

Lab Exercise 3

Membrane Transport Mechanisms and Osmosis

The Cell Cycle

Textbook Reference: See Chapter 3

What you need to be able to do on the exam after completing this lab exercise:

- Be able to define Brownian motion.
- Be able to explain the criteria for passive transport and how certain factors influence it.
- Be able to name and explain the different types of passive transport.
- Be able to explain osmosis and explain which way water will move in or out of a cell.
- Be able to define hypotonic, hypertonic, and isotonic, and explain what happens to a cell when placed in each of these solutions.
- Be able to explain the results of the osmosis demonstration.
- Be able to name all phases in the cell cycle and explain what happens in each phase.
- Be able to identify the phases of the cell cycle on the lab models.
- Be able to identify the specified cell structures on the lab models.
- Be able to identify the phases of the cell cycle on microscope slides.

Membrane Transport Mechanisms & Osmosis

Brownian Motion

In 1827, the English botanist Robert Brown noticed that pollen grains suspended in water jiggled about under the lens of the microscope, following a zigzag path. This zigzag motion of the particles resulted from collisions with smaller particles and is referred to as **Brownian motion**.

Although the individual molecules cannot be seen, the random motion of the molecules colliding with one another can be observed. The higher the temperature of the liquid, the faster the movement of the particles.

We will not be observing Brownian motion in this lab, but know the definition of Brownian motion.

→ Answer Questions 1 – 2 on your “Membrane Transport & Osmosis” homework sheet.

Passive Transport

Passive transport involves the movement of particles from an area of higher concentration to an area of lower concentration, without the input of energy.

Simple diffusion: Simple diffusion is a type of passive transport. Molecules simply move from where they are more concentrated to where they are less concentrated, down the concentration gradient (the difference between high and low concentration).

In simple diffusion, molecules may move through a solid (such as gel-like agar), a liquid (such as water or blood), or a gas (such as the air).

Several factors can affect the rate of diffusion. Some of these include temperature, particle size, molecular weight, and concentration of particles.

The higher the temperature, the faster the rate of diffusion.

The smaller the particle size, the faster the rate of diffusion.

The smaller the molecular weight, the faster the rate of diffusion.

The more concentrated the particles, the faster the rate of diffusion.

→ Answer Questions 3 – 4 on your “Membrane Transport & Osmosis” homework sheet.

Observing the Simple Diffusion of Dye Through Water

Your instructor will do this activity as a class demonstration.

1. Your instructor will place a few crystals of potassium permanganate dye into a large beaker of water.
2. Observe the movement of the dye in the water. At first you can differentiate between the dye and the water. As the dye continues to diffuse through the water (down the concentration gradient), the dye particles move from where they are more concentrated to where they are less concentrated, without an input of energy.
3. Observe the beaker again toward the end of the lab period. The water should appear a uniform pinkish-purple color. The dye molecules should have reached **equilibrium** and are now equally concentrated throughout the water.
4. How does the temperature of the water affect the movement of the dye molecules?

Facilitated Diffusion

To travel in or out of a cell, molecules must move across the **plasma membrane**.

The plasma membrane is **selectively permeable**. It acts as a barrier to the movement of some molecules, but allows other molecules to pass through. You could say it “selects” what ions/molecules can enter and leave the cell.

If the ions/molecules require the aid of a membrane-protein to move in or out of the cell, we call the movement **facilitated diffusion**. The movement of the ions/molecules is facilitated (or “helped”) by membrane-proteins.

→ Answer Question 5 on your “Membrane Transport & Osmosis” homework sheet.

Osmosis

The diffusion of water through a selectively permeable membrane is called **osmosis**. Water will move from where it is more concentrated to where it is less concentrated across the plasma membrane.

Since most solvents contain water, osmosis is the movement of solvent across the membrane. Water moves from the side of the membrane that has a **lower solute concentration** to the side of the membrane that has a **higher solute concentration**.

