

Water Relations and Mineral Nutrition

Cells are the basic units in both plants and in animals. Guided by heredity information and environmental stimuli, they absorb materials and manufacture substances required for growth and development.

In all these activities cells depend on the controlled uptake of water and nutrients from the environment. In today's lab we will explore some of the factors that influence water and nutrient uptake into cells. We will also spend time considering the roles of various mineral nutrients in the growth and development of plants.

Water Potential in Plant Cells

Will a plant take water from the environment or will it lose water? The answer to this question depends on a number of factors including:

1. Whether the cell is bounded by a differentially permeable membrane.
2. What the water potential of a cell is in relation to its immediate environment.

Let's consider the notion of water potential first.

Obtain a 6-inch piece of dialysis tubing. The dialysis tubing contains many very small microscopic pores. For the tubing used today, the pores are large enough for water molecules to pass through, but too small to permit the passage of larger molecules like the sucrose molecule. Tie off one end of the dialysis tubing with an overhand knot. When securing the knot, be careful to hold the knot with one hand, and with your other hand, pull on the short, loose end of tubing to tighten. **Do not pull on or grasp the long end of the dialysis tubing to tighten the knot, as this will stretch the tubing and result in enlarging the pores, which may allow sucrose molecules to pass through.** Since only certain molecules can diffuse through the dialysis tubing wall, this wall is said to be differentially permeable. Fill the tubing with 2M sucrose containing the dye, Congo red. Insert a 0.1 ml pipette into the open end of the dialysis tube so that the sucrose solution meniscus can be seen near the bottom of the pipette. Secure the tubing to the pipette by using the wire ties provided (**wrap wire tie around tubing/pipette twice before twisting to secure**). Any leaks will impair the function of the set-up. Clamp the pipette to a ring stand so that the dialysis tubing is suspended in the beaker containing pure water. After 30 and 60 minutes, note which direction the meniscus has moved.

You should observe a rise in the level of the meniscus in the pipette indicating that the water is diffusing across the dialysis tubing wall from the beaker into the bag. This diffusion of water across a differentially permeable wall (membrane) is called osmosis.

But why does the water move across the dialysis tubing wall? To answer this question, consider what would happen if you put a drop of the Congo red solution directly into a beaker containing pure water. Initially the red drop would be very compact and the color easily seen. Soon, however, the color would fade as the red diffused into other regions of the beaker. This diffusion occurs because the dye is moving from a region of high concentration (the initial drop) to a region where it is not highly concentrated, such as everywhere else in the beaker. The dye is essentially diffusing along a concentration gradient.

So why does water move into the dialysis bag? In the beaker, the water is pure; nothing is dissolved into the water and thus the only molecules present in the beaker are water molecules. The dialysis bag, however, contains, in addition to water, sucrose (also known as solute, which is a general term for anything dissolved in water). In the dialysis bag, therefore, water is less pure, or less concentrated. So when the dialysis bag is placed into the beaker of pure water, in a similar manner for dye movement, water is going to “want” to move from where it is more concentrated (from the beaker) to where it is less concentrated, diffusing down a concentration gradient, just as for the dye. As more water enters the bag, the meniscus begins to rise. We call the potential of water to move from one region to another the water potential, and designate it by the Greek letter Ψ (psi).

The device you have constructed is a simple osmometer. If it were possible to add weights to the open end of the water column (and keep the dialysis tubing from breaking) we could determine the force or pressure generated within the dialysis bag as a result of water moving into the bag. We designate this pressure which develops in cells as turgor pressure, and abbreviate it Ψ_p .

Turgor pressure (Ψ_p) develops because of water pushing against the cell membrane. Such pressure can be substantial—but plant cells do not explode—because they have a cell wall which resists the pressure. For an analogy to illustrate turgor pressure, take a balloon and blow it up. The air you blow into a balloon causes the balloon to inflate because of the pressure of the air on the balloon rubber wall. This air pressure is essentially the same as water pressure, Ψ_p . In the case of the balloon however, greatly increasing the air pressure will cause the balloon to explode.

