Freeze Avoidance in a Mammal: Body Temperatures below 0° C in an Arctic Hibernator
Author(s): Brian M. Barnes
Published by: American Association for the Advancement of Science
Stable URL: http://www.jstor.org/stable/1704133
Accessed: 21-01-2016 18:35 UTC

REFERENCES
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can be obtained from the structural relations model ([P. Sprent, J. R. Stat. Soc. (B) 28, 278 (1966); Models in Regression and Related Topics (Methuen, London, 1969); J. M. V. Rayner, J. Zool. 206, 415 (1985); B. Kuby and L. F. Marcus, Syst. Zool. 26, 201 (1977)]. This model requires that the ratio (denoted by λ) of the error variance in the y observations to that in the x observations is known. Model 1, model 2, and reduced major axis regression estimates are simply special cases of this model: model 1 estimates are found by assuming that λ is zero, model 2 is found by assuming that λ is 1.0, and reduced major axis by assuming that λ equals the ratio of the true variances in χ and y, which has previously been estimated as 0.19 [M. D. Pagel and P. H. Harvey, in [5]]. λ can also be estimated by a different procedure: the variance in mean brain and body weight was calculated in 117 species for which two or more mean values were reported from different sources. This direct estimate of the amount of variation in species, mean brain and body weights reported in the literature produces λ of 0.22. A used in this study is the mean of the two values (0.205), although either used alone gives similar results.

14. About 10% of the slopes calculated within genera were extreme outliers (defined as slopes with absolute values outside of ± 3, which is more than 3 standard deviations from the mean). These were overwhelmingly (88%) from genera for which only two data points were represented in the sample, and so they were excluded from the within-genus calculations. However, the species values were used in the calculation of higher order means.
16. All analyses of variance reported used a two-way classification of orders by taxonomic level. Subfamilies, families, superfamilies, and suborders were combined in order to achieve a more even distribution of degrees of freedom. However, slopes increased with taxonomic level even in the uncombined data (Table 1). The degrees of freedom for the mean square associated with taxonomic levels is one instead of two because a test was conducted for a linear trend in the means. This is a much more powerful test for a trend in the means than is the test of the overall mean square for taxonomic level. The latter is roughly half the value of the linear effect because it has two degrees of freedom, and yet the linear effect with only one degree of freedom accounts for about 98% of the variation among the means.
17. The presence of an association cannot be taken as evidence that the particular ecological factor identified is the cause of the difference in encephalization, but it does suggest that a single group with a different ecology than the rest is responsible for the trend. Any other factor that differs between the single group and the remaining taxa could also be responsible for the differences in encephalization.
23. We thank R. Lande and A. Read for comments. M. P. was supported by NSF fellowship BSR-8600171 and by the Royal Society.
3 January 1989; accepted 1 April 1989

Freeze Avoidance in a Mammal: Body Temperatures Below 0°C in an Arctic Hibernator

BRIAN M. BARNES

Hibernating arctic ground squirrels, Spermophilus parryii, were able to adopt and spontaneously arouse from core body temperatures as low as −2.9°C without freezing. Abdominal body temperatures of ground squirrels hibernating in outdoor burrows were recorded with temperature-sensitive radiotransmitter implants. Body temperatures and soil temperatures at hibernaculum depth reached average minima during February of −1.9°C and −6°C, respectively. Laboratory-housed ground squirrels hibernating in ambient temperatures of −4.3°C maintained above 0°C thoracic temperatures but decreased colonic temperatures to as low as −1.3°C. Plasma sampled from animals with below 0°C body temperatures had normal solute concentrations and showed no evidence of containing antifreeze molecules.

Hibernation in mammals is expressed by a fall in body temperature (TB) to the ambient temperature of the hibernaculum. Torpid animals maintain low TB's for up to several weeks until a brief (<24 hours) spontaneous arousal to high TB occurs, after which animals recoul. The lowest TB's previously reported for natural hibernation in a variety of mammalian hibernators are between 0.5°C and 2°C and in ambient conditions of 0°C to 3°C (1). In experimental conditions, slowly lowering ambient temperatures below 0°C leads either to an increase in an animal's metabolism and stabilization of TB or an "alarm arousal" after which the animal, upon returning to torpor, will actively regulate TB at 2°C to 3°C (2). Some ectothermic vertebrates can endure subzero TB's either by avoiding or tolerating freezing. For example, many species of polar and north temperate fish, through use of blood antifreeze proteins or glycoproteins, live at temperatures of −1.9°C (3), and painted turtles and four species of frogs can pass the winter frozen at ambient temperatures of −3°C to −7°C (4). Accounts of endotherms surviving subzero TB's are either anecdotal (5) or describe the artificial induction of subzero body temperatures, a condition from which the animal could not independently arouse (6). I report telemetric and direct evidence of the regular, prolonged, and spontaneously reversible adoption of core TB of as low as −2.9°C in the arctic ground squirrel, Spermophilus parryii, hibernating in outdoor enclosures.

