

Transcutaneous Blood Gas Monitoring in the Rat

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Transcutaneous blood gas (TCBG) analysis is a noninvasive alternative method of estimation of blood gas tensions. The objective of the study reported here was to validate this method against standard blood gas (STBG) analysis in adult and juvenile Sprague-Dawley rats. We sought to establish the optimal TCBG probe site and temperature, to establish probe temperatures that would not cause thermal burns, to evaluate correlations between blood gas values ($P_a\text{CO}_2$ and $P_a\text{O}_2$) determined by use of TCBG and STBG, and to evaluate the sensitivity of the TCBG unit to changes in arterial blood gas partial pressures. Our results indicated that: in general, the xyphoid area was the optimal site for probe placement, with 44.5°C being the optimal probe temperature for the highest correlation, but thermal burns may be a problem; probe temperatures of 42.5°C (adults) and 42.0°C (juveniles) do not cause thermal burns when left in place for three hours; probe temperatures of 44°C (adults) and 42°C (juveniles) resulted in moderate correlation between $P_a\text{CO}_2$ and $P_{tc}\text{CO}_2$; and the TCBG unit adequately responded to changes in arterial blood gas partial pressures. Neither $P_{tc}\text{CO}_2$ or $P_{tc}\text{O}_2$ reflect actual values of $P_a\text{CO}_2$ or $P_a\text{O}_2$, respectively. We concluded that TCBG analysis may be used as an indicator of change in $P_a\text{CO}_2$ with sufficient animal numbers under tightly controlled conditions, but not as an indicator of change in $P_a\text{O}_2$ in adult and juvenile rats.

Current methods for assessing O_2 or CO_2 partial pressures or saturation within the blood involve use of three common techniques, standard blood gas analysis (SBGA), pulse oximetry (PO), and capnography. A fourth technique, transcutaneous blood gas (TCBG) analysis, with more limited use, has been used in human clinical and research settings for almost 30 years (1, 2).

In support of ongoing efforts to establish the juvenile rat as a model for sudden infant death syndrome (SIDS), there was a need for a technique that would allow us to monitor blood gas tensions over time in response to compounds that are reported to depress respiration. The ideal technique would also require minimal, if any blood sampling. Pulse oximetry would be a candidate for trend monitoring without a blood sample; however, only O_2 is monitored. Additionally, PO is not as sensitive in the flat upper portion of the hemoglobin dissociation curve (3, 4). Capnography measures the end-tidal partial pressure of CO_2 ($P_{et}\text{CO}_2$) without a blood sample, which in general accurately reflects the arterial partial pressure of CO_2 ($P_a\text{CO}_2$); however, patients are usually intubated and when not, has limited usefulness (5). Remote telemetry would be ideal for these types of experiments where anesthesia or restraint could be avoided. At this time, telemetric devices that measure the arterial partial pressure of O_2 ($P_a\text{O}_2$) or $P_a\text{CO}_2$ are not available. The research model (the suckling rat) has a limited blood volume, making serial sampling for SBGA difficult. Additionally, arterial access is technically difficult. Lastly, SBGA provides information only at specific time points; thus, transient changes might be missed. The objective of the study reported here was to validate the TCBG method in the rat.

Briefly the TCBG analysis instrument utilizes a heated (37 to 45°C) probe that is applied to the skin. The probe is comprised of a Stowe-Severinghaus electrode, which measures the partial pres-

sure of CO_2 (PCO_2), as well as a Clark-type polarographic electrode, which measures the partial pressure of O_2 (PO_2). The heat supplied by a thermistor, causes an increase in blood flow to the probe site, which increases the amount of O_2 that is brought to and released at the probe site (6, 7). The heat also shifts the O_2 and CO_2 dissociation curves to the right, thus increasing the partial pressures of both gases (7, 8). In humans, normal transcutaneous partial pressure of O_2 ($P_{tc}\text{O}_2$) readings should be slightly below the $P_a\text{O}_2$ and the transcutaneous partial pressure of CO_2 ($P_{tc}\text{CO}_2$) should be slightly above the $P_a\text{CO}_2$. The $P_{tc}\text{O}_2$ is lower due to heat-induced increases in metabolic demand by the cells and to a diffusion gradient established by the O_2 consumed by the polarographic electrode (9). The $P_{tc}\text{CO}_2$ is higher, also due to heat-induced increases in cell metabolism (7).

Use of TCBG analysis in species other than humans appears to be growing. The TCBG analysis has been used in a variety of species, including dogs (10-12), mice (13), rats (14, 15), cats (16), rhesus monkeys (17, 18), sheep (19), guinea pigs (20), rabbits (21, 22), swine (23, 24), and horses (25, 26).

Furset and coworkers (15) used TCBG analysis to monitor the $P_{tc}\text{CO}_2$ in rats within a hyperbaric chamber. Their $P_{tc}\text{CO}_2$ measurements correlated well with the $P_a\text{CO}_2$; however, $P_{tc}\text{CO}_2$ measurements were always higher than $P_a\text{CO}_2$ measurements (15). Additionally, the $P_{tc}\text{CO}_2$ response time was slower than that of $P_a\text{CO}_2$ when inspired amounts of CO_2 were changed (15). In another report (14), TCBG analysis was assessed for use during magnetic resonance imaging (MRI) of the rat. The technique was found to be acceptable for trend monitoring of CO_2 during MRI procedures with no adverse effects on imaging; however, TCBG readings again lagged behind changes measured by use of STBG analysis. These findings agree with those of other published reports (2, 15). Correlation between $P_{tc}\text{CO}_2$ and $P_a\text{CO}_2$ was high, but the $P_{tc}\text{O}_2$ and $P_a\text{O}_2$ correlated poorly (14).

Materials and Methods

Animals. Sprague-Dawley rats were bred and housed in the

Received: 4/17/01. Revision requested: 5/09/01 and 8/06/01. Accepted: 9/28/01. Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana 70803.

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vivarium of the School of Veterinary Medicine at Louisiana State University. The facility is operated by the Division of Laboratory Animal Medicine and is approved by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The study reported here was approved by the Institutional Animal Care and Use Committee.

Rats were housed in polycarbonate cages on corncob bedding (Bed-O-Cobs, The Andersons, Maumee, Ohio), with a 12/12-h light/dark cycle. Room temperature was maintained between 20 to 22.2°C and humidity between 40 and 60%. Feed (Lab Diet 5001, PMI International, Inc., Brentwood, Mo.) and water (via water bottles) were provided ad libitum. Sentinel rats housed within the facility were tested quarterly for Sendai, sialodacryoadenitis, and Kilham's rat viruses, pneumonia virus of mice, and *Mycoplasma pulmonis*. In addition, animals were tested annually for murine bacteria, as well as internal and external parasites.

Transcutaneous blood gas monitoring. A transcutaneous blood gas monitor (Model TINA TCM3, Radiometer, Copenhagen, Denmark) was used for the validation study. Manufacturer specifications include a range of 0 to 800 mmHg (PO₂) and 0 to 200 mmHg (PCO₂), as well as 1 mmHg accuracy at 25°C for both parameters. The probe (Model E5280 Radiometer), is a combined transcutaneous PO₂ and PCO₂ probe reported by the manufacturer to have a drift of < 1 mmHg/h and a 90% response time of 20 sec for PO₂ and 50 sec. for PCO₂ when used on humans.

Prior to calibration, two membranes were applied to the probe surface to exclude ions and charged molecules. Two drops of electrolyte solution containing propanediol, propanetriol, potassium chloride, potassium hydrogen carbonate, and deionized water (Radiometer) were applied to the ventral surface of the probe. The membranes were then applied, followed by inspection for trapped air bubbles. If any air bubbles were observed, the probe was re-membraned. The probe was re-membraned every two weeks during this study per manufacturer's recommendations. The TCBG monitor was calibrated against a standard calibration gas supplied by the manufacturer that contained 5.0% CO₂ and 20.90% O₂, with the balance being N₂. The unit was calibrated at start up and each time the probe site or probe temperature was changed. Calibration values for O₂ and CO₂

were programmed daily during testing. Values were derived from standard tables supplied by the manufacturer, and corresponded to barometric pressures taken and recorded daily.

For experiment one, probe site preparation consisted of initial clipping of the hair followed by application of a depilatory agent (Nair, Carter-Wallace, Inc., New York, N.Y.). Nair was applied and left in place for approximately 2 min. After Nair removal, the probe site was cleaned with alcohol and dried to facilitate adhesion of the fixation ring. Later experiments included only hair clipping. The calibrated probe was attached to the skin by use of a fixation ring (Radiometer). Once the fixation ring was applied on a flat, horizontal area of skin, three to five drops of contact liquid, 1, 2-propanediol and deionized water, (Radiometer) were placed within the ring. The calibrated probe was then attached to the ring by a 45° clockwise turn, which locked the probe to the fixation ring (Fig. 1). Thereafter, the animal can be moved if needed without leakage of the contact fluid. However, in our study, a position change was not made. Daily, after testing and removal of the probe, antibiotic ointment (Fougera Triple Antibiotic Ointment, Melville, N.Y.) was applied to the probe site to lubricate the skin and prevent infections.