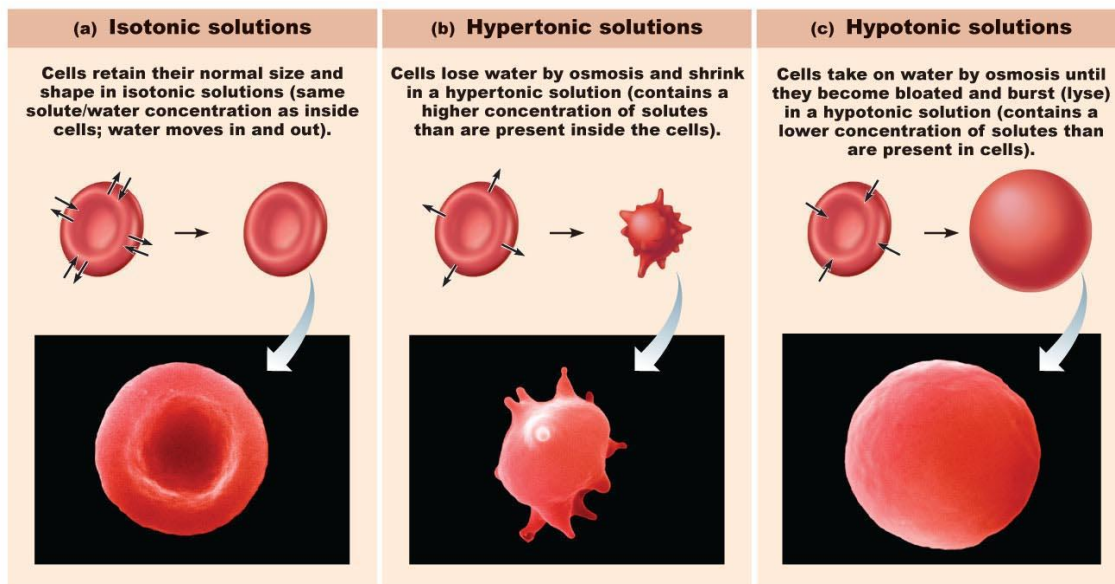
Hypotonic describes the solution with a **lower solute concentration**; higher water concentration
Hypertonic describes the solution with a **higher solute concentration**; lower water concentration
Isotonic describes the solution with an **equal solute concentration**; equal water concentration

Water moves from a hypotonic solution to a hypertonic solution (**Hypo** → **Hyper**) across a selectively permeable membrane.

If you place a cell (70% water/30% solute) in a **hypotonic** solution (90% water/10% solute), the **cell will gain water and swell**. Water moves by osmosis from the solution (which has a higher water concentration) into the cell (which has a lower water concentration).

If you place a cell (70% water/30% solute) in a **hypertonic** solution (40% water/60% solute), the **cell will lose water and shrink (crenate)**. Water moves by osmosis from the cell (which has a higher water concentration) into the solution (which has a lower water concentration).

If you place a cell (70% water/30% solute) in an **isotonic** solution (70% water/30% solute), the **cell will not gain or lose water and will stay the same**. Water moves in and out of the cell at the same rate, so there is no net gain or loss of water.



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Observing Osmosis Through a Selectively Permeable Membrane

Your instructor will do this activity as a class demonstration. You will record the data on your “Membrane Transport & Osmosis” homework sheet (Question #7).

Procedure:

1. On the front desk, you will see three beakers with the following solutions:

Beaker #1 – 0% sucrose solution

Beaker #2 – 40% sucrose solution

Beaker #3 – 0% sucrose solution

2. Your instructor will fill the first bag of dialysis tubing (a selectively permeable membrane) with 40% sucrose solution. The bag will be weighed on the balance before the instructor places it into Beaker #1. Record the weight of the bag on your homework sheet.
3. Next, your instructor will fill the second bag with a 0% sucrose solution. The bag will be weighed on the balance before being placed into Beaker #2. Record its weight.
4. Finally, your instructor will fill a third bag with 0% sucrose solution. The bag will be weighed on the balance before being placed into Beaker #3. Record its weight.
5. Your instructor will leave the bags in the beakers for approximately 1 hour.
6. After 1 hour, your instructor will take each bag out of the beaker one-by-one, dry off the excess water on the outside of the bag, and weigh each bag on the balance. Record the weight of each bag in the appropriate box on your homework sheet.
7. Calculate and record the change in weight for each bag.

→ Answer Questions 6 & 8 – 11 on your “Membrane Transport & Osmosis” homework sheet.

