Thermal energy exchange between organism and environment, PG SEM-4

1. Thermal energy exchange

The effects of weather are manifested in an organism-environment relationship through the medium of thermal or h eat exchange_ Meteorological parameters alone may be quite meaningless when used in interpreting animal responses because weather instruments do not respond to the thermal regime of the atmosphere in the same way that a living organism does _ Further, weather instruments are often placed in standard weather shelters at standard heights so that as many variables as possible are eliminated_ A living organism, however, is exposed to changing weather conditions, and it also has its own physiological and behavioral variables. Knowledge of the basic principles of heat exchange enables the biologist to understand the functional relationships between weather and the living organism. An organism is coupled to energy-exchange processes by certain specific properties of its own (Gates 1963). The amount of heat exchange by radiation, convection, conduction, or evaporation depends on the thermal characteristics of the atmosphere and substrate, such as soil, rock, or snow, and the thermal characteristics of an organism. If a surface is highly reflective to radiant energy, then there can be little thermal effect from radiation. A cylinder with a very small diameter is a very efficient convector and slight air movement can result in a large amount of heat loss. Conversely, a large cylinder is a poor convector. An object covered with a layer of good insulation loses little heat by conduction, and an object with no water or other fluid that can be vaporized can have no heat loss by evaporation.

The thermal characteristics of an organism are related to its physiological and behavioral characteristics also, and these may change drastically in a short period of time. A deer, for example, may be bedded quietly until frightened, when it literally leaves its bed on the run! This results in very abrupt changes in the thermal regime. It has been traditional to categorize animals as either homeothermic or poikilothermic, but the distinction between the two is not entirely clear. Reptiles can regulate their body temperatures somewhat by behavioral thermoregulation, including changes in activity, location, and posture . Mammals do not have the same body temperature throughout their entire bodies. Deep body temperature is quite constant, with the temperatures of appendages more closely coupled to the external thermal environment. Hibernating mammals regulate their body temperatures by increasing heat production when their body temperatures approach the freezing point. They are in a sense homeothermic, but at a lower set point.

2. The concept of homeothermy

Warm-blooded or homeothermic animals are usually described as animals that maintain a constant body temperature. This very simple idea is often presented to students in elementary grades, and it is commonly said that the body temperature of humans is 98.6°F. All humans do not have the same body temperature though, and all parts of the human body are not at the same temperature. Careful consideration of the basic concept of homeothermy leads to a conclusion that is much more basic than

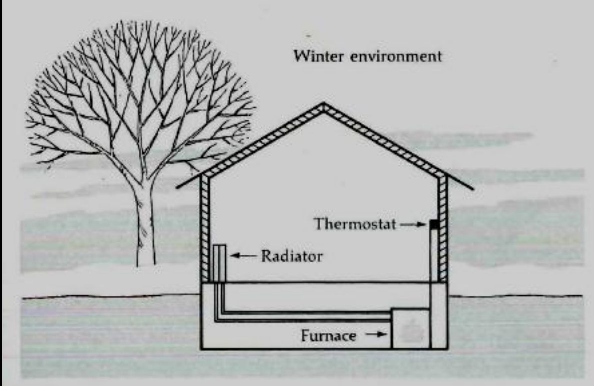


FIGURE 13-1. A heated home is like a homeothermic animal; a balance between the heat energy produced in the heat source (furnace) and the heat energy lost to the heat sink (the winter environment) is regulated by a thermostat so that the house temperature remains quite constant.

the simple statement that the body temperature remains "constant." Heat energy is produced when food is "burned," and heat is lost to the environment when the environment is colder than the animal. Homeothermic animals regulate the balance between heat production and heat loss. The effect of this balance is a relatively constant body-core temperature. The actual heat exchange between a homeothermic animal and its environment is very complex. The concept is simple, however, and can be illustrated by the example of a heated home in the winter (Figure 13-1). Fuel is burned in the furnace, and the heat energy is distributed throughout the house by water or steam pipes or by air ducts. The amount of heat energy distributed throughout the house is controlled by a thermostat that can be set at a desired temperature. The fue l in the furnace (oil, gas, coal, or wood) is a source of energy like the food eaten by an animal. The water pipes, steam pipes, or air ducts are like the blood vessels. The thermostat can be compared to the hypothalamus in an animal-a part of the brain that regulates heat production. If the weather gets colder, the fire in the furnace will burn longer in order to maintain a balance between the amount of heat energy released from the furnace and the amount of heat lost from the house. The net result is the maintenance of a constant house temperature. The Astrodome in Houston, Texas, operates in a similar manner, with air conditioning to keep it cool in the summer and furnaces to keep it warm in the winter. There are instruments or "nerve endings" on the outside surfaceof the Astrodome that perceive the amount of energy striking it from the sun, just as a person feels heat energy on the skin surface in bright sunlight!

3. Regions of thermal exchange

The exchange of heat between animal and environment occurs in three distinct regions, including the internal thermal region, the boundary region, and the external thermal region. The external thermal region includes both the atmosphere and the substrate (Figure 13-2). The internal thermal region includes all of the body tissue except the hair. Heat flow through body tissue is primarily by conduction and convection, with additional heat exchange by evaporation in the respiratory tract. The rate of conduction in this region depends on the thermal conductivity of the different kinds of body tissue, including muscle, fat, bones, and other tissue. The circulation of blood results in the distribution of heat within th e body by conduction and convection. The sites of active metabolism may have an excess of heat energy, which can be removed by the flow of blood and dissipated in areas in which the body tissue is cooler. Thus the circulatory system is a thermal transport system as well as an oxygen and carbon dioxide transport system. The boundary region is a thermally active region in which the influence of the animal's surface is exerted, both physically and thermally, on the external thermal region. Temperature differences between the internal thermal region of animals and the external thermal regron (the atmosphere) often exist, resulting in temperature gradients that characterize the boundary layer. Further, the boundary region surrounding birds and mammals includes the feather-air and hair-air interfaces, respectively.