Catheter placement. Adult animals were anesthetized in a chamber into which 5% isoflurane (IsoFlo, Abbott Laboratories, Chicago, Ill.) flowed, using 100% O₂ as the carrier gas. Once recumbent, animals were removed from the chamber and anesthesia was maintained via a nose cone supplying O₂ and 2.5% isoflurane. Buprenorphine HCl (Buprenex, Rickett & Coleman Products, Hull, UK) was administered subcutaneously at a dosage of 0.05 mg/kg of body weight for postoperative analgesia. Hair over the incision sites (left femoral triangle, dorsal interscapular area) was clipped, and the skin was prepared for aseptic surgery. Using the method of Wayneforth and Flecknell (27), femoral artery catheters were placed, using either a 2-F (0.3 mm ID × 0.6 mm OD) or 3-F (0.6 mm ID × 0.9 mm OD) polyurethane catheter (Access Technologies, Skokie, Ill.) filled with heparinized saline (62 IU/ml).

Juvenile animals were anesthetized with isoflurane (2.5%) via nose cone, using room air (0.6 L/min) as the carrier gas. Once the animal was anesthetized, the skin was prepared for aseptic surgery. The left carotid artery was visualized via a 1-cm incision and

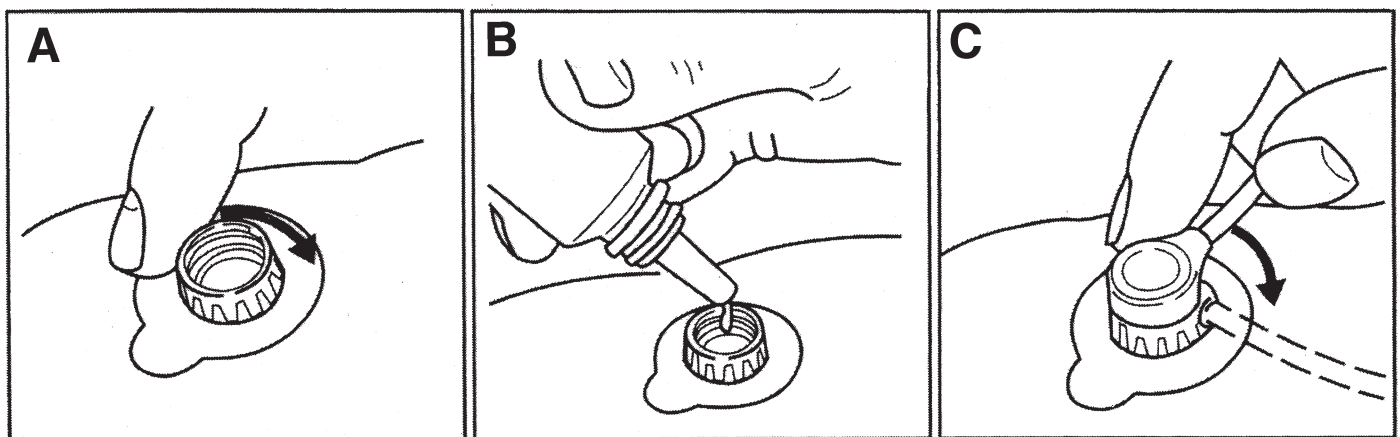


Figure 1. Illustration of the transcutaneous probe application procedures. The adhesive ring is applied (A), followed by placement of five to seven drops of contact liquid within the well formed by the ring (B). The probe is then screwed into the threads of the well by a 45° clockwise motion (C). The well design excludes air, retains the contact fluid, and provides solid attachment of the probe. Illustration reprinted with permission of Radiometer America Inc., Westlake, Ohio.

blunt dissection, followed by insertion of a 1-F (0.18 mm ID × 0.41 mm OD) polyurethane catheter (Access Technologies) filled with heparinized saline (62 IU/ml), and secured in place with 6-0 silk suture. Buprenorphine HCl was not administered because these were terminal surgeries, postoperative analgesia was not needed, and the drug has been documented to lower blood pressure in animals under isoflurane anesthesia (28).

Inspired gases. Room air or 100% O₂ were used as the primary anesthetic carrier gases for all experiments. They were chosen to simulate what might be encountered under standard gas anesthesia conditions, or normally breathed in the environment. To each of the primary gases was added O₂ or CO₂ to assess the ability of the transcutaneous instrument to reflect changes within the blood as assessed by STBG. Additionally, the various concentrations of CO₂ reflect concentrations that may be easily attained under normal conditions while infants are laying prone on soft bedding material and appear to be associated with SIDS (29). Specific carrier gases and mixtures were addressed for each experiment.

Standard blood gas analysis. For adult animals, blood samples (0.2 ml) for SBGA were collected into a heparinized 1-ml syringe. Pre-samples of 0.2 ml were collected, then were re-injected after the SBGA sample was taken. Finally, the catheter was flushed with heparinized saline (0.1 ml). The collected blood sample was analyzed within 15 min of collection to prevent equilibration of the blood in the syringe with room air. The SBGA unit (Model 238 pH/Blood Gas Analyzer, Ciba-Corning, Medfield, Mass.) was calibrated daily by use of two-point calibration, and hourly by use of single point calibration. Blood samples and pre-samples for juvenile animals were 0.12 ml each and were analyzed and handled similarly as were those from the adults.

Experiment 1. The purpose of this experiment was to establish the optimal site for the transcutaneous probe. The optimal site would be the location at which P_{tc}O₂ and P_{tc}CO₂ closely approximated or equaled their arterial reference counterparts. Two male (mean body weight, 568 g; mean age, 40 weeks) and two female (mean body weight, 322 g, mean age, 56 weeks) retired Sprague-Dawley breeder rats were used. Animals were anesthetized with Isoflurane mixed in 100% O₂ as previously described. Anesthesia depth was monitored and adjusted as needed to maintain a light plane of anesthesia.

Three probe sites were tested. Site 1 was 2 cm lateral to the umbilicus on the right ventral aspect of the abdomen. Site 2 was 2 cm lateral to the linea alba just caudal to the rib cage. Site 3 was just caudal to slightly overlapping the xyphoid process at midline. The P_{tc}O₂ and P_{tc}CO₂ readings were taken at probe temperatures of 37 and 43°C, with calibration between temperature changes. The P_{tc}O₂ values recorded at 37 and 43°C were combined for statistical analysis as were P_{tc}CO₂ values. At a given probe temperature, values were recorded when the readings from the instrument stabilized, usually between 12 and 20 min. Once values were taken for one site, the probe was moved to the next site, followed by recalibration and data acquisition. The process was repeated until all three sites had been tested.

Experiment 2. The purpose of this experiment was to establish the optimal probe temperature at which P_{tc}O₂ and P_{tc}CO₂ values most closely approximated their arterial reference counterparts and to evaluate any carryover effects that may have resulted from daily use of the same animal and probe site.

Four female (mean body weight, 213 g) and four male (mean

body weight, 306 g) Sprague-Dawley rats (mean age, 9 weeks) were tested by use of a single probe temperature once a day for 10 days. Testing was done at the same time each day. One of five probe temperatures (41, 43, 43.5, 44, and 44.5°C) were randomly selected daily and assigned to each animal. Using 100% O₂ as the carrier gas, rats were anesthetized as previously described and were positioned in dorsal recumbency. The xyphoid region was selected as the probe site. The hair was clipped by use of a standard clipper followed by an electric razor to remove as much hair as possible from the probe site. On subsequent days, the probe site was shaved as needed, using an electric razor. The probe was applied each day, and transcutaneous values were collected an average of 33 min after probe application. After data collection, the rats were allowed to recover from anesthesia and were monitored in their cages. After a five-day rest period, testing was repeated; however, room air was used as the carrier gas in place of O₂.

Experiment 3. The purpose of this experiment was to establish the maximal probe temperature that could be applied for three to four hours without damaging the skin of the animal. Initially, two adult Sprague-Dawley rats were tested. The animals were anesthetized, and the skin was prepared as previously described. Buprenorphine HCl was given subcutaneously at a dosage of 0.05 mg/kg for analgesia. The probe temperature was set at the highest temperature (44.5°C), then was calibrated. The probe was then applied and left in place for the allotted time. The following day, one animal was humanely euthanized via CO₂ asphyxiation and tissues were harvested for histologic examination. The second animal was observed for two more days to watch for lesion development at the probe site. If lesions were detected grossly or microscopically, two more rats were tested at the next lower probe temperature, stepping down in 0.5°C decrements until a probe temperature was reached at which lesions were not observed grossly or microscopically. Ten adult rats with mean age of 12 weeks were used in this experiment.

For juvenile animals (mean age, 11 days) it was assumed that probe temperatures that damaged the skin of adults would damage juvenile skin too. Using the probe temperature found in adults not to cause damage at 3 h, juveniles were tested like the adults and were similarly examined for skin damage. Additional juvenile rats were tested as needed until a probe temperature was found that would not damage the skin during the allotted application time frame. Six juvenile rats were used in this experiment.

