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COVER ILLUSTRATION

An icy stare

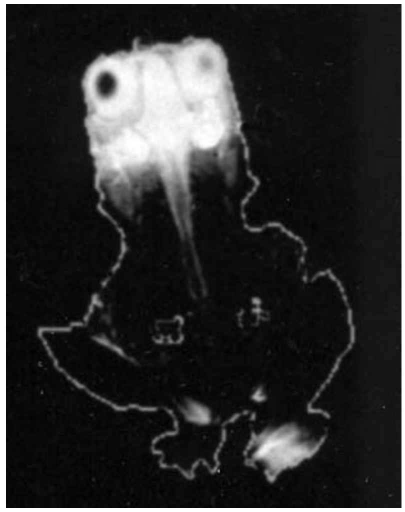
Polar latitudes have among the harshest of climates on earth, and those ill prepared for these extremes sometimes pay with their lives. Witness those who were bent on finding the northwest passage in the early 1800s. Explorers such as Parry and Franklin, among others, lost ships, supplies, men, and in some cases their own lives in their quest for a route to the orient. They simply weren't well enough prepared. Surprisingly, some vertebrates prepare for such extreme conditions in a manner many would consider wholly inconsistent with life. These creatures allow up to 65% of their body water, including their eyes, to freeze over winter!

Rana sylvatica, the North American wood frog, pictured on the front cover, is found across most of the northern tier of North America. While the range of this frog does extend south of Canada into the northeast United States and even further south into northern Georgia, its stronghold is the Canadian shield, extending northwest to include Alaska. It is the only frog found north of the Arctic Circle. This ectothermic anuran survives there—a feat that is nothing short of extraordinary.

Winter comes early north of 66° and with the first sign of freezing, perhaps in September, the wood frog quickly begins its descent into a cyrobiological state. The first formation of ice crystals on the skin of the wood frog triggers genetic expression of a gene in the liver that participates in the production of the cryoprotectant—glucose.

As the glucose levels rise, the cells lose free water, and even shrink to some extent. The peripheral limbs freeze first beginning with the fingers and toes. Freezing progresses centrally until it reaches the core. All organs freeze in an almost scripted fashion usually taking a maximum of 24 hours, and the three principal organs—liver, heart, and brain—are the last to freeze, and do so simultaneously.

But, as difficult as it is to imagine an animal freezing as a form of hibernation, re-entry to the world of the living is even more bizarre. How the recovery occurs is not a mystery, although the initiating cue remains obscure. If the periphery thaws first, it will undergo necrosis without the blood supply from the frozen cardiovascular system.



Magnetic resonance image of almost completely frozen frog. Note frozen lens, but still metabolically active brain.

Hence, the heart, brain, and liver must thaw concurrently and the entire thawing process must proceed in reverse. Amazingly, that is exactly what happens, although the details are not completely understood. The extremities do not thaw until there is a vascular supply to support them.

Each of the individual eucaryotic cells in the frog's body must travel the lonely road of suspended frozen animation by itself. Any rupture in the cell membrane or alteration in cellular metabolism, be that cell an astrocyte, a corneal endothelial cell, or a hepatocyte, would result in that cell's death. So, a cryoprotectant is essential to assist cells to pass this biological Rubicon. In essence, the frog becomes diabetic by converting glycogen stores to glucose and flooding the system with a glucose storm with levels 100-fold greater than normal. Cardioacceleration during the freezing process assures penetration of the cryoprotectant to the cellular periphery. Glucose makes an excellent protectant because it is so readily available and can be quickly generated from glycogen, even within minutes. It is not clear how the insulin metabolism is circumvented to maintain high glucose levels in circulation. Each cell, then, crenates, losing water molecules that as crystals of ice would disrupt the cell membrane. The concentration of glucose rises even further in the core organs, and these organs actually shrink in size (Storey KB, *Am J Physiol Regul Integr Comp Physiol* 1987;**253**:292). All cell functioning ceases—completely. As astonishing as this process is, there are limits. If the temperature goes to −30°C, this degree of freezing is lethal. In the Arctic, this is entirely possible. But, before beginning this process, the frog burrows beneath leaf litter to a few inches below the surface leaf litter and snow where the temperatures are more constant and less severe, even if frozen. The reverse process begins with the core organs, which thaw at a relatively lower temperature than those with a lower level of glucose. Heartbeat is the first physiological function to be restored. The whole process is much quicker than freezing and once it starts all tissues are thawed nearly concurrently, perhaps in as little as 1–2 hours.

The eyes freeze as well, but not uniformly. The lenses freeze first but late in the overall freezing progression. The brain, heart, and liver, as previously indicated, freeze last and nearly simultaneously. Although not known for certain, it would appear from magnetic resonance images that the retinas are among the last tissues to freeze. In that respect, the retina is very much like the brain (Rubinsky ST, *et al*, *Am J Physiol Regul Integr Comp Physiol* 1994; **266**:1771).

As the lenses freeze they become cloudy and opaque and do not become clear again until the thaw is nearly completed, often after heartbeat and breathing are re-established. The ocular melting process is not a mirror image of the freeze as the lenses thaw later than would be expected. Remarkably, this freezing and thawing of living cells, tissues, and organs has implications for organ and tissue survival, storage, and transplantation. Careful cellular studies document that a critical cellular volume of water must remain. Possibly, once the ice begins to melt, and vision returns to the frog, it must see the world though a very icy stare.

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