Effects of Insulation and Antifreeze/Glycerol on Thermoregulation of Simulated Animals Living in Cold Conditions

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Abstract

The survival of all living organisms depends on their physiological and behavioral adaptations to their environment, especially in extreme conditions. Endothermic animals maintain their dynamic body temperature via internal heat production and appropriate insulation, such as fat and fur. The survival of ectothermic animals living in temperatures considerably below the freezing point of their body fluids depends on unique compounds, such as supercooling agents, glycerol, and glycoprotein. These "antifreeze" compounds found in blood and tissue fluid prevent the fatal formation of ice in their body tissues. To study the thermoregulation mechanisms of endotherms in cold temperatures, 160-ml aluminum cans were filled with 37°C normal saline to simulate endothermic animals' internal environment. The first objective of this study was to determine the effectiveness of fat and/or fur as insulators on thermoregulation at ambient temperatures of 22°C and 8°C. The second objective was to investigate the effectiveness of glycerol, antifreeze, and sodium chloride on

thermoregulation of ectothermic animals at freezing ambient temperatures. The results of the experiment demonstrate that fur is a better insulator than fat. However, both fat and fur decrease the fluctuation of internal temperature and assist in maintaining a relative stable internal temperature for a longer period of time compared to the control with no external insulation. The findings support the concept that animals in cold ambient temperatures develop a thicker layer of subcutaneous fat to help them maintain a more stable internal temperature without the expenditure of vast amounts of energy generated by shivering. In the simulation of ectothermic animal's internal environment, various concentrations of glycerol, glucose, sodium chloride, and antifreeze are shown to lower the freezing point. The freezing point depression of the fluids increases linearly with their concentrations. The presence of antifreeze has the greatest effect in preventing the formation of ice under prolonged subzero temperature conditions. Utilizing antifreeze compound in the blood and tissue fluid permits animals to survive and swim in seawater at a temperature of -1.8^{o} C.

Student Outline

Introduction:

Ambient and internal body temperatures play a very important role in an animal's survival. All living organisms have limits to the temperature range in which they can survive, whether they maintain a relatively constant internal temperature or conform to their environmental temperature. The degree of environmental temperature tolerance varies with the duration of exposure, seasons and/or their stages of development. The endothermic animals can tolerate only a narrow range of body temperature, but they are able to live in a wide range of environmental temperatures. The ambient temperature range within which the metabolic heat production of an animal is unaffected is considered as the animal's ***thermoneutral zone*** (Withers, 1992). It is advantageous for an animal to modify its behavior and physiological adaptations, such as thicker insulation, to remain in the thermoneutral zone. While in the thermoneutral range, the animal's resting metabolic rate remains fairly constant and effort for thermoregulation is reduced to a minimum. Thermoregulatory heat production is enormously expensive and makes up the single largest component of the energy budget in endotherms. When ambient temperature is below the thermoneutral zone, the resting metabolic rate of an animal has to be increased by shivering to replenish heat that was lost to the environment. The ambient temperature that initiates changes in the animal's resting metabolic rate is called the ***lower critical temperature***. Most tropical mammals have critical temperatures between $+20^{o}$C and $+30\ ^{o}$C, and Arctic animals have much lower critical temperatures. A well-insulated animal such as an Arctic fox does not increase its metabolic rate significantly until the air temperature is below -40^{o}C (Schmidt-Nielsen, 1997), a point at which it starts shivering.

In situations where ambient temperature (Ta) is below the body temperature (Tb), heat is transferred from the animal to the environment by conduction and radiation. Endothermic animals adjust their rate of metabolic heat production (H) to equal the rate of heat loss (Q). The heat balance of an animal can be expressed by the following equation:

$$\mathbf{H \alpha Q \alpha C (Tb\text{-}Ta)}$$

The loss of heat is mediated by the gradient between the (Ta) and (Tb). The greater the difference between the ambient and body temperatures, the faster and greater is the heat loss. For example, camels increase their Tb to reduce the gradient between the Ta and Tb, thus reducing heat loss to their environment.

