

EXERCISE

2

Transport Mechanisms in the Body

OBJECTIVES

After completing these activities, you should be able to:

- o Understand the differences between passive and active processes of transport
- o Define diffusion, osmosis, and filtration
- o Explain how and where these processes occur in the body
- o Explain the effects of isotonic, hypotonic and hypertonic solutions on cells
- o Define active transport, pinocytosis, phagocytosis and exocytosis
- o Give an example of where these active processes occur in the body

BACKGROUND

Many substances, both solids and liquids, must move within and throughout your body. These substances will move between fluid compartments of the body, for example between the plasma of blood and interstitial fluid around cells, and between interstitial fluid and cytosol. The fluids of the body are categorized as either *extracellular* (interstitial fluid, plasma and lymph) or *intracellular* (cytosol). These fluids are composed of water plus some solid molecules. Water is considered the *solvent*, the molecules dissolved in the water are considered the *solutes*. The combination of solvent plus solute creates a *solution*. In cells, the plasma membrane performs the important function of regulating the movement of these substances into and out of the cell. This is possible because the membrane is *selectively permeable*, allowing some substances to pass through and excluding others. The tendency of some solutes to pass through while others are excluded establishes a difference in solute concentration between the two sides of the membrane. This difference is known as a *concentration gradient*.

Movement of substances within the body can either be passive or active. **Passive processes** are those where molecules randomly move from areas of high concentration to areas of low concentration (“down” their concentration gradient), due to *kinetic energy*. Kinetic energy is due to the vibration of molecules; all molecules possess kinetic energy. **Active processes** require the cell’s use of ATP. Movement must occur across a living cell membrane and substances can move against their concentration gradient (“uphill”). Both active and passive transport mechanisms are described in Table 2.1. Notice from the table that **diffusion, facilitated diffusion, osmosis, and filtration** are passive processes. **Active transport** and both forms of **vesicular transport** are active processes.

Table 2.1: Movement of Materials in the Body			
PROCESS	ENERGY SOURCE	METHOD OF MOVEMENT	EXAMPLES
Diffusion	Passive, by kinetic energy	Down a concentration gradient	Movement of oxygen and carbon dioxide
Facilitated diffusion	Passive, by kinetic energy	Down a concentration gradient via a carrier protein	Movement of glucose and amino acids into cells
Osmosis	Passive, by kinetic energy	Water movement down a concentration gradient	Movement of water molecules through pores in a membrane
Filtration	Passive, by hydrostatic pressure	Down a pressure gradient	Movement of water and small solutes across capillary wall membranes
Active transport	Cellular energy (ATP)	Against a concentration gradient involving a carrier protein (ion pump)	Movement of ions such as Na ⁺ and K ⁺ across a plasma membrane
<i>Vesicular transport:</i> Endocytosis	Cellular energy (ATP)	Engulfing of substances by cell membrane <i>into</i> the cell	<i>Receptor-mediated endocytosis</i> for specific molecules; <i>phagocytosis</i> of dead cells and bacteria by white blood cells; <i>pinocytosis</i> of fluid by various cells
Exocytosis	Cellular energy (ATP)	Export of substances <i>out</i> of a cell	Secretion of proteins, hormones and various products by cells

PASSIVE PROCESSES OF TRANSPORT

You have just learned that passive processes of transport include those that do not require an input of energy, but movement of substances passively will only occur from areas of high concentration to lower concentration. In the following exercises, you will observe these processes by performing the following activities.

Exercise 2.1: Diffusion of dye through water

You will observe the diffusion of the purple dye, potassium permanganate, through water and record your results. This activity represents the movement of solid molecules, such as glucose, amino acids, or ions, diffusing within the interstitial fluid surrounding cells.