Experiment 4. The purpose of this experiment was to compare partial pressure gas values of O₂ and CO₂ measured by use of TCBG analysis versus SBGA in adult and juvenile rats while varying probe temperature. Ten male (mean body weight, 278 g) and 14 female (mean body weight, 196 g) Sprague-Dawley rats (mean age, 8 weeks) were assigned to three groups. Group 1 consisted of 12 rats (five males, seven females) in which a probe temperature of 42.5°C was used. Group 2 contained six rats (two males, four females) subjected to a probe temperature of 43°C, and group 3 consisted of six rats (three males and three females) exposed to a probe temperature of 44°C. Testing began five to seven days after catheter implantation, as previously described. Room air contains 20.95% O₂ at sea level and will be rounded to 21.00% hereafter. Room air contains 0.03% CO₂ at sea level and will be referred to as 0.00% hereafter. A single pair of animals were tested daily, over six days with two of three pos-

sible inspired gas mixtures delivered under isoflurane (2%) anesthesia (treatment 1: room air at 0.6 L/min, 21.00% fraction of inspired O₂ (FIO₂), 0.00 % fraction of inspired CO₂ (FICO₂); treatment 2: room air at 0.6 L/min plus 50 ml of O₂/min, 27.00% FIO₂, 0.00% FICO₂; treatment 3: room air at 1 L/min plus 50 ml of CO₂/min, 19.88% FIO₂, 4.76% FICO₂).

For the first three days of testing, the pair were anesthetized by use of treatment 1, and a blood sample was collected for SBGA approximately 30 min after probe application. The TCBG values were recorded at the time of blood collection. Thereafter followed initiation of treatment 2, and approximately 20 min later, TCBG values and a blood sample were collected. The following three days (the same pair of animals), treatments 1 and 3 were applied and data were collected, followed by euthanasia on day six. The next pair of animals was then tested with treatments 1 and 3 for three days, followed by a three-day rotation with treatments 1 and 2. Each subsequent pair was tested switching the aforementioned treatment order as described previously.

Sixteen male and 14 female (mean body weight, 25 g; age, 10 days) juvenile rats were assigned to three groups of 10 by probe temperature (42, 43, and 44°C). Five animals in each group received treatment 1 followed by treatment 2, and the other five animals received treatment 1 followed by treatment 3. Treatments 1-3 were the same as those applied to the adult animals. Animals were initially anesthetized with isoflurane (2.5%) via nose cone, using room air (0.6 L/min) as the carrier gas. After catheter placement, the isoflurane concentration was decreased to 1.5%, followed by application of the probe to the abdomen at midline at the level of the umbilicus. After simultaneous blood sample and TCBG data collection for each treatment (time points as in adults), animals were humanely euthanized by cervical dislocation.

Experiment 5. The purpose of this experiment was to define the sensitivity of the TCBG instrument by comparison of TCBG data and STBG data while testing progressively lower concentrations of inspired CO₂ in adult and juvenile animals. Five female (mean body weight, 167 g; mean age, 7.5 weeks) and five male adult Sprague-Dawley rats (mean body weight, 196 g; mean age, 7.5 weeks) were tested daily for five days, using a probe temperature of 44°C. Animals were anesthetized as described in experiment 4, using room air (0.6 L/min) as the carrier gas. At approximately 30 min, TCBG and STBG readings were taken, followed by application of one of five randomized CO₂/room air concentrations (A = 5.00% FICO₂, 19.95 % FIO₂; B = 2.50% FICO₂, 20.47% FIO₂; C = 1.25% FICO₂, 20.74% FIO₂; D = 0.62% FICO₂, 20.85% FIO₂; and E = 0.31% FICO₂, 20.94% FIO₂) at the rate of 1 L/min. Approximately 20 min after application of the second treatment, TCBG and STBG readings were taken, followed by recovery of the animal. On the last treatment day after data collection, the animals were humanely euthanized via CO₂ inhalation followed by skin specimen collection of the probe site for histologic examination.

Twenty-four female (mean body weight, 21 g) and 26 male (mean body weight, 22 g) juvenile Sprague-Dawley rats, 10 days old, were tested at a probe temperature of 42.0°C. Animals were tested in groups of 10 and were anesthetized initially with isoflurane and room air as previously described for juveniles in experiment 4. Approximately 30 min later, TCBG and STBG readings were taken. Groups then received one of the five treatments previously mentioned for the adults (group 1, treatment A; group 2, treatment B; group 3, treatment C, group 4, treat-

ment D; and group 5, treatment E), followed by data collection for TCBG and STBG analysis approximately 20 min later. Animals were then humanely euthanized by cervical dislocation.

Statistical analysis. For experiment 1, mean P_{tc}O₂ and P_{tc}CO₂ values were compared between probe sites by use of analysis of variance (ANOVA). The Bonferoni post-hoc test was used to examine differences between each probe site. Mean P_{tc}O₂ and P_{tc}CO₂ values for each probe site were compared with mean P_aO₂ and P_aCO₂ reference values derived from samples collected in preliminary trials from 17 adult rats (eight male, nine female, 97 blood gas samples) under the same anesthetic and carrier gas conditions. Mean ± SEM P_aO₂ reference values while breathing 100 % O₂ are 370.53 ± 4.59 mmHg. Mean ± SEM P_aCO₂ reference values while breathing 100% O₂ are 39.51 ± 0.56 mmHg.

For experiment 2, mean P_{tc}O₂ data were compared by probe temperature by use of ANOVA along with Bonferoni's post-hoc test to examine differences between probe temperatures. P_{tc}CO₂ data were similarly examined. Summary statistics are presented in Table 1. Mean P_{tc}O₂ and P_{tc}CO₂ for each probe temperature while animals were breathing 100% O₂ were compared with reference values established for experiment 1. Mean P_{tc}O₂ and P_{tc}CO₂ for each probe temperature while animals were breathing room air were compared with reference values established from data collected in experiments 4 and 5 with those for anesthetized adult animals breathing room air only (15 male, 17 female, 166 blood gas samples). For P_aO₂ reference values while breathing room air, mean ± SEM values were 78.03 ± 0.72 mmHg. For P_aCO₂ reference values while breathing room air, mean ± SEM values were 37.76 ± 0.50 mmHg. The effect of probe temperature, prior probe temperature, and treatment (O₂ or room air) on P_{tc}O₂ and P_{tc}CO₂ values was evaluated for carryover effects, using a mixed effect linear model where the random variance of rat and anesthesia time were included with the aforementioned fixed effects. Proc Mixed (SAS version 6.12, SAS Institute, Cary, N.C.) was used for analysis.

For experiment 4, P_aO₂ and P_{tc}O₂ as well as P_aCO₂ and P_{tc}CO₂ data were subjected to regression analysis for each probe temperature, and Pearson correlation coefficients (*r*) are reported. Using the General Linear Model (GLM) with P_aCO₂ as the dependent variable, we looked for the effects of P_{tc}CO₂, anesthesia time, sex,

Table 1. Summary statistics of transcutaneous gas data collected at five probe temperatures (41.0, 43.0, 43.5, 44.0 and 44.5°C) from rats (n = 8) under isoflurane anesthesia with 100% O₂ or room air as the carrier gas

		41°C	43°C	43.5°C	44.0°C	44.5°C
100% O ₂ P _{tc} O ₂ (mmHg)	Mean	96.18 ^a	84.18 ^a	105.12 ^a	197.64 ^b	260.00 ^c
	SEM	±12.69	±8.87	±16.67	±20.46	±16.60
	Range	35–216	36–199	39–318	73–340	172–400
P _{tc} CO ₂ (mmHg)	Mean	63.94 ^a	61.18 ^a	58.44 ^{a,b}	56.21	53.12 ^b
	SEM	±1.62	±1.33	±2.50	±2.04	±2.00
	Range	54–76	51–68	44–86	43–69	39–71
Room air P _{tc} O ₂ (mmHg)	Mean	61.50 ^a	59.94 ^a	72.43 ^{a,b}	68.90 ^{a,b}	86.92 ^b
	SEM	±5.33	±5.88	±5.48	±7.93	±5.02
	Range	25–99	17–103	26–113	24–116	65–128
P _{tc} CO ₂ (mmHg)	Mean	65.93 ^a	66.87 ^a	57.79 ^a	59.60 ^a	56.31 ^a
	SEM	±1.73	±3.11	±2.27	±5.31	±2.72
	Range	55–76	48–91	48–77	49–105	44–71

P_{tc}O₂ = Transcutaneous partial pressure of O₂, P_{tc}CO₂ = transcutaneous partial pressure of CO₂. Within a row, differences could not be detected between treatment means with superscripts in common (*P* > 0.05).

body weight, and treatment for each probe temperature and multivariate correlation coefficients (R) are reported. Significant variables were then further analyzed by use of Tukey's post-hoc test. Using GLM with P_aO_2 as the dependent variable we tested for the effects of $P_{tc}O_2$, anesthesia time, sex, body weight, and treatment for each probe temperature, with Tukey's post-hoc test applied where appropriate. Summary statistics (Tables 2 and 3) are reported, along with differences between means determined by use of ANOVA, with treatment as the independent variable, along with Bonferoni's post-hoc test.

For experiment 5, P_aO_2 and $P_{tc}O_2$ as well as P_aCO_2 and $P_{tc}CO_2$ data were analyzed by use of regression analysis by group, and correlation coefficients (r) are reported. Groups were assigned according to the second treatment applied, and analysis of a given group's data contained the first and second treatments on a given day. The GLM procedure was used as described in experiment 4; however, either group or treatment was used in a model, and probe temperature was never used in either model. Tukey's post-hoc test was applied where appropriate. Summary statistics are reported (Tables 4 and 5), with differences between means determined by use of ANOVA, with treatment as the in-

dependent variable, along with Tukey's post-hoc test. Probability of $P \leq 0.05$ was considered significant in all experiments. Statistical analysis was performed, using Systat (Version 9, SPSS, Inc., Chicago, Ill.) unless otherwise indicated.