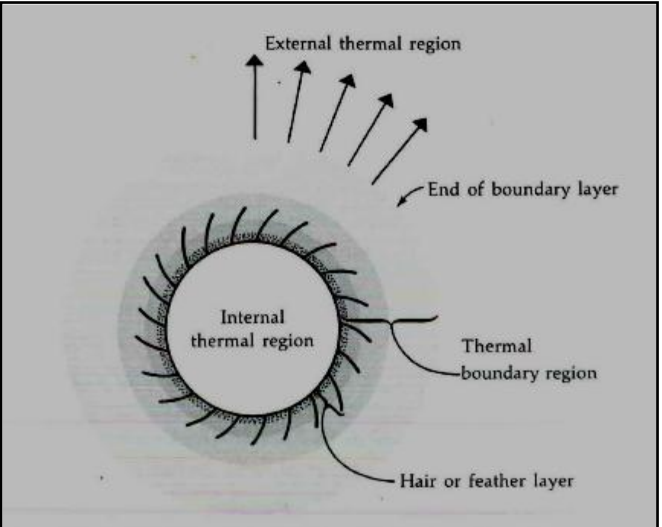


FIGURE 13-2. The three regions of heat exchange between animal and environment: internal thermal region, boundary region, and external thermal region.

The hair-air interface is a fibrous and porous medium with a density gradient from the base to the tips of the hairs, while a feather-air interface has a density gradient and a considerable amount of lateral overlap (Figure 13-3). These physical characteristics result in a very complex heat transfer that is made even more complex by the pliability of the hair or feather coat. Movement of the hairs or feathers increases the rate of heat transfer because it disturbs the hair-air or feather-air geometry. The relative importance of each mode of heat transfer varies across the interface layer. Conduction is the most important mode at the base of the hair because the hairs are tightly packed together and there is little air movement. Heat is conducted along the shafts of the hairs (or feathers) and through the air that is trapped between them. The space between the hairs in the outer portions of the hair-air interface is greater and the hair shafts are more exposed to the environment. Thus there is radiation exchange between the surfaces of the hair shafts and the surroundings. The decreased density of the hair permits a larger amount of air movement so convection processes accelerate. Each hair functions as a little convection cylinder exposed to both free and forced convection, and the proportion of free and forced convection is dependent on the air motion at the site of convection and the amount of penetration by the wind. The overlapping feathers function in a similar manner, with some modifications due to differences in physical morphology. Evaporative heat exchange through the interface results if energy is involved in the conversion of secretions of the skin or of rain or snow from a liquid to a gaseous phase. The rate at which this change of phase occurs is dependent on the movement of water molecules in the interface. A very dense hair layer will trap water molecules at the skin surface and will also prevent the penetration of rain or melted snow. The oily characteristic of an animal's coat further enhances its protective characteristics. The destruction of normal feather structure by oil on the surface of ducks destroys its insulative and waterproof characteristics, resulting in a high rate of heat flow through the feather-air interface.

4. Analyses of homoeothermic relationships

A basic concept that emerges from the consideration of all of the thermal relationships between animal and environment is that the thermal exchange of an animal is centered on the heat flow through the boundary region. This region, including hair or feather insulation, is a barrier to both heat absorption and heat dissipation. If the functional thermal characteristics of the boundary region are known, then the calculated heat flow through that layer will be in balance with the heat production of the animal and with the heat loss from its surface, with the additional consideration of the heat lost by respiratory evaporation. There are two approaches to the study of homeothermy. One is to measure the heat production of an animal in a chamber, which can be assumed to be equal to the heat loss as long as the body temperature remains stable. The other is to measure the heat loss, which can be assumed to be equal to the heat production if body temperature remains stable. The use of heat-production measurements as analogs to heat loss is valid, but the means by which heat is lost are usually not considered in this type of analysis. An animal held in a refrigerated metabolism chamber exhibits an increase in heat production as ambient conditions in the chamber become colder. This provides very useful information on the metabolic potential of the animal, but the chamber temperature does not represent outdoor conditions in which so many other thermal factors are present. The use of a mask on an animal held outdoors provides a more realistic exposure of the animal to natural weather conditions, but the limitations imposed by the mask prevent the animal from exhibiting normal behavioral responses. The calculation of heat loss is very complex owing to the complicated geometry of the anima l and the very labile nature of the thermal energy regime that is commonly referred to as weather. The heat loss must be equivalent to the heat production except for transient fluctuations over short periods of time. The synthesis of heat-production and heat-loss measurements into a single unified concept of heat exchange is the most logical approach to the analysis of the energy requirements of a free-ranging animal. The animal and its thermal environment are a thermal system, a homeostatic one; and analyses of the relationships between the two should include the recognition of every system component.

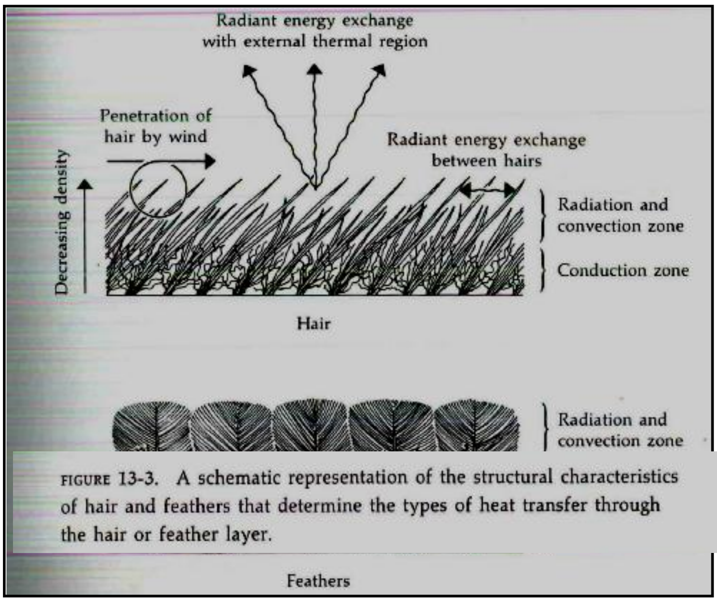


FIGURE 13-3. A schematic representation of the structural characteristics of hair and feathers that determine the types of heat transfer through the hair or feather layer.

