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ARTICLE in CANADIAN JOURNAL OF ZOOLOGY · FEBRUARY 2011
Impact Factor: 1.35 · DOI: 10.1139/z92-015

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Freezing survival of the garter snake *Thamnophis sirtalis parietalis*

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Received January 15, 1991
Accepted August 7, 1991


Cold hardiness was evaluated for the red-sided garter snake, *Thamnophis sirtalis parietalis*. Snakes collected in the autumn near communal den sites showed an ability to supercool (Supercooling point, SCP = −5.5°C). However, by midwinter, supercooling capacity was reduced and snakes cooled only to −0.8 to −1.2°C before freezing. Survival of freezing and body ice contents were determined over a time course of freezing exposure at −2.5°C. Snakes recovered fully after freezing exposures of 3 h or less that produced ice contents of up to 40% of total body water. After longer periods and with ice contents of over 50%, survival was reduced. Only 50% of snakes survived 10 h of freezing and no snakes recovered after 24 or 48 h with a maximal ice content of 70% of body water. Putative cryoprotectants were assessed in seven organs (liver, kidney, muscle, intestine, brain, lung, heart) as well as the eggs after 5 h of freezing at −2.5°C. Glucose content increased 4-fold in liver, and lactate rose by 50% in heart, but levels of these and other possible cryoprotectants did not increase in other organs during freezing. However, a high free amino acid pool, including 14–24 μmol/g wet weight taurine, was present in the organs. The data suggest that long-term freezing survival is not part of the winter hibernation strategy of this species, but tolerance of brief freezing exposures may be adaptive in dealing with overnight frosts when the animals are active above ground.


La résistance au froid a été mesurée chez des couleuvres rayées *Thamnophis sirtalis parietalis*. Les couleuvres capturées l’automne au voisinage des refuges communaux étaient capables de survivre (point de survivre, SCP = −5.5°C). Cependant, au milieu de l’hiver, la capacité de survivre était réduite et les couleuvres ne pouvaient subir de baisse de température au-delà de −0.8 à −1.2°C avant de geler. La survie au gel et l’importance de la quantité de glace dans le corps ont été déterminées à intervalles à la suite d’une exposition à −2.5°C. Les couleuvres pouvaient se remettre parfaitement des effets d’une exposition de 3 h ou moins à un gel qui engendrait la formation de glace dans le contenu hydrique du corps, jusqu’à concurrence de 40% du contenu total. La survie diminuait à la suite d’expositions plus longues ou après formation de glace dans 50% du contenu hydrique. Seulement 50% des couleuvres ont survécu après 10 h de gel et aucune couleuvre n’a pu survivre à une exposition de 24 ou de 48 h au gel avec contenu maximal de glace de 70% du contenu hydrique. Les substances cryoprotectrices possibles ont été mesurées dans sept organes (foie, rein, muscle, intestin, cerveau, poumon, cœur) et dans les œufs, après 5 h de gel à −2.5°C. Le contenu en glucose a quadruplé dans le foie et le lactate a augmenté de 50% dans le cœur, mais les concentrations de ces produits et des autres substances cryoprotectrices possibles n’ont pas augmenté dans les autres organes au cours du gel. Cependant, un important pool d’acides aminés libres, notamment de la taurine à raison de 14–24 μmol/g masse fraîche, a été trouvée dans les organes de la couleuvre. Les données indiquent que la survie à un gel de longue durée ne fait pas partie de la stratégie d’hibernation de cette espèce, mais la tolérance à de courtes expositions au gel peut permettre à l’animal de survivre au cours des nuits de gel pendant la période où les animaux sont actifs à la surface du sol.