Results

Experiment 1. Site 3 (xyphoid area) was determined to be the probe location of choice when 100% O_2 was used as the carrier gas for the anesthetic. Mean \pm SEM $P_{tc}O_2$ at site 3 (191.89 ± 50.36 mmHg) most closely approximated mean reference P_aO_2 (370.53 ± 4.59 mmHg). Mean $P_{tc}PO_2$ at site 3 differed (higher) from that at sites 1 and 2 (Fig. 2). Differences were not evident between probe sites for $P_{tc}CO_2$, although mean $P_{tc}CO_2$ at site 3 (68.44 ± 2.42 mmHg) most closely approximated mean reference P_aCO_2 (39.51 ± 0.56 mmHg).

Experiment 2. The optimal probe temperature was determined to be $44.5^\circ C$ where $P_{tc}O_2$ and $P_{tc}CO_2$ most closely approximate arterial reference blood gas values. When 100% O_2 was used as the carrier gas, as probe temperature increased mean $P_{tc}O_2$ values also increased (Fig. 3). Mean $P_{tc}O_2$ at $44.5^\circ C$ (260.00 ± 16.61 mmHg) most closely approximated mean refer-

Table 2. Summary statistics of transcutaneous and arterial gas data collected at three probe temperatures ($42.5^\circ C$, n = 12; $43.0^\circ C$, n = 6; $44.0^\circ C$, n = 6) in adult rats under isoflurane anesthesia (2%) while varying carrier gas FIO_2 and $FICO_2$ (treatments 1-3)

	42.5°C Treatment			43.0°C Treatment			44.0°C Treatment		
	1	2	3	1	2	3	1	2	3
P_aO_2 (mmHg)									
Mean	80.46 ^a	86.81 ^a	80.32 ^a	80.09 ^a	90.30 ^b	88.67 ^b	68.16 ^a	86.00 ^b	75.79 ^c
SEM	± 1.29	± 2.54	± 2.95	± 1.48	± 1.57	± 2.81	± 1.31	± 2.04	± 2.38
Range	52-96	68-113	48-110	65-98	78-98	72-106	52-83	68-100	62-92
$P_{tc}O_2$ (mmHg)									
Mean	54.27 ^a	60.63 ^a	61.60 ^a	53.12 ^a	55.76 ^a	69.87 ^b	53.03 ^a	75.50 ^b	72.71 ^b
SEM	± 2.35	± 3.79	± 4.36	± 3.64	± 5.69	± 5.29	± 2.74	± 4.91	± 4.78
Range	20-104	25-110	23-105	12-100	10-82	37-118	13-89	45-131	28-98
P_aCO_2 (mmHg)									
Mean	36.00 ^a	37.78 ^a	43.28 ^b	39.62 ^a	41.13 ^a	53.27 ^b	43.22 ^a	46.00 ^a	57.14 ^b
SEM	± 0.86	± 1.18	± 1.55	± 1.06	± 1.88	± 2.01	± 1.774	± 1.55	± 1.88
Range	23-56	28-50	29-59	28-50	29-53	36-63	31-59	28-56	47-68
$P_{tc}CO_2$ (mmHg)									
Mean	57.08 ^a	59.89 ^a	69.60 ^b	59.00 ^a	61.47 ^a	77.76 ^b	64.94 ^a	68.29 ^a	86.27 ^b
SEM	± 1.43	± 2.11	± 3.02	± 1.63	± 3.07	± 4.06	± 1.77	± 2.70	± 2.77
Range	32-87	42-78	45-107	39-73	40-85	30-91	45-92	40-89	72-106

P_aO_2 = Arterial partial pressure of O_2 ; $P_{tc}O_2$ = transcutaneous partial pressure of O_2 ; P_aCO_2 = arterial partial pressure of CO_2 ; $P_{tc}CO_2$ = transcutaneous partial pressure of CO_2 ; FIO_2 = fraction of inspired O_2 ; $FICO_2$ = fraction of inspired CO_2 ; treatment 1 = 21% FIO_2 , 0.00% $FICO_2$; treatment 2 = 27.00% FIO_2 , 0.00% $FICO_2$; treatment 3 = 19.88% FIO_2 , 4.76% $FICO_2$. Within a probe temperature and row, differences could not be detected between treatment means with superscripts in common ($P > 0.05$).

Table 3. Summary statistics of transcutaneous and arterial gas data collected at three probe temperatures ($42.0^\circ C$, n=10; $43.0^\circ C$, n = 10; $44.0^\circ C$, n = 10) in juvenile rats under isoflurane anesthesia (1.5%) while varying carrier gas FIO_2 and $FICO_2$ (treatments 1-3)

	42.0°C Treatment			43.0°C Treatment			44.0°C Treatment		
	1	2	3	1	2	3	1	2	3
P_aO_2 (mmHg)									
Mean	44.20 ^a	52.00 ^a	49.40 ^a	47.40 ^a	46.60 ^a	62.20 ^b	43.80 ^a	56.20 ^a	44.00 ^a
SEM	± 2.59	± 2.10	± 4.82	± 3.33	± 4.06	± 3.15	± 2.99	± 2.20	± 5.79
Range	32-56	45-57	35-61	29-60	35-55	55-70	23-54	48-61	27-58
$P_{tc}O_2$ (mmHg)									
Mean	1.50 ^a	1.60 ^a	1.00 ^a	2.60 ^a	6.40 ^a	3.00 ^a	14.20 ^a	36.40 ^a	12.80 ^a
SEM	± 0.72	± 0.75	± 0.55	± 1.01	± 2.60	± 2.02	± 4.83	± 8.08	± 7.79
Range	0-7	0-4	0-3	0-9	1-15	0-11	0-46	19-59	1-43
P_aCO_2 (mmHg)									
Mean	32.90 ^a	37.40 ^a	44.60 ^a	34.40 ^a	39.80 ^a	40.40 ^a	35.10 ^a	33.40 ^a	45.40 ^a
SEM	± 2.48	± 2.98	± 5.69	± 1.77	± 2.96	± 2.66	± 2.02	± 1.96	± 4.67
Range	21-49	31-48	34-66	27-44	32-47	35-50	27-48	30-41	29-54
$P_{tc}CO_2$ (mmHg)									
Mean	79.50 ^a	93.60 ^a	95.40 ^a	79.50 ^a	83.00 ^a	85.20 ^a	64.70 ^a	58.20 ^a	85.40 ^b
SEM	± 2.83	± 5.82	± 7.71	± 3.03	± 6.29	± 3.22	± 5.08	± 4.32	± 5.83
Range	67-94	80-115	82-124	66-94	62-93	76-94	48-103	51-75	70-103

Within a probe temperature and row, differences could not be detected between treatment means with superscripts in common ($P > 0.05$). See Table 2 for key.

Table 4. Summary statistics of transcutaneous (44°C probe temperature) and arterial gas data collected in adult rats (n=10) under isoflurane anesthesia (2%) while varying carrier gas FICO₂ (treatments: room air-E)

	Room Air	Treatment				
		A	B	C	D	E
P_aO₂ (mmHg)						
Mean	80.50 ^a	88.89 ^b	85.10 ^{a,b}	82.18 ^{a,b}	80.30 ^{a,b}	81.70 ^a
SEM	±0.89	±1.84	±2.18	±1.87	±2.41	±1.97
Range	60–92	82–97	75–95	71–92	70–91	72–93
P_{tc}O₂ (mmHg)						
Mean	22.90 ^a	40.89 ^{a,b}	42.50 ^b	44.18 ^b	35.50 ^{a,b}	34.60 ^{a,b}
SEM	±2.38	±8.25	±6.60	±5.30	±6.69	±5.44
Range	3–64	16–81	14–89	11–64	10–66	12–68
P_aCO₂ (mmHg)						
Mean	34.92 ^a	44.00 ^b	39.20 ^{a,b}	38.73 ^{a,b}	35.70 ^a	34.40 ^a
SEM	±0.62	±2.13	±1.70	±1.39	±1.81	±1.02
Range	25–44	35–55	32–47	34–50	28–47	30–40
P_{tc}CO₂ (mmHg)						
Mean	63.66 ^a	73.77 ^b	68.70 ^{a,b}	60.27 ^a	62.80 ^{a,b}	59.20 ^a
SEM	±1.29	±2.89	±3.48	±2.64	±2.93	±2.49
Range	29–88	58–90	52–84	45–79	51–76	43–72

Room air = 21% FIO₂, 0.00% FICO₂; A = 19.95% FIO₂, 5.00% FICO₂; B = 20.27% FIO₂, 2.50% FICO₂; C = 20.74% FIO₂, 1.25% FICO₂; D = 20.85% FIO₂, 0.62% FICO₂; E = 20.94% FIO₂, 0.31% FICO₂. Within a row, differences could not be detected between treatment means with superscripts in common (*P* > 0.05). See Table 2 for key.

