Freezing-induced changes in the heart rate of wood frogs (*Rana sylvatica*)

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LAYNE, JACK R., JR., RICHARD E. LEE, JR., AND THOMAS L. HEIL. *Freezing-induced changes in the heart rate of wood frogs (Rana sylvatica).* Am. J. Physiol. 257 (Regulatory Integrative Comp. Physiol. 26): R1046–R1049, 1989.—During the first few hours of freezing the cardiovascular system must distribute cryoprotectant throughout the body of freeze-tolerant frogs. This study presents initial documentation of the changes in heart rate of wood frogs (*Rana sylvatica*) during nonlethal freezing. Heart rate was determined by measuring the electrocardiogram of frogs. Within 1 min of the onset of freezing the heart rate nearly doubled to ~8.0 beats/min. The heart rate began to slow after the first hour of the freeze, and the heart completely stopped beating near the completion of ice formation ~20 h later. Recordings from a single frog revealed that the heart beat resumes within 1 h after thawing and near-normal function is achieved after only a few hours. The release of the latent heat of fusion caused a rise in body temperature (1.7°C) for a few hours and was closely correlated with an increase in the heart rate. However, other factors such as reduction in blood volume, increase in blood viscosity, and progressive hypoxia may prominently influence cardiac function indirectly. Regardless, the heart functions long enough to distribute glucose throughout the body during the first few hours of the freeze.

cryobiology; freeze tolerance; overwintering adaptation

FOUR SPECIES of terrestrially hibernating frogs are freeze tolerant (for a review see Ref. 10). These frogs rapidly mobilize glucose via glycogenolysis for cryoprotection of body tissues within minutes after the onset of ice formation; however, only the tree frog *Hyla versicolor* extends this process by synthesizing glycerol from glycogen (10). Frog survival is linked to adequate mobilization of cryoprotectant (8), yet frogs may endure freezing of body fluids with no more than a fewfold increase in the concentration of these substances (1). Cryoprotectant mobilization and maintenance depend on changes in the activity of certain enzymes of glycogenolysis and glycolysis (10), but higher level controls remain to be elucidated.

Freezing of body fluids in frogs is extensive and may have pronounced consequences on function at both cellular and systemic levels. Ice may accumulate to 66% of the total body water content in *Rana sylvatica* frozen at −2.5°C (1). Ice accumulation is relatively fast, and even large frogs reach an equilibrium ice content in <24 h at −2.5°C (1). Presumably only extracellular water freezes; visible ice crystals occupy the abdominal cavity, subcutaneous space, and spaces within organs. Frozen frogs show no breathing movements or heartbeat, and they are thus forced to rely on anaerobiosis to sustain their energetic needs (10).

Cryoprotectant production in frogs is largely restricted to the liver (4, 5). Survival of body tissues requires timely delivery of glucose or glycerol, and, aside from diffusion, the cardiovascular system is the only means for delivery of cryoprotectants to body tissues. Freezing of body fluids, however, presents obstacles to continued cardiovascular function, because there is a loss of fluid volume to ice, and progressive hypoxia in body tissues diminishes the capacity for activities requiring high levels of energy including the contraction of the heart. To distribute glucose from the liver successful freeze tolerance requires maintenance of cardiac function during the early stages of freezing. This study documents the time course of changes in cardiac function during ice accumulation in freeze-tolerant frogs.

METHODS

Six adult male *R. sylvatica* were collected from a small breeding pond in Athens County, Ohio, during March, 1988. An adult male wood frog (11.5 g) from Ontario County, New York, was collected and used during December, 1988. The body mass of these frogs ranged from 10.6 to 14.0 g (mean = 11.9 g). Frogs were acclimated at 3°C for 1–2 wk and then tested.

Heart rate was recorded using a Physiograph MK III recorder. A recording electrode was connected to the proximal portion of each forelimb. A ground electrode was attached proximally to the left hindlimb. The frog was wrapped in a single layer of cheesecloth that held it in a sitting posture. The loose ends of the cheesecloth were then closed with tape so that the electrodes were held securely.

The protocol for freezing was similar to procedures used by Layne and Lee (1). Each frog was placed in a plastic centrifuge tube (50 ml) and cooled in a Neslab RTE 210A refrigerated bath. A thermocouple passed through the cap of each tube and came to rest against the abdomen of a frog. Temperature was compiled by an Omega RD-106 multichannel thermocouple recorder.