Behavioral Thermoregulation

1. Introduction

Why do lizards sunbathe? Why do jackrabbits have huge ears? Why do dogs pant when they're hot? Animals have quite a few different ways to regulate body temperature! These **thermoregulatory** strategies let them live in different environments, including some that are pretty extreme.

Polar bears and penguins, for instance, maintain a high body temperature in their chilly homes at the poles, while kangaroo rats, iguanas, and rattlesnakes thrive in Death Valley, where summertime highs are over 100°F.

Let's take a closer look at some behavioral strategies, physiological processes, and anatomical features that help animals regulate body temperature.


2. Mechanisms of thermoregulation

As a refresher, animals can be divided into endotherms and ectotherms based on their temperature regulation.

- **Endotherms**, such as birds and mammals, use metabolic heat to maintain a stable internal temperature, often one different from the environment.
- **Ectotherms**, like lizards and snakes, do not use metabolic heat to maintain their body temperature but take on the temperature of the environment.

Both endotherms and ectotherms have **adaptations**—features that arose by natural selection—that help them maintain a healthy body temperature. These adaptations can be behavioral, anatomical, or physiological. Some adaptations increase heat production in endotherms when it's cold. Others, in both endotherms and ectotherms, increase or decrease exchange of heat with the environment.

The three broad categories of thermoregulatory mechanisms are:

- Changing behavior
- Increasing metabolic heat production
- Controlling the exchange of heat with the environment

2.1. Behavioral strategies

How do *you* regulate your body temperature using behavior? On a hot day, you might go for a swim, drink some cold water, or sit in the shade. On a cold day, you might put on a coat, sit in a cozy corner, or eat a bowl of hot soup.

Nonhuman animals have similar types of behaviors. For instance, elephants spray themselves with water to cool down on a hot day, and many animals seek shade when they get too warm. On the other hand, lizards often bask on a hot rock to warm up, and penguin chicks huddle in a group to retain heat.

Some ectotherms are so good at using behavioral strategies for temperature regulation that they maintain a fairly stable body temperature, even though they don't use metabolic heat to do so.

Examples of behavioral temperature regulation, from top left: basking in the sun, cooling off with water, seeking shade, and huddling for warmth.

2.2. Increasing heat production—thermogenesis

Endotherms have various ways of increasing metabolic heat production, or **thermogenesis**, in response to cold environments.

One way to produce metabolic heat is through muscle contraction—for example, if you shiver uncontrollably when you're very cold. Both deliberate movements—such as rubbing your hands together or going for a brisk walk—and shivering increase muscle activity and thus boost heat production.

Nonshivering thermogenesis provides another mechanism for heat production. This mechanism depends on specialized fat tissue known as **brown fat**, or brown adipose tissue. Some mammals, especially hibernators and baby animals, have lots of brown fat. Brown fat contains many mitochondria with special proteins that let them release energy from fuel molecules directly as heat instead of channeling it into formation of the energy carrier ATP.

2.3. Controlling the loss and gain of heat

Animals also have body structures and physiological responses that control how much heat they exchange with the environment:

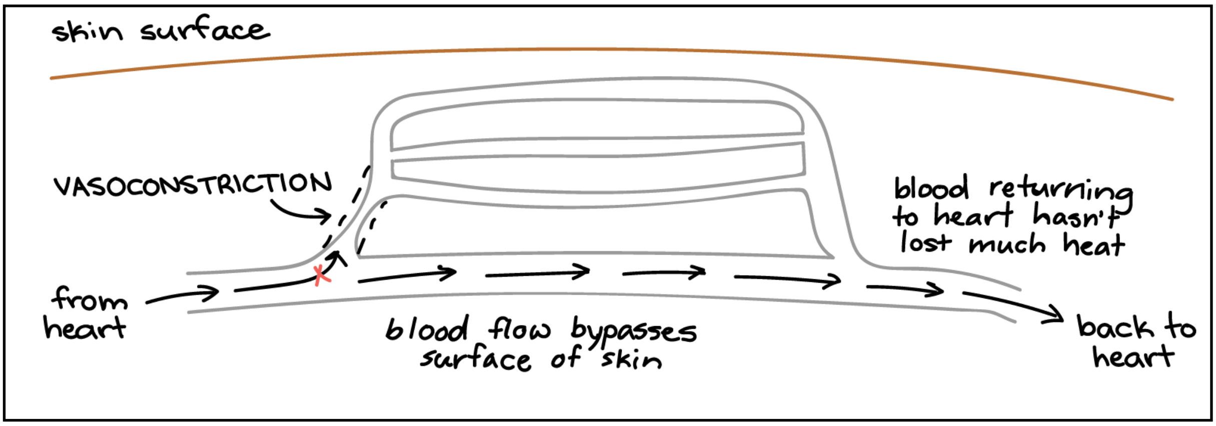
- Circulatory mechanisms, such as altering blood flow patterns
- Insulation, such as fur, fat, or feathers
- Evaporative mechanisms, such as panting and sweating