[Intaduit par la rédaction]

**Introduction**

The garter snake *Thamnophis sirtalis* is the most northerly distributed reptile in North America; the range of the species extends as far as 60°N, to James Bay in the east and to northern Alberta in the west (Behler and King 1979; Cook 1984). Individuals from northern populations frequently migrate considerable distances between summer grounds and communal dens, where they hibernate in large numbers (Gregory 1982). Indeed, the availability of suitable den sites may be one factor that limits the distribution of garter snakes in northern regions (Gregory 1982; Macartney et al. 1989). Presumably temperature, moisture content, and ventilation are criteria important in the choice of hibernation sites.

Low environmental temperatures have perhaps the greatest influence on the winter survival of ectothermic animals. The majority of species elude exposure to temperatures below the freezing point of their body fluids, typically by choosing appropriate hibernacula. Many other species, however, have faced the challenges of life below 0°C and perfected one of two strategies of cold hardiness (Storey and Storey 1989). Freeze avoidance is characterized by the use of antifreeze proteins and other adaptations to inhibit ice crystal growth within body fluids, as well as high concentrations of cryoprotectants that push the supercooling point (SCP) or crystallization temperature, *T*<sub>c</sub>, of body fluids to a value well below the normal environmental temperature minima (Storey and Storey 1989). Freeze tolerance includes the use of ice nucleators to stimulate the formation of extracellular ice at relatively mild subzero temperatures, as well as adaptations that control ice growth and preserve cell structure and function during freezing episodes (Storey and Storey 1988).

Early considerations of reptilian hibernation presumed that these animals would be killed by freezing exposure. The limited northerly ranges of most species, their choice of hibernation sites under water or underground, and the ability of most species to supercool to between −4 and −7°C all supported the view that reptiles survived the winter by avoiding freezing, choosing hibernacula where temperatures rarely, if
ever, fell below 0°C, and enduring short-term exposures to mild subzero temperatures with their moderate supercooling capacities (Gregory 1982; Lowe et al. 1971). However, studies in recent years have identified several species of amphibians (4 frogs and 1 salamander) that survive natural freezing exposures while overwintering in terrestrial hibernation sites (Schmid 1982; Berman et al. 1984; Storey and Storey 1986a; Storey 1990). For example, wood frogs, which hibernate under forest leaf litter, can survive at least 2 weeks frozen at −2.5 °C, and 50% survived 11 days at −6 °C (reviewed by Storey 1990). These studies have prompted recent assessments of the freeze tolerance of some northern reptiles. Two species of turtles, hatchlings of *Chrysemys picta* that winter in terrestrial nests and box turtles (*Terrapene carolina*) that hibernate in shallow burrows, survive substantial freezing exposures (Storey et al. 1988; Costanzo and Claussen 1990; Churchill and Storey 1992). Furthermore, Costanzo et al. (1988) reported freezing survival of garter snakes from central Wisconsin under two test conditions, 6 h at −3.3 °C or 48 h at about −1 °C. Ice contents of these snakes ranged from 18 to 36% of total body water, values considerably lower than the 50–65% ice that is survivable by various frog and turtle species (Schmid 1982; Layne and Lee 1987, 1989; Storey 1990; Storey et al. 1988; Costanzo and Claussen 1990; Churchill and Storey 1992).

The present study consists of a detailed examination of the capacity for freezing survival of garter snakes. We used red-sided garter snakes, *T. s. parietalis*, from a population in central Canada that hibernates in communal dens to explore both physiological and metabolic responses to freezing. The relationships between the length of freezing, percent ice, and survivorship were assessed, along with seasonal changes in the ability of the animals to supercool. In addition, the production of carbohydrates and amino acids that could act as cryoprotectants was examined in snake organs.