Materials (per table)

- o 25-ml graduated cylinder
- o flat bottomed clear bowl
- o potassium permanganate crystals
- o forceps
- o plastic metric measuring ruler
- o white sheet of paper

Procedure

1. Fill the 25 ml graduated cylinder with tap water and pour it into the bowl. The water should create a thin layer covering the bottom of the bowl.
2. Lay the white paper on your table, the ruler on the paper and place the water-filled bowl on top of the plastic ruler, so that you are able to see the numbers through the water (see Figure 2.1). Let the water settle before performing the next step.
3. Using the forceps, pick out *one* crystal of potassium permanganate. Carefully place the crystal into the center of your bowl. Note the initial marking on the ruler at which the crystal landed. This will be the mark from where you will begin measuring diffusion.
4. Record the time that you dropped the crystal into the bowl as time zero (0), and record the starting mark as 0 mm, in Table 2.2. Each successive measurement will be in millimeters from this initial mark.
5. Continue to measure the distance (diameter) the potassium permanganate crystal dye diffuses outward in millimeters (mm) at time increments of 3 minutes for a total time of 15 minutes. Keep the bowl on the table and avoid shaking. Record all your data in Table 2.2.

Table 2.2	
Time in minutes	Distance potassium permanganate diffused in millimeters (mm)
0	0 mm
3	
6	
9	
12	
15	

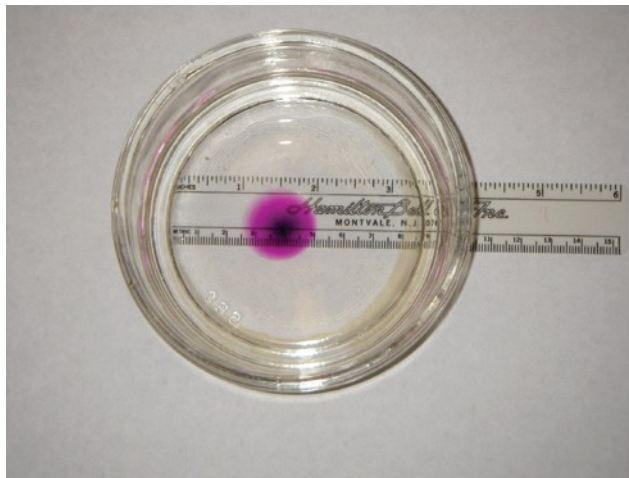


Figure 2.1: Diffusion of dye through water.

Exercise 2.2: Diffusion of dye through agar gel

You will compare the rates of diffusion within agar gel between two dyes with different molecular size. Potassium permanganate is a smaller molecule with a molecular weight of 158 amu*; methylene blue is a larger molecule with a molecular weight of 320 amu*. The agar gel has the same consistency as cytosol within cells.

* amu = atomic mass units.

Materials (per table)

- o agar filled Petri dish, with two small wells punched out
- o clear plastic metric ruler
- o dropper bottle of 1% potassium permanganate solution
- o dropper bottle of 1% methylene blue solution

PROCEDURE

1. Place the agar filled Petri dish on top of a piece of white paper.
2. Measure the initial size of the diameter of each well in the agar using the metric ruler, and record the diameter in millimeters at time 0. (This is your initial size measurement.)
3. Carefully place two drops of potassium permanganate solution in one punched out well, and two drops of methylene blue solution into the other well. These solutions should be added simultaneously. Record the time the solutions were added as time zero.
4. With the metric ruler, measure the zone of diffusion (diameter) in millimeters at 15 minute intervals for a total of one hour. Record your results in Table 2.3 below.

Time in minutes	Diffusion of potassium permanganate in millimeters (mm)	Diffusion of methylene blue in millimeters (mm)
0	(initial size)	(initial size)
15		
30		
45		
60		

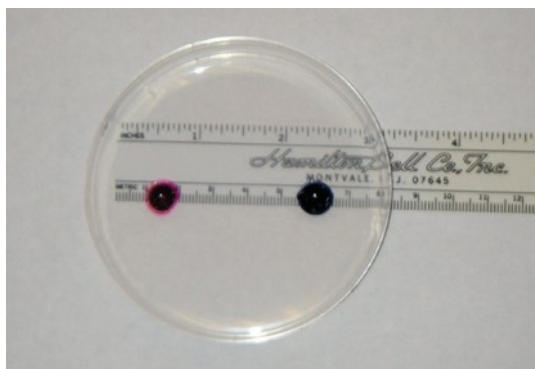


Figure 2.2: Diffusion of dye through agar gel.