Conductance (C) represents heat flow from the body to the environment or vice versa. To reduce their thermal conductance, animals rely on behavioral adaptations and the efficiency of their insulation such as fat, blubber, and fur. As insulation is increased, conductance decreases, heat loss is minimized, and the animals remain in their thermoneutral zones with minimal expenditure of energy to regulate their Tb. In aquatic environments, fur is not as advantageous as it loses its insulation value when wet. This is one reason most aquatic mammals have a thick layer of blubber instead of fur.

Arctic animals that live in ambient temperatures below freezing have to avoid the fatal formation of ice in their body tissue. These animals depend on physiological as well as biochemical adaptations in order to live. High concentration of glycerol is found in the blood of the Antarctic fish, *Trematomus borchgrevinki.* Glycerol increases the cold tolerance and lowers the freezing point of the tissue fluid, preventing the deadly formation of ice crystals in the blood and tissues, thus enabling the fish to live in seawater at a temperature of –1.8°C (Schmidt-Nielsen, 1997). Another chemical that is present in animals living in cold environment is a glycoprotein compound, an "antifreeze" complex that resists ice formation by preventing the addition of water molecules to the crystal lattice of ice. Yet another method of coping with subzero temperature for most freeze tolerant insects is the presence of nucleating agents, large hydrophilic protein molecules, promoting ice formation in the hemolymph (Withers, 1992). Formation of ice crystal in the hemolymph increases the osmotic pressure of the tissue fluid, creating a hyperosmotic environment for the cell. Water exits the cell, leaving the internal environment with higher osmolality, which in turn lowers the freezing point, and reducing the chance of ice formation within the cell.

Objective

The purpose of this investigation is to use simulated conditions to study non-metabolic temperature regulation mechanisms for both endo- and ectothermic animals in cold environments. Part one of the experiment studies the effectiveness of fat and/or fur to reduce conductance of heat at Ta of 20^{o}C and 10^{o}C. Part two of the experiment determines the effectiveness of glycerol, antifreeze and various concentrations of physiologic solutions (glucose and NaCl) on freezing point depression.

Materials:

- Five 162-ml aluminum can (Treetop apple juice cans)
- 10 thermometers
- 3 Ziploc bags
- 3 cups Crisco shortening
- 2 rabbit fur pelts
- Funnels
- Balance
- 5 1L beakers
- 5 ice buckets
- Rubber bands
- Wax papers
- 4 Styrofoam containers
- Cotton balls to cushion the small test tubes
- Glycerol
- 0.9%, 1.8% sodium chloride solution
- 5 %, 10% dextrose solution
- Commercial antifreeze solution
- Rock Salt
- 4 large test tubes
- 4 small test tubes (fit inside the large ones)
- 4 stirrers
- Crushed ice

Procedures

Part 1

Prepare fat by weighing two 8-oz. portions of Crisco shortening on wax papers. Place the shortening in two different Ziploc bags. Spread the shortening to an even thin layer, and zeal the bags. Label 4 aluminum cans, 1-4. Punch a small hole on the top and insert a thermometer in each can. Wrap can #1 with an 8-oz. fat-filled bag, can #2 with a rabbit fur pelt, can #3 with both 8-oz. fat filled bag and fur. The last can, #4, is the control; it has neither fat nor fur.

Heat 1L of 0.9% saline to 37^{o}C. Use a small funnel to fill the cans with warmed saline. Attach the wrapped fat and/or fur linings to the can with a rubber band. Make sure the entire can is covered with fat and/or fur, or both. Place all four cans at room temperature: 22^{o}C. Record the core temperature of the cans every 5 minutes for an hour, starting at 37^{o}C. Repeat the experiment at 8^{o}C ambient temperature.