**Materials and methods**

**Chemicals and animals**

All chemicals and biochemicals were purchased from Sigma Chemical Co., St. Louis, Mo., or Boehringer Mannheim Corp., Montréal, Que. Adult red-sided garter snakes were collected in the Interlake region of Manitoba, Canada, by Dr. M. Mendonca and co-workers during the first week of September, 1988 (within the short government-licensed collecting period). The snakes had gathered near the entrances to winter dens prior to entering hibernation, as described by Joy and Crews (1987). Snakes were air freighted to our laboratory at −15 °C in constant darkness. Animals were held in cloth bags with pieces of damp sponge added for moisture (Joy and Crews 1987). Low temperature acclimation followed the recommendation of Dr. M. Mendonca (personal communication) for successful laboratory hibernation of snakes. The ambient temperature was lowered 1 °C per day until 3 °C was reached and then maintained at 3 ± 1 °C for 2–3 weeks prior to beginning experimentation. Mean weights of the snakes were 16.4 ± 0.6 g (n = 25) for males and 64 ± 9 g (n = 18) for females.

**Freezing and supercooling point determination**

For determining the supercooling point, a thermistor was taped to the abdomen of a snake (within the middle one-third of the body length) and then the animal was placed in a cooled position inside a styrofoam cup. The cup had holes punched in it for ventilation and the thermistor wire ran out through a hole in the lid. The snake was then placed in an incubator set at −2.5 °C and the body surface temperature was monitored as the snake cooled at a rate of −0.3 to −0.5 °C · min⁻¹ until it was within 0.5 °C of the incubator temperature. The incubator temperature was then adjusted downwards to −4.0 °C (and subsequently, if necessary, to −6.0 or −8.0 °C) and snakes were again cooled to within 0.5 °C of incubator temperature or until the SCP was reached and freezing began. Thermistors were attached to a YSI telethermometer with a multichannel switch box. Typically, snakes were monitored at any one time; recording switched between channels every 15 s. Output was to a Kipp and Zonen linear recorder. The whole-animal SCP was taken as the lowest body surface temperature recorded before the sharp rise in temperature associated with nucleation. This elevated body surface temperature was maintained at a constant value for the first 30–60 min of freezing and is termed the rebound temperature.

**Time course of ice formation**

Male snakes (mean weight 16 ± 2 g) were used for this experiment. Snakes were placed individually in large styrofoam cups and then placed in an incubator set at a constant −2.5 °C. Body temperature was monitored by a telethermometer probe taped to the abdomen and hooked up to a linear recorder. When the body temperature had equilibrated to −2.5 °C, freezing was initiated by quickly touching the animal (in the middle third of the body) with a metal rod that had been cooled in liquid nitrogen. Nucleation was apparent from the immediate rebound of body temperature (the exotherm) and the length of freezing exposure was timed from this point. After set intervals, ranging up to 48 h of freezing exposure, snakes were rapidly removed from the incubator and thawed in an insulated container of water. The percentage of body water as ice was determined by calorimetry using the method of Lee and Lewis (1985): experimentally measured parameters for these garter snakes were as follows: specific heat of the dry mass, 0.3905 ± 0.0357 cal · g⁻¹ · °C⁻¹ (n = 4), water content, 70.5 ± 0.7% of body mass (n = 5), and F factor for the calorimeter, 1.03 ± 0.01 (n = 6). After thawing, snakes were immediately assessed for survival by testing righting ability, tail twitch in response to pinching, and capacity for locomotion. Animals were then placed at 3°C inside plastic boxes with damp moss and survival was again assessed after 1 day, 1 week, and 4 weeks.

**Metabolite analysis**

To assess changes in metabolite levels in response to freezing, thermistors were attached to snakes and the animals were then placed in the incubator at a constant −2.5 °C. Female snakes of average weight 64 g were used for this study. When the body temperature had equilibrated to −2.5 °C, freezing was again initiated by seeding, as above. After 5 h of freezing, snakes were removed from the incubator and killed by decapitation. Seven organs plus eggs were rapidly excised from the still frozen animal and plunged into liquid nitrogen. Tissues were then transferred to −80 °C for storage until processing. Control snakes were sampled directly from the 3°C incubator.