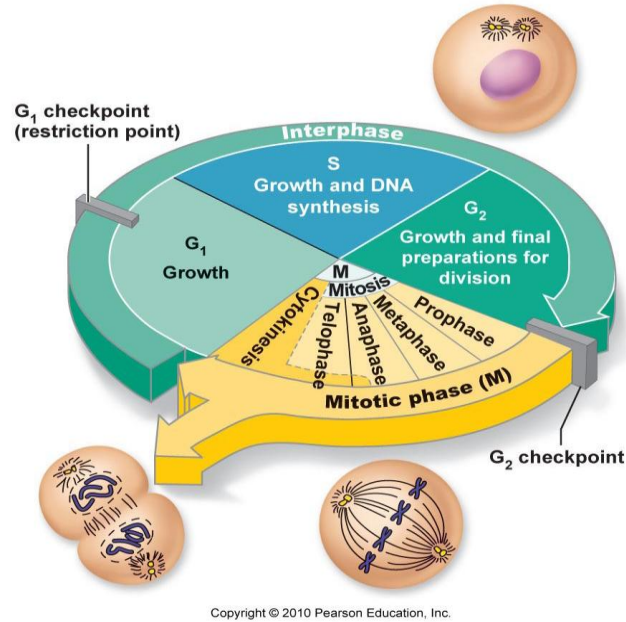
Filtration

Filtration is a passive transport process where water and solutes move from an **area of higher hydrostatic (fluid) pressure** to an **area of lower hydrostatic (fluid) pressure**. The water and solutes will move through pores in the membrane, down a pressure gradient.

Example: Coffee moves by filtration through the pores of a coffee filter from the side of the filter that has the highest (gravitational) pressure to the side of the filter that has the lowest pressure.

The Cell Cycle

Most of your cells divide for growth and repair. The stages that a cell goes through from the time it is formed until it divides is called the **cell cycle**.



Two main stages of the cell cycle include:

- Interphase**
- Mitotic Phase**

Interphase is sub-divided into 3 stages:

- G₁** (gap 1)
- S** (synthesis)
- G₂** (gap 2)

In **G₁** of interphase, the cell is growing and doing its job – making proteins and undergoing routine metabolism

In **S** of interphase, DNA replicates. Each chromosome makes an exact copy of itself.

The duplicated chromosomes are held together by a structure called a **centromere**. As long as the duplicated chromosomes are held together by the centromere, they are referred to as **sister chromatids**.

In G_2 of interphase, the cell continues to make proteins, undergo routine metabolism, and also prepares the cell for division by making more cytoplasm and replicating the centrioles.

The **mitotic phase** includes:

Mitosis
Cytokinesis

Mitosis is sub-divided into 4 stages:

Prophase
Metaphase
Anaphase
Telophase

Prophase	centrioles move to opposite poles chromatin condenses into visible chromosomes mitotic spindles (microtubules) form nuclear envelope and nucleolus disappear
Metaphase	sister chromatids line up at the metaphase plate (equator) of the cell
Anaphase	sister chromatids separate and are pulled toward opposite poles of the cell
Telophase	chromosomes relax to form chromatin spindle microtubules disappear nuclear envelope and nucleolus reappear cleavage furrow forms, which is a “pinching-in” of the plasma membrane that will eventually separate the two daughter cells

Cytokinesis is the division of the cytoplasm. As the cleavage furrow pinches the cell in two, the cytoplasm divides so that each new daughter cell has enough cytoplasm to begin its life as a new cell.

The formation of the cleavage furrow and cytokinesis begin at the end of anaphase and continue to the end of telophase.

Identifying Interphase and the Mitotic Stages on Mitosis Models

In the lab are interphase and mitosis models. Know the name of the stage represented by each model.

Be able to identify the following cell structures on the models:

Cytoplasm

Nucleus

Nucleolus

Chromosomes

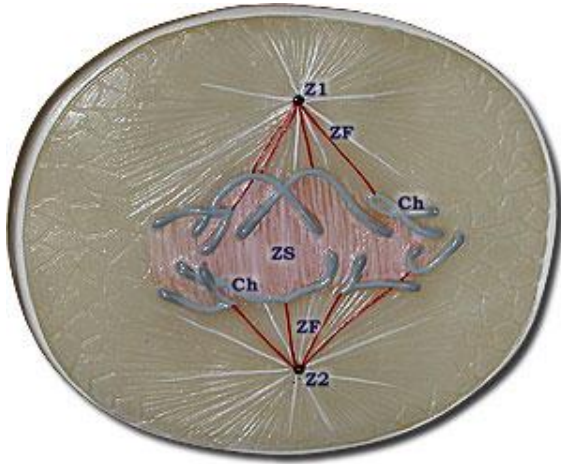
Centrosomes (Centrioles)

Mitotic Spindles (Spindle Microtubules)