So water potential of a cell, Ψ , has, as one of its components, turgor pressure. Another component or contributing factor to the water potential of a cell is the concentration of solutes within the cell. As you have just demonstrated with your osmometer, water will cross a semi-permeable membrane if the solute concentration differs on the two sides of the membrane. We designate the contribution of the solutes to the overall water potential as Ψ_π , and call this the solute or osmotic potential. The water potential of a cell Ψ , therefore, consists of at least two components; turgor pressure Ψ_p , and the solute potential, Ψ_π . These two components are related to the water potential in the following formula:

$$\Psi = \Psi_p + \Psi_\pi, \text{ where } \Psi_p \text{ is usually positive, and } \Psi_\pi \text{ is never positive.}$$

During this laboratory exercise, you will experimentally determine the components of the water potential equation.

Determination of Water Potential (Ψ) of Tissue

There are several methods which have been devised to measure the Ψ of plant tissues and, in this laboratory, we will use the Shardaikov Procedure.

It requires only very simple materials and the procedure can be learned easily. Shardaikov developed the method originally to determine the appropriate time for irrigation of cotton crops. It also has been referred to as the Russian Dye Method or, in brief, Dye Method.

In this experiment, you will be provided with a series of sucrose **test solutions** in the range of 0.05 to 1.0 molarities, differing by 0.05 M. (*This experiment will have been conducted prior to lab in order to reduce the range of sucrose solutions needed. For example, the experimental range in your lab may only be 0.05 M to 0.5 M, or a range of 10 sucrose test solutions in 0.05 M steps*). A corresponding series of **control solutions** in the same range of molarities will also be provided. The control solutions differ only in having a small amount of powdered methylene blue dye added.

Prior to beginning the experiment, obtain one large and one small test tube for each test solution that is available (for example, if there are 10 test solutions, get 10 large and 10 small test tubes). Make sure the test tubes are dry; use a Kimwipe® if necessary. Label the large test tubes such that you have one for each corresponding test solution molarity. Do the same for the small test tubes, (one each for every corresponding control solution).

Obtain fresh leaves from onion bulbs, blot them with paper towels, and then cut each leaf into 1/2 cm squares. The material is then packed into as many test tubes as there are test solutions, and submerged in 1.5 to 3 ml of test solutions. Care should be taken to introduce as much material as possible into the test tubes so that the volume of the plant material per volume of sucrose solution is as large as is practical, in order to induce changes as large as possible in the densities of the solutions. However, make sure you have space for 1.5 - 3 ml of test solution. Also, do not forget to agitate tubes at frequent intervals to ensure mixing.

Those test solutions which have a water potential more negative than the plant tissue will be diluted by water moving out of the tissue, and those test solutions which have a water potential less negative than the plant tissue will become more concentrated. Only that test solution which is in equilibrium with the plant tissue from the beginning will show no change in its water potential (no change in density). Plant tissue which is cut into pieces and blotted dry will allow a faster exchange of water than material covered with cuticle (e.g., whole leaves).

After incubation (60-90 minutes) as much liquid as possible is removed by carefully pouring the solute from the large test tubes into the small, labeled test tubes (excluding pieces of onion). Changes in density, with respect to the initial density, are detected by very gently depositing a drop of the corresponding colored control solution with a clean Pasteur pipette in the vertical middle of the test solution. (**Do not cross contaminate any of the solutions**). Care should be taken not to produce any movement in the test solution, and not to “squirt” the control solution into the test solution. Test solutions which lost water to the plant material will have a density higher than initially so that the colored drop will rise. Conversely, the colored drop will sink in the test solutions which gained water from the plant material. If the colored drop neither rises nor sinks, the known osmotic potential of the test solution is considered to be equal to the average water potential of the plant tissue.