If a cold room is not available, place the can with fat/fur in a large beaker and then place the entire assembly in an ice bucket with ice and water. Placing the fat/fur wrapped can in a large beaker is to ensure even distribution of temperature around the aluminum can. Use a thermometer to monitor the temperature inside the beaker. Adjust the amount of ice and water until the temperature in the beaker registers around 8^{o}C. The apparatus for measuring internal temperature of the cans is shown in Appendix A.

Part 2

Prepare an ice-salt bath using layers of crushed ice and rock salt. Alternate the layers of ice and salt until the Styrofoam container is full. Stir the ice-salt mixture. Clean and dry the smaller test tube and add to it 10 ml of glycerol. Insert the stirrer and thermometer into the tube. Make sure the thermometer is immersed in the solution, and the stirrer moves up and down easily. Immerse the tube into the ice-salt bath, stir the solution with the stirrer, and allow the solution to cool to about 5^{o}C. Remove the smaller test tube from the bath and place it inside the larger test tube. Immerse the entire test tube assembly into the ice-salt bath. Placing the smaller tube in a larger one ensures even distribution of temperature around the small tube and prevent cold spots outside the small tube. Stir the solution in the test tube continuously at a uniform rate with the wire stirrer. Stirring the solution avoids development of cold spots and ensures the temperature of the solution is the same throughout the small test tube. Continue recording temperature measurements every two minutes until six successive readings are equal. Stir the solution with the stirrer and measure the temperature of the solution. Subsequent freezing point of tap water, deionized (DI) water, 1:1 ratio of saline and antifreeze, 0.9% saline, and 2.7% saline are obtained using the same procedure. The apparatus for measuring freezing point of solutions is shown in Appendix B.

Results

Part 1

1. The time versus temperature data is arranged in tabular form for each of the three trials, at 22^{o}C, at 8^{o}C, and with twice the shortening as insulation. See table below.
2. Prepare the graphs from the data tables.
3. Determine the greatest and the least heat loss relative to insulation.

4. Determine the greatest and the least heat loss relative to ambient temperature.

Time (Min.)	Temp -^{o}C Can – no insulation	Temp -^{o}C Can with fat	Temp -^{o}C Can with fur	Temp -^{o}C Can with fat and fur
0	37	37	37	37
5				
10				
15				
20				
25				
30				
35				
40				
45				
50				
55				
60				

Part 2

1. The time versus temperature data should be arranged in tabular form for each of the six trials: water, glycerol, saline and antifreeze, 0.9% and 2.7% concentrations of saline.
2. Prepare cooling curve for water and the other solutions from the data tables.
3. Calculate the freezing point of water – both tap and DI water.
4. Calculate the freezing point depression for each of the solutions

Time (Min.)	Temp -^{o}C Glycerol	Temp -^{o}C Saline and antifreeze 1:1 ratio	Temp -^{o}C 0.9% Saline	Temp -^{o}C 1.8% saline	Temp -^{o}C Tap water	Temp -^{o}C DI water
0	5	5	5	5	5	5
2						
4						
6						
8						
10						
12						
14						
16						
18						
20						
22						
24						
26						
28						
30						

Discussion Questions

1. In the simulated conditions for endotherms, which aluminum can has the greatest conductance of heat? Which has the lowest? What is the relationship between conductance and insulation?
2. What are the differences in rate of heat loss amongst the four solutions between 22^{o}C and 8^{o}C? Explain the result using the heat loss and heat gain equation.
3. What are the two variables that an animal can adjust, without any changes in the metabolic rate, in order to reduce heat loss to the environment,
4. Why is thicker insulation advantageous to the survival of Arctic animals?
5. How do these terms "thermoneutral zone" and "lower critical temperature" associate with the results of this experiment?
6. How do glycerol, antifreeze, and various concentrations of NaCl and dextrose affect freezing point?