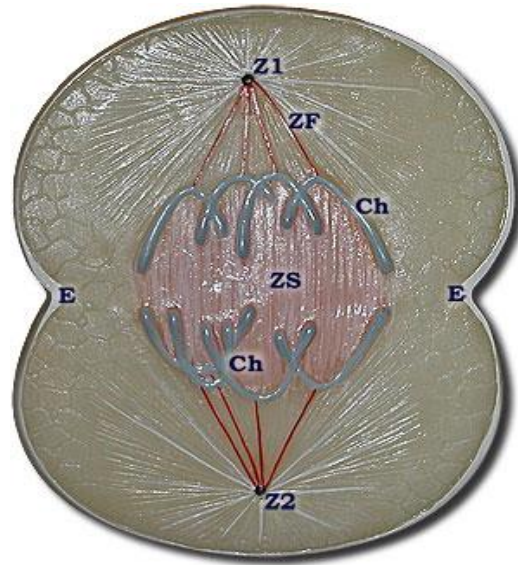
Cleavage Furrow



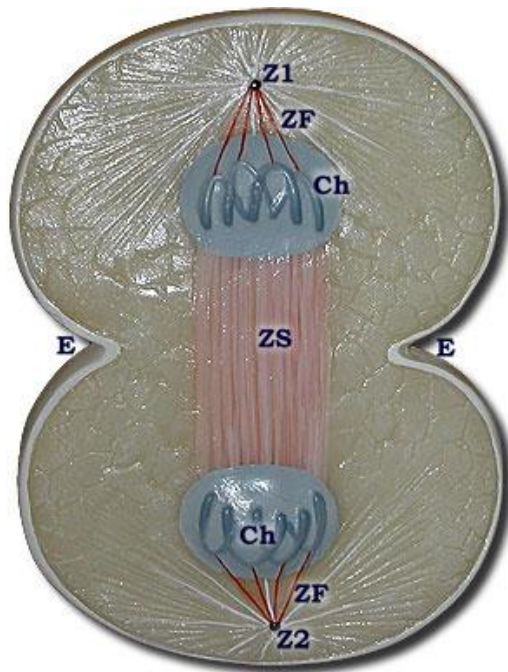
Early Anaphase



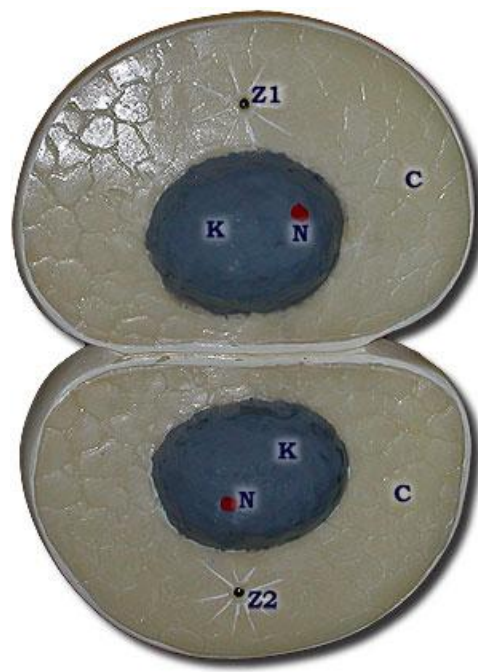
Late Anaphase



Telophase



Interphase



C = Cytoplasm
Ch = Chromosome
E = Cleavage Furrow
K = Nucleus

N = Nucleolus
Z1, Z2 = Centrosome (Centrioles)
AF, AS = Mitotic Spindle (Spindle Microtubules)

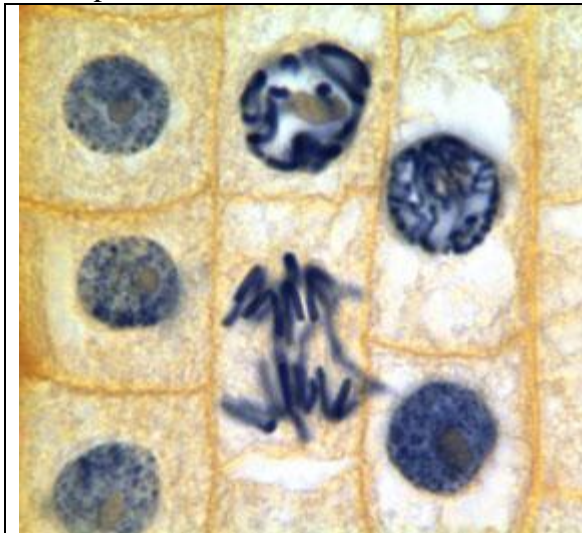
Identifying Interphase and the Mitotic Stages on Onion Root (*Allium*) Tip Slides

Procedure:

1. Place the onion root tip slide on the microscope and position the specimen over the opening in the stage.
2. Using the **scanning lens (4X)**, bring one of the root tips into focus (there may be 2 -3 onion root tips on your slide). It is best to look toward the bottom of your onion root tip, but not at the very tip.
3. You should see vertical columns of cells. Each small box-like structure is a cell. The cells will be in various stages of the cell cycle.
4. Position the root tip in the center of your field of view and switch to the **low power lens (10X)**. You should be able to see the cells a bit more clearly now. The dark structures around the center of each cell (box) is either the nucleus or the visible chromosomes, depending upon the stage.
5. Position the cells in the center of your field of view and switch to the **high power lens (40X)**.
6. Use the fine adjustment to make your image as clear as possible.
7. Locate cells in each of the following stages on your slide: interphase, prophase, metaphase, anaphase, telophase.