Exercise 2.3: Osmosis and diffusion across a nonliving membrane

As described in Table 2.1, osmosis is the movement of water molecules across a selectively permeable membrane. The water molecules move along a concentration gradient; in other words, they move from a region of high water concentration to a region of low water concentration. In our bodies, the different compartments on either side of the plasma mem-

brane (*interstitial fluid* and *cytosol*), may contain different amounts of water depending on the amount of solutes dissolved in them. The compartment with a higher concentration of solutes (lower water concentration) is said to be **hypertonic**, whereas the compartment with a lower concentration of solutes (higher water concentration) is **hypotonic**. When the amounts of solutes are equal (equal amount of water) on both sides of the membrane the solutions are said to be **isotonic**.

In this exercise you will observe osmosis (water movement) and diffusion (solute movement) across a nonliving selectively permeable membrane made from *dialysis tubing*. The dialysis tubing will simulate the function of the plasma membrane, by allowing water and small molecules to pass through, but limiting the movement of large molecules. In this exercise, you will be using glucose and albumin (protein) solutions, which have different molecular weights (size). You will determine how different solutions will affect osmosis, which substances can pass through the dialysis tubing, and in which direction movement will occur. Diastix test strips will be used to indicate the presence of glucose. The Diastix indicator will turn brown in the presence of glucose. Albustix test strips will be used to test for albumin, and the indicator will turn green if albumin is present.

Materials (per 2 students)

- o Dialysis tubing 15 cm in length, which has been soaked in deionized water
- o Thread
- o Scissors
- o 25-ml graduated cylinder
- o 250-ml beaker
- o Albustix indicator strip (if assigned beaker 4)
- o Diastix indicator strip (if assigned beaker 1)
- o Wash bottle of distilled water
- o Beaker solutions (depending upon your assignment): Distilled water, 10% glucose solution, 20% glucose solution
- o Dialysis sac solutions (depending upon your assignment): 10% albumin solution, 10% glucose solution
- o Metric balance at the teacher's table

PROCEDURE

1. Working in groups of two, obtain a previously soaked **dialysis tube** from the activity station.
2. Twist one end of the tubing and using the thread, tie the twisted end securely. Rub the untied end of the dialysis bag end between your fingers to open it. (Be sure that your fingers are wet in order to pry it open.)
3. Using the **graduated cylinder**, carefully fill the dialysis bag with **20 ml** of your assigned solution. Each pair of students will be assigned either Beaker 1 or 2 or 3 or 4.
4. Use Figure 2.3 or Table 2.4 to determine the contents of your sac.