Perchloric acid extracts of tissue samples were prepared as described by Storey and Storey (1984) and stored at −80 °C until use. Metabolites were quantified in coupled enzyme assays by the following methods: glycerol, Keppler and Decker 1974; glyceraldehyde, Eggstein and Kuhlmann 1974; and glucose, lactate, and alanine, Lowry and Passonneau 1972. For analysis of free amino acid levels, frozen tissue samples were homogenized in 0.5% sulphosalicylic acid and then centrifuged at 6000 × g in an IEC bench-top centrifuge. Amino acids were analyzed in aliquots of the supernatant by means of a Waters HPLC after precolumn derivatization with orthophthalaldehyde. All data are given as the mean ± SE for samples from different individual snakes. Statistical analysis was performed using Student's t-test.

**Water content**

Five snakes that did not survive the 24- to 48-h freezing exposures were used for the determination of body water content. Wet weights were measured and then animals were dried to a constant weight over 2 weeks at 60°C. To determine whether any dehydration occurred during the freezing episode itself, four snakes were weighed before, during, and after freezing exposure for 10 h at −2.5°C.

**Results**

**Supercooling and freezing**

When snakes were tested for supercooling ability in the autumn (during October after 2 weeks to cool from 15 to 3°C
followed by 2–3 weeks’ acclimation at 3°C), animals displayed considerable supercooling ability, with an average SCP of 
\[-5.5 \pm 0.3 \text{°C} \] (n = 4), similar to the value reported by Lowe et al. (1971) for another species of garter snake. At nucleation, body temperature jumped to \[-0.8 \pm 0.1 \text{°C} \] (n = 13; in this and subsequent experiments), a rebound temperature that is slightly lower than the expected freezing point of vertebrae body fluids. After measuring SCP, these snakes were left for 2 h of freezing exposure (air temperature approximately \[-6\text{°C}\] ) and then returned to 3°C to thaw for 24 h. None survived this treatment.

Snakes maintained under hibernating conditions at 3°C (in the dark) until midwinter (January 15 – February 15) and then tested for supercooling showed a different response. Supercooling capacity was reduced. Three animals froze when the body temperature reached \[-0.8 \text{°C} \] without showing a visible exotherm, one froze at \[-1.0 \text{°C} \], and one at \[-1.2 \text{°C} \] (mean \[-0.92 \pm 0.07 \text{°C} \], significantly higher according to Student’s t-test, \(P < 0.005\), than the autumn SCP).

To monitor the progress of ice formation in garter snakes and assess survival of freezing, autumn-collected snakes were cooled individually to \[-2.5 \text{°C} \], seeded by touching with a cold metal rod (rebound temperature \[-0.8 \text{°C} \]), and then sampled after timed intervals of freezing at \[-2.5 \text{°C} \]. Figure 1 shows the percentage of body water as ice and the survival rate for freezing times ranging from 30 min to 48 h. Ice formed rapidly in the snakes, reaching the half-maximal amount of approximately 35% of total body water after about 2.5 h of freezing at \[-2.5 \text{°C} \]. After 24 h at \[-2.5 \text{°C} \] the average ice content was 59.5% and at 48 h the average was 70%. Survival of snakes was excellent when freezing times were short. Snakes recovered fully when freezing time was 3 h or less and ice content averaged about 40% or less. Survival decreased, however, when freezing was extended to 5 h or more. Only 50% of snakes recovered after 10 h of freezing and no snakes survived 24 or 48 h of freezing. Eighteen out of 20 snakes that revived after freezing showed strong signs of life immediately after thawing in the calorimeter; their tails twitched in response to pinching and most were also able to move across the bench or right themselves when placed on their back. Two others required a longer recovery time but all survivors appeared completely normal 1 day, 1 week, and 4 weeks after returning to 3°C. All others, from the longer freezing exposures, were unresponsive after removal from the calorimeter and dead when assessed after 1 day or 1 week at 3°C.

Because rapid thawing in the calorimeter might be more stressful than the slower thawing that would occur in nature, additional tests of freezing survival were undertaken. Snakes that were chilled and seeded as above, frozen for 5 h, and then transferred to 3°C to thaw all recovered fully (n = 4).