Notes for the Instructor

There are two variables that an animal may adjust to reduce heat loss to the environment: (1) increase insulation, and (2) decrease the difference between ambient temperature and body temperature (Tb-Ta), either by a higher ambient temperature (e.g., animals living in burrows where ambient temperature is higher than the surface environment), or by changing body temperature. These hypotheses may be tested by:

1. Altering the layer of fat: instead of using 8 oz. of shortening, use 16 oz. or more.
2. The ambient temperature may be changed to 15 ^{o}C from 8^{o}C.
3. The temperature of the saline in the cans may be lowered to 34^{o}C instead of 37^{o}C.

The freezing point of deionized water is below zero (the deionized water is supercooled). Tap water freezes at 0^{o}C; it has more nucleating agents and is not as pure as the deionized water. Part 2 of Procedure can be repeated using different solutions such as:

1) 1:2 concentrations of saline and glycerol
2) Increase ratio of antifreeze to saline
3) 5% dextrose
4) 10% dextrose
5) 15 % dextrose

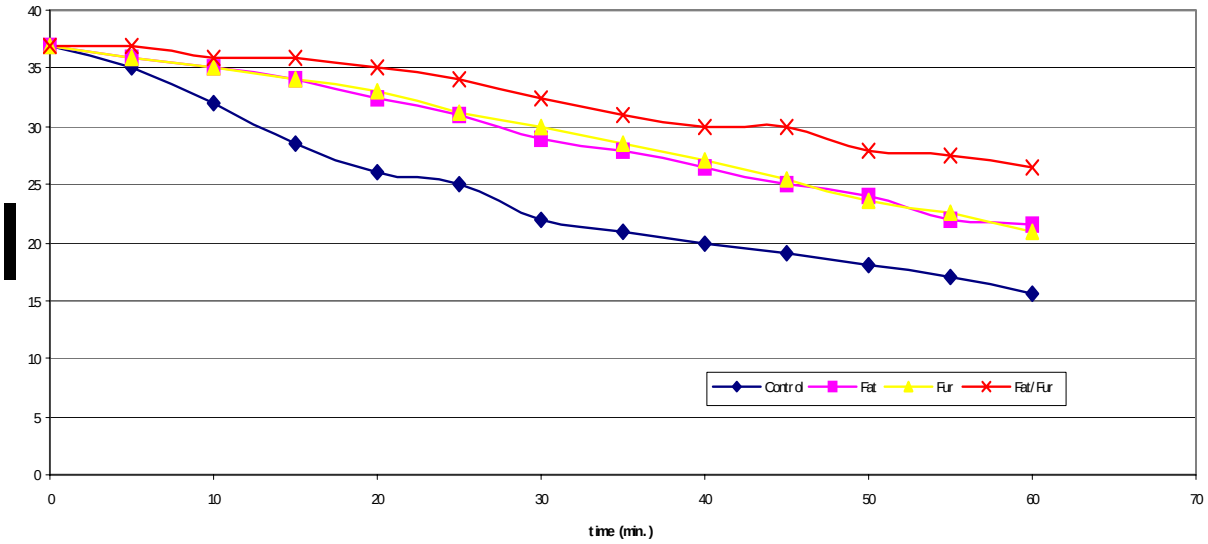
Sample Student Discussion of Results

While the cans were in the freezer without the layer of fat, the heat transfer flowed out of the can directly into the air space. After cooling, the internal temperature of the cans was below ambient temperature and the heat transfer on the counter flowed back into the cans. Because the temperature gradient was greater in the freezer (an average 60° difference) than on the counter (an average 15°

difference), the change in temperature over 30 minutes was greater in the freezer. In the oven, there was an even greater temperature gradient (an average 124° difference), and the internal temperatures of the cans increased rapidly. The return to ambient temperature after heating showed an average 20° gradient and therefore a slower cooling.

