BIOLOGY 211: HUMAN ANATOMY & PHYSIOLOGY

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**TRANSPORT ACROSS THE PLASMA MEMBRANE**

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Reference: Saladin, KS: Anatomy & Physiology, The Unity of Form and Function, 8th ed. (2018).

**INTRODUCTION:**

 Each cell of the human body is completely surrounded by a plasma membrane. In addition to maintaining the integrity of the cell, this membrane can selectively allow different substances to pass through it in both directions. This allows the cell to take in some materials while eliminating or secreting others, thus allowing it to maintain very different concentrations of these substances inside and outside the cell. As you will recall from your reading, with the exception of the outermost cells in the epidermis of the skin all cells of the body are completely surrounded by extracellular or interstitial fluid. Thus,

* Atoms and molecules passing into the cell through its plasma membrane are passing from the extracellular fluid into the cytoplasm of the cell, while
* Atoms and molecules passing out of the cell through its membrane are moving from the cytoplasm into that extracellular fluid.

 All fluids of the body have two components - the liquid in which materials are dissolved is called the ***solvent***, and all of the atoms and molecules dissolved in that solvent are called the ***solutes***. In fluids of the human body, and in fact in all living organisms, the only solvent is water but there are literally thousands of different solutes. These include nutrients such as amino acids and sugars; ions such as sodium, potassium, chloride, bicarbonate or phosphate; gases such as oxygen and carbon dioxide; waste products such as lactic acid and urea; hormones; antibodies; enzymes; proteins; and many other things. Both the cytoplasm and the extracellular fluid also contain large molecules and particles which do not dissolve in the water but are instead suspended in it. All of these materials, including the water, the solutes, and the undissolved particles, can move across the plasma membrane by different mechanisms.

 Some types of membrane transport are considered to be ***active*** because the cell must use energy (most commonly in the form of adenosine triphosphate or ATP), and other types of membrane transport are ***passive*** because no energy is used. The most common of these are discussed in your textbook:

**Passive Membrane Transport Processes (do not require the cell to use energy)**

**Simple Diffusion** is the movement of an ion or molecule from an area of higher concentration to an area of lower concentration (“down its concentration gradient”). This can cause the movement of a solute through its solvent, or it can cause the movement of a solute across a membrane. In all cases, the “driving force” causing this movement is the kinetic energy of the solute molecules. For example, oxygen diffuses from the plasma of your blood (higher concentration) into red blood cells (lower concentration) where it can bind onto hemoglobin.

**Osmosis (Figure 3.15)** is the diffusion of one specific molecule, **water**, through a membrane due to its kinetic energy. For example, water moves by osmosis from the plasma of your blood into the extracellular fluid surrounding capillaries all over the body.

**Facilitated Diffusion (Figure 3.18)** is the movement of an ion or molecule across a membrane from an area of higher concentration to an area of lower concentration (“down its concentration gradient”) **with the assistance of a transmembrane protein**. For example, glucose molecules cross the plasma membranes of your muscle cells by facilitated diffusion to enter those cells.

**Filtration (Figure 3.14)** is the movement of both solvent (water) and solutes (ions and molecules) through small openings, or pores, of the plasma membrane. **This always occurs from an area of higher pressure to an area of lower pressure** (“down its pressure gradient”). For example, your blood pressure forces fluid out of capillaries in the kidneys into tubular structures (nephrons) where urine is formed.

**Active Membrane Transport Processes (require the cell to use energy)**

**Active Transport (Figures 3.19 & 3.20)** is the movement of an ion or molecule across a **membrane from an area of lower concentration to an area where it is already in higher concentration** (“up its concentration gradient”) with the assistance of a transmembrane protein. **This specifically requires the cell to use energy** to “force” those ions or molecules to move in the direction opposite to the way they would normally diffuse. For example, in order to conduct an electrical impulse along a nerve cell. the cell must first be electrically “polarized” using active transport to force sodium and potassium ions in directions which are “up” their concentration gradients. Like most processes in the body that require energy, the source of this energy is ATP.

**Vesicular Transport** is actually a group of related processes for moving large numbers of molecules or solid particles across the plasma membrane of a cell. If something is moving into the cell it is called **endocytosis**. If something is moving out of the cell it is called **exocytosis**. What they all have in common is that they use a vesicle within the cell to contain the m aterial being moved.