Table 5. Summary statistics of transcutaneous (42°C probe temperature) and arterial gas data collected in juvenile rats (n=50) under isoflurane anesthesia (1.5%) while varying carrier gas FICO₂ (treatments: room air-E)

	Room Air	Treatment				
		A	B	C	D	E
P_aO₂ (mmHg)						
Mean	64.16 ^a	47.80 ^{a,b}	77.80 ^{a,c}	60.80 ^a	69.80 ^a	64.00 ^a
SEM	±2.72	±4.54	±4.95	±7.42	±2.73	±4.82
Range	30–100	13–60	51–106	32–100	57–80	42–81
P_{tc}O₂ (mmHg)						
Mean	1.36 ^a	2.40 ^a	0.50 ^a	0.30 ^a	1.70 ^a	1.10 ^a
SEM	±0.29	±0.75	±0.17	±0.30	±0.99	±0.55
Range	0–8	0–6	0–1	0–3	0–8	0–5
P_aCO₂ (mmHg)						
Mean	29.68 ^a	45.50 ^b	33.70 ^a	34.90 ^a	29.10 ^a	33.10 ^a
SEM	±0.84	±2.36	±1.37	±2.31	±1.28	±1.41
Range	19–43	37–60	29–43	23–45	24–36	29–43
P_{tc}CO₂ (mmHg)						
Mean	82.08 ^a	101.00 ^b	90.90 ^{a,b}	98.70 ^{b,c}	82.70 ^{a,c}	89.80 ^{a,c}
SEM	±1.54	±3.47	±3.91	±6.59	±2.46	±4.36
Range	57–110	87–119	76–117	52–127	70–96	72–112

Within a row, differences could not be detected between treatment means with superscripts in common (*P* > 0.05). See Tables 2 and 4 for key.

ence P_aO₂ (370.53 ± 4.59 mmHg). Mean P_{tc}O₂ values at 44 and 44.5°C were different (higher) from those at the other three temperatures tested. When room air was used as the carrier gas, only mean P_{tc}O₂ at 44.5°C (86.92 ± 5.02 mmHg) differed (higher) from values at the other temperatures.

The optimal probe temperature for mean P_{tc}CO₂ (53.13 ± 1.99 mmHg) was 44.5°C where transcutaneous values most closely approached mean reference P_aCO₂ (39.51 ± 0.56 mmHg). At 44.5°C, P_{tc}CO₂ values were different (lower) from readings taken at the other probe temperatures tested with 100% O₂. While breathing room air, a difference could not be detected between probe temperatures for P_{tc}CO₂ means. Results for all carrier gases and probe temperatures are presented in Table 1. Regarding carryover effect from the preceding day's treatment, an effect for prior probe temperature, or prior treatment (O₂ or room air) was not apparent for P_{tc}CO₂ or P_{tc}O₂ (data not shown).

Experiment 3. The highest probe temperature that could be used for at least three hours without damage to the skin of an adult rat was 42.5°C. In juvenile rats, the maximal probe temperature for a three-hour exposure was 42.0°C. Damage to the

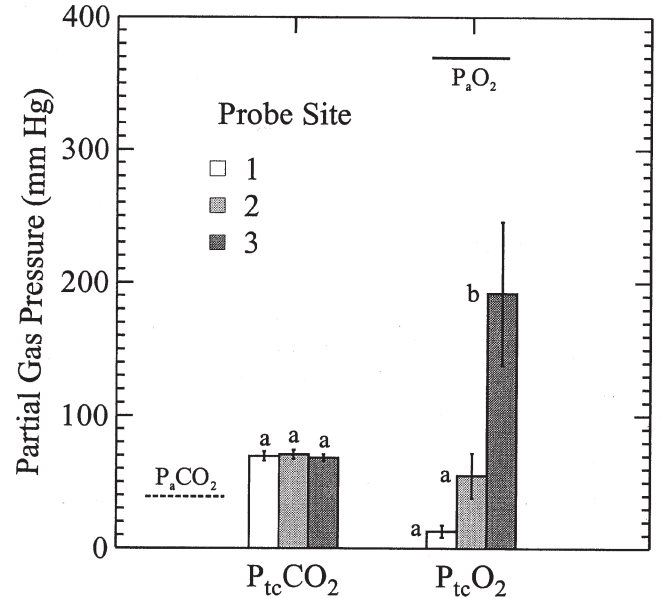


Figure 2. Comparison of transcutaneous partial pressures at selected probe sites. Bars represent mean (± SEM) P_{tc}O₂ and P_{tc}CO₂ at each probe site. Four anesthetized rats were tested while breathing 100% O₂. Data were collected for two probe temperatures (37 and 43°C) at each of three probe sites, with calibration between each temperature or probe site change (6 data points/animal). Mean reference values for P_aO₂ = 370.53 mmHg (solid line), mean P_aCO₂ = 39.51 mmHg (dotted line). Site 1: 2 cm lateral to the umbilicus on the right, site 2: 2 cm lateral to the linea alba on the right side just caudal to the rib cage, site 3: just caudal or slightly overlapping the xyphoid process on midline. Differences in partial gas pressures could not be detected for probe sites with superscripts in common within a given transcutaneous parameter.

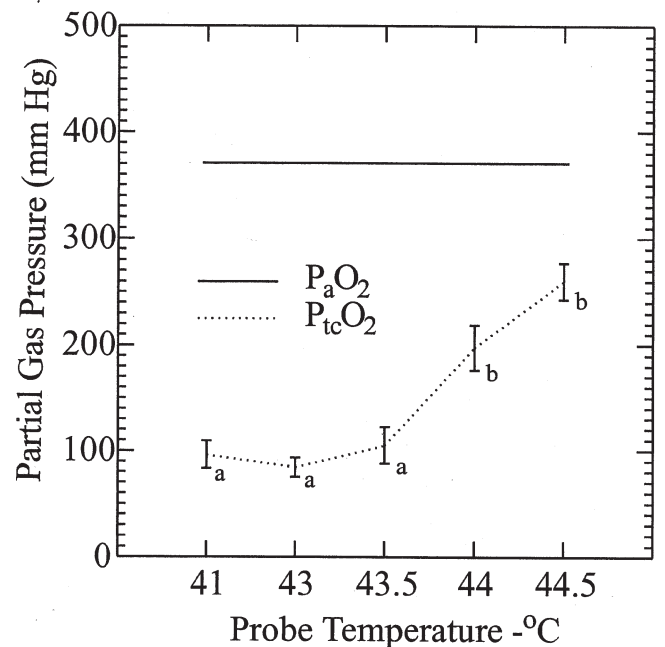


Figure 3. Comparison of mean P_{tc}O₂ at five probe temperatures (42, 43, 43.5, 44, and 44.5°C), using 100% O₂ as the anesthetic carrier gas. The dotted line represents mean (± SEM) P_{tc}O₂ at each probe temperature. Mean P_aO₂ reference value (370.53 mmHg) is represented by a solid line and is shown only for comparison. Eight adult rats were tested daily with a single probe temperature randomly selected each day for five days. Differences could not be detected for probe temperatures with subscripts in common.

skin was not always grossly apparent immediately after probe removal. In adults, lesions when present were limited to edema and erythema. Gross lesions were more apparent 24 h after probe removal. At 24 h after probe removal (44.5°C, four-hour treatment), an eschar covered the entire probe site. Gross lesions diminished as probe temperature and application time decreased, in subsequent animals, until they were absent when the probe was applied at 43.0°C for three hours. Testing in juveniles started at 43°C for three hours revealed only slight erythema at 24 h after treatment. Gross lesions were not observed 24 h after a three-hour test period at 42.5°C.

Microscopic lesions in adults consisted of coagulative necrosis, with occasional ulceration of the epidermis (44.5°C, four hours). A sharp line of demarcation was present at the borders of the probe site. Occasional sebaceous glands and blood vessels exhibited coagulative necrosis deeper in the dermis where mild hemorrhage was also present. Inflammatory cells consisted principally of neutrophils and were most often seen in ulcerated areas. When the probe was applied for three hours at 43.0°C, microscopic lesions were restricted to the epidermis and consisted of mild karyorrhexis and some karyolysis of the basal cells. The basement membrane remained intact. Similar mild lesions were observed in juveniles at low probe temperatures (42.5°C, three hours).

Experiment 4. Comparison of correlation coefficients for TCBG versus STBG values while varying probe temperature yielded the following results. In adult rats at a probe temperature of 42.5°C, the correlation coefficient for P_aO_2 versus $P_{tc}O_2$ was poor ($r = 0.344$). Good correlation was found between P_aCO_2 and $P_{tc}CO_2$ ($r = 0.812$). Inflammation was not evident at the probe site in any animal. Animals were anesthetized for an average of 92 min, and the probe was in place for an average of 69 min. At probe temperature of 43°C, P_aO_2 correlated poorly with $P_{tc}O_2$ ($r = 0.148$); however, P_aCO_2 versus $P_{tc}CO_2$ correlation improved ($r = 0.873$).

Occasionally, erythema was seen at the probe site; however, this did not persist from day to day. In that phase of the study, rats were anesthetized for 90 min, and the probe was in contact with the animal for a mean time of 73 min. Using a probe temperature of 44°C, P_aO_2 versus $P_{tc}O_2$ correlation was modest ($r = 0.530$), whereas P_aCO_2 versus $P_{tc}CO_2$ correlation was high ($r = 0.924$) (Fig. 4). Mean anesthesia and probe contact time at 44°C were 90 and 72 min, respectively. Differences between treatments by probe temperature for adult animals are presented in Table 2.