Arctic ground squirrels were captured during late August 1987 in the northern foothills of the Brooks Range, Alaska, near the Toolik Field Station of the University of Alaska Fairbanks (68°38'N, 149°38'W; elevation 809 m) and transported to Fairbanks. Animals were implanted abdominally with miniature temperature-sensitive radiotransmitters that had been previously calibrated (7). On 19 September 1987, seven males and five females were released in Fairbanks into outdoor wire cages (0.9 by 0.9 by 1.8 m, buried to 1.3 m) where they dug burrows and remained for the next 8 months (8). Each cage was fitted with copper wire loop antennas (two or four each) housed in plastic pipe and connected to coaxial leads. Each lead was connected to a radio receiver with an interface to a computerized data acquisition system (9). Bandpass filters were used to overcome radio interference from a local AM radio station, and data collection began in mid-February 1988. In spring, after each animal emerged from the hibernaculum, transmitters were recovered and recalibrated (10). Soil and air temperatures at the site were recorded with thermocouples and a thermocouple thermometer. To determine the temperature regimes arctic ground squirrels experience during hibernation in the environment at which they were collected, soil temperatures at a depth of 1.0 m at two natural burrow sites near the Toolik Field Station were recorded over winter on automated remote recorders (11).

Minimum TB's of six hibernating ground squirrels occurred in February and March and averaged −1.9°C ± 0.3°C (range −2.9°C to −1.1°C). The TB of the individual that reached the lowest TB (−2.9°C) is shown...
whereas temperatures at the same depth in natural burrow locations on the North Slope of Alaska recorded over the winter of 1988 were similar in all six animals studied. These patterns were measured in ground squirrels with subzero deep torpor, Tb in the region of the transmitter did not vary more than 1°C, and it remained between 10 and 30°C above the soil temperature until mid-April when the hypothalamic set point of Tb in hibernation, the cooling rate of Tb slowed significantly after reaching ~1°C, and it remained between 1°C and 3°C above the temperature of the soil until mid-April when Tb approximated the temperature of the warming soil just before the final arousal. Several days before each spontaneous arousal, Tb began to slowly rise, increasing by approximately 0.5°C before arousal ensued. Early indications of arousals have also been shown by a rise in abdominal temperatures (12). These patterns were similar in all six animals studied.

Soil temperatures at a depth of 1.0 m at natural burrow locations on the North Slope of Alaska recorded over the winter of 1987–88 reached a minimum of ~18°C, whereas temperatures at the same depth in the caged burrows in Fairbanks never fell lower than ~7°C. Nest chambers where animals hibernated in the experimental burrows were excavated: the spherical nests were constructed of straw, approximately 30 cm in diameter, and usually located in a corner of the cage at 1.2 ± 0.05 m depth. Depth of natural hibernacula are limited by the permafrost table; ground squirrels appear not to dig into frozen ground. The permafrost table lies between 25 and 100 cm deep over most of Northern Alaska. To reproduce conditions of freeze avoidance under laboratory conditions, arctic ground squirrels were housed in an environmental chamber whose temperature was gradually reduced in fall 1988 from 5°C to ~−4.3°C during 1 month. Body temperatures of hibernating animals were measured at several locations, and blood was sampled by cardiac puncture from individuals that exhibited subzero rectal temperatures. Plasma was separated from blood cells, measured for solute concentration (15), and screened for the presence of antifreeze properties by testing for thermal hysteresis of melting and freezing points (16).

In ambient temperatures of ~−4.3°C arctic ground squirrels adopted colonic, foot, and subcutaneous temperatures that ranged from ~1.3°C to 10°C, and maintained oral and thoracic temperatures of ~0.70°C to 0.7°C. Subzero body parts seemed fully perfused as subdermal wounds inflicted on toes and abdominal skin bled promptly. In six animals with colonic temperatures averaging ~0.63°C, concentrations of plasma solutes were normal (302 ± 4.4 mmol/kg), and spontaneous freezing and melting points of plasma were not different (~0.59°C ± 0.02°C and ~0.56°C ± 0.01°C, respectively) and were similar to equilibrium freezing points of blood in nonhibernating mammals (17).