* **Receptor Mediated Endocytosis (Figure 3.22)** is the movement of molecules into a cell by the formation of a new vesicle from the plasma membrane. This requires those molecules to first bind onto protein receptors which are part of the plasma membrane, which then folds into the cell to form a vesicle. For example, many cells of your body use receptor mediated endocytosis to take in cholesterol molecules. Formation of the vesicle and its internalization into the cell require the cell to use energy provided by ATP.
* **Pinocytosis**, sometimes called “cell drinking”, is a form of endocytosis which cells use to take in small amounts of fluid (both water and solutes) from the extracellular fluid. This is nonspecific – that is, the cell takes in whatever molecules are in the extracellular fluid around it. Formation of the vesicle and its internalization into the cell require the cell to use energy provided by ATP.
* **Phagocytosis (Figure 3.20)** is a form of endocytosis in which the plasma membrane of a cell surrounds a solid particle and then internalizes it within a vesicle. For example, macrophages use phagocytosis to engulf bacteria and pieces of other cells. Movement of the membrane, formation of the vesicle, and its internalization into the cell require the cell to use energy provided by ATP.
* **Exocytosis (Figure 3.24)** is the movement of substances out of a cell via fusion of secretory vesicles with the plasma membrane. For example, endocrine cells use exocytosis to release the hormones they have produced, and macrophages use exocytosis to release the residue of bacteria they have destroyed. Formation of the vesicle and its internalization into the cell require the cell to use energy provided by ATP.

In this lab exercise we will examine some of these processes individually. However, keep in mind that the plasma membrane is part of a living cell, and both passive and active processes occur simultaneously to meet the needs of the cell.

You will do some of these exercises within your own lab groups of three or four. Others will be done with the assistance of the lab instructor in groups of 6 to 8. Others may be done as demonstrations by the instructor for the entire lab.

**EXERCISE 1: PASSIVE TRANSPORT BY SIMPLE DIFFUSION**

In simple diffusion there is a net movement of substances from a region of higher concentration to a region of lower concentration. Diffusion can occur in solids, liquids, or gas, and across the plasma membrane of cells.

In this activity, we will observe the diffusion of two colored molecules (dyes) through agar. Methylene blue has a molecular weight of 320, and potassium permanganate has a molecular weight of 158. When placed on the agar, crystals of these dyes will dissolve and the two solutes will diffuse at different rates through the agar, which is made up mostly of water.

 *Based on their molecular weights, would you predict that methylene blue*

 *or potassium permanganate will diffuse more quickly through the agar?* \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Obtain an agar plate and use forceps to place one crystal

 each of methylene blue and potassium permanganate on

 it, well separated as shown.

2. Every 15 minutes for the next hour while you are doing the

 other exercises, use a small millimeter ruler to measure

 the diameter of the diffusion ring around each crystal and

 record it in the space provided.

3. At the end of the hour, calculate the diffusion rate for each

 of the two solutes as millimeters per minute

4. Clean up as directed by your instructor.

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| **DIFFUSION RESULTS** |
| TIME (MINUTES)  | DIFFUSION DIAMETER OF METHYLENE BLUE IN MM  | DIFFUSION DIAMETER OF POTASSIUM PERMANGANATE IN MM |
| 15 |  |  |
| 30 |  |  |
| 45 |  |  |
| 60 |  |  |

Diffusion rate of methylene blue in mm / min \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Diffusion rate of potassium permanganate in mm / min \_\_\_\_\_\_\_\_\_\_\_\_\_

***Items For Discussion With Your Lab Partners:***

 *Was your prediction correct about which solute diffused more quickly through the agar?*

 *What factors cause solutes to diffuse more quickly or more slowly, and how this affects*

 *ions and molecules moving through different fluids of your body.*

**EXERCISE 2: PASSIVE TRANSPORT BY OSMOSIS WITH RED BLOOD CELLS**

(Your lab instructor will help you do this exercise in groups of 6-8)

When two different concentrations of solutes are on either side of a selectively permeable membrane, there is a **concentration gradient** of the solute. The side with a lower concentration of solutes is called **hypotonic**, and the side with the higher concentration of solutes is called **hypertonic**. Unless the membrane prevents it, water will move from the hypotonic side to the hypertonic side, in effect diluting it until the concentration of solutes is equal on both sides, in which case they are called **isotonic**. The movement of water through a membrane from a hypotonic solution to a hypertonic solution is called **osmosis.** Note that the terms hypotonic, hypertonic, and isotonic are only used to compare two solutions to each other.

In this activity we will use red blood cells (RBCs) to

observe the effects of osmosis because their shape

changes dramatically when they are exposed to hypotonic

or hypertonic solutions and either lose or gain water.

Their normal shape is a round biconcave disk with a

smooth plasma membrane. Under the microscope,

they look two-toned, with the center being lighter

because of the concavity and thinness of the center.

If the cell loses most of its water by osmosis when put

in a hypertonic solution, it becomes crenated or shriveled

with spiked edges. If the cell gains a significant amount

of water by being placed in a hypotonic solution, it swells

and may eventually burst – a process called hemolysis

(*hemo-* = blood; -*lysis* = break down).