When juveniles were tested at 42.0°C, correlation for P_aO_2 and $P_{tc}O_2$ was poor ($r = -0.135$). Correlation between P_aCO_2 and $P_{tc}CO_2$ was much higher ($r = 0.892$). During this stage, mean anesthesia time was 76 min and mean probe application time was 53 min. At 43.0°C, juvenile P_aO_2 and $P_{tc}O_2$ still correlated poorly ($r = 0.122$); however, P_aCO_2 and $P_{tc}CO_2$ correlation decreased ($r = 0.465$). At 43.0°C, juvenile rats were anesthetized for a mean 88 min, and mean probe application time was 60 min. Finally, at 44.0°C with a mean anesthesia time of 88 min and mean probe application time of 59 min, P_aO_2 and $P_{tc}O_2$ again correlated poorly but improved ($r = 0.531$). The P_aCO_2 correlated well with $P_{tc}CO_2$ ($r = 0.899$), but was only slightly better than results at 42.0°C. Differences between treatments by probe temperature for juvenile animals are presented in Table 3.

When all probe temperature data were combined for adult animals, $P_{tc}O_2$, anesthesia time, probe temperature, sex, and

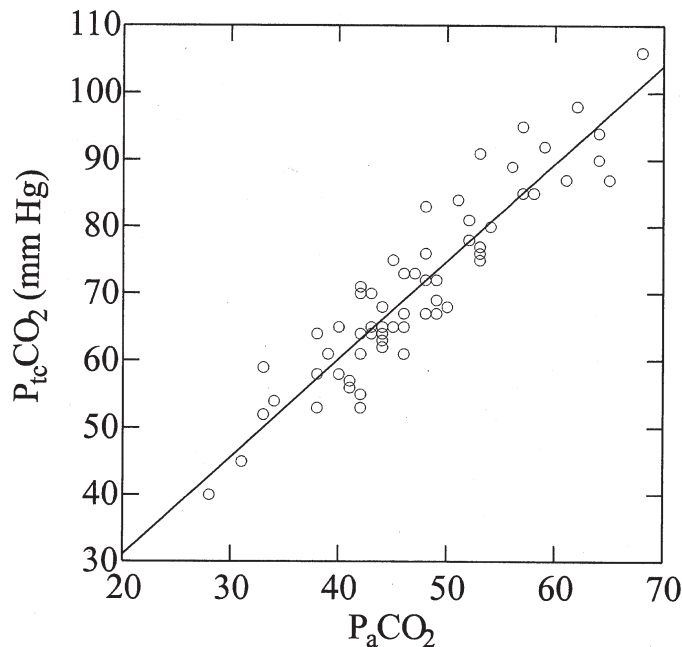


Figure 4. Scatterplot with regression line, $P_aCO_2 = 5.748 + 0.585(P_{tc}CO_2)$, using a probe temperature of 44.0°C ($R = 0.924$). Six adult rats were subjected daily for six days to two of three inspired gas treatments that differed in concentration of O_2 and CO_2 (treatment 1: room air; treatment 2: 27% FIO_2 , 0.00% $FICO_2$; or treatment 3: 19.88% FIO_2 , 4.7% $FICO_2$). Transcutaneous data and STBG samples were collected simultaneously for a total of 64 samples.

treatment affected prediction of P_aO_2 ($R = 0.603$), whereas body weight did not. The $P_{tc}CO_2$, probe temperature, anesthesia time, and treatment affected prediction of P_aCO_2 ($R = 0.899$). Effects of sex or body weight in adults could not be documented.

When all probe temperature data were combined for juveniles, the variables, P_aO_2 , probe temperature, anesthesia time, and treatment, affected prediction of $P_{tc}O_2$ positively ($R = 0.541$) whereas sex and body weight did not. The $P_{tc}CO_2$, probe temperature, and body weight were useful in prediction of P_aCO_2 and improved correlation ($R = 0.833$). Effects could not be documented for anesthesia time, sex, or treatment in prediction of P_aCO_2 for juvenile animals.

Experiment 5. Sensitivity assessment of TCBG technology indicated that P_aO_2 versus $P_{tc}O_2$ correlation in adults was poor for individual groups (A: $r = 0.025$, B: $r = 0.065$, C: $r = 0.056$, D: $r = 0.225$, and E: $r = 0.270$) and when all groups were combined ($R = 0.099$). Correlation coefficients for P_aCO_2 versus $P_{tc}CO_2$ for adult animals varied between groups (A: $r = 0.440$, B: $r = 0.466$, C: $r = 0.643$, D: $r = 0.489$, and E: $r = 0.344$). Overall P_aCO_2 versus $P_{tc}CO_2$ correlation for all groups ($r = 0.493$) was moderate. Mean anesthesia time and probe contact time were 66 and 53 min, respectively for all groups combined. Differences between treatments for adult animals are presented in Table 4.

The P_aO_2 versus $P_{tc}O_2$ correlation coefficient in juveniles varied for individual groups (A: $r = 0.172$, B: $r = 0.497$, C: $r = 0.130$, D: $r = 0.289$, and E: $r = 0.560$), and when all groups were combined ($R = 0.083$) was low. The P_aCO_2 versus $P_{tc}CO_2$ correlation coefficients for juvenile animals also varied among groups (A: $r = 0.869$, B: $r = 0.310$, C: $r = 0.767$, D: $r = -0.043$, and E: $r = 0.826$). Mean anesthesia time and probe contact time were 75 and 53 min, respec-

tively; overall $P_a\text{CO}_2$ versus $P_{tc}\text{CO}_2$ correlation for all groups ($R = 0.634$) was moderate to good. Differences between treatments for juvenile animals are presented in Table 5.

Using GLM with all groups combined, correlation was poor ($R = 0.225$), however improved with only body weight serving as a predictor for $P_a\text{O}_2$ in adults. $P_a\text{CO}_2$ correlation improved ($R = 0.629$), with $P_{tc}\text{CO}_2$, anesthesia time, and group serving as useful predictors. When juveniles were evaluated with GLM, correlation improved to a moderate level ($R = 0.579$), with only group being useful for prediction of $P_a\text{O}_2$. For $P_a\text{CO}_2$, correlation improved ($R = 0.791$), with $P_{tc}\text{CO}_2$ and group serving as useful predictors. When GLM was applied using the same variables with the exception of swapping treatment for group, results were similar.

Discussion

The objective of this study was to validate non-invasive methods to assess changes in arterial blood values of O_2 and CO_2 in the rat. Analysis by use of STBG requires blood samples; determination of $P_{et}\text{CO}_2$ ideally requires intubation, and PO does not provide $P_a\text{CO}_2$ assessment. Our future experimental animal model (suckling rat) has a small blood volume, and difficult venous access. The TCBG method correlates well with $P_a\text{O}_2$ and $P_a\text{CO}_2$ in humans, particularly infants, as well as providing trend data over time without need for venous access (3, 30).

Several factors, such as skin thickness, presence of hair, skin lesions, and perfusion, influence diffusion of O_2 and CO_2 through the skin (31). Skin thickness is inversely related to diffusion of gases through the skin (32). Skin thickness influences O_2 diffusion more than CO_2 diffusion since O_2 is 16 times less liposoluble than is CO_2 (20). In quadrupeds such as the rat, the skin is thinner on the ventrum and medial aspects of the limbs. For this reason, we chose the three probe sites tested in experiment 1. Additionally, each of the sites chosen provide a flat surface for probe attachment. Differences were found in probe sites for pony foals (25) and humans (31), and were attributed to skin thickness variation between sites probe locations.

The presence of hair presents another problem for gas diffusion through the skin of the rat since the only glabrous areas are the soles of the feet, nose, tail, and ears, none of which are sufficiently large for probe placement. Hair can be removed as was done in these experiments; however, the hair above the skin surface may not be all of the problem. Takiwaki (31) reported much lower $P_{tc}\text{O}_2$ and modestly higher $P_{tc}\text{CO}_2$ readings taken from the human cheek (male), compared with glabrous sites. He speculated the presence of pilo-sebaceous glands, which release oily secretions, may inhibit diffusion of O_2 to the skin surface. We also speculate that hair follicles, which are extensions of the epidermis deep into the dermis, may also inhibit the flow of gases to the surface. While the surface hair can be removed, we still have the remaining hair shaft within the follicle, the pilo-sebaceous glands, and the dense number of follicles extending into the dermis, all of which would inhibit gas flow to the surface, especially O_2 . In experiment 1, Nair was used to remove hair from the surface. This method of depilation was discontinued after finding that this depilatory agent caused cutaneous inflammation that became apparent on the second day after application. Depilatory use did not adversely affect experiment 1 since all data were collected on the day of depilatory application. Inflammation principally affected $P_{tc}\text{O}_2$ readings, but $P_{tc}\text{CO}_2$ values also were altered (data not shown). The use of de-

pilatory agents is not recommended for studies in which $P_{tc}\text{O}_2$ or $P_{tc}\text{CO}_2$ is measured from day to day in the same animal. In two other studies concerned with correlation of STBG and TCBG, depilatory agents were used with good results for $P_{tc}\text{CO}_2$; however, in those studies, rats were not tested serially at the same probe site (14, 15).