The frogs were cooled until they reached an equilibrium with the surrounding environment (−1.8 to

−2.8°C). They were held supercooled at the equilibrium temperature for at least 1 h before freezing was triggered by external seeding with small crystals of ice. Electrocardiograms (ECGs) were recorded on the Ohio frogs at time intervals immediately before seeding (0 min) and at 1, 30, 60, 90 min and 2, 3, 5, 8, 11, 14, and 21 h after the onset of freezing. The ECG of the New York specimen was recorded at comparable intervals during the freezing episode. Heart rate was also measured before freezing at a body temperature nearly equivalent to the peak temperature attained at the onset of freezing. Furthermore, the resumption of cardiac activity in this frog was followed for up to 6 h after thawing at 5°C. Individual recordings were made to obtain 6–8 heartbeats for most time intervals. During the later states of freezing, recordings lasted for as long as 30 min to determine whether the heart was still beating. Statistical comparison of heart rates at different time intervals during the freezing process was done using a randomized block analysis of variance and Tukey's test.

RESULTS

Freezing was marked by a sudden rise in the body temperature due to the release of the latent heat of fusion (Figs. 1*A* and 2). The body temperature rose from −2.4 to −0.7°C in the first minute of the freeze. The gradual decline in temperature near the end of the freeze made it difficult to gauge the completion time for the completion of the exotherm, but probably averaged near 20 h.

All frogs completely recovered after freezing with no evidence of injury, which is consistent with previous findings for *R. sylvatica* from Ohio after their emergence for breeding in late winter or early spring (1). Three of the Ohio frogs had completed or nearly completed exotherms after freezing for 14 h, whereas the remaining three frogs required 21 h to complete or nearly complete their exotherms (Fig. 1*A*). The frog from New York completed its exotherm in 15 h (Fig. 2). Based on data from our previous study (1), the frogs should have ~50% of their body water frozen after 14 h, whereas an equilibrium ice content of 66% was reached after 20 h.

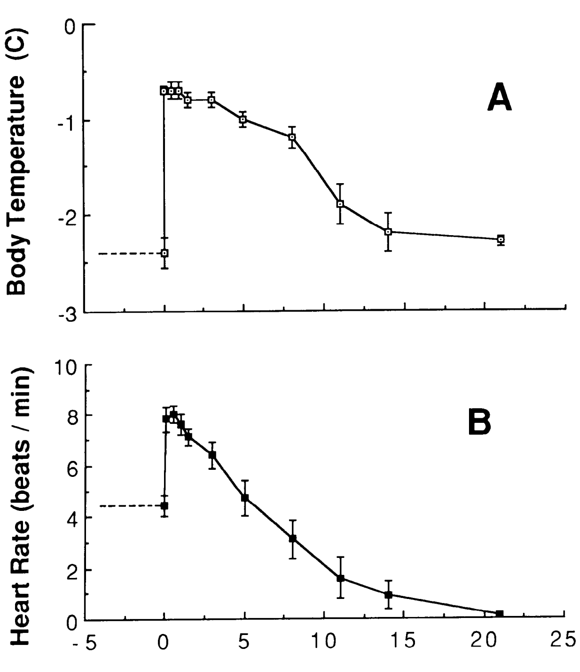

FIG. 1. Body temperature (*A*) and heart rate in beats/min (*B*) of 6 frogs subjected to freezing at −1.8 to −2.4°C. Symbol, mean values; vertical lines, SE. Lines represent a simple visual connection of successive points and are not result of a derived equation.

The quality of the ECG pattern showed some variation among individual frogs. The QRS complex was consistently seen in all seven frogs except late in the freezing process, when the heart progressively entered an arrested state. The P wave was not easily observed in all frogs, since the high-sensitivity setting needed for these recordings often exaggerated low-level interference. Accumulation of ice in the body fluids did not disrupt the electrical signal; in fact, some frogs showed an increase in the amplitude of the ECG.

Ohio frogs supercooled to −2.4°C had an average heart rate of 4.4 beats/min. Initiation of freezing triggered a sudden and significant ($P < 0.05$) cardioacceleration that was apparent within 1 min after the onset of ice nucleation and had peaked 30 min into the freeze at 8.0 beats/min (Fig. 1*B*). For 90 min after freezing began the heart rate was significantly ($P < 0.05$) higher than it was immediately before ice nucleation. The heart rates obtained 3–8 h into the freeze were not significantly ($P > 0.05$) different from the heart rate before freezing. All heart rates obtained 5 h and later into the freeze were significantly ($P < 0.05$) lower than values obtained in the first hour. By 11 h the heart rate of frozen frogs was significantly ($P < 0.05$) lower than their corresponding rate in the supercooled state. Cessation of the heartbeat was seen in one frog after 11 h and in two of three frogs after 21 h.