Circulatory mechanisms

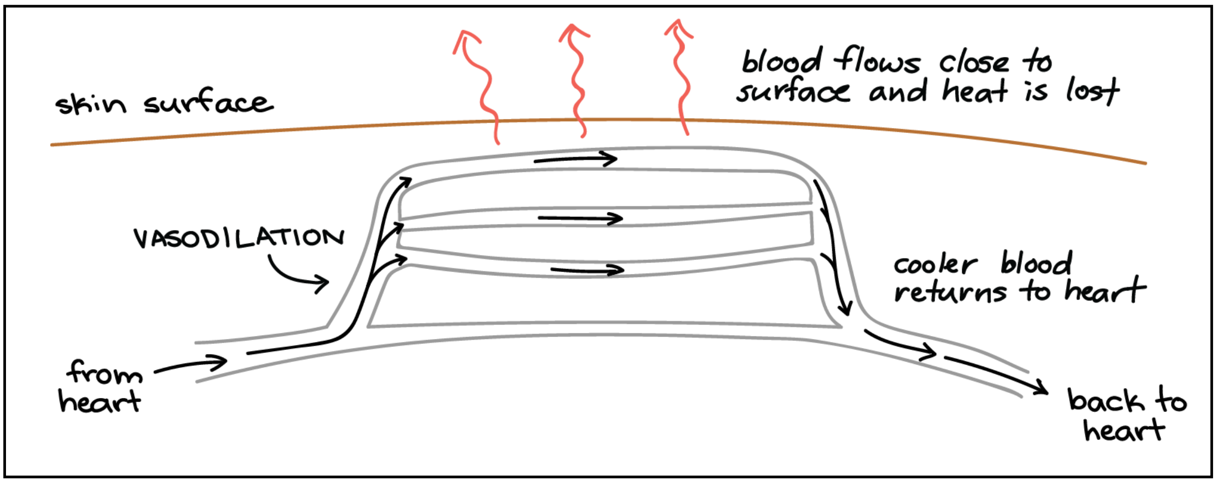
The body's surface is the main site for heat exchange with the environment. Controlling the flow of blood to the skin is an important way to control the rate of heat loss to—or gain from—the surroundings.

Vasoconstriction and vasodilation

In endotherms, warm blood from the body's core typically loses heat to the environment as it passes near the skin. Shrinking the diameter of blood vessels that supply the skin, a process known as **vasoconstriction**, reduces blood flow and helps retain heat.

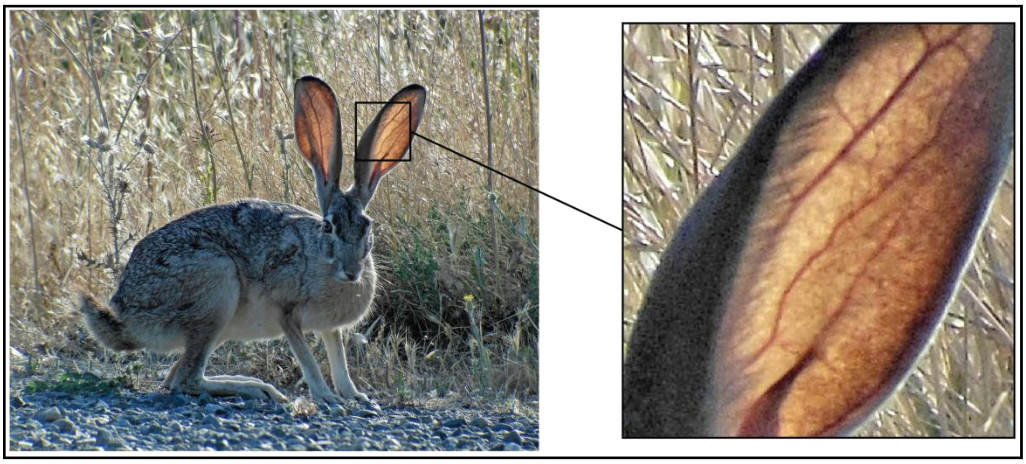


On the other hand, when an endotherm needs to get rid of heat—say, after running hard to escape a predator—these blood vessels get wider, or dilate. This process is called **vasodilation**. Vasodilation increases blood flow to the skin and helps the animal lose some of its extra heat to the environment.



Furry mammals often have special networks of blood vessels for heat exchange located in areas of bare skin. For example, jackrabbits have large ears with an extensive network of blood vessels that allow rapid heat loss. This adaptation helps them live in hot desert environments.

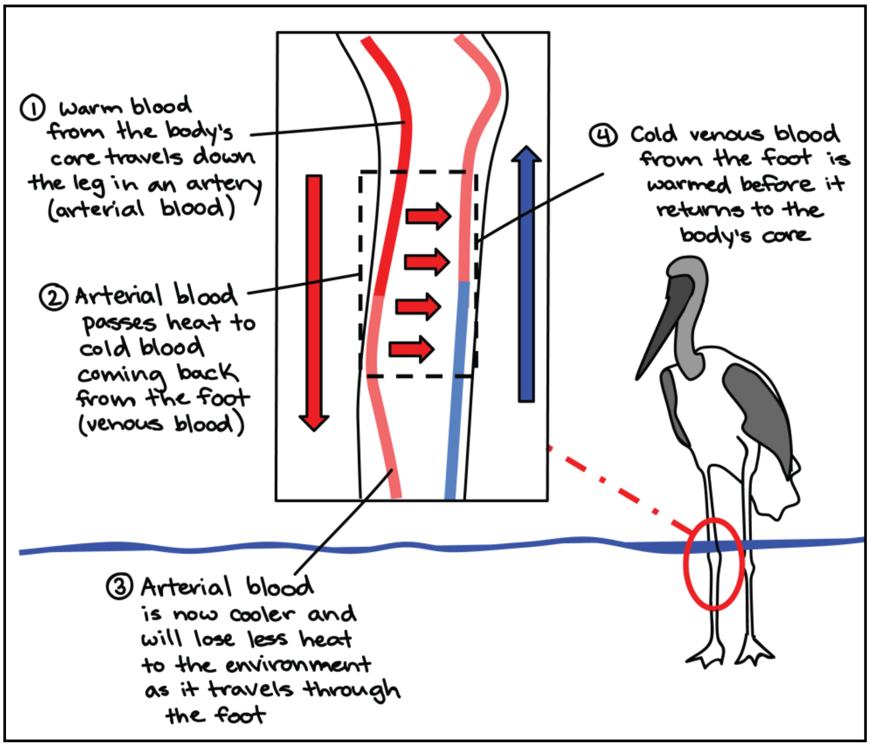
Some ectotherms also regulate blood flow to the skin as a way to conserve heat. For instance, iguanas reduce blood flow to the skin when they go swimming in cold water to help retain the heat they soaked up while on land.