Perfusion of the skin can also alter TCBG values (9). Heat from the probe increases perfusion to the probe site, and additionally, shifts the O_2 and CO_2 dissociation curves to the right, thus increasing the partial pressures of both gases (7, 8), both of which serve to enhance accuracy of the instrument. The link between TCBG technology and skin perfusion can be used to advantage in certain situations, such as skin graft assessment and diabetic limb ischemia (22, 33, 34). On the other hand, skin perfusion can also be a problem in patients with low blood pressure due to cardiac failure, severe dehydration, or hypovolemia. In those situations, TCBG technology should be used with caution. Additionally, many commonly used pharmacologic agents may alter blood pressure, cardiac output, or skin perfusion (28, 35). While establishing methods for this experiment, atropine sulfate (Atropine SA, Butler, Columbus, Ohio) and glycopyrrolate (Robinul-V, Fort Dodge, Fort Dodge, Iowa) were used to control mucous secretions that might adversely affect respiration. However, reduction in $P_{tc}\text{O}_2$ was occasionally observed after their use. Further evaluation of literature revealed that anticholinergics may alter skin vasodilation in rate and magnitude in response to heat (36), and cutaneous blood flow in response to noxious stimuli (37). Therefore, use of anticholinergics was discontinued. Other drugs, such as halothane and nitrous oxide, commonly used in anesthesia, are reduced at the polarographic O_2 electrode of the TCBG probe and falsely increase $P_{tc}\text{O}_2$ values (8). We did not find reported direct effects of isoflurane on the polarographic O_2 electrode. However, isoflurane is a respiratory depressant and will lower blood pressure at increased values, as reported by the manufacturer (IsoFlo, Abbott Lab.; Chicago, Ill.). The potential effects of any pharmacologic agent on skin perfusion should be investigated prior to use when TCBG analysis will be implemented and the appropriate controls should be in place.

Experiment 3 was conducted to evaluate the thermal burn potential of the heated probe. To our knowledge, data on thermal burn potential in relationship to TCBG probe temperature and contact time in the rat have not been published; however, they have been reported in humans (6, 7). We have documented that 42 and 42.5°C probe temperatures can be successfully used for three hours in juvenile and adult rats, respectively, without damage to the skin. These findings are important for future research applications, wherein daily TCBG analysis will be performed. Others have reported thermal burns in adult rats using a 44°C probe temperature (15). Those authors attributed the burns to hypovolemia due to multiple blood sampling, but did not report the probe contact time.

In experiment 4, we examined correlations between TCBG and STBG, while evaluating various probe temperatures and carrier gas mixtures. In adult rats, $P_{tc}\text{O}_2$ and $P_a\text{O}_2$ correlated poorly, and this is in agreement with another reports (14). The $P_{tc}\text{O}_2$ and $P_a\text{O}_2$ also correlated poorly in juvenile rats at all probe temperatures. Tables 2 and 3 reveal the wide range and SEM of $P_{tc}\text{O}_2$, compared with $P_a\text{O}_2$ values. It is unclear why the mean $P_{tc}\text{O}_2$ values in juvenile animals are so low. Although the $P_a\text{O}_2$ values are also low in juvenile animals, the difference is greater between juvenile $P_a\text{O}_2$

and $P_{tc}O_2$ values than those in the adult. It is possible that skin perfusion is still undergoing some degree of development. This should be investigated in future studies.

In general, $P_{tc}CO_2$ and P_aCO_2 correlation was much better than that of $P_{tc}O_2$ and P_aO_2 , regardless of probe temperature. In adult rats, the correlation improved as probe temperature increased. At 43°C, our correlation of 0.873 was lower than that of another study, in which a correlation coefficient of 0.93 at 43°C was reported (15). It should be pointed out that their animals were ventilated and were subjected to only one inspired gas concentration per day, which would serve to decrease variability and increase correlation. We chose spontaneous ventilation and at least three gas mixtures in anticipation of future studies with drugs that may alter ventilatory drive in the rat.

We believe this study to be the first report to validate TCBG use in juvenile rats. In juvenile animals, $P_{tc}CO_2$ to P_aCO_2 correlation was almost identical at 42 and 44°C; however, it was lower at 43°C. The reason for the decrease in correlation at 43°C is unclear. Since correlation was still very good at 42°C, it was chosen over 44°C to eliminate the possibility of thermal burns in future studies.

In experiment 5, we tested the sensitivity of the TCBG unit to reflect P_aO_2 and P_aCO_2 changes by incrementally lowering inspired CO_2 concentrations in a spontaneously ventilated rat. Figure 5 depicts the differences between P_aCO_2 and $P_{tc}CO_2$ at variable $FICO_2$ values in juvenile rats. Again, $P_{tc}O_2$ and P_aO_2 correlations were extremely poor for adults and juveniles. In adult rats, $P_{tc}CO_2$ and P_aCO_2 correlation decreased from that seen in experiment 4, with correlation varying between inspired gas concentrations. The fluctuation was more pronounced in juvenile rats. This was unexpected and stimulated further review of the data. In adults and juveniles, we found differences in anesthesia time and probe contact time from experiment 4 to experiment 5, in which each parameter decreased. Furthermore, there was a downward trend for anesthesia time in juvenile animals within experiment 5 between groups, as CO_2 concentration decreased (data not shown). Apparently, as our technique improved over time, catheterization as well as instrumentation became faster, and the juvenile animals did not have as much time to stabilize before the first sample was taken. Previous rodent studies did not mention an anesthesia stabilization period; however, TCBG instrument stabilization was accounted for (14, 15). In future studies, an anesthesia stabilization period should be incorporated before application of the probe, especially when using the TCBG unit on spontaneously breathing animals, and probe positions kept constant.

An additional question was raised at the close of the experiment concerning the poor correlation of P_aO_2 and $P_{tc}O_2$. Specifically, must mean P_aO_2 or P_aCO_2 values differ by treatment for their transcutaneous counterparts to correlate reasonably well? The answer appears to be no for P_aCO_2 , and possibly for P_aO_2 . Tables 4 and 5 present means, SEM, and ranges for the variables in question. We see, in Table 4, statistical differences between P_aO_2 means for some of the treatments even though the actual changes in inspired O_2 were small. In this instance, correlation was poor with $P_{tc}O_2$. On the other hand, similar differences were seen in P_aCO_2 means between treatments, yet correlation with $P_{tc}CO_2$ was higher. Table 5 reinforces this argument using juvenile animals. Only treatment A yielded a significantly different mean P_aCO_2 , yet correlation with $P_{tc}CO_2$ was better than that for P_aO_2 and $P_{tc}O_2$. Tables 2 and 3 present

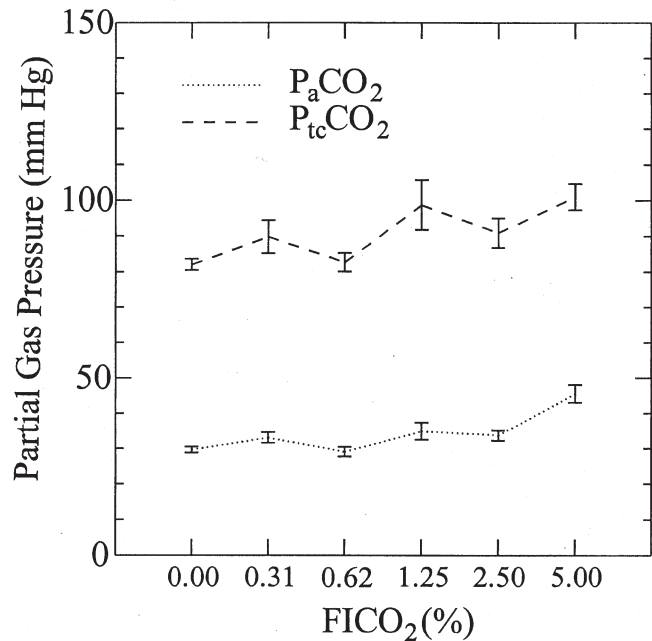


Figure 5. Comparison of mean P_aCO_2 and $P_{tc}CO_2$ (mmHg) at various $FICO_2$ values, in anesthetized 10-day-old Sprague-Dawley rats. Data represent simultaneously collected STBG samples and TCBG data for each animal (24 males and 26 females). Lines represent mean (\pm SEM) P_aCO_2 and $P_{tc}CO_2$.

similar data for experiment 4. Only in Table 2 at a probe temperature of 44°C was correlation modest for P_aO_2 and $P_{tc}O_2$ in adult animals. In this instance, not only were there differences between treatment means, but they were rather large. P_aCO_2 means for adults (Table 2) were different for treatment 3 as expected. However for juveniles, means were not different (Table 3). Yet in each instance, correlation was better for P_aCO_2 and $P_{tc}CO_2$.

In conclusion, we can say that $P_{tc}CO_2$ correlates better with its arterial counterpart and appears to be considerably more sensitive than is $P_{tc}O_2$ in rats. Further use of TCBG as a tool to evaluate small changes in P_aO_2 in the rat appears unwarranted. However, it may be of use for other research, such as skin graft assessment (38, 39) or perfusion after detorsion of an organ (40), where alterations in blood flow cause large differences in O_2 delivered to the tissue.

Use of TCBG analysis is warranted in rats under controlled conditions. The correlation between $P_{tc}CO_2$ and P_aCO_2 is not sufficiently strong for use as a clinical tool in individual animals. However, this technology is capable of trend monitoring, which enables the investigator to gather information that would otherwise be unobtainable in situations where blood volume would limit or exclude use of conventional methods. Additionally, the technology is non-invasive, which allows daily data collection. This attribute opens up the possibility of repeated measures analysis, thus minimizing animal numbers needed for experimentation.

Acknowledgments

This manuscript was submitted by Rhett W. Stout in partial fulfillment of the Ph.D. degree. We thank Michael T. Kearney and Giselle Hosgood for their help with statistical analysis. We would also like to thank Catherine L. Christensen, Essie B. Mack, and Natalie T. Simpson for their help with blood gas analysis.

