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Abstract approved: _____
Robert T. Mason

Variability in the depth and duration of anesthesia in individuals within a species is frequently observed, yet few studies have investigated its causes in reptiles. To evaluate the potential causes of variability in reptile anesthesia, I conducted experiments to test for effects of body temperature, body composition and time post-feeding on the duration of brevital sodium anesthesia in the red-sided garter snake (*Thamnophis sirtalis parietalis*). Mean times to righting ability of snakes anesthetized at 31°C were twice as high as snakes at 21°C. Fat snakes (i.e. those with higher mass/SVL ratios) had a mean time to righting ability that was 60% lower than did thin snakes. Time post-feeding did not have a statistically significant effect on the time to righting ability in snakes that were anesthetized one, three and ten days after consuming 30% of their body mass in food. Recommendations for producing more predictable results when using injectable anesthetics in reptiles are given and an equation to predict the effective dosage of sodium brevital based on body temperature and body condition in *T .s. parietalis* is proposed.

Key Words: red-sided garter snake, *Thamnophis sirtalis parietalis*, injectable anesthetic, surgery, body temperature, body composition

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Sources of Variability in Duration of Anesthesia with Brevital Sodium in Snakes

by

Daniel L. Preston

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Daniel L. Preston, Author

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INTRODUCTION

Surgical procedures requiring anesthesia are often necessary to study aspects of reptilian anatomy, physiology and behavior (e.g. Miller and Gutzke, 1999; Blouin-Demers and Weatherhead, 2001; Krohmer et al., 2004). A safe and effective anesthetic protocol is necessary to facilitate surgery and minimize stress and injury to experimental subjects. An ideal anesthetic agent should take effect rapidly, create a sufficient depth of anesthesia for the desired procedure, and allow reasonably fast and predictable recovery after surgery has ended. A suitable anesthetic for reptile research must also be easy to administer under laboratory and field conditions and should not create side effects that may impact experimental data.

A wide variety of injectable and inhalant anesthetic agents have been used in reptile anesthesia (Mosley, 2005; Bertelsen, 2007). Among the most commonly used anesthetics in reptile research is the barbiturate brevital sodium (methohexital sodium). Brevital sodium is associated with rapid induction and recovery times and is effective when administered subcutaneously (Wang et al., 1977).

Brevital sodium has been used to induce general anesthesia in frogs (Malvin and Walker, 2001), tuatara (Gorniak et al., 1982), lizards (Smith, 1982; Gaztelu et al., 1991), snakes (Nichols and Lamirande, 1994; Miller and Gutzke, 1998) and turtles (Gaztelu et al., 1991; Jackson et al. 2000). Brevital sodium has also been used as an induction agent prior to halothane gas anesthesia in alligators (Gatesy, 1990). Variable effects across different reptile species have been observed with brevital sodium and the effective

dosages have ranged from 5 mg/kg in *Rhinophis* to 20 mg/kg in *Anolis* (Wang et al., 1977). Colubrid snakes have been effectively anesthetized with brevital sodium at dosages from 9 mg/kg (Nichols and Lamirande, 1994) to 15 mg/kg (Wang et al., 1977).

Variable anesthetic effects between individuals of the same species have been observed in reptile anesthesia studies and have been anecdotally attributed to possible differences in temperature, body size, body condition, sex, stress level, stage of ecdysis, drug administration route and elapsed time since previous dosings (Karlstrom and Cook 1955; Cooper, 1974; Wang et al., 1977; Hill and Mackessy, 1997; Blouin-Demers et al., 2000). Few studies have experimentally evaluated causes of variability in anesthetic duration within a species. Only body temperature has been shown to have an effect on ketamine anesthesia in skinks (Arena et al., 1988) and isoflurane anesthesia in desert iguanas (Dohm and Brunson, 1998). Both studies found lower body temperatures resulted in prolonged duration of anesthesia.