As a basis to compare various solutions to blood, the

salt (NaCl) content of blood is 0.9%, and a solution with

this concentration is called ‘physiologic saline’.

**Safety Precautions for working with human and Blood and Body Fluids:**

Like all body fluids, blood can contain viruses and bacteria -- pathogens that can cause disease. The viruses which cause AIDS (HIV) and hepatitis are just two examples that you are probably familiar with. You need not worry about exposure to these contaminants from your own blood -- you are already exposed. But you must be much more diligent when working with the fluids from someone else by wearing gloves and being sure that contaminated objects such as lancets, needles, or paper towels are not reused. Dispose of all possibly contaminated materials in the red receptacles provided.

You will expose red blood cells to three solutions: Distilled water, 0.9% saline, and 5% saline

***Items For Discussion With Your Lab Partners:***

 *Identify whether each of these solutions is isotonic, hypertonic, or hypotonic in comparison to*

 *the fluid inside the red blood cells (which is the same as the blood in which they are suspended)*

 *What do you predict will happen to red blood cells placed in each of these solutions?*

 *• In a isotonic solution, the RBCs will swell, crenate, or not change shape (circle one).*

 *• In a hypotonic solution, the RBCs will swell, crenate, or not change shape. (circle one).*

 *• In a hypertonic solution the RBCs will swell, crenate, or not change shape. (circle one).*

**Slide # 1: 0.9% saline solution**



 Use a plastic dropper to obtain a drop of blood from your lab instructor

 and place this on a slide.

 With a different dropper, add a drop of 0.9% saline solution to the blood.

 Tilt the slide to mix the two solutions, and cover with a coverslip.



 Using high power, observe the slide for changes in cell shape. Blood cells are

 small and difficult to see under low power, so you will want to increase

 the magnification until you can see the cells.

***Items For Discussion With Your Lab Partners:***

 *Is 0.9% saline hypotonic, hypertonic, or isotonic to the red blood cells?*

 *Did the RBCs will swell, crenate, or not change shape?*

 *Was your prediction correct about what happens to red blood cells in 0.9% saline?*

**Slide # 2: 5% saline solution**

Use a dropper to obtain another drop of blood from your lab instructor and place this on a clean slide.

With a different dropper, add a drop of 5% saline solution to the blood. Tilt the slide to mix the two solutions, and cover with a coverslip. Observe the slide for changes in cell shape as you did above.

***Items For Discussion Your Lab Partners:***

 *Is 5% saline hypotonic, hypertonic, or isotonic to the red blood cells?*

 *Did the RBCs swell, crenate, or not change shape?*

 *Was your prediction correct about what happens to red blood cells in 5% saline?*

**Slide # 3: Distilled Water**

Use a dropper to obtain another drop of blood from your lab instructor and place this on a clean slide.

With a different dropper, add a drop of distilled water to the blood. Tilt the slide to mix the two solutions, and cover with a coverslip. Observe the slide for changes in cell shape as you did above.

***Items For Discussion Your Lab Partners:***

 *Is distilled water hypotonic, hypertonic, or isotonic to the red blood cells?*

 *Did the RBCs swell, crenate, or not change shape?*

 *Was your prediction correct about what happens to red blood cells in distilled water?*

**EXERCISE 3: PASSIVE TRANSPORT BY OSMOSIS AND DIFFUSION ACROSS**

 **A DIALYSIS MEMBRANE**

In this exercise, we will observe another situation in which a selectively permeable membrane separates two solutions which are **hypertonic** and **hypotonic** to each other, but this time both the solvent and the solutes may be able to move.



The membrane in this exercise will be dialysis tubing with has small pores which allow small molecules to pass through but prevent larger ones from doing so. If a molecule is large and pore is small, its diffusion is ‘selectively’ excluded because it cannot fit through the pore. The dialysis tubing used in this lab has pores that exclude particles with a molecular weight of about 12,000 or larger.

A solution of 40% sucrose and 5% starch will be added to the inside of the tubing, which will be sealed off at both ends to form a dialysis bag. This will then be immersed in a beaker of distilled water, and we will measure whether water is entering or leaving the bag (osmosis) by weighing it at regular intervals. If water enters the bag, the weight of the bag will increase. If water leaves the bag, its weight will decrease.

Water has a molecular weight of 18 g/mole, sucrose has a molecular weight of 342 g/mole, and starch has a molecular weight above 14,000 g/mole. As noted above, the dialysis tubing has pores that exclude particles with a molecular weight of about 12,000 or larger. At the end you will add iodine, which has an atomic weight of 127.

***Items For Discussion Your Lab Partners:***

 *Identify which solution (the sucrose and starch inside the dialysis bag or the distilled water outside*

 *of the bag) is hypertonic to the other, and which solution is hypotonic.*

 *Hypertonic: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

 *Hypotonic: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

 *Which molecule(s) will be able to pass through the dialysis membrane?*

 *Which molecule(s) will NOT be able to pass through the dialysis membrane?*

1. Obtain a dialysis bag which has been filled with a solution of 40% sucrose and 5% starch. This

 has been stored in a solution of the same concentration.

