings suggested 3 d of treatment were sufficient to control postoperative pain.

One animal in the buprenorphine group had an elevated postoperative heart rate and stood less frequently during the postoperative period. These findings suggest that buprenorphine was not an effective postoperative analgesic for this baboon. In humans, variability in pharmacokinetics among patients may result in an opioid providing insufficient pain relief to some.<sup>23</sup> Variability in pharmacokinetics is a possible explanation for this 1 baboon's reaction to buprenorphine. Any single analgesic likely will not be effective in all animals, as was the case for the 1 animal in the buprenorphine group. Because of the small sample size (n = 4), this animal influenced the data analysis for the entire buprenorphine group. However, review of the raw data revealed that the other 3 animals in the buprenorphine group apparently had adequate analgesia. In the carprofen and buprenorphine+carprofen groups, all 4 animals in each group had similar postoperative and baseline data, indicating that the animals received adequate analgesia.

Assessing analgesia in baboons has several inherent difficulties. Evaluating pain in NHPs is a challenge in that often they will not display signs of mild to moderate pain to an observer. An observer cannot enter the room and watch an animal without agitating the animal to some degree. As a result, differentiating between whether an animal is in pain or is reacting to a human observer is difficult. In addition, baboons must be sedated prior to handling, making it difficult to assess reactions such as pain on palpation of an incision site.

In conclusion, this study is the first report of which the authors are aware that attempts to evaluate the postoperative analgesic effectiveness of buprenorphine, carprofen, and buprenorphine+carprofen in baboons by use of physiologic and behavioral parameters. Although the sample size is small and demonstrates, in part, the difficulty of obtaining large numbers of NHPs in a research environment, we found that a twice-daily, 3-d dosing regimen for carprofen and buprenorphine+carprofen provided adequate and reliable analgesia for all animals in their respective groups. Buprenorphine provided adequate analgesia for 3 of the 4 animals in that group; the remaining baboon had elevated heart rates and decreased standing throughout the postoperative period, indicating inadequate analgesia. On the basis of our limited data, we recommend carprofen and buprenorphine+carprofen as more effective alternatives to buprenorphine for postoperative analgesia. Additional studies should be considered to further assess the reliability of buprenorphine for analgesia in baboons and to evaluate other NSAIDs, in particular those that can be administered orally or once daily.

## Acknowledgments

We thank Maria Muyot (Illinois Statistics Office) for her assistance with statistical submissions. The University of Omaha at Nebraska Endocrine Bioservices Laboratory is supported in part by grant HD 42882 from the National Institutes of Health to JAF.