Research conducted by Mason and collaborators has utilized brevital sodium in the field and laboratory for over 20 years to anesthetize red-sided garter snakes (*Thamnophis sirtalis parietalis*) prior to surgical procedures (e.g. Nelson et al., 1987; Shine et al., 2001; Krohmer et al., 2004; Lutterschmidt et al., 2006). Other injectable anesthetics including ketamine, propofol, medetomidine and midazolam have been used in *T. s. parietalis* and none were determined to be superior to brevital sodium in terms of ease of administration, depth of anesthesia and time to recovery (D. Preston, unpublished data). While brevital sodium is likely the most effective injectable agent in *T.s.parietalis*, a high degree of variability in the duration of anesthesia has been observed between

individuals. No studies have been done to assess the causes of variability in anesthesia in snakes.

Three separate experiments were conducted to asses the factors that may cause variability in duration of anesthesia in *T. s. parietalis*. The effects of 1.) body temperature, 2.) body composition and 3.) time post-feeding on duration of brevital sodium anesthesia were evaluated. Recommendations for modifying anesthetic protocols to produce more predictable results when using injectable anesthetics in reptiles are given and an equation is proposed to predict effective dosages of sodium brevital in garter snakes based on the parameters body condition and body temperature.

MATERIALS AND METHODS

Collection and Captive Maintenance of Garter Snakes

Thirty male and 45 female *T. s. parietalis* were collected from over-wintering hibernacula near Inwood, Manitoba during May of 2007 and 2008. Garter snakes were transported to Oregon State University in Corvallis, Oregon and maintained in 10g glass aquaria in a microprocessor controlled environmental chamber. From May until September snakes were maintained at 24°C during the day and 14°C at night with 14L:10D photophase. Forty watt incandescent bulbs provided a thermal gradient within aquaria. From October to April snakes were maintained at 4°C, 0L:24D to simulate hibernation. During the summer months, garter snakes were fed an alternating diet of earthworms and trout weekly. Water was provided *ad libitum*.

Brevital Sodium

Brevital sodium (methohexital sodium, JHP Pharmaceuticals, Parsippany, NJ) was diluted to a 1% solution (10 mg/ml) in deionized water. The 1% solution was further diluted to a 0.5% solution in sterile 10 ml vials of 0.9% NaCl. Brevital sodium was stored at 4°C and was used over a period of several months.

Anesthetic Protocol

One hour prior to anesthesia snakes were removed from the environmental chamber and placed individually into ten gallon glass aquaria lined with newspaper. This allowed the body temperatures of the snakes to reach the desired temperature for each

experiment. Dosages of 15 mg/kg (equivalent to 0.003 ml of 0.5% brevital sodium/g body mass) were administered subcutaneously between the dorsal and ventral scales at a distance approximately 20 cm from the head of the snake. Injections were made with 1.00 CC syringes and ¾ 26G needles. Snake body temperatures were recorded using a thermocouple thermometer (Digi-Sense ThermoLogR Thermistor, Cole-Parmer) inserted 3 cm into the snake's cloaca and were measured every 30 min during anesthesia and recovery. Quantifying the duration of anesthesia was accomplished by measuring the time after anesthetic administration at which each snake regained its ability to right its entire body when placed on its back (hereafter called righting ability). Time to righting ability was the most appropriate measure of anesthetic duration because nearly every snake lost righting ability and it was the least subjective measure taken.

Experiment 1: Body Temperature

Twenty male *T. s. parietalis* (43 to 50 cm SVL, 27 to 47 g body mass) were anesthetized at body temperatures of approximately 21°C, 26°C and 31°C in a repeated measures experimental design. Snakes were anesthetized 6 to 8 days after feeding. At each temperature the twenty snakes were anesthetized over two consecutive days. Ten snakes were randomly assigned to be anesthetized on day one and ten on day two. Snakes at 21°C received no supplemental heat. Snakes at 26°C and 31°C were warmed with incandescent light bulbs throughout anesthesia and recovery.