2. Rinse the bag to remove excess sucrose/starch solution, dry the bag, check to see that no liquid is

 leaking out of either end, and weigh it. Record the beginning weight at “0 minutes” in the table below.

3. Submerge the dialysis bag in a beaker of distilled water. Record the time in the table below.

4. After 15, 30, 45, and 60 minutes you should dry and weigh the dialysis bag again, Record each weight

 in the table below and calculate the change from the “0 minute” measurement.

5. After your last weighing at 60 minutes, add three drops of iodine solution to the beaker. After five

 minutes, record the colors of the solution in the beaker and the solution in the dialysis tubing.

6**.** Clean up as directed by your instructor.

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| **DIALYSIS BAG RESULTS** |
| TIME (MINUTES) | WEIGHT OF DIALYSIS BAG (GRAMS | CHANGE IN WEIGHTOF BAG (GRAMS) |
| 0 min:  | Time = |  | **0** |
| 15 min |  |  |
| 30 min |  |  |
| 45 min |  |  |
| 60 min |  |  |

|  |  |
| --- | --- |
| Color of solution in beaker |  |
| Color of solution in dialysis bag |  |

***Items For Discussion Your Lab Partners:***

 *Did* ***water*** *move across the dialysis membrane?*

 *If so, in what direction?*

 *If so, why? If not, why not?*

 *Did* ***sucrose*** *move across the dialysis membrane?*

 *If so, in what direction?*

 *If so, why? If not, why not?*

 *Did* ***starch*** *move across the dialysis membrane?*

 *Note: If starch is present in a solution, iodine will react with it to form a dark blue/black color*

 *If so, in what direction?*

 *If so, why? If not, why not?*

 *During the time of this experiment, did the solution inside the dialysis bag ever become*

 *isotonic with the solution in the beaker?*

 *If so, why? If not, why not?*

**EXERCISE 4: PASSIVE TRANSPORT - FILTRATION**

(Your lab instructor will help you do this exercise in groups of 6 - 8)

Filtration is the separation of substances passing through a membrane from a region of higher pressure to a region of lower pressure. Small molecules, both solvent and solutes, pass through the pores of a membrane while larger molecules are kept on the original side.

In this activity, we will observe selectivityof a filtration membrane and how the rate of filtration changes when a mixture of water, copper sulfate (blue), and powdered charcoal are placed into a funnel with a piece of filter paper.

***Items For Discussion Your Lab Partners:***

 *Predict which of those substances will pass through the filter*.

  *Predict which of those substances will NOT pass through the filter*.

1. Obtain a funnel, ringstand, beaker, and piece of filter paper.

2. Fold the circular filter paper in half twice, and open it to form a

 cone. Place this cone in the funnel and place the funnel in a

 ring on the ring stand. Put the beaker under the funnel.

3. Stir the solution of water, copper sulfate, and powdered

 charcoal and pour it into the funnel to approximately two-thirds

 full (not all the way full). Be sure that the edges of the filter paper

 do not collapse and that the solution doesn’t go over the top.

4. As soon as the liquid slows down enough to count individual

 drops, count the number of drops in 15, 30, 45, 60, and 90

 seconds. Record that in the table below.

5. Observe the filtrate liquid in the beaker. If it is blue, the copper

 sulfate was filtered; if it is black, the charcoal was filtered.

.

6.Clean up as directed by your instructor.

|  |
| --- |
| FILTRATION RESULTS |
| TIME (SECONDS) | NUMBER OF DROPS | FILTRATION RATE (DROPS/SEC) |
| 15 |  |  |
| 30 |  |  |
| 45 |  |  |
| 60 |  |  |
| 90 |  |  |
| COLOR OF FILTRATE: |

***Items For Discussion Your Lab Partners:***

 *Was your prediction correct about which substances would and would not pass through the filter?*

 *What cell structure does the filter paper represent?*

 *(That is, what structure does filtration occur across?)*

 *What determines which substances cross the filtration paper?*

 *What is the force that drives the filtration in this experiment?*

 *What is the force the drives the filtration of molecules out of capillaries in the body?*

 *Did you see a difference in the filtration rate during the activity? Explain why or why not.*

**EXERCISE 5: ACTIVE VESICULAR TRANSPORT - PHAGOCYTOSIS**

Your lab instructor will do this as a demonstration using a video of an amoeba taking a smaller particle or organism into itself by phagocytosis. You can find many similar videos with a simple web search.

Observe how the amoeba changes shape as it moves and uses extensions of itself to surround whatever it is engulfing.