Observations of the internal organs were made during dissection of some individuals after freezing exposure. After 5 h of freezing, the snakes were externally rigid due to much ice in the skin and skeletal muscle layers. Some ice was found in the abdominal cavity but the internal organs were unfrozen, and heart beat and blood circulation were apparent in the larger specimens. The appearance of the eyes changed from clear to milky white after 5–10 h of freezing and the skin color changed from dark greenish-black to bluish when frozen. After 24 h of freezing, ice was found throughout the abdominal and thoracic cavities, all organs were hard to the touch, and there was no heart beat or bleeding.

The average body water content of the garter snakes was 70.5 ± 0.7% (n = 5) of total body weight. To be certain that body water content remained constant over the course of a freezing–thawing exposure, the body weight of four snakes was measured before freezing, after 10 h freezing at \[-2.5 \text{°C} \], and then again 1 week after returning to 3°C. Weights were 23.9 ± 2.4, 23.8 ± 2.5, and 23.5 ± 2.3 g, respectively.

**Cryoprotectants**

The accumulation of high concentrations of low molecular weight cryoprotectants such as glucose or glycerol is a key element of freeze tolerance for many species (Storey and Storey 1988). To determine whether snakes produced cryoprotectants, animals were given a survivable freezing exposure, 5 h
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at $-2.5^\circ C$, and then dissected immediately (without thawing). Metabolite levels were measured in seven organs plus the eggs. Table 1 shows that freezing resulted in a 4-fold increase in glucose content (to $3.4 \, \mu mol/g$ wet weight) in liver. Glucose levels in other organs, however, were not affected by freezing exposure and were less than $1 \, \mu mol/g$ in all cases. Glycerol concentration was less than $1.8 \, \mu mol/g$ wet weight in all organs; levels tended to be lower in organs of frozen snakes but were not statistically different from control values in any instance. Sorbitol, fructose, and mannose concentrations were also measured; levels of each compound did not exceed $0.55 \, \mu mol/g$ and no changes in concentration with freezing were found for any organ. Lactate levels increased significantly in heart during freezing and decreased in skeletal muscle but were not altered in any other organs. Table 1 also shows that freezing had no significant effect on the glycogen content of any of the eight organs assessed.

Table 2 shows the mean levels of free amino acids (values for control and frozen samples are combined) in seven organs of garter snakes. The major amino acids in all organs, present in concentrations of about $1 \, \mu mol/g$ higher, were glutamate, glutamine, arginine, taurine, and aspartate (asparagine, where present, was less than 10% of the value for aspartate). Valine and $NH_4^+$ coeluted, and the peak levels of 0.4–4.5 $\mu mol/g$ may largely represent the $NH_2$ content of the tissues. Taurine was outstanding as the major component of the free amino acid pool, representing 34 and 76% of the total pool size. In general, there were no significant changes in the levels of amino acids during freezing, with three notable exceptions (see footnote to Table 2). In liver the levels of glutamate and glutamine decreased significantly during freezing, by 25 and 58%, respectively, whereas in skeletal muscle, taurine content decreased by 56% in frozen animals.

However, they appear to be unable to maintain a supercooled state for long; nucleation occurred within a few minutes in this study (T. Churchill, personal observations) and within three-quarters of an hour in the study by Costanzo et al. (1988).

If snakes cannot sustain a supercooled state, then freeze tolerance is the only viable alternative if the snakes routinely encounter subzero temperatures. The present study shows that garter snakes have a significant ability to withstand the short term freezing of body fluids. Snakes fully recovered from 30-min to 3-h freezing exposures at an air temperature of $-2.5^\circ C$ with ice contents that ranged up to about 40% of total body water. After 5 h freezing, survival was 100% for slowly thawed individuals and 80% for snakes thawed in the calorimeter. Costanzo et al. (1988) reported similar short-term survival for Wisconsin garter snakes: 6 h at an air temperature of $-3.3^\circ C$ with 17–36% of body water as ice ($n = 6$). Ice formation in snakes followed a typical hyperbolic curve relating percent ice to time frozen (Fig. 1). The time required for half-maximal ice formation was estimated to be about 2.5 h at $-2.5^\circ C$, somewhat less than the approximately 4 h half-time for R. sylvatica held at the same temperature and of similar body mass (Layne and Lee 1987).