## References

1. **Cambridge AJ, Tobias KM, Newberry RC, Sarkar DK.** 2000. Subjective and objective measurements of postoperative pain in cats. *J Am Vet Med Assoc* **217**:685–690
2. **Carlton SM, Lekan HA, Kim SH, Chung JM.** 1994. Behavioral manifestations of an experimental model for peripheral neuropathy induced by spinal nerve ligation in the primate. *Pain* **56**:155–166.
3. **Chudler EH, Dong WK, Kawakami Y.** 1986. Cortical nociceptive responses and behavioral correlates in the monkey. *Brain Res* **397**:47–60.
4. **Cooper BY, Vierck CJ.** 1986. Vocalizations as measures of pain in monkeys. *Pain* **26**:393–407.
5. **Crews JC, Sehlhorst CS.** 1991. Response to maintenance of tourniquet inflation in a primate model. *Reg Anesth* **16**:195–198.
6. **Curry SL, Cogar SM, Cook JL.** 2005. Nonsteroidal antiinflammatory drugs: a review. *J Am Anim Hosp Assoc* **41**:298–309.
7. **Dong WK, Hayashi T, Roberts VJ, Fusco BM, and Chudler EH.** 1996. Behavioral outcome of posterior parietal cortex injury in the monkey. *Pain* **64**:579–587.
8. **Ehlers CL, Killiam EK.** 1980. Circadian periodicity of brain activity and urinary excretion in the epileptic baboon. *Am J Physiol* **239**:35–41.
9. **Firth AM, Haldane SL.** 1999. Development of a scale to evaluate postoperative pain in dogs. *J Am Vet Med Assoc* **214**:651–659.
10. **Fite JE, French JA.** 2000. Pre- and postpartum sex steroids in female marmosets (*Callithrix kuhlii*): is there a link with infant survivorship and maternal behavior? *Horm Behav* **38**:1–12.
11. **Flecknell PA.** 2001. Analgesia of small mammals. *Vet Clin North Am Exot Anim Pract* **4**:47–56.
12. **French JA, Brewer KJ, Shaffner CM, Schalley J, Hightower-Merritt DL, Smith TE, Bell SM.** 1996. Urinary steroid and gonadotropin excretion across the reproductive cycle in female Wied's black tufted ear marmosets (*Callithrix kuhlii*). *Am J Primatol* **40**:231–245.
13. **French JA, Koban T, Rukstalis M, Ramirez SM, Bardi M, Brent L.** 2004. Excretion of urinary steroids in pre- and postpartum female baboons. *Gen Comp Endocrinol* **137**:69–77.
14. **Gillingham MB, Clark MD, Dahly EM, Krugner-Higby LA, Ney DM.** 2001. A comparison of two opioid analgesics for relief of visceral pain induced by intestinal resection in rats. *Contemp Top Lab Anim Sci* **40**(1):21–26.
15. **Hubbell JA, Muir WW.** 1996. Evaluation of a survey of the diplomats of the American College of Laboratory Animal Medicine on use of analgesic agents in animals used in biomedical research. *J Am Vet Med Assoc* **209**:918–921.
16. **Jablonski P, Howden BO, Baxter K.** 2001. Influence of buprenorphine analgesia on post-operative recovery in two strains of rats. *Lab Anim* **35**:213–222.
17. **Katz WA.** 2002. Cyclooxygenase-2-selective inhibitors in the management of acute and perioperative pain. *Cleve Clin J Med* **69**(Suppl 1):S165–S175.
18. **Leece EA, Brearley JC, Harding EF.** 2005. Comparison of carprofen and meloxicam for 72 hours following ovariohysterectomy in dogs. *Vet Anaesth Analg* **32**:184–192.
19. **Liguori A, Morse WH, Bergman J.** 1996. Respiratory effects of opioid full and partial agonists in rhesus monkeys. *J Pharmacol Exp Ther* **277**:462–272.
20. **Manning AA, Vierck CJ.** 1973. Behavioral assessment of pain detection and tolerance in monkeys. *J Exp Anal Behav* **19**:125–132.
21. **Mollenhoff A, Nolte I, Kramer S.** 2005. Anti-nociceptive efficacy of carprofen, levamethadone and buprenorphine for pain relief in cats following major orthopaedic surgery. *J Vet Med A Physiol Pathol Clin Med* **52**:186–198.

22. **Molony V, Kent JE.** 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. *J Anim Sci* **75**:266–272.
23. **Morgan GE, Mikhail MS.** 1996. Pain management. In: Morgan GE, Mikhail MS, Murray MJ, editors. *Clinical anesthesiology*. Stamford (CT): Appleton and Lange. p 274–316.
24. **National Research Council.** 1996. *Guide for care and use of laboratory animals*. Washington (DC): National Academy Press.
25. **Pioli EY, Gross CE, Meissner W, Bioulac BH, Bezard E.** 2003. The deafferented nonhuman primate is not a reliable model of intractable pain. *Neurol Res* **25**:127–129.
26. **Roughan JV, Flecknell PA.** 2001. Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain* **90**:65–74.
27. **Roughan JV, Flecknell PA.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Lab Anim* **36**:322–343.
28. **Roughan JV, Flecknell PA.** 2003. Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *Eur J Pain* **7**:397–406.
29. **Roughan JV, Flecknell PA.** 2004. Behaviour-based assessment of the duration of laparotomy-induced abdominal pain and the analgesic effects of carprofen and buprenorphine in rats. *Behav Pharmacol* **15**:461–472.
30. **Smith TE, French JA.** 1997. Psychosocial stress and urinary cortisol excretion in marmoset monkeys (*Callithrix kuhli*). *Physiol Behav* **62**:225–232.
31. **Srivastava A, Kahan M.** 2006. Buprenorphine: a potential new treatment option for opioid dependence. *Can Med Assoc J* **174**:1835–1836.
32. **Thomas DA, Anton F, Kenshalo DR, Williams GM, Dubner R.** 1993. Noradrenergic and opioid systems interact to alter the detection of noxious thermal stimuli and facial scratching in monkeys. *Pain* **55**:63–70.
33. **Thomas DA, Oliveras JL, Maixner W, Dubner R.** 1992. Systemic morphine administration attenuates the perceived intensity of noxious heat in the monkey. *Pain* **49**:129–135.
34. **Yeomans DC, Cooper BY, Vierck CJ.** 1996. Effects of systemic morphine on responses of primates to first or second pain sensations. *Pain* **66**:253–263.
35. **Yoder B, Martin H, McCurnin DC, Coalson, JJ.** 2002. Impaired urinary cortisol excretion and early cardiopulmonary dysfunction in immature baboons. *Pediatr Res* **51**:426–432.