Countercurrent heat exchange

Many birds and mammals have **countercurrent heat exchangers**, circulatory adaptations that allow heat to be transferred from blood vessels containing warmer blood to those containing cooler blood. To see how this works, let's look at an example.

In the leg of a wading bird, the artery that runs down the leg carries warm blood from the body. The artery is positioned right alongside a vein that carries cold blood up from the foot. The descending, warm blood passes much of its heat to the ascending, cold blood by conduction. This means that less heat will be lost in the foot due to the reduced temperature difference between the cooled blood and the surroundings and that the blood moving back into the body's core will be relatively warm, keeping the core from getting cold.



Insulation

Another way to minimize heat loss to the environment is through **insulation**. Birds use feathers, and most mammals use hair or fur, to trap a layer of air next to the skin and reduce heat transfer to the environment. Marine mammals like whales use blubber, a thick layer of fat, as a heavy-duty form of insulation.

In cold weather, birds fluff their feathers and animals raise their fur to thicken the insulating layer. The same response in people—goosebumps—is not so effective because of our limited body hair. So, most of us wear a sweater!

Left, a pigeon fluffs its feathers for warmth; right, human goosebumps are an attempt to increase insulation by trapping air near the skin

Evaporative mechanisms

Land animals often lose water from their skin, mouth, and nose by evaporation into the air. Evaporation removes heat and can act as a cooling mechanism.

For instance, many mammals can activate mechanisms like sweating and panting to increase evaporative cooling in response to high body temperature.

- In sweating, glands in the skin release water containing various ions—the "electrolytes" we replenish with sports drinks. Only mammals sweat.
- In panting, an animal breathes rapidly and shallowly with its mouth open to increase evaporation from the surfaces of the mouth. Both mammals and birds pant, or at least use similar breathing strategies to cool down.^88start superscript, 8, end superscript

In some species, such as dogs, evaporative cooling from panting combined with a countercurrent heat exchanger helps keep the brain from overheating.

Left, wolf panting to lose heat; right, beads of sweat on a human arm.

Super-cooling and Anti-freezing

- Supercooling is the process of lowering the temperature of a liquid or a gas below its freezing point without it becoming a solid. Aqueous solutions are progressively cooled and don't freeze not even below its freezing point. But this is an unstable state and the supercooled solution can suddenly freeze. Despite animals can voluntarily produce their supercooling, they can modify the probability of freeze spontaneously. To do it, they eliminate the nucleating agents of ice, substances that are the focus of the development of freezing.

- Antifreeze substances are dissolved substances present in body liquids to decrease the freezing point (temperature from which a liquid freeze). These substances can work in two ways. On the one hand, their presence in a liquid increases the concentration of substances and this produce a decrease of freezing point, but it is not for their chemical properties. On the other hand, these substance can present chemical attributes that produce a reduction of the freezing point. In specific, they join to ice crystals and avoid their growing.

1. Freeze Avoidance

Many biologists would be surprised to learn that a solution or organism does not necessarily freeze at its FP (freezing Point), but under certain conditions can cool much further whilst remaining unfrozen (liquid), or "supercooled." Indeed, a small volume of pure water can be chilled to nearly -40°C before it spontaneously freezes at its so-called "supercooling point," or, more accurately, temperature of crystallization (T_c).

To exploit the purely physical phenomenon of supercooling in a freeze-avoidance strategy, an organism must remain free of potent ice-nucleating agents (INAs), any of various inorganic particulates, microorganisms, proteins, and organic residues that can organize water molecules into a crystalline arrangement. Ubiquitous in nature, INAs of various potencies occur in diverse habitats, including the overwintering sites of molluscs (Ansart *et al.* 2010), insects (Zachariassen & Kristiansen 2000), amphibians (Costanzo *et al.* 1999), and reptiles (Costanzo *et al.* 2000), and may enter the body through orifices or be inadvertently ingested with food. Many freeze-avoiding ectotherms prepare for dormancy by eliminating ingested INAs, and also by masking or inhibiting endogenous ice-nucleating proteins (Costanzo *et al.* 2003, Duman 2001). It is crucial that they avoid physical contact with ice, which potentially can invade the body and initiate freezing. Species that rely on supercooling for winter survival can reduce the risk of such "inoculative freezing" by selecting hibernacula that limit their exposure to environmental ice. Some harbor in their tissues antifreeze proteins (AFPs) that effectively inhibit inoculation (Duman 2001).

To avoid ice nucleation, many cold-hardy ectotherms accumulate one or more cryoprotectants in advance of winter (Zachariassen & Kristiansen 2000). Representing several classes of compounds, these solutes vary by species, but all are of low molecular mass and benign in high concentrations (Table 1). They not only colligatively depress the organism's FP, much as automotive antifreeze (e.g., ethylene glycol) prevents radiator fluid from freezing, but also can enhance supercooling (Figure 3). Supercooling capacity is further increased by partial dehydration of the body, which occurs preparatory to winter in many insects (Lee 2010).

Table: 1 Cryoprotectants used in animal freeze-avoidance and freeze-tolerance

Class	Examples	Known From
Carbohydrates	polyhydric alcohols (glycerol, sorbitol, ethylene glycol); sugars (glucose, trehalose); cyclitols (*myo*-Inositol)	bacteria, marine and terrestrial invertebrates, amphibians, reptiles
Amino acids & derivatives	taurine, glycine, proline, alanine, asparagine, glutamic acid, lysine	bacteria, marine and terrestrial invertebrates
Methylamines	glycine betaine, glycerophosphorylcholine, trimethylamine oxide	bacteria, marine invertebrates, beetles
Urea		Terrestrial gastropods, amphibians, reptiles

Permutations of the freeze-avoidance strategy include vitrification, a process in which body fluids form a glass when cooled to extreme temperatures (Sformo *et al.* 2010), and cryoprotective dehydration, a survival adaptation of various invertebrates that overwinter in frozen substrata (Holmstrup *et al.* 2002, Sørensen & Holmstrup 2011). Because the vapor pressure of supercooled water exceeds that of ice, water tends to leave the unfrozen body until the internal vapor pressure reaches that of its frozen environment. Concomitantly, tissue FP drops as solutes become concentrated in a reduced water volume (and, commonly, as cryoprotectants are synthesized), thereby totally eliminating the risk of freezing. For this mechanism to be effective-and for ice inoculation to be avoided-dehydration must proceed quickly enough that the organism's FP consistently remains near the temperature of its cooling environment. Consequently, this strategy can be useful only to small ectotherms with a highly-permeable integument and profound desiccation tolerance.