When the duration of freezing was extended, however, freezing survival declined rapidly. Only 50% of animals recovered after 10 h at $-2.5^\circ C$ and no snakes survived for 24 h or longer when ice contents rose to 60–70%. At lower temperatures, freezing was even more rapidly lethal: none of the snakes revived after just 2 h at $-6^\circ C$. However, at about $-1^\circ C$, slightly below the freezing point of body fluids, snakes ($n = 3$) were able to survive 48 h of freezing (ice content was 34%, $n = 1$) (Costanzo et al. 1988). Obviously, as is true of all freeze-tolerant animals, time and temperature interact in determining freezing survival, owing to their effects on both the rate and amount of ice formed. For garter snakes, however, survival time declines very rapidly with decreasing temperature, providing a very narrow window within which the freeze tolerance of this species could have ecological relevance as a viable hibernation strategy. One potential use for freeze tolerance in snakes may be for enduring overnight frosts during the autumn (or spring) when snakes are active above ground. From the above information it appears that snakes could readily endure freezing (as a result of seeding by environmental ice, since SCPs are low in autumn) if exposed to a night at about $-1^\circ C$, but even the very small drop to a nighttime temperature of $-2.5^\circ C$ could result in substantial mortality. However, given that nighttime minimum temperatures cannot be predicted by the snakes and that a frozen snake cannot move once a moderate amount of ice forms, it seems likely that the first line of defence for the animals would be to seek thermally

### Table 1. Concentrations of Some Metabolites ($\mu mol/g$ Wet Weight)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.84±0.26</td>
<td>3.43±0.72*</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.11±0.22</td>
<td>0.79±0.05</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.88±0.54</td>
<td>4.57±0.18</td>
</tr>
<tr>
<td>Glycogen</td>
<td>148±34</td>
<td>233±51</td>
</tr>
</tbody>
</table>

Table 1 shows that freezing resulted in a 4-fold increase in glucose content (to $3.4 \, \mu mol/g$ wet weight) in liver. Glucose levels in other organs, however, were not affected by freezing exposure and were less than $1 \, \mu mol/g$ in all cases. Glycerol concentration was less than $1.8 \, \mu mol/g$ wet weight in all organs; levels tended to be lower in organs of frozen snakes but were not statistically different from control values in any instance. Sorbitol, fructose, and mannose concentrations were also measured; levels of each compound did not exceed $0.55 \, \mu mol/g$ and no changes in concentration with freezing were found for any organ. Lactate levels increased significantly in heart during freezing and decreased in skeletal muscle but were not altered in any other organs. Table 1 also shows that freezing had no significant effect on the glycogen content of any of the eight organs assessed.

### Discussion

Garter snakes in Manitoba hibernate for about 7 months of the year (Joy and Crews 1987). They return to their communal dens in late August and remain active, but usually not eating, in the vicinity of the hibernacula for several weeks until cued, probably by low ambient temperatures, to enter the dens and begin winter hibernation (Gregory and Stewart 1975). The results of the present study show that autumn-collected garter snakes have effective strategies for dealing with slightly sub-zero ambient temperatures. These snakes supercooled to $-5.5^\circ C$. This capacity could give snakes some ability to elude freezing when temperatures fall below the freezing point of body fluids (about $-0.5$ to $-0.6^\circ C$ for most vertebrates). This might be adaptive for dealing with occasional overnight frosts when the snakes are still free-ranging above ground.
in garter snake organs and eggs after freezing at $-2.5^\circ C$ for 5 h