The addition of the dye to the control solutions, in order to make these later visible in the test solutions, should be as limited as possible in order not to change the density of the solution. Theoretically, the immersion should be as short as possible to prevent any significant uptake and metabolism of sucrose by the plant tissue. An error may be introduced by the contents of cut cells, which contaminate the test solution and change its density. At longer incubation times, however, these changes will be minor in respect to the exchange of water from the tissue to the solution, or vice versa.

Clean up your workbench and all materials when finished. All glass pipettes should be disposed of in the “broken glass” disposal box. The pipettes used to construct an osmometer are plastic and can be put in the regular trash. All test tubes should be washed with soap and rinsed thoroughly with water. **Invert all cleaned test tubes in their test tubes rack to drain off residual water.** Remove pen markings on test tubes by wiping with ethanol and a paper towel. Wash and dry all other materials and return to their proper place. **DO NOT LEAVE UNCLEARED MATERIALS FOR THE NEXT LAB!**

Determination of the Osmotic Potential (Ψ_{π}) of Plant Tissue

For these measurements you will observe plasmolysis in plant tissues. Plasmolysis can be defined as occurring when the cell loses enough water so that it begins to shrink. Actually, the cell cannot shrink because of the rigid cell wall, but the protoplast can. Plasmolysis, therefore, can be said to begin just when the protoplast pulls away from the cell wall. **For this experiment, we can define plasmolysis as beginning when about 50% of the cells in the lowest concentration of sucrose show the protoplast moving away from the cell wall (that is, the cell wall no longer has an internal pressure exerted on it). Botanists call this 50% point “incipient plasmolysis.”** When incipient plasmolysis occurs, $\Psi_p = 0$ and therefore, $\Psi_{\pi} = \Psi$. In this experiment, epidermal strips of the red onion are used because the large central vacuoles of these cells contain pink-colored pigment, making the changes in protoplast volume easy to observe.

Remember, incipient plasmolysis is a method (device) for determining osmotic potential. We assume that when we withdraw a small amount of water that the osmotic potential within the cell has not changed from what it is at full turgor. Clearly, if you use external solutions with high osmotic potentials then you will withdraw a lot of water from the cell and then the solute concentration in the cell becomes more negative. In this case this higher concentration of solute within the cell is artificial and not a true representation of what is the solute concentration in a cell within the onion sitting on the bench.

Remove several of the outer dried leaves from the bulb of a red onion and obtain a portion of one fleshy leaf base (note that the inside [upper] epidermis is clear and the outer [lower] epidermis is red). Procure a small patch (1-2 mm square is adequate) of the red epidermis by one of the following procedures: 1) with a sharp razor blade, make a very thin slice parallel with the epidermis; make sure the section is very thin (ideally containing only the epidermis); or 2) fold the fleshy leaf in half so the red epidermis is on the inside of the fold (clear epidermis will break apart) then carefully peel away

the two broken halves of the bulb, leaving the red epidermis unbroken and obtain a patch of red epidermis. Without letting the peels dry out, **quickly**, place them individually into one of a graded series of solutions of sucrose, 0 to 1M (a few drops per slide), and place a cover glass over the peel. Look at the highest concentration first so that you will see what plasmolysis looks like. Then look at lower concentrations and determine whether plasmolysis has occurred.

Every 5-10 minutes (using the high power [40x] objective of your microscope), observe the peels. Note the slide in which incipient plasmolysis is first observed. What does this indicate about the solute concentration of the cells in the peel (isotonic, hypotonic, hypertonic)? Record this value. If the onions supplied to you were very sweet, would you expect plasmolysis to occur at low or high concentrations of sucrose? Using the Shardaikov and incipient plasmolysis methods, you have determined the Ψ and Ψ_{π} respectively, of onion leaves. Having done so, can you calculate the Ψ_p of cells in these leaves?