Experiment 2: Body Condition

Forty five female *T. s. parietalis* (60 to 70 cm SVL, 57 to 118 g body mass) were divided into three groups based on body condition. Mass/SVL ratio was chosen to

quantify body condition due to the narrow range of snake lengths used in the experiment and the relatively small sample size. The 15 snakes with the lowest mass/SVL ratio were placed in the thin group. Snakes with the middle 15 mass/SVL ratios were placed in the medium group and the remaining 15 snakes in the fat group. Snakes were anesthetized within one month of being collected and were not fed in captivity before being anesthetized (*T. s. parietalis* is aphagic during the mating season). The 45 snakes were anesthetized in groups of nine on five days. Three snakes from each group were randomly selected to be anesthetized each day. Snake body temperatures were maintained at approximately 21°C during anesthesia.

Experiment 3: Time Post-Feeding

Ten male *T. s. parietalis* (50 to 55 cm SVL, 43 to 60 g body mass) and ten female *T. s. parietalis* (61 to 66 cm SVL, 93-107 g body mass) were anesthetized one, three and ten days after feeding in a repeated measures experimental design. Snakes were anesthetized in groups of ten and were given as much food as they could consume in one day prior to the first day of anesthesia. The dosage of sodium brevital administered to each snake was calculated based on the snake's mass one day prior to feeding. Snake masses were measured daily for ten days after feeding and snake body temperatures were maintained at approximately 21°C during anesthesia.

Statistical Analysis

One-way repeated-measures analysis of variance (ANOVA) was used to compare differences in time to righting between temperature groups and between time post-

feeding groups. One-way ANOVA was used to compare differences between body composition groups. Time to righting was log transformed for all experiments to normalize the data. Posthoc Tukey tests were used to compare individual groups within all three experiments. Sigma Stat was used for statistical analyses and Microsoft Excel was used to make graphics.

RESULTS

Experiment 1: Body Temperature

Mean snake body temperatures during anesthesia for the three groups were 21.5°C (+/- 0.067 SE), 26.3° C (+/- 0.073 SE) and 31.5°C (+/- 0.13 SE). At 21°C and 26°C all 20 snakes lost righting ability. At 31°C one snake did not lose righting ability and was not included in the analysis. Higher temperatures resulted in shorter times to righting ability (Fig.1, ANOVA, $F_{2,17} = 12.71$, $P<0.001$). The mean time to righting ability at 31°C was nearly twice as high 21°C (Fig. 1, posthoc Tukey, $P<0.001$) and the difference in mean times to righting ability was statistically significant between all three temperatures (Fig. 1, posthoc Tukey, 21°C vs 26°C, $q = 3.637$, $P = 0.037$ and 26°C vs 31°C, $q = 3.562$, $P = 0.042$).