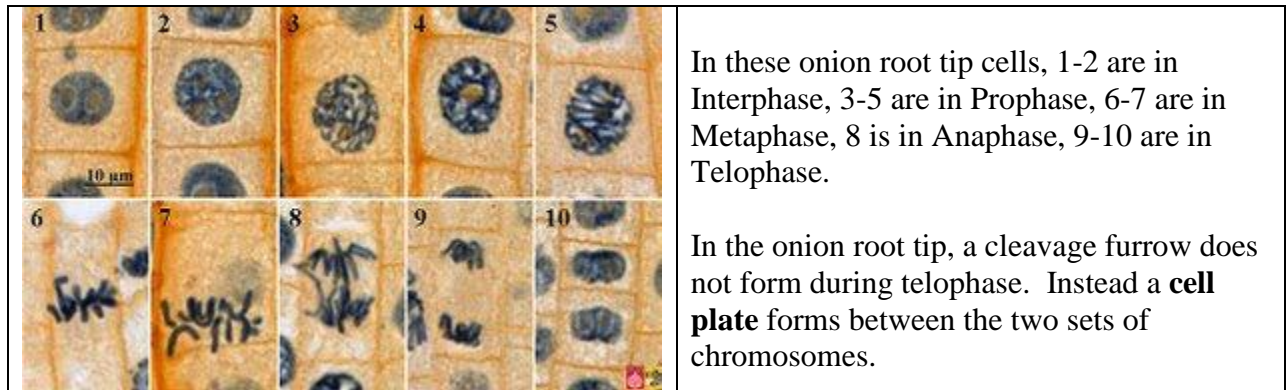
Note: You may have to move your slide around while viewing it to find all 5 phases. You should be able to find all 5 phases on one root tip, but if not, look at the other root tips on your slide.

Examples:



In this onion root tip, 4 cells are in Interphase, 2 cells are in Prophase, and 1 cell is in Anaphase.

Can you spot the phases?



8. Make a drawing of a cell from your onion root tip slide in each of stages of the cell cycle.

<u>Interphase</u>	<u>Prophase</u>	<u>Metaphase</u>
<u>Anaphase</u>	<u>Telophase</u>	

Membrane Transport & Osmosis Homework

Due at the start of next lab

1. The zigzag motion of particles resulting from collisions with smaller particles is called:
2. What effect would *decreasing* temperature have on Brownian motion?
3. Define diffusion; include in your definition whether this process involves the movement of the solute or solvent
4. For molecules with a given amount of kinetic energy, small molecules move more rapidly than do large molecules. This difference is even more pronounced when diffusing through a semi-solid substance, such as agar, that can hinder the movement of molecules: small molecules can slip through the gaps more easily than large molecules.
 - a. With this in mind, which solution, potassium permanganate (molecular weight = 158), methylene blue (mw = 320), or congo red (mw = 697) would you predict to move the fastest (and thus go furthest in a given time period) through the agar?
 - b. How would temperature affect diffusion rates?
5. A _____ membrane acts as a barrier to the movement of some molecules but allows other molecules to pass through.
6. Define osmosis; include in your definition whether this process involves the movement of the solute or solvent.

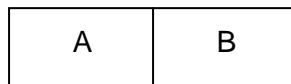
7. Record the results from your instructor's demonstration of osmosis in the table below:

Sucrose Concentration in Dialysis Bag	Sucrose Concentration in Beaker	The bag solution is ___* to the beaker solution.	Weight at Start	Weight at End	Change in Weight

* isotonic, hypotonic, or hypertonic

8. Which bag(s) did you predict should gain weight and which should lose weight?

9. Do the observed results match your prediction? If not, what are some possible reasons?



10.

The two artificial cells shown above are separated by a selectively permeable membrane that is permeable to water but impermeable to sodium chloride (NaCl). The solution inside cell A contains a 30% NaCl solution, while cell B contains a 20% NaCl solution.

a. Cell A is **hypotonic** / **hypertonic** / **isotonic** (circle one) compared to cell B.

b. Draw an arrow on the diagram to indicate in which direction water would move.

11. If you were hospitalized and needed to be given fluids intravenously (IV), would you want that fluid to be hypertonic to your cells? Explain what would occur if you were to receive such a fluid.