Animals withstand body temperatures below the freezing point of water by being freeze tolerant (4), by solute-dependent freezing point depression (18), by using antifreeze molecules (3), or by supercooling (19). There was no evidence of an exotherm (thermal heat of fusion) at subzero Tb's, which indicates that body water did not freeze. Plasma solute concentrations measured in ground squirrels with subzero deep body temperatures would have offered protection from freezing to temperature of approximately ~0.6°C, but for the core temperature measured of ~1.3°C to ~2.9°C a further mechanism of freeze avoidance must be offered. Antifreeze molecules depress freezing points relative to melting points by providing resistance to the growth of ice crystals (3). Freezing and melting points of plasma taken from ground squirrels hibernating at ambient temperatures of ~−4.3°C were equal, indicating that, under these conditions, antifreeze substances are not present. By exclusion this leaves supercooling, which is a metastable state of below freezing temperatures that persists in the absence of a nucleator which would readily initiate crystallization (3). Freezing and melting points of plasma were not different (~0.59°C ± 0.02°C and ~0.56°C ± 0.01°C, respectively) and were similar to equilibrium freezing points of blood in nonhibernating mammals (17).

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usually shorter and recorded hibernaculum temperatures remain above freezing (22). Supercooling to near –3°C should offer energetic advantages over maintaining greater than 0°C Tb’s to ground squirrels hibernating at ambient temperatures substantially below –0°C. Few metabolic measurements have been made of hibernators maintained in subzero conditions and none have been made for animals with below 0°C Tb. However, extrapolating from existing data on the greatly elevated metabolic costs of hibernators that maintain above 0°C Tb’s at ambient temperatures of 0°C and –2°C (23) suggests that supercooling to –3°C might save ten times the energy expended by maintaining above 0°C Tb (24). Any metabolic savings accrued over the hibernation season would be advantageous to ground squirrels—presumably in the forms of increased overwinter survivorship and of energetic advantages over maintaining above 0°C overall temperature throughout the short but frenetic reproductive season that begins at emergence from hibernation.

REFERENCES AND NOTES


7. Transmitters (model VM-FH disk, Mini-Mitter Co., Inc., Sunriver, OR) were implanted in animals anesthetized with methoxyfluran (Metofane, Pitman-Moore, Washington Crossing, NJ). Animal care was in accordance with University of Alaska Animal Use and Care Committee guidelines.

8. Ten of 12 animals survived to emergence in spring, but signals from only six transmitters were consistently received. Absent signals were due to battery failures in three cases or nest locations that were not close enough to an antenna for signal reception in two cases.


10. Transmitters were recalibrated in a refrigerated alcohol-water bath over the range of −4°C to +2°C within 24 hours of recovery from each animal. Computed temperatures based on initial calibration values were monitored from each animal’s own receiver simultaneously with temperature on a mercury thermometer relative to International Practical Temperature Scale 1968. Transmitter values were monitored from each animal’s own receiver simultaneously with temperature on a mercury thermometer relative to International Practical Temperature Scale 1968. Transmitter values averaged 0.09°C high (range −0.39°C to 0.60°C, n = 6). Data presented are corrected to reflect the recalibration values.


15. Plasma volumes of 10 μl were measured for solute concentrations with a Wescor 5050 Vapor Pressure Osmometer (Logan, UT).

16. Ice crystal melting temperatures and ice crystal growing temperatures were measured in plasma volumes of 20 μl after a small seed crystal was introduced by spray freezing. Procedures are described by A. L. DeVries (Methods Enzymol. 127, 293 (1986)).


24. This experiment results from extrapolating metabolic costs of torpor shown from Geiser and Kenagy (23, figure 2) to an ambient temperature of –10°C and a Tb of either 0°C or –3°C. At ambient temperatures below Tb greater than 0°C effects (see K. Schmidt-Nielsen, Animal Physiology: Adaptation and Environment (Cambridge Univ. Press, Cambridge, 1979), p. 207) on metabolism are seen as animals must produce heat to maintain a gradient between body and ambient temperatures. The extent of energy savings due to supercooling would depend on the proportion of metabolically active tissue that attains the supercooled state. Since measurements of body temperatures in hibernators at an ambient temperature of –3.4°C suggest that only posterior regions supercool, and since the most metabolically active tissues during torpor likely reside in the anterior of the body (heart, brain, brown adipose tissue), then energetic advantages of partial supercooling over maintaining above 0°C temperatures throughout the body may be significantly less than this estimate.

25. Supported by NIH grant HD 23383. I thank H. A. Maier, A. S. Porchet, and A. D. York for assistance, D. Borchert for the drawing, and A. D. York, G. J. Kenagy, L. K. Miller, and R. Elsner for reading the manuscript.

8 February 1989; accepted 26 April 1989