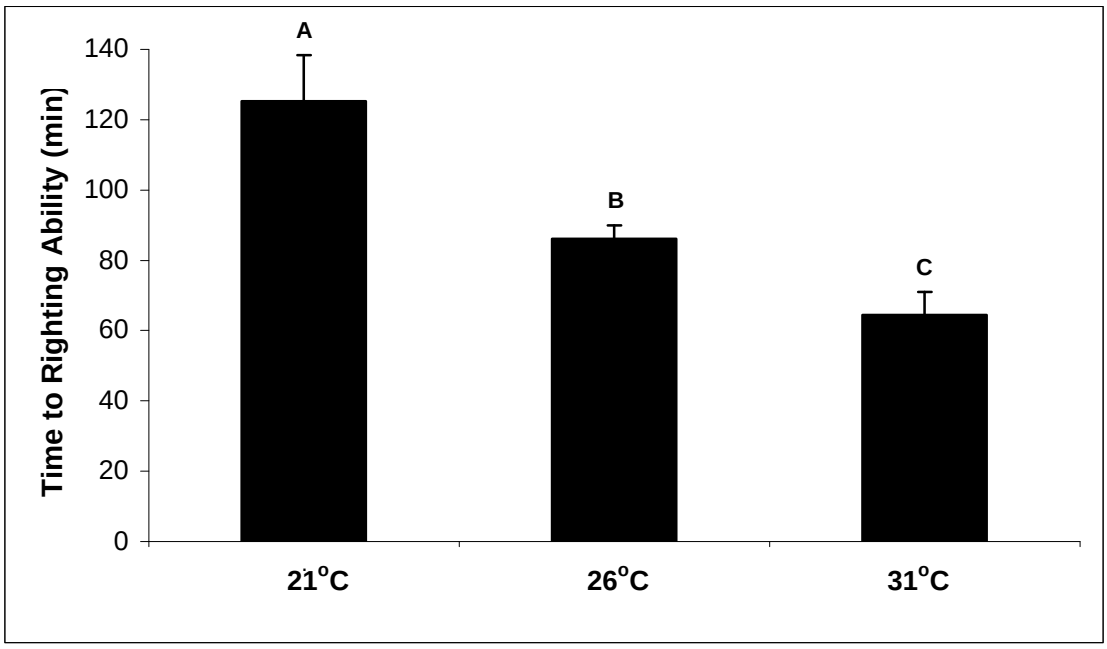

Figure 1. Mean time to righting ability (+/- SE) for T. s. parietalis (n=20 per temperature, repeated measures) at 21°C, 26°C and 31°C.

Experiment 2: Body Condition

Mean snake body temperatures during anesthesia were 21.1°C (+/- 0.37 SE) for thin snakes, 21.2°C (+/- 0.37 SE) for medium snakes and 21.1°C (+/- 0.38 SE) for fat snakes. As mass/SVL ratio increased, times to righting ability decreased (Fig. 2, r^2 = 0.252, P<0.001). Differences in time to righting ability were statistically significant between body condition groups (Fig. 3, ANOVA, $F_{2,42} = 5.026$, P = 0.011). Fat snakes regained righting ability 60% faster on average than thin snakes (Fig. 3, posthoc Tukey, q = 4.482, P = 0.008). Medium snakes regained righting ability over 20 minutes faster than thin snakes and over 20 minute slower than fat snakes on average, however these differences were not statistically significant (Fig. 3, posthoc Tukey, medium vs fat, q = 2.536, P = 0.230; medium vs thin, q = 2.126, P = 0.300).

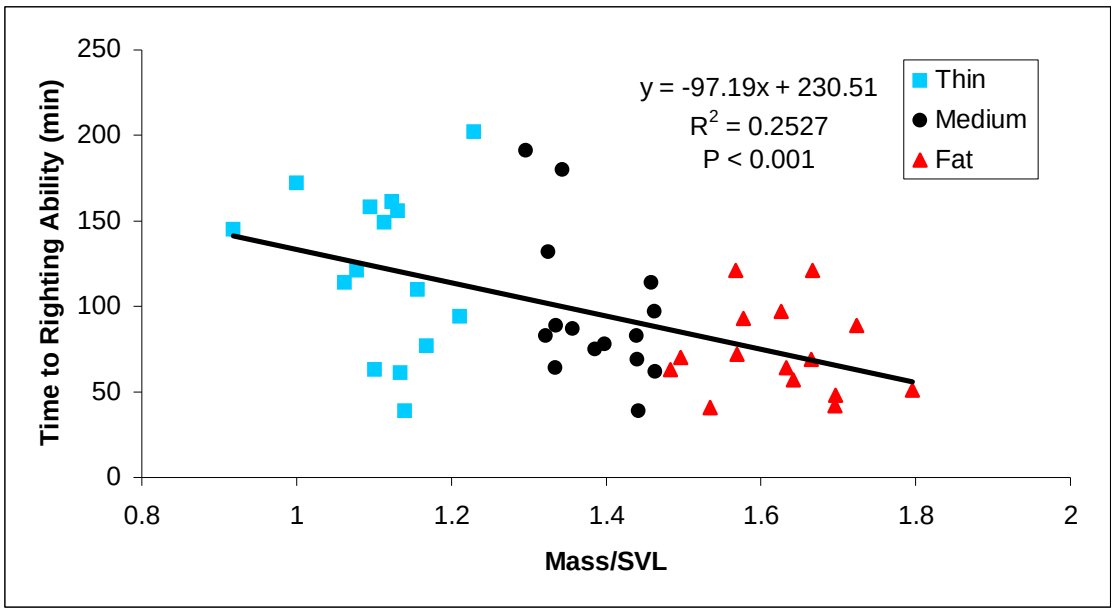

Figure 2. Time to righting ability versus mass/SVL ratio of *T. s. parietalis*. Squares represent snakes in the thin group, circles represent snakes in the medium group and triangles represent snakes in the fat group (n=15 per group).