The body temperature and heart rate profile for the frog from New York generally paralleled the previous group (Fig. 2). The heart rate peaked at 7.1 beats/min after freezing for 30 min, which was slightly lower than its heart rate at nearly the same body temperature (−0.5°C) but in an unfrozen state. Cardiac arrest was observed 15 h after the onset of freezing. This specimen showed resumption of its heart beat after thawing for 1 h at 5°C (Fig. 2). After 6 h the heart rate rose to 13.6 beats/min as body ice melted and its body temperature rose to 5°C. Before the freezing episode this frog had a heart rate of 15 beats/min at this body temperature.

Body temperature and heart rate showed a considerable degree of parallel changes during the time course of a freezing episode (Figs. 1 and 2). Both body temperature and heart rate showed a substantial and concurrent rise immediately after the onset of freezing. Freezing-induced cardioacceleration nearly doubled heart rate while body temperature rose 1.7°C during the 30 min immediately after the onset of freezing. Subsequent changes in body temperature and heart rate that were associated with freezing closely followed an exponential relationship (Fig. 3). The heart rate declined by >60% during the first 8 h of the freeze while body temperature fell only 0.5°C; however, the rate of change in the heart beat

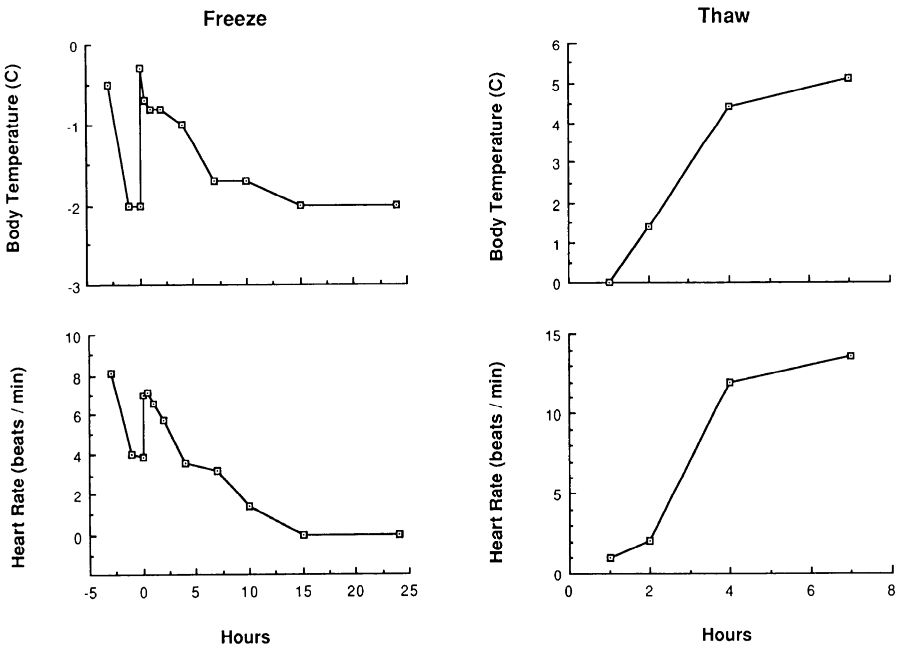

FIG. 2. Body temperature and heart rate in beats/min for wood frog from New York subjected to freezing at −2.0°C and subsequently thawed at 5°C.

slowed relative to the change in body temperature during the remaining hours of the freeze.

DISCUSSION

The present data are consistent with an earlier report on freeze tolerance and freezing times for *R. sylvatica* from a nearby southern Ohio population (1). In both studies, frogs were taken following their emergence for breeding in the late winter/early spring. Freezing temperatures commonly occur for up to a few weeks following emergence of the frogs, and thus maintenance of freeze tolerance is required even in frogs from the southern portion of their range (1).

Cardiac events monitored here can be extrapolated to a known time course of freezing for *R. sylvatica* (1). Even with the freezing process ~60% completed after 8 h (or ~40% of their body water frozen) frogs still had a heart rate of 3.1 beats/min. Cessation of the heartbeat did not occur until the completion or near completion of the freezing process. However, cardiac output is probably minimal before the cessation of heart beat, since most extracellular fluids are sequestered into ice (1).

The circulatory system must distribute cryoprotectant throughout the body as it is being released from the liver.