As resistance to heat fluctuation, the layer of fat slowed the heat loss from the cans in the freezer by about half, as the average heat loss in 30 minutes was only 11° F. Back at ambient temperature, the fat resisted more heat absorption into the cans as their internal temperature continued to drop slightly. In the oven, the transfer of heat into the cans with the fat insulation was slowed as the cans only gained 1/3 the heat (11.6°) in 30 minutes, compared to 30° in 30 minutes without the insulation. Since the fat layer was still retaining heat the insulated cans continued to gain heat for 15 minutes when placed in the ambient air. After that, the fat layer prevented further heat loss and the temperatures remained constant for the final 15 minutes.

The most unexpected result was the rise in initial temperature of the can containing antifreeze. At room temperature of 71° F, the distilled water and sucrose/NaCl cans both began at a slightly lower temperature of 69° F. At the same time, the antifreeze solution was 81° F. Because the result of antifreeze material in an animal's blood is to lower the freezing point, there may be some mechanism that accomplishes this by accepting heat from the environment without allowing the transfer of heat back out at the same point.



Effect of insulation on core temperature

Conclusion

The layer of fat did help to maintain a more stable internal temperature against a temperature gradient for all three solution mixes. This supports the idea that a layer of fat helps thermoregulating animals to maintain a more stable internal temperature without having to expend vast amounts of energy to generate heat. The addition of antifreeze to the solution did not appear to have any affect on the overall range of temperature fluctuation. As an adaptation to prevent ice formation, the antifreeze solution may need to be tested against sub-zero conditions rather than a wide range of relatively low and high temperatures.

The results of the experiment demonstrated that a layer of fat around the can (or body) decreased the amount of fluctuation and maintained a relative stable internal temperature. The findings supported the concept that a layer of fat helps animals to maintain a more stable internal temperature without having to expend vast amounts of energy to generate heat. In the simulation of an ectothermic animal's internal environment, various concentrations of solution of glucose, sodium chloride and antifreeze lowered the internal temperature fluctuation and freezing point. The freezing point depression of the fluids increased linearly with their concentration. The presence of antifreeze had the greatest effect under prolonged subzero temperature condition.

Antifreeze solution

Freezing point depression observed with various concentrations of glucose and sodium chloride and the antifreeze. Both NaCl and Glucose the freezing point depression increases linearly with the concentration, but NaCl, because of its dissociation into Na^+ and Cl^-, as twice the freezing point depression of glucose. The antifreeze prevents the formation of ice in water several hundred times as effectively as other solutes

References

Schmidt-Nielsen, Knut. Animal Physiology adaptation and environment, 5th edition. Cambridge University Press. New York. 1997.

Withers, Philip C. 1992 Comparative Animal Physiology. Saunders College Publishing. San Diego. 122-191.