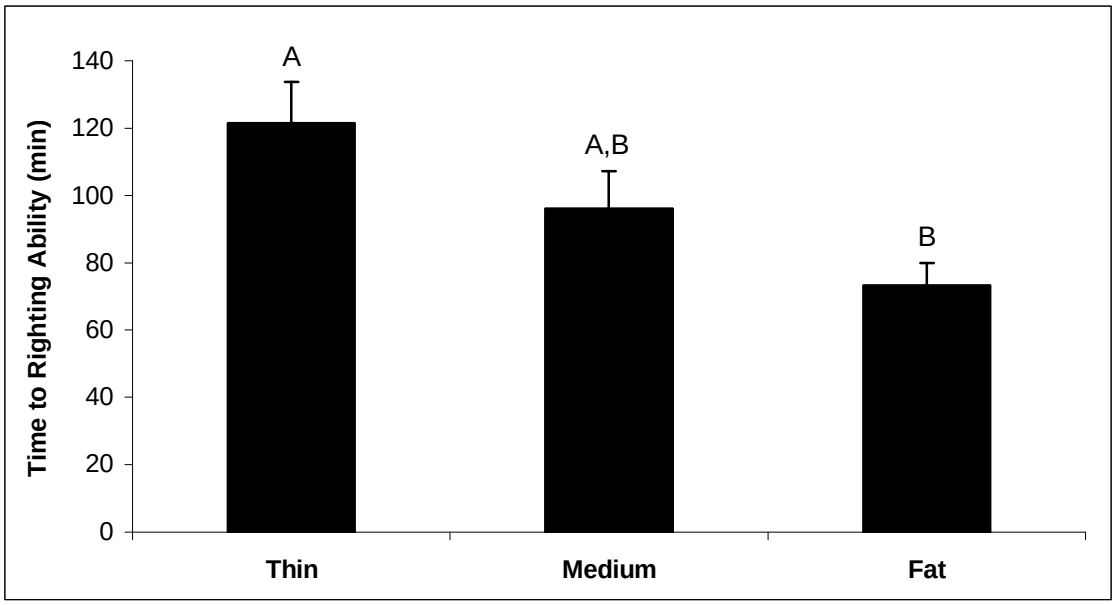

Figure 3. Mean time to righting ability (+/- SE) of *T. s. parietalis* in three body condition groups (n=15 per group). Body condition groups are based on mass/SVL ratios.

Experiment 3: Time Post-Feeding

Mean body temperatures for snakes during anesthesia were 21.8°C (+/- 0.13 SE) one day post-feeding, 22.0°C (+/- 0.18 SE) three days post-feeding and 21.5°C (+/- 0.13 SE) ten days post-feeding. On average, snakes ate 31% of their body mass in food (Fig. 4). Snakes had digested the majority of their meals by day three post-feeding and gradually decreased within 3% of their initial masses by day ten post-feeding (Fig. 4). There was not a statistically significant difference between the time to righting ability on day 1, 3 or 10 (Fig. 5, ANOVA, $F_{2,17} = 3.091$, $P = 0.057$).