Excessively deep or prolonged chilling can result in transient or permanent neuromuscular dysfunction or cold narcosis ("chill coma"), and even death (Salt 1961). Underlying mechanisms of chilling injury are not well understood, but probably include disturbance of ion homeostasis and metabolic functions, oxidative stress, and adverse phase changes in membranes (Kostal *et al.* 2006, MacMillan & Sinclair 2011).

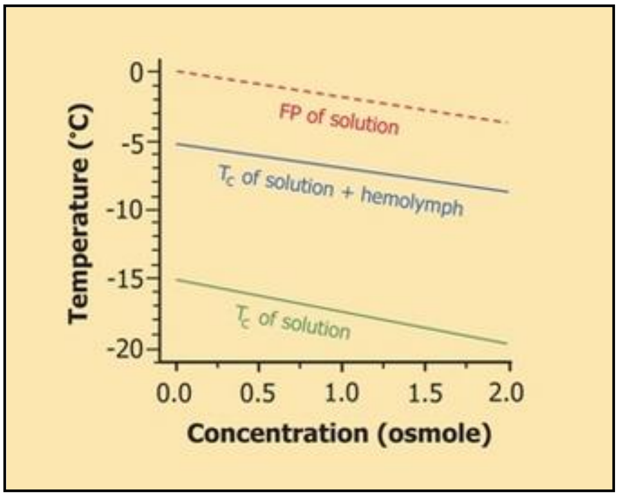


Figure: Effect of solute concentration on a solution's freezing point (FP) and supercooling capacity, as represented by its temperature of crystallization (T_c)

Upper (red) line depicts the theoretical FP depression of -1.86°C per osmole. Lower lines are regressions fitted to empirically determined Tc values for 5-μl samples of solutions containing various quantities of solute, with or without hemolymph (5% v/v) from the cold-hardy beetle, *Eleodes blanchardi*. Note that a potent ice-nucleating agent in the hemolymph markedly raised the solution's T_c but did not alter the effect of solute concentration on supercooling capacity. Modified from Lee *et al.* (1981).

2. Freeze Tolerance

Found in a small number of arthropods, molluscs, nematodes, annelids, amphibians, and reptiles, freeze tolerance is an adaptation for the survival of tissue freezing under ecologically-relevant thermal and temporal conditions. Freezing and thawing of the body, whilst potentially lethal, can be managed by mounting a diverse array of molecular and physiological responses that limit injury to cells and tissues.

For many species, freeze tolerance is expressed seasonally, usually developing in autumn in response to environmental cues and becoming most robust during the coldest months. In the laboratory, capacity for freezing survival can be enhanced by acclimation to high salinity and low environmental temperature, oxygen tension, and water potential (Lee 2010, Murphy 1983).

Control of the freezing process is key to survival in freeze-tolerant ectotherms (Lee & Costanzo 1998). In many invertebrates, which are prone to supercool owing to their small size, freezing is initiated by INAs in the hemolymph or other tissues. In other taxa, particularly species (e.g., amphibians) having a permeable integument, inoculation by external ice ensures that tissues freeze at relatively high temperatures (Costanzo & Lee 1996, Layne *et al.* 1990). Slow cooling enhances freezing survival, perhaps by allowing the organism adequate time to mount adaptive responses and by restricting ice to extracellular spaces; intracellular freezing is lethal for all but a few species (Lee 2010, Murphy 1983).
Ectotherms vary markedly in their limits of freeze tolerance. Known from laboratory studies, freeze endurance extends from perhaps a few days to several months of continuous freezing, although multiple freeze-thaw episodes are commonly experienced in nature (Layne *et al.* 1999). Depending on the taxon, the minimum survivable temperature ranges from a degree or two below 0°C to about -80°C (Table 2). Despite this variation, apparently no ectotherm can withstand the freezing of more than 50-80% of their body water.

Table 2: Thermal limits of freeze tolerance in animals

Taxon	Examples	Lower Lethal Temperature (°C)	Reference
Marine invertebrates (intertidal)	barnacles, bivalves, gastropods	-20	Loomis 1991, Murphy 1983
Terrestrial annelids	earthworms, enchytraeids	-20	Holmstrup 2007, Slotsbo et al. 2008
Terrestrial molluscs	slugs, snails	-5	Ansart and Vernon 2003
Other terrestrial invertebrates	free-living nematodes, centipedes, flies, beetles, butterflies and moths, wasps	-80	Lee 1991, Wharton 2002
Amphibians	salamander, frogs	-40 (frogs, -6)	Storey and Storey 1992
Reptiles	turtles, lizard, snake	-4	Storey and Storey 1992

Molecular and physiological adaptations to freezing and thawing help prevent cell death from dehydration, mechanical distortion, damage to macromolecules and membranes, metabolic perturbation, and ischemia/reoxygenation (Storey & Storey 1992). Dehydration is a particularly onerous problem. As ice accumulates in extracellular spaces, water is lost from the supercooled cells to the interstitium where solutes, rejected by the forming ice crystals, become increasingly concentrated in the as yet unfrozen solution. The rising osmotic potential of this solution withdraws water from inside the cells, progressively shrinking them and concentrating cytosolic solutes to potentially harmful levels (Mazur 1984). Oxidative damage, precipitated by the return of oxygen to thawed tissues, also contributes to cryoinjury (Storey & Storey 2010, Swartz 1972).