As you have just demonstrated, cells contain solutes within their protoplasts. With regard to maintenance of cell shape, these solutes serve a very important function. As water moves into a cell, the cell begins to experience the development of an internal pressure. As more water moves in, the pressure can become quite large. This pressure resulting from water within the cell is turgor pressure, Ψ_p , and accounts for the maintenance of the shape of many plants. When a plant looks wilted, it is because too much water has been lost from the plant to maintain sufficient turgor pressure within the cells. Turgor pressure also has another important role—namely, it is the force or the power which causes cells to enlarge, and hence, can be considered the driving force for plant growth.

Now you might ask, what is to prevent the turgor pressure from becoming so great so as to cause the cell to burst? In plants, part of the answer is that the cell wall prevents the cell from exploding.

Actual direct measurements for turgor pressure Ψ_p are more difficult to do than are measurements for either Ψ or Ψ_{π} . Therefore, Ψ_p is usually calculated once, both Ψ and Ψ_{π} are measured, as you have just done for onions.

As always, clean all materials that you have used when you are finished with your experiments. **Wash and dry all microscope slides** and return them to the proper box. **DO NOT LEAVE MICROSCOPE SLIDES IN WASH BASIN.** Throw away plastic coverslips in trash container.

Summary of Water Relations

From this portion of the lab you should understand that water diffuses across a semipermeable membrane as a result of a gradient in water potential Ψ . As a first approximation, the water potential of cells appear to be determined mainly by the Ψ_{π} of the individual cells, but the turgor pressure also affects how much water is in a cell and, therefore, the water potential.

Mineral Nutrition and Nutrient Deficiency Symptoms

The final portion of today's lab will deal with plant requirements for particular minerals.

In addition to carbon, hydrogen and oxygen, which comprise about 98% of the fresh weight of the plant, 13 other chemical elements are known to be essential for plant growth. Six of these elements, nitrogen, potassium, calcium, phosphorus, magnesium, and sulphur, are required in relatively large amounts and are known as **macronutrients**. The remaining essential elements, iron, chlorine, copper, manganese, zinc, molybdenum, and boron, are required in minute quantities (as little as a few parts per million) and are known as **micronutrients**.

Deficiencies of one or more of the essential elements result in abnormal plant growth and development. Plants suffering from the deficiency of these elements exhibit deficiency symptoms, such as pale green or yellow leaf color (chlorosis), localized death of tissues (necrosis), abnormal anthocyanin formation and stunting of growth.

In order to determine what mineral or combinations of minerals the plant needs for growth, plants are commonly grown in liquid solution cultures, a technique known as hydroponics. Only under such conditions can the composition of the mineral medium be accurately known. As early as 1860 the German physiologist Sachs used hydroponics to demonstrate the mineral requirements of plants.

In the experiment today, you will be provided with a series of sunflower plants which have been growing for about 6 weeks in the greenhouse in liquid solutions supplemented with the minerals indicated. You will also be supplied with plants grown for two weeks initially in complete solution and then subsequently transferred to media lacking particular mineral elements. Observe the shoot portions of these plants and record the characteristics of plants growing in media supplemented with various combinations of minerals. Note leaf color, the positions of healthy leaves on the plants (i.e., are the healthy leaves the newly formed ones or the older leaves?), the condition of the terminal apex, as well as the overall height of the plant and internode length. What other sorts of measurements could you make, assuming that you could do anything you wanted with the plants provided?

In summarizing your results, estimate whether the deficiency elements are relatively mobile, immobile, or intermediate in this respect.

Name _____

Worksheet #1: Water Relations

1. Osmometer results:

- A. Did water move into or out of the pipette?
- B. At what rate (ml/minute) did the water move?
- C. What would happen to the rate and direction of water movement if salt or sugar was added to the water in the beaker?