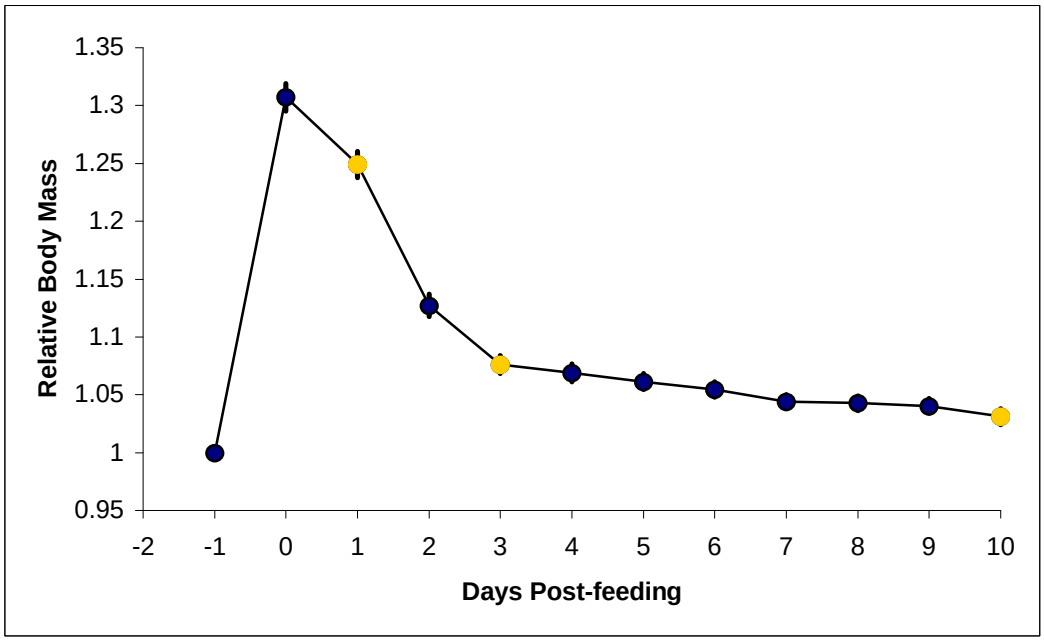

Figure 4. Relative body masses (+/- SE) of *T. s. parietalis* (n=20) from one day prior to feeding to 10 days post-feeding. Snakes were fed on day zero. Grey points indicate days when snakes were anesthetized. Snakes were given dosages of brevital sodium based on their mass one day prior to feeding.

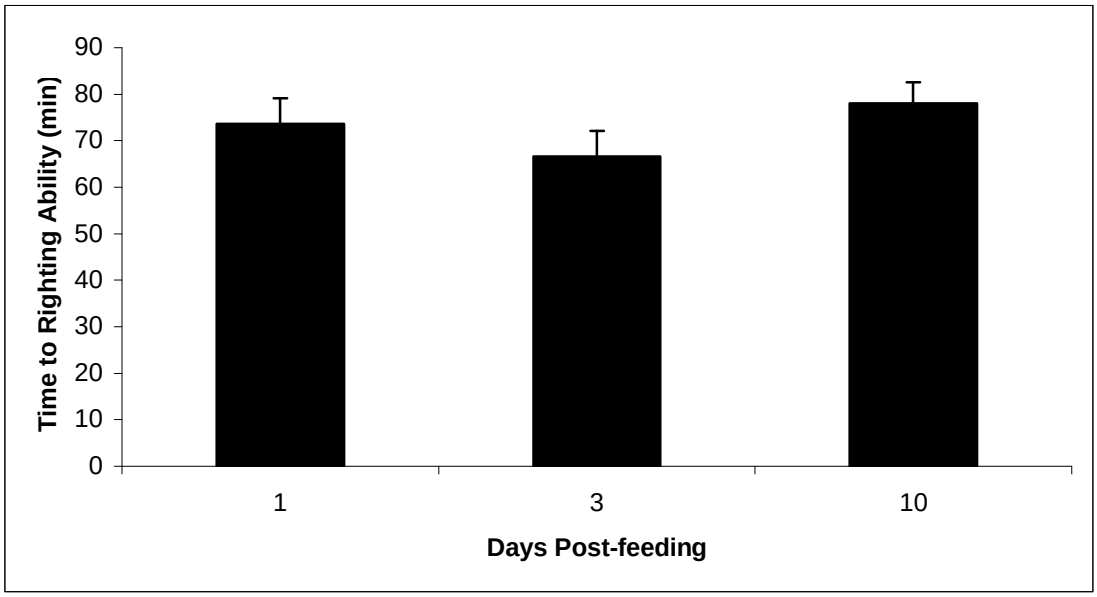

Figure 5. Mean time to righting ability (+/- SE) of *T. s. parietalis* one, three and ten days post-feeding (n=20 per group, repeated measures).