Freezing/thawing stress can be ameliorated by invoking mechanisms of anoxia tolerance, depressing metabolism, and accumulating one or more cryoprotectants, either in anticipation of or during freezing. Cryoprotectants used in freezing adaptation include a host of sugars and sugar alcohols, amino acids, and even the "waste product," urea (Table 1). By colligatively depressing the FP of body fluids, these solutes permit the cytoplasm to remain supercooled whilst the extracellular fluids freeze, and also limit ice formation (Storey 1997). Many play supportive roles in antioxidation, energy supply, macromolecular stabilization, and counteraction of perturbing solutes (Yancey 2005).

3. Freeze Avoidance or Freeze Tolerance?

Freeze avoidance is an effective survival strategy in small ectotherms that either use dry hibernacula or possess an impermeable integument. Although exposure to extreme cold or prolonged chilling can be harmful, cryoinjury can be minimized if freezing is avoided. Accordingly, freeze-avoiding species generally tolerate a relatively broad range of subzero temperatures. On the other hand, overwintering in the supercooled state is a precarious proposition, as death is the likely consequence of an inadvertent nucleation event.

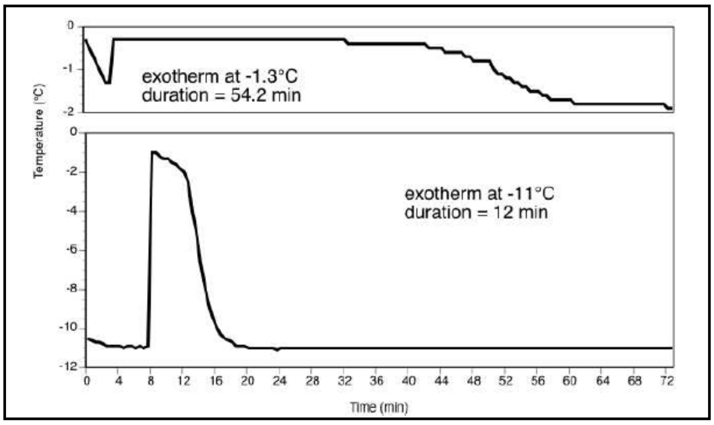
Freeze-tolerant ectotherms may be of any size and utilize myriad hibernacula, including ones that harbor ice. For those overwintering in an icy environment, tissue freezing eliminates the vapor pressure gradient across the integument and thereby prevents the organism from desiccating. On the downside, freezing/thawing stresses can be highly deleterious and, therefore, survival is possible over a relatively limited range of temperatures and exposure durations.

Although most species rely on freeze avoidance or freeze tolerance, a very few are uniquely adapted to employ either strategy, where the mode used during any particular chilling episode depends on prevailing physiological and/or environmental conditions (Costanzo *et al.* 2008, Sformo *et al.* 2009, Sinclair *et al.* 2004). Intensive study of these species may provide new insights into how the various selective pressures that shape life history traits drive the evolutionary development of cold-hardiness strategies (Sinclair *et al.* 2003).

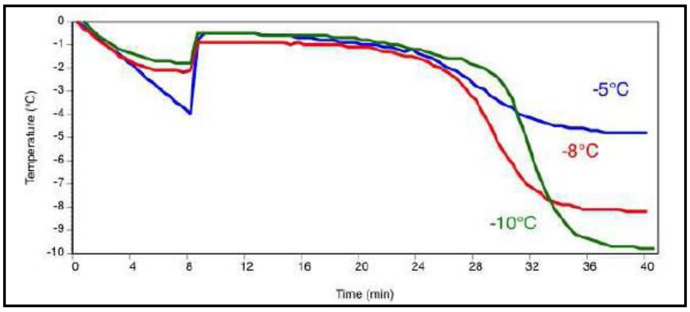
4. Most freezing tolerant animals limit supercooling

In contrast to freeze avoiding animals (see Ramløv, this volume), most freezing tolerant animals prevent extensive supercooling and encourage ice formation at a high subzero temperature. A clue to the reason why they do this can be seen from the relationship between the rate of ice formation and temperature. Figure 1 shows that the rate at which water freezes (or in this case a suspension of nematodes in water; with the duration of the exotherm produced during the freezing of the sample used as a measure of the freezing rate) decreases dramatically as the temperature approaches its melting point. Figure 2 compares exotherms in 50µl of nematode suspension where freezing was initiated at -1.3°C and -11.0°C. The exotherm duration is about 4.5 times longer when freezing is initiated at -1.3°C than at -11°C. Perhaps of more significance is that at -1.3°C the temperature becomes elevated to the melting point of the suspension (-0.3°C) and remains there until the freezing process is completed, indicating that the spread of ice through the sample is slow. At -11°C the temperature fails to reach the melting point of the suspension and declines rapidly from the maximum temperature reached (-0.9°C), indicating the rapid spread of ice through the sample.

In the moderately freezing tolerant insect Celatoblatta quinquemaculata cooled to different temperatures (Fig. 3), exotherms are similar since freezing is initiated at a relatively high subzero temperature by ice nucleators in the gut or haemolymph of the cockroach (Worland et al., 1997). The temperature at which the insect freezes spontaneously (the whole body supercooling point, SCP or temperature of crystallization, Tc) is not significantly different between animals frozen to -5, -8 or -12°C, with a mean SCP of -4.0±0.2°C (mean±se, N = 12) and the temperature becomes elevated to the melting point of the animals' body fluids in each case (-0.5 to -1.4°C) (Worland et al., 2004). Across a range of freezing tolerant animals freezing tends to occur at high subzero temperature, the rate of ice formation is slow and it takes a long time for the exotherm associated with the freezing event to be completed (Table 1). Perhaps not surprisingly these parameters are broadly correlated with the size of the animal.



Exotherms from 50µl suspensions of the Antarctic nematode *Panagrolaimus davidi*, with freezing initiated at -1.3°C (top) and -11.0°C (bottom) (data from Wharton et al., 2003).



Exotherms from the New Zealand alpine cockroach cooled to -5, -8 or -10°C at 0.5°C min-1 (data from Worland et al., 2004).