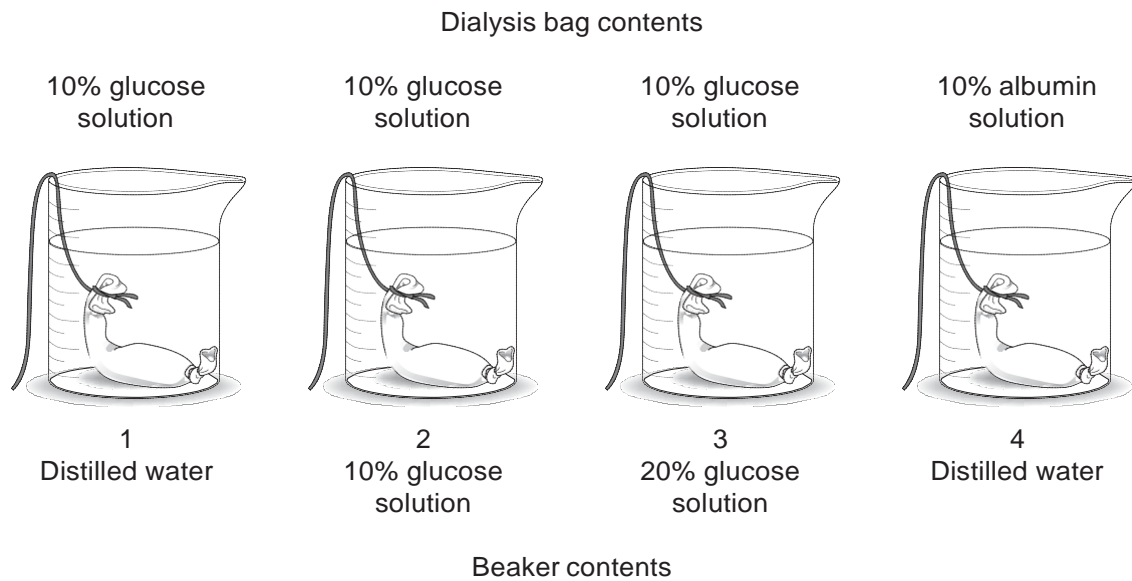


Figure 2.3

4. Push any remaining air out of the bag, and twist the open end, tying it firmly with the thread. The bag should look like the submerged bags in Figure 2.3. Check both ends to make sure the seals are tight.
5. Rinse the filled dialysis bag with distilled water from a wash bottle to remove excess solution, blot it gently using a paper towel, and weigh it using the metric balance on the instructor's desk. Record the initial weight in Table 2.4.
6. Fill a 250 ml **beaker** with **200 ml** of your corresponding beaker solution. This should be enough solution to fully submerge the dialysis bag. See Figure 2.3 and Table 2.4 for contents of beaker solutions.
7. **Gently submerge the dialysis bag into your beaker**, and make note of the time.

Question: Are your beaker solutions *isotonic*, *hypotonic*, or *hypertonic* in relation to the dialysis bag solution?

Record your answers for each of the four beakers in Table 2.4.

Predict whether your dialysis bags will *lose* weight, *gain* weight or *stay the same*. Explain.

Record your prediction for all of the four beakers in Table 2.4.

Table 2.4							
Contents of beaker	Contents of sac	Beaker Solution (hypertonic, hypotonic, isotonic)	Initial weight (g)	Prediction of weight change	Final weight (g)	Actual weight change	Test for beaker fluid (only Beakers # 1 & 4)
Beaker 1 Distilled water	10% glucose solution						Diastix +/-
Beaker 2 10% glucose solution	10% glucose solution						-----
Beaker 3 20% glucose solution	10% glucose solution						-----
Beaker 4 Distilled water	10% albumin solution						Albustix +/-

8. **After one hour**, remove the bag, blot it, weigh it, and record the weight in the data table. The class data will be transferred to the board so that it can be shared.
9. For students assigned **beaker #1** – After the **final** weighing of the dialysis bag, dip a Diastix test strip into the beaker water to test for the presence of glucose. Record your results in Table 2.4.
10. For students assigned **beaker #4** – After the **final** weighing of the dialysis bag, dip an Albustix test strip into the beaker water to test for the presence of albumin in the beaker. Record your finding in Table 2.4.
11. Make sure to share your results with the other groups who performed different beaker set-ups, and get their results as well.

Exercise 2.4: Osmosis in live cells: Observation of the effects of various solutions on red blood cells

Osmosis is the primary process of water movement into and out of cells. In this exercise, you will observe a demonstration in which prepared slides containing blood samples were exposed to three solutions. One solution is hypotonic to the red blood cells, a second solution is hypertonic to the red blood cells, and a third solution (the control) is isotonic to the red blood cells. These slides are being viewed through a microscope at the highest power possible.

A 0.9% saline solution, also called physiological (or normal) saline, is balanced to have an equivalent solute concentration with body cells. Therefore, this solution represents your control, since the solution is _____ to the cells. The red blood cells in the 0.9% saline solution should appear normal (round and biconcave). If water flows into the cells due to exposure to a hypotonic solution, this will cause some cells to swell. Some cells may burst in response to the inflow of water, a phenomenon known as **hemolysis**. Water will flow out of cells when they are placed in a hypertonic solution, causing cells to shrivel; a phenomenon known as **crenation**.

Materials

- o Slide 1: the blood is mixed with 0.9% saline solution
- o Slide 2: the blood is in a 9% saline solution
- o Slide 3: the blood is in distilled water

Based on your understanding of the movement of water in cells from **Exercise 2.3**, discuss with your lab group which of the solutions are hypertonic, hypotonic, and isotonic to the