References

- Huch, R., A. Huch, and D. W. Lubbers. 1973. Transcutaneous measurement of blood P_{O_2} (tc P_{O_2})—Method and application in perinatal medicine. *J. Perinatol. Med.* **1**:183-191.
- Carter, R., and S. W. Banham. 2000. Use of transcutaneous oxygen and carbon dioxide tensions for assessing indices of gas exchange during exercise testing. *Respir. Med.* **94**:350-355.
- Brudin, L., S. Berg, P. Ekberg, and J. Castenfors. 1994. Is transcutaneous PO_2 monitoring during exercise a reliable alternative to arterial PO_2 measurements? *Clin. Physiol.* **14**:47-52.
- Leasa, D. J. 1992. Noninvasive blood gas monitoring: a review for use in the adult critical care unit. *Can. Med. Assoc. J.* **146**:703-712.
- Szafarski, N. L. 1996. Emerging technology in critical care: continuous intra-arterial blood gas monitoring. *Am. J. Crit. Care.* **5**:55-65.
- Huch, R. 1995. Review: perinatal monitoring. *Acta. Anaesthesiol. Scand. Suppl.* **39**:91-94.
- Rithalia, S. V. S. 1991. Developments in transcutaneous blood gas monitoring: a review. *J. Med. Eng. Technol.* **15**:143-153.
- Rochat, M. C., and F. A. Mann. 1994. Transcutaneous blood gas monitoring. *Compend. Contin. Educ. Pract. Vet.* **16**:1181-1186.
- Huch, A. 1995. Transcutaneous blood gas monitoring. *Acta. Anaesthesiol. Scand. Suppl.* **39**:87-90.
- Gottrup, F., S. Gellet, L. Kirkegaard, E. S. Hansen, and G. Johannsen. 1988. Continuous monitoring of tissue oxygen tension during hyperoxia and hypoxia: relation of subcutaneous, transcutaneous, and conjunctival oxygen tension to hemodynamic variables. *Crit. Care Med.* **16**:1229-1234.
- Brothers, T. E., T. W. Wakefield, L. A. Jacobs, and S. M. Lindenaer. 1993. Effects of lumbar sympathectomy on canine transcutaneous oxygen tension. *Surgery* **113**:433-437.
- Wilhelm, J. A., P. Veng-Pedersen, T. B. Zakszewski, E. Osifchin, and S. J. Waters. 1995. Duration of opioid antagonism by nalmeferone and naloxone in the dog. A nonparametric pharmacodynamic comparison based on generalized cross-validated spline estimation. *Int. J. Clin. Pharmacol. Ther.* **33**:540-545.
- Kamler, M., H. A. Lehr, J. H. Barker, R. K. Saetzler, T. J. Galla, and K. Messmer. 1993. Impact of ischemia on tissue oxygenation and wound healing: intravital microscopic studies on the hairless mouse ear model. *Eur. Surg. Res.* **25**:30-37.
- Yamamoto, T., and I. Kida. 1996. Application of transcutaneous blood carbon dioxide monitoring to the magnetic resonance imaging of rat. *J. Med. Eng. Technol.* **20**:164-168.
- Furset, K., L. Aanderud, K. Segadal, and I. Tyssebotn. 1987. Transcutaneous measurement of pCO_2 at high ambient pressure (41 bar). *Undersea Biomed. Res.* **14**:51-62.
- Mann, F. A., C. C. Wagner-Mann, and K. R. Branson. 1998. Transcutaneous oxygen and carbon dioxide monitoring in normal cats. *J. Vet. Emerg. Crit. Care* **7**:99-109.
- Fall, O., M. Johnson, B. A. Nilsson, and G. Rooth. 1979. Continuous transcutaneous oxygen tension measurements in experiments with juvenile rhesus monkeys and pregnant ewes. *Birth Defects Orig. Arctic. Ser.* **15**:629-632.
- Mueller-Heubach, E., and A. F. Battelli. 1982. Variable heart rate decelerations and transcutaneous PO_2 during umbilical cord occlusion in fetal monkeys. *Am. J. Obstet. Gynecol.* **144**:796-802.
- Bergmans, M., G. Stevens, H. Keunen, and T. Hasaart. 1997. Transcutaneous and arterial carbon dioxide tension at various conditions of fetal stress in lambs. *Gynecol. Obstet. Invest.* **43**:1-5.
- Braems, G., U. Lang, and W. Kunzel. 1996. Skin blood flow influences the transcutaneous PCO_2 (tc PO_2) during stress in the guinea pig. *J. Perinatol. Med.* **24**:155-161.
- Ovadia, Z., R. Kornowski, B. Gavish, D. Chayen, R. Walden, H. Varda-Bloom, A. Battler, and M. Eldar. 1995. Noninvasive evaluation of microcirculatory hemodynamic changes during hemorrhage followed by saline or blood transfusion. *Shock* **4**:96-101.
- Raposio, E., and P. L. Santi. 1998. Pharmacological enhancement of cutaneous flap survival with topical dimethyl sulphoxide and hydrogen peroxide. *Br. J. Plast. Surg.* **51**:551-554.
- Barker, S., J. Hyatt, C. Clarke, and K. Tremper. 1991. Hyperventilation reduces transcutaneous oxygen tension and skin blood flow. *Anesthesiology* **75**:619-624.
- Sundin, B. M., M. A. Hussein, S. Glasofer, M. H. El-Falaky, S. M. Abdel-Aleem, R. E. Sachse, and B. Klitzman. 2000. The role of allopurinol and deferoxamine in preventing pressure ulcers in pigs. *Plast. Reconstr. Surg.* **105**:1408-1421.
- Warren, R., A. Webb, P. Kosch, and L. Coons. 1984. Evaluation of transcutaneous oxygen monitoring in anaesthetised pony foals. *Equine Vet. J.* **16**:359-361.
- Wagner, A. E., R. M. Bednarski, and W. W. Muir, 3rd. 1990. Hemodynamic effects of carbon dioxide during intermittent positive-pressure ventilation in horses. *Am. J. Vet. Res.* **51**:1922-1929.
- Wayneforth, H. B., and P. A. Flecknell. 1992. Chronic catheterization of blood vessels. p. 212-234. *In* Experimental and surgical technique in the rat, 2nd ed. Academic Press, Inc., San Diego, Calif.
- Martinez, E. A., S. M. Hartsfield, L. D. Melendez, N. S. Matthews, and M. R. Slater. 1997. Cardiovascular effects of buprenorphine in anesthetized dogs. *Am. J. Vet. Res.* **58**:1280-1284.
- Carleton, J. N., A. M. Donoghue, and W. K. Porter. 1998. Mechanical model testing of rebreathing potential in infant bedding materials. *Arch. Dis. Child.* **78**:323-328.
- Tobias, J. D., W. R. Wilson, and D. J. Meyer. 1999. Transcutaneous monitoring of carbon dioxide tension after cardiothoracic surgery in infants and children. *Anesth. Analg.* **88**:531-534.
- Takiwaki, H. 1994. Transcutaneous PO_2 and PCO_2 measurement in dermatology. *Acta. Dermatol. Venereol. Suppl. (Stockh.)* **185**:21-25.
- Falstie-Jensen, N., E. Spaun, J. Brochner-Mortensen, and S. Falstie-Jensen. 1988. The influence of epidermal thickness on transcutaneous oxygen pressure measurements in normal persons. *Scand. J. Clin. Lab. Invest.* **48**:519-523.
- Peirce, S. M., T. C. Skalak, and G. T. Rodeheaver. 2000. Ischemia-reperfusion injury in chronic pressure ulcer formation: a skin model in the rat. *Wound Repair Regen.* **8**:68-76.
- Petrakis, I. E., and V. Sciacca. 2000. Spinal cord stimulation in diabetic lower limb critical ischaemia: transcutaneous oxygen measurement as predictor for treatment success. *Eur. J. Vasc. Endovasc. Surg.* **19**:587-592.
- Cowan, A., J. C. Doxey, and E. J. Harry. 1977. The animal pharmacology of buprenorphine, an oripavine analgesic agent. *Br. J. Pharmacol.* **60**:547-554.
- Kellogg, D. L., P. E. Pergola, K. L. Piest, W. A. Kosiba, C. G. Crandall, M. Grossman, and J. M. Johnson. 1995. Cutaneous active vasodilation in humans is mediated by cholinergic nerve co-transmission. *Circ. Res.* **77**:1222-1228.
- Low, A., and R. A. Westerman. 1989. Neurogenic vasodilation in the rat hairy skin measured using a laser doppler flowmeter. *Life Sci.* **45**:49-57.
- Frick, A., R. G. H. Baumeister, M. D. Menger, B. Vollmar, U. Wohllaib, and B. Wiebecke. 1999. Secondary ischaemia in experimental free flaps -treatment by long acting prostacyclin analogues. *Br. J. Plast. Surg.* **52**:392-398.
- Rochat, M. C., E. R. Pope, J. T. Payne, L. W. Pace, and C. C. Wagner-Mann. 1993. Transcutaneous oxygen monitoring for predicting skin viability in dogs. *Am. J. Vet. Res.* **54**:468-475.
- Kram, H. B., E. Miyamoto, J. Rajfer, P. L. Appel, and W. C. Shoemaker. 1989. Testicular oximetry: a new method for the assessment of tissue perfusion and viability following torsion and detorsion. *J. Pediatr. Surg.* **24**:1297-1302.