<table>
<thead>
<tr>
<th></th>
<th>Intestine</th>
<th>Lung</th>
<th>Brain</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.45 ± 0.14</td>
<td>0.44 ± 0.19</td>
<td>0.33 ± 0.21</td>
<td>0.58 ± 0.18</td>
</tr>
<tr>
<td>Frozen</td>
<td>0.51 ± 0.07</td>
<td>1.75 ± 0.22</td>
<td>3.06 ± 1.78</td>
<td>1.59 ± 0.32</td>
</tr>
<tr>
<td>Control</td>
<td>0.98 ± 0.17</td>
<td>1.33 ± 0.11</td>
<td>—</td>
<td>0.96 ± 0.19</td>
</tr>
<tr>
<td>Frozen</td>
<td>4.02 ± 0.13</td>
<td>4.44 ± 0.19</td>
<td>58 ± 7</td>
<td>3.78 ± 0.51</td>
</tr>
<tr>
<td>Control</td>
<td>3.98 ± 0.41</td>
<td>6 ± 2</td>
<td>91 ± 20</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Frozen</td>
<td>9 ± 3</td>
<td>16 ± 3</td>
<td>76 ± 13</td>
<td>76 ± 13</td>
</tr>
</tbody>
</table>

buffered shelters that minimize both the risk of freezing and the depth of freezing exposure should it occur.

Indeed, for long-term hibernation this is precisely the strategy used by garter snakes, since their ability to tolerate either a high ice content or a long freezing time is so poor. The maximum ice content tolerated without injury is about 35–40% of total body water (Fig. 1; Costanzo et al. 1988). As the ice content rose to nearer 50%, however, survival decreased and long-term freezing at $-2.5^\circ C$ that brought ice content up to 60–70% was lethal in all cases (Fig. 1). These tolerable amounts of internal ice in snakes are considerably lower than the amounts endured by freeze-tolerant frogs and turtles (which readily survive 50–65% ice) (Schmid 1982; Layne and Lee 1987; Storey 1990; Storey et al. 1988; Costanzo and Claussen 1990; Churchill and Storey 1992).

Survival times are also much shorter for snakes; at $-2.5^\circ C$, snakes survive only a few hours of freezing, whereas the duration of freezing survival ranges up to at least 11 days for painted turtle hatchlings and 14 days for wood frogs at the same temperature (Storey 1990; Churchill and Storey 1992). During the months of winter hibernation, then, the best strategy is probably to avoid all exposure to subzero temperatures, since if the temperature fell low enough to initiate the freezing of body fluids, it would be difficult to imagine circumstances in which ice formation would not proceed over time to an equilibrium ice content that was lethal. Indeed, garter snakes do choose thermally-buffered den sites, either underground or under water (Macartney et al. 1989; Costanzo 1989); these are much more protected than the hibernacula of the terrestrial frogs (under forest leaf litter) or painted turtle hatchlings (nests less than 10 cm deep) that display much greater freeze tolerance. Actual temperature measurements in garter snake dens back this up. Macartney et al. (1989) found that temperatures in a communal den in northern Alberta never fell below 0°C, even though outside air temperatures dropped as low as $-25$ to $-35^\circ C$. Bailey (1949) assessed survival at different depths (6–24 in; 1 in. = 25.4 mm) in an artificially constructed snake pit. He found that all snakes survived at a depth of 24 in., where the soil temperature never fell below $-0.5^\circ C$, but mortality increased at the 18-in. depth, where the temperature sometimes fell to $-1.5^\circ C$. All snakes died at all shallower levels, where extended exposures to $-2^\circ C$ or lower were recorded (Bailey 1949). Thus, it appears that freeze tolerance probably has little or no relevance for the winter hibernation of garter snakes. The degree of freezing survival of this species suggests that its freeze tolerance is a "back-up" system for enduring brief exposures to slightly subzero temperatures when its primary strategy, retreat from subzero exposure, fails.

