CRYOPRESERVATION LAB

Using osmosis to increase freezing survival

In this lab, you'll be doing an experiment in **cryobiology**—the science of how living things survive cold temperatures.

For quite a few years now, humans have been trying to find ways to FREEZE THE HUMAN BODY in a state of "suspended animation"—we've managed to do this with sperm, eggs, even human embryos—but not with anything more complex than that. The main problem is this: ICE FORMATION MESSES UP COMPLEX CELLS AND TISSUES. When ice crystals form, they often **split the cell membrane**—ever tried to freeze a can of pop and had it split open? Same deal. When a cell membrane splits, **the stuff in the cell leaks out**, the cell dies, and the organism is damaged. Also, **closely packed tissues are sometimes pried apart by ice formation**, damaging the tissues. **This is why humans aren't able to survive freezing.**

However, some **insects and other animals are capable of surviving freezing**, and we're starting to figure out why. It has to do partly with **OSMOSIS**! Just before they freeze, these insects manage to **DEHYDRATE** their cells by osmosis (remember the potato slices in salt water?)—that way there's less water in the cell to freeze, and less damage occurs! Scientists are now investigating the advantages of placing tissues in **HYPERTONIC SOLUTIONS** before freezing.

BEETS are a vegetable recognized by the deep red juice of their below-ground roots. Beet cells are great for investigating cryopreservation, because **when their cells are damaged, a RED LIQUID leaks out**, which we can easily see and measure

In this lab, you'll be **soaking beet slices in different solutions and freezing them**, to test their FREEZING TOLERANCE—that is, how well they survive freezing.

EXPERIMENTAL PROCEDURE
PART I

MATERIALS NEEDED: sliced beets
Ziploc bags
Solutions: **strong and weak sugar water, strong and weak salt water**
Sharpies
A freezer

1. You will need **4 ziploc baggies**. Label them with YOUR NAMES, and with the name of the solution that will be in them—here are the different solutions:
   A. Salt water
   B. Sugar water
   C. Distilled water
   D. No water
2. Have one member of your group put **10 eyedroppers** full of each solution into its corresponding baggie. Another group member should get **six small pieces of beet** and carry them to your table on a paper towel.
3. Place one piece of beet into each of the LABELED Ziploc baggies. WE WILL THEN FREEZE THE BAGGIES OVERNIGHT.

QUESTIONS:

1. Why did one bag have NO solution? _____

2. What are we trying to find out in this experiment? _____
   _____

3. What is a HYPERTONIC SOLUTION? _____
   _____

4. Do you think it's OK for people to have their bodies frozen after they die, hoping that medical science will find a way to successfully thaw and revive them in the future? EXPLAIN WHY OR WHY NOT. _____
   _____
   _____
   _____

5. Hypothesize: Which of these solutions do you think will be best for CRYO-PRESERVATION? _____ WHY? _____
   _____
   _____

PART II

Procedures:

1. Warm your samples with your hands until the solutions have **completely melted**.
2. Use a probe to **remove the beet** from each baggie. FEEL each beet for firmness, and RANK the beets from FIRMEST to LEAST FIRM.

FIRMEST _____
_____
_____

LEAST FIRM _____

3. Why are the firmnesses different? _____
_____

4. RANK your solutions from MOST RED to LEAST RED, **holding each one up to a sheet of white paper to compare**.

MOST RED _____
_____
_____

LEAST RED _____

4. COMPARE your solutions to other groups who had the SAME types of solutions. Are your results similar to theirs? _____ If not, how are they different? _____
_____

QUESTIONS

1. Which **solute** was least effective in reducing cellular damage?
_____
2. Why? _____
_____
3. Which solute was most effective in **reducing cellular damage**? _____
4. Why? _____
_____
5. Describe a solution that might help REHYDRATE cells during thawing.
_____
6. As a researcher, you might be interested in more than the release of pigments from beet cells, which also contain other organic molecules, such as sugars. **Predict the results of a test for the presence of sugars** released from beet cells in the SALT, SUGAR, and DISTILLED solutions _____
_____
_____
7. Why is **salt** placed on roads when they are icy? How does it work? _____
_____
_____
8. Predict the **effect of the salt on plants** that grow on roadsides._____
_____
_____