Ice nucleation in freezing tolerant animals

In contrast to freeze avoiding animals, that eliminate or mask sources of ice nucleation, freezing tolerant animals allow and encourage ice nucleation. Some freezing tolerant insects produce proteins or lipoproteins that have ice nucleating activity. These ensure that freezing occurs at a relatively high subzero temperature. They may also control the site of ice formation so that it occurs in the haemocoel, preventing potentially fatal intracellular freezing (Duman et al., 2010). The freezing tolerant Southern Hemisphere frog Litoria ewingi has ice nucleators in its skin secretions (Rexer-Huber et al., 2011), which ensure that this winter-active and largely terrestrial frog will freeze at a very high subzero temperature (-1.7°C) even on a dry substrate (Bazin, et al., 2007). Moderately freezing tolerant insects continue to feed during the winter, ensuring the yearround presence of food and microorganisms in their gut that could act as ice nucleators. Celatoblatta quinquemaculata and H. maori have ice nucleators in their haemolymph, gut contents and faeces (Wilson & Ramløv, 1995; Worland, et al., 1997). The nucleating activity of the faeces is greater than that of the haemolymph (Sinclair et al., 1999; Worland, et al., 1997). This suggests that the gut is the primary site of ice nucleation, with nucleators in the haemolymph providing a back-up system if the gut is empty (Worland, et al., 1997). The strongly freezing tolerant insect E. solidaginis forms a non-feeding dormant larval stage overwinter, surviving within the gall it induces in the stem of its host plant (Baust & Nishino, 1991). When the water content of the gall is high the larvae freeze by ice inoculation from the surrounding plant tissue (Lee & Hankison, 2003). As the autumn and winter progresses the galls dry out, inoculative freezing decreases and the insects rely on endogenous nucleators. These include calcium phosphate spherules that accumulate in the Malpighian tubules of overwintering larvae. These spherules, and the insect's fat body cells, have ice nucleating activity that ensure that the larvae freeze at -8°C to -10°C (Mugnano et al., 1996). Although some nematodes can survive desiccation, for growth and reproduction to occur at least a film of water must be present. Nematodes, and animals that live in similar habitats (such as tardigrades and rotifers), are likely to be faced with the risk of inoculative freezing from ice in their surroundings. Few species have been examined in this respect, but in those that have (Panagrolaimus davidi and Panagrellus redivivus) they have little ability to resist inoculative freezing (Hayashi & Wharton, 2011; Wharton & Ferns, 1995; Wharton et al., 2003). This also is the case in the infective larvae of the insect parasitic nematode Steinernema feltiae (Farman & Wharton, unpublished results) and the free-living Antarctic nematode Plectus murrayi (Raymond, 2010). In P. davidi inoculative freezing occurs via body openings, especially the excretory pore (Wharton & Ferns, 1995) and endogenous ice nucleators are absent (Wharton & Worland, 1998). However, if freezing of the media occurs at a high subzero temperature (-1°C) inoculative freezing does not occur in P. davidi and the nematode can survive by cryoprotective dehydration (Wharton, et al., 2003). In P. redivivus, however, inoculative freezing occurs in some individuals even at -1°C and the small amount of cold tolerance that this species possesses is largely due to freezing tolerance, although those few nematodes that remain unfrozen survive by cryoprotective dehydration (Hayashi & Wharton, 2011). Other freezing tolerant animals can use cryoprotective dehydration as an alternative strategy to freezing tolerance, especially under conditions where the chances of inoculative freezing is reduced (such as in soil of low water content). This has been reported in freezingtolerant earthworms (Pedersen & Holmstrup, 2003) and in the Antarctic midge, Belgica antarctica (Elnitsky et al., 2008). Some freezing tolerant arthropods appear to rely on inoculative freezing for survival. The centipede Lithobius forficatus freezes at a temperature just below the melting point of their haemolymph (about -1°C) by inoculative freezing when in contact with ice and survives, but if it supercools to -7°C and below it dies when it freezes (Tursman, et al., 1994). Caterpillars of the moth Cisseps fulvicolis also require inoculative freezing at a high subzero temperature to tolerate freezing (Fields & McNeil, 1986). Diapausing larvae of the fly Chymomyza costata can survive to low temperatures better if freezing is initiated by inoculative freezing at -2°C, than if they are allowed to supercool (Shimada & Riihimaa, 1988). Inoculative freezing may also be an important factor in the cold tolerance mechanisms of vertebrate ectotherms. The skin of frogs has a high permeability to water and if they are cooled in contact with a moist natural substrate (such as soil or leaf mould) they freeze by inoculative freezing when ice forms in the substrate. Soil contains abundant ice nucleators that initiate freezing at a high subzero temperature (Costanzo et al., 1999). The skin of hatchling turtles is much less permeable to water than that of frogs but inoculative freezing can still occur via body openings, such as the eyes, ears, nose, cloaca, umbilicus, mouth and anus. Given the high levels of ice nucleators in their natural substrate this necessitates a level of freezing tolerance (Costanzo, et al., 2008) and inoculative freezing may be required to ensure survival over winter (Baker et al., 2006).

Intracellular freezing

The use of ice nucleators to induce freezing at high subzero temperature in the body fluids of many freezing tolerant animals is thought to ensure that freezing occurs extracellularly (Duman, et al., 2010). Intracellular freezing is thought to be fatal due to the mechanical disruption of cells by the expansion of water as it freezes, the puncturing of membranes by ice crystals or the redistribution of ice crystals (recrystallization) after freezing and during thawing (Acker & McGann, 2001; Muldrew et al., 2004). However, some examples of survival of intracellular freezing have been discovered in particular cells and tissues of some freezing tolerant animals (Sinclair & Renault, 2010). The only animal shown to survive extensive intracellular freezing throughout its body is the Antarctic nematode P. davidi (Wharton & Ferns, 1995). Some other nematodes have now been shown to have at least some ability to survive intracellular freezing, including Steinernema feltiae (Farman & Wharton, unpublished results) and Plectus murrayi (Raymond, 2010).