DISCUSSION

The shortened duration of sodium brevital anesthesia at higher temperatures in garter snakes is consistent with previous reports in lizards anesthetized with ketamine (Arena et al., 1988) and isoflurane gas (Dohm and Brunson, 1998). Elevated body temperatures likely reduce duration of anesthesia in all reptile species, whether inhalant or injectable anesthetics are used. The respiration and heart rates of reptiles increase with body temperatures (Greenwald, 1971) and this increases rates of distribution and metabolism of injectable anesthetics and decreases their duration of action.

Anesthetic protocols in veterinary medicine recommend maintaining reptiles at or near their preferred body temperature (PBT) for the duration of anesthesia and recovery (Mosley, 2005; Bertelsen 2007). PBT refers to the body temperature selected by an individual when given a thermal gradient to choose from. This practice is used to maintain optimal immune response and elevated metabolic rates in reptiles, which may reduce risks associated with anesthesia and surgery. The PBT of *Thamnophis sirtalis parietalis* ranges from 26°C to 30°C depending on the digestive state of the snake (Lysenko and Gillis, 1980). In our experience, red-sided garter snakes anesthetized near 30°C require over 20 mg/kg of brevital sodium and rarely reach a level of anesthesia suitable for surgery. We have obtained the most effective anesthesia by maintaining snakes near 21°C from one hour prior to anesthetic administration until the completion of surgical procedures. Snakes are warmed to their PBT immediately after surgery. This results in a deeper plane of anesthesia, but still allows rapid recovery times after surgery has ended. We have not observed any detrimental effects from anesthetizing healthy garter snakes at temperatures below their PBT.

Thinner garter snakes took longer to recover than heavier snakes (Fig. 3). This finding contrasts with a study by Nichols and Lamirande (1994) that found prolonged recovery times in heavy-bodied kingsnakes (*Lampropeltis calligaster*) compared to slender-bodied Brown Tree Snakes (*Boiga irregularis*). However, the species-specific differences observed by Nichols and Lamirande may be the result of variables other than relative amounts of adipose tissue, such as metabolic rate or amount muscle tissue. Studies using brevital sodium in garter snakes (Wang et al., 1977) and crotalines (Miller and Gutzke, 1998) suggest that reduced dosages are sufficient for anesthesia in neonatal snakes compared to adults. This observation may reflect the lower relative amounts of body fat in juvenile snakes compared to adults. Studies involving the dissociative agent ketamine in turtles (Holz and Holz, 1994) and in snakes (Glenn et al., 1972) found a similar pattern as that with brevital sodium where smaller individuals required smaller relative dosages.

The effect of body condition, and perhaps the effects of body size found in other studies, may be the result of the affinity for adipose tissue of many injectable agents. Barbiturate anesthetics are known to distribute sequentially from the blood pool into viscera, lean tissue and adipose tissue and are metabolized hepatically (Gillis et al, 1976; Bickel, 1984). In humans, concentrations of brevital sodium in adipose tissue reached 2 to 6 times concentrations in plasma after 4 hours (Brand, et al. 1963). The pharmacokinetics of brevital sodium in reptiles has not been studied, however a similar pattern of distribution as seen in mammals is likely. The shorter duration of anesthesia in fatter snakes may be the result of a greater percentage of the dosage becoming rapidly bound in adipose tissue shortly after giving the subcutaneous injection. Other injectable

agents used in reptiles, such as the lipophilic agent propofol, are known to distribute into adipose tissue to some degree and may show a similar increased potency (i.e. longer duration of anesthesia and longer recovery times) in thin reptiles as do the barbiturates.