Maria Powell, Kelli Lawrence, Marady Nay Zoology 4032 April 18, 2002

Appendix A

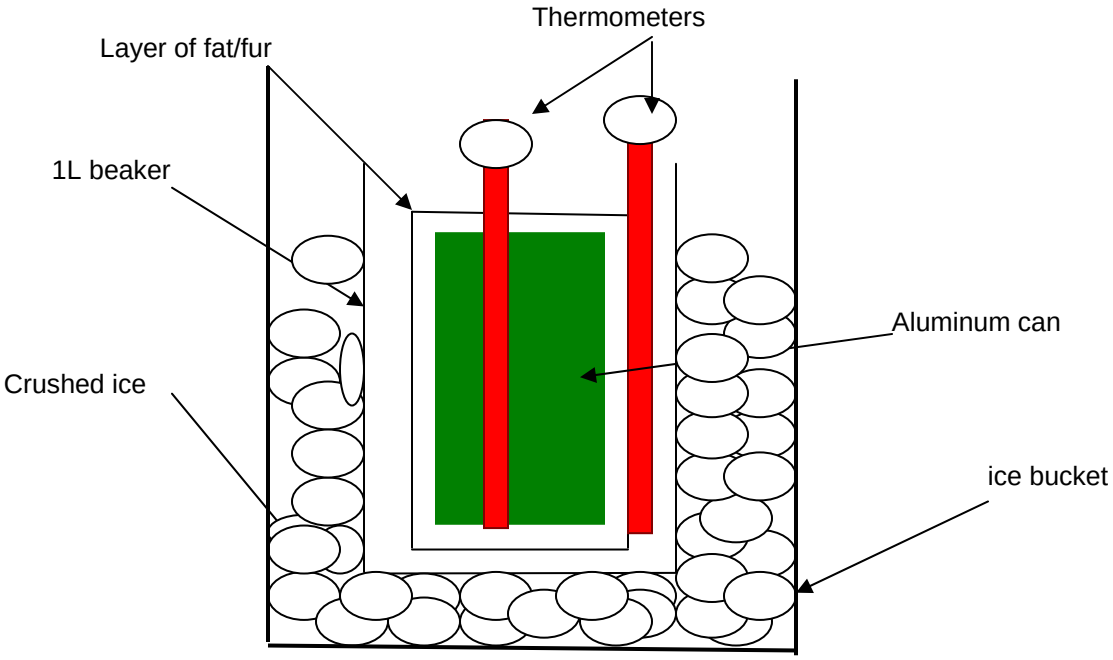


Figure 1. Apparatus for measuring internal temperature of fluid in the can at ambient temperature of 8^{o} C

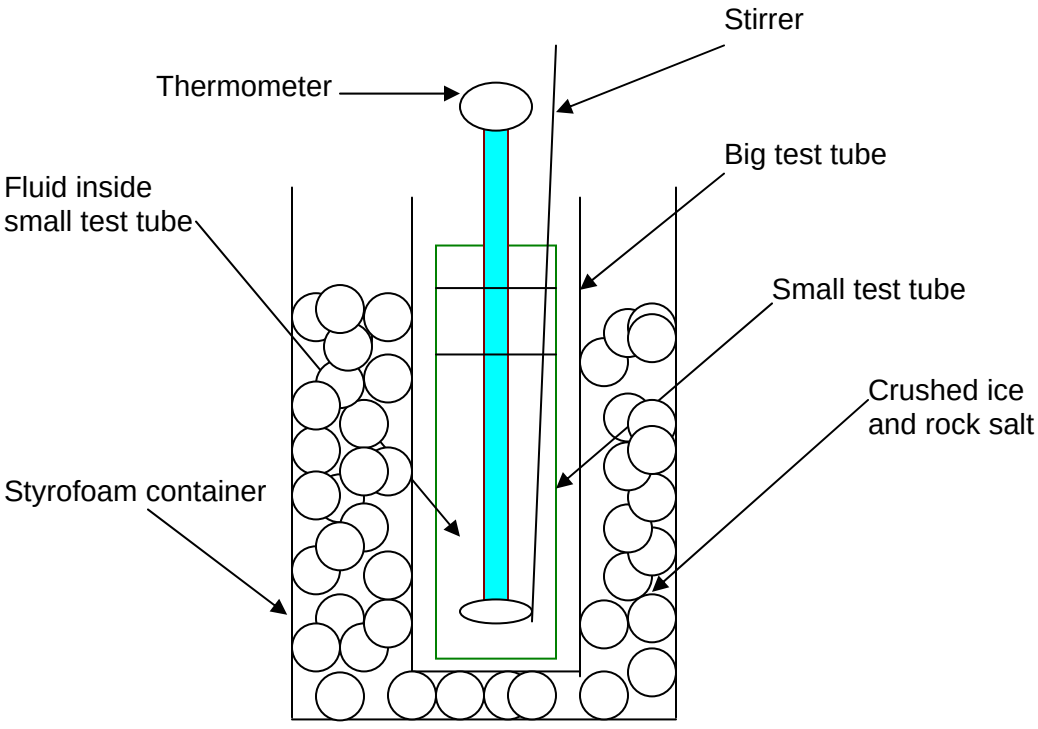


Figure 2. Apparatus for measuring freezing point of fluid in the small test tube