2. Shardakov procedure results:

- A. What was the water potential of the onion cells in moles/liter?
- B. Convert this value to megapascals using the following formula:

$$\Psi = -iMRT, \text{ where:}$$

$i = 1$ (ionization constant for sucrose)

$M =$ moles/liter (your result)

$R = .0083$ MP_A liter/°K mole (the gas constant)

$T =$ temperature in °K (273 + room temp. in °C)

3. Incipient plasmolysis results:

- A. What was Ψ (in moles/liter) of onion cells at incipient plasmolysis? (i.e. the concentration of the sucrose solution at which you observed incipient plasmolysis)
- B. Convert this value to MP_A using $\Psi = -iMRT$.
- C. Using what you know about Ψ_p at incipient plasmolysis, calculate Ψ_π .

4. Using the values for Ψ (water potential of cell) and Ψ_π (solute potential) above, calculate Ψ_p (turgor pressure) for onion cells.

Worksheet #2: Results

Your GSI will have each group put their results for the Water Relations experiments on the chalk board. This is to check to see if you all have approximately the same values. Fill out the table below with the combined results.

	Ψ (Shardakov)	Ψ_{π} (Plasmolysis)	Ψ_p (Calculate)
Group # _____			
Group # _____			
Group # _____			
Group # _____			
Group # _____			
Group # _____			

**minus
calcium** 0 weeks

2 weeks

**minus
iron** 0 weeks

2 weeks

**minus
micro-
nutrients** 0 weeks

2 weeks

2. Which deficiency elements are mobile and which are immobile? How can you tell?

Worksheet #3: Mineral Deficiencies in Plants

1. Record your observations of the sunflowers in the table below.

Treatment	Plant age at treatment	Plant height	Internode lengths	Condition of old leaves	Condition of young leaves	Other
complete nutrients	0 weeks					
	2 weeks					
<hr/>						
minus nitrogen	0 weeks					
	2 weeks					

Water Relations Lab – Problem Set

(5 points)

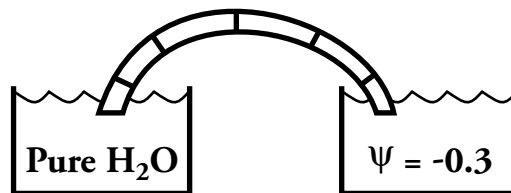
Prof. L. Feldman

For All These Problems:

A. The general formula: $\Psi = \Psi_{\pi} + \Psi_p$ applies.

B. The Ψ of pure water is zero.

1. A leaf falls from a tree onto the surface of a pond. The Ψ_{π} of the pond is -0.5 MPa (megapascals). The Ψ_p and Ψ_{π} of the leaf cells are 0.2 and -0.9 MPa respectively. What is the Ψ and Ψ_p of the pond water? What is the Ψ of the leaf cells? Will water move into or out of the leaf?
2. A filament of algal cells has a Ψ_p of 0.25 MPa and a Ψ_{π} of -0.45 MPa. The filament is arranged so that one end dips into a solution with Ψ of -0.3 MPa and the other end of the filament is dipping into pure water (see diagram).



Evaporation is prevented and the volume of water in the two beakers is very large compared to the size of the algal cells. Will any movement of water occur? Explain.

3. Pollen grains of cotton and many other species germinate readily on the stigma, but burst rapidly if floated upon pure water or a dilute sugar solution. Explain. Assume the Ψ_p and Ψ_{π} of the pollen grains were 0.2 and -0.6 MPa respectively. What are some reasonable values for Ψ in cells of the stigma and of the dilute sucrose solution?
4. The Ψ_p of a cell at incipient plasmolysis is _____ MPa. Therefore:
 - a) $\Psi_p = -\Psi_{\pi}$
 - b) $\Psi = \Psi_{\pi}$
 - c) $\Psi_{\pi} + \Psi = \Psi_p$
 (Indicate which choice is correct).
5. Water in the xylem is normally under tension. Which of the following apply?
 - a. Both the Ψ_p and Ψ_{π} are negative numbers.
 - b. Only the Ψ_{π} is negative, but the Ψ_p is likely a positive number.
 - c. Neither the Ψ_p or Ψ_{π} is a negative number.
 - d. The Ψ_p is a positive number.