Time post-feeding did not appear to have an impact on duration of anesthesia despite the considerable physiological changes snakes undergo during digestion (Fig. 5). Metabolic rate, blood flow to digestive organs and the size of the heart, liver and intestinal wall are known to increase during digestion in snakes (Secor et al. 2000; Wang et al. 2001; Andersen et al. 2005). In garter snakes, the size of hepatocytes and enterocytes increases after feeding (Starck and Beese, 2002) and the increases in cell sizes are due to elevated blood flow to these organs (Starck and Wimmer, 2004). Although sodium brevital is metabolized hepatically, it is redistribution from the blood pool that terminates the action of the anesthetic. This may explain why changes in liver activity did not appear to affect anesthetic duration. However, it is still not recommended to anesthetize snakes shortly after feeding since the added mass of food in the gut may result in calculating a dosage based on an inaccurate body mass. It has also been suggested that reptiles not be anesthetized after recent feeding due to the compression of lung tissue caused by large food items in the gut (Malley, 1997).

Snakes with the same mass/SVL ratio anesthetized at the same temperature still show considerable differences in time to righting ability which suggests that other factors, such as the route of drug administration, may contribute to variability between individuals. Subcutaneous and intramuscular drug administration are considered to be the least precise routes to deliver anesthetic agents since the percentage of the dosage entering the blood pool is essentially unknown and may vary between individuals

(Mosley, 2005). Subcutaneous injections, however, are the most practical route of drug administration when the accessibility or size of blood vessels in small lizards, snakes and turtles does not allow easy intravenous access. Unlike other barbiturates, there is no tissue irritation associated with subcutaneous injections of brevital sodium.

In order to produce more consistent durations of anesthesia between individual garter snakes, a linear model to predict the dosage for surgical anesthesia based on body temperature and body condition was created:

$$\text{Dose} = 5.2 + 0.4T + 2.4(M/SVL)$$

where dose is in mg/kg, T is body temperature in degrees Celsius, M is mass in grams and SVL is snout-vent length in cm. The model predicts that male *T. s. parietalis* at 21°C with a mass/SVL ratio of 0.7 require a dosage of 15 mg/kg, which is the same dosage found effective by Wang et al. (1977). As body temperature and mass/SVL ratio increase, the predicted effective dosage increases linearly to 24 mg/kg for *T. s. parietalis* at 31°C with a mass/SVL ratio of 2.75. The goal of producing an effective dosage was to create a time to righting ability of between 90 and 120 minutes, which results in a duration of surgical anesthesia suitable for procedures lasting around 45 minutes. The predicted dosages based on this model were tested in 30 snakes ranging in mass/SVL ratio from 0.7 to 1.8 and at temperatures of 21°C to 29°C. Results of these trials suggest that the model predicts safe dosages that produce more consistent duration of anesthesia than using 15 mg/kg in all snakes regardless of body temperature or body condition. For snakes with mass/SVL ratios below 0.6 and for gravid, neonatal, sick or injured snakes it is recommended to use a dose of 12.5 mg/kg, as the reactions of these snakes to brevital sodium tends to be unpredictable and the risk of anesthetic overdose may be higher.

During over 200 anesthesias involving more than 75 different snakes, only one animal has been killed by anesthetic overdose using brevital sodium in the author's experience. The snake that died was given 15 mg/kg and was a small, underweight male that had parasitic flukes present in the mouth.

In our experience, brevital sodium is the most effective anesthetic agent for reptiles under many circumstances. Other agents, such as ketamine, require long recovery periods and may not create sufficient muscle relaxation (Throckmorton, 1981; Holz and Holz, 1994). Propofol, a popular fast-acting injectable anesthetic in reptile veterinary medicine, must be administered intravenously and is therefore impractical in many small snake species (Bertelsen, 2007). Gas agents such as isoflurane and sevoflurane require precision vaporizers to accurately control dosages. Vaporizers are often unavailable to researchers, especially when conducting fieldwork, and the breath holding abilities of certain taxa (e.g. many turtles) makes the use of gases problematic in some situations. For these reasons, sodium brevital may be the best option to create safe and effective anesthesia under many circumstances, especially if dosages are modified to account for differences in body temperature and body composition between individual reptiles.

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