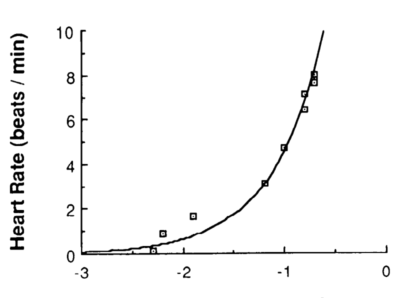

FIG. 3. An exponential plot of relationship between heart rate in beats/min vs. body temperature during course of freezing for Ohio frogs. Equation for this plot is $y = 33.6 \times 10^{0.862}$ ($r = 0.92$).

The persistence of the heartbeat during the freeze indicates that cryoprotectant delivery to many body tissues can continue for many hours. This corresponds with a report that glucose levels continue to rise in certain body tissues many hours after the onset of freezing (5). Cardiac function during the first few hours of freezing undoubtedly is essential to survival of freezing; however, additional studies are required to determine the effects of progressive freezing on cardiac output and tissue perfusion.

The release of the latent heat of fusion raises the body temperature of a frog up to a few degrees Celsius for several hours before body temperature gradually returns to the ambient temperature (1–3, 6). Previously Lotshaw (2) reported a link between freezing and cardioacceleration, but he gave no further details. It is reasonable to assume that thermally dependent events such as heart rate are affected by the release of the latent heat of fusion. This is well evidenced here by the pronounced cardioacceleration at the onset of freezing and by the subsequent and concurrent decline in body temperature and heart rate (Figs. 1 and 2).

Temperature-induced changes are insufficient to explain all events observed in this study. As freezing neared completion, all frogs had lower heart rates than when they were supercooled despite equivalent or even higher body temperatures in the freezing frogs. Ultimately, frogs entered nonlethal cardiac arrest as a consequence of freezing. Thus it is reasonable to conclude that changes in cardiac function are influenced by numerous freezing-induced events such as cryoprotectant mobilization, changes in fluid volume, the concentration of solutes and electrolytes in unfrozen body fluids, and tissue hypoxia.

Restoration of the heartbeat occurs soon after the onset of thawing (within 1 h) and a near-normal rate is achieved after only a few hours (Fig. 2). The recovery is very rapid compared with the time required for freezing-induced cardiac arrest. Rapid restoration of cardiac function seems necessary, since metabolic demands undoubtedly increase with the thawing of body fluids and rise in body temperature. Frogs, like other vertebrates, sustain themselves by aerobic metabolism under normal conditions, and cardiovascular transport of oxygen is crucial. The time scale for reperfusion of specific tissues is not known and may be an important component for recovery during thawing.

Freezing induces changes in cadiovascular function, probably as a consequence of several interacting factors. Further studies are required to increase our understanding of how these vertebrates survive ice accumulation in body fluids.

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REFERENCES

1. Layne, J. R., Jr., and R. E. Lee, Jr. Freeze tolerance and the dynamics of ice formation in wood frogs (*Rana sylvatica*) from southern Ohio. *Can. J. Zool.* 65: 2062–2065,1987.
2. Lotshaw, D. L. Temperature adaptation and effects of thermal acclimation in *Rana sylvatica* and *Rana catesbeiana*. *Comp. Biochem. Physiol. A Comp. Physiol.* 56A: 287–294, 1977.
3. Schmid, W. D. Survival of frogs in low temperature. *Science Wash. DC* 215: 697–698, 1982.
4. Storey, J. M., and K. B. Storey. Biochemical adaptation for freezing tolerance in the wood frog, *Rana sylvatica. J. Comp. Physiol.* 155: 29–36, 1984.
5. Storey, J. M., and K. B. Storey. Triggering of cryoprotectant synthesis by the initiation of ice nucleation in the freeze tolerant frog, *Rana sylvatica. J. Comp. Physiol.* 156: 191–195, 1985.
6. Storey, K. B. Freeze tolerance in the frog, *Rana sylvatica. Experientia Basel* 40: 1261–1262, 1984.
7. Storey, K. B., and J. M. Storey. Freeze tolerant frogs: cryoprotectants and tissue metabolism during freeze-thaw cycles. *Can. J. Zool.* 64: 49–56, 1986.
8. Storey, K. B., and J. M. Storey. Evaluation of the persistence of freeze tolerance in terrestrially hibernating frogs after spring emergence. *Copeia* 1987: 720–726, 1987.
9. Storey, K. B., and J. M. Storey. Organ-specific metabolism during freezing and thawing in a freeze-tolerant frog. *Am. J. Physiol.* 253 (*Regulatory Integrative Comp. Physiol.* 22): R292–R297, 1987.
10. Storey, K. B., and J. M. Storey. Freeze tolerance in animals. *Physiol. Rev.* 68: 27–84, 1988.