One of the critical factors in freezing survival is the regulation of cell volume reduction. Natural freeze tolerance includes adaptations that limit the dehydration of the cell during extracellular ice formation and maintain a critical minimum cell volume. Typically this is the function of low molecular weight cryoprotectants; high concentrations of these solutes limit cell volume reduction via colligative actions. Freeze-tolerant wood frogs accumulate 0.25–0.5 M glucose in their organs during freezing for this purpose, whereas the grey tree frog and the Siberian salamander produce equally high amounts of glycerol (Schmid 1982; Berman et al. 1984; Storey and Storey 1984, 1985; Storey 1990). The presence of high concentrations of one or more low molecular weight solutes and (or) their synthesis during the freezing process is a good indicator, therefore, of a species that experiences substantial natural freezing exposures. The two cryoprotectants most common among other vertebrates, glucose and glycerol, were not produced in any substantial amounts by T. s. parietalis during freezing, however. Liver glucose increased to 3.4 μmol/g after 5 h of freezing but glucose levels in other organs were not increased by freezing. By contrast, the equivalent 5-h freezing exposure at $-2.5^\circ C$ given to autumn wood frogs raised the liver glucose concentration to 100 μmol/g and increased the glucose concentration in blood and other internal organs to about 50 μmol/g (Storey and Storey 1986b). Garter snakes also showed no change in the glycerol, sorbitol, fructose, or mannose levels in the organs during freezing, and the constant levels of glycogen in the organs were further evidence that no other carbohydrate cryoprotectant was being produced during freezing. In addition, the low glycogen content in snake organs, particularly the liver, indicated that there was little scope for the synthesis of substantial levels of carbohydrate cryoprotectants. Freeze-tolerant frogs, by contrast, have a liver glycogen content that is at least 5 times higher when beginning winter hibernation (Storey and Storey 1984, 1986b).

The lack of accumulation of traditional cryoprotectants in snakes could be due to the production of unusual cryoprotectants, perhaps specific to reptiles. Indeed, our studies with hatching painted turtles also show low glucose or glycerol accumulation but substantial production of lactate (up to 40 μmol/g) during freezing, along with free amino acid pools that contain high levels of taurine (up to 8 μmol/g) in the organs (Storey et al. 1988; Churchill and Storey 1992). In snakes, however, lactate content increased only in heart during freezing exposure, probably due to a shift towards glycolytic ATP production brought about by the progressive hypoxia–anoxia imposed by the freezing process. Free amino acid levels were also largely unaffected during freezing exposure, although the taurine content of skeletal muscle dropped.
by one-half in freezing-exposed animals (Table 2). The absolute taurine levels in snake organs were quite high (8–24 μmol/g), however, as were the total free amino acid pools (21–35 μmol/g). Taurine is widely used as an adjustable osmolyte by euryhaline marine organisms and has been shown to have a cryoprotective role in freeze-tolerant molluscs (Loomis et al. 1988). Thus, taurine and the free amino acid pool in general represent a fairly large permanent osmolyte pool in snake organs that could have a role, albeit fairly small, in cell volume regulation in the event of freezing.

In summary, then, garter snakes appear to be well equipped to deal with short-term exposure to subzero temperature (e.g., overnight frosts) during the autumn months, with a substantial supercooling capacity and an ability to tolerate brief freezing exposures. However, the capacity for long-term freezing survival does not appear to be a part of the winter hibernation strategy of this species. Winter survival in underground dens probably depends upon avoiding prolonged exposures to temperatures below the freezing point of body fluids.

**Acknowledgements**

We are very grateful to Dr. D. Crews and Dr. M. Mendonca, University of Texas, Austin, and their support from the Texas Advance Research Program, for collecting and shipping snakes and providing information on their care. We also thank the Manitoba Department of Natural Resources for their permission to collect animals and cooperation in mediating the collection process. We appreciate the critical comments of J. M. Storey. This work was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) Canada, National Institutes of Health grant GM43796-01 to K.B.S., and an NSERC postgraduate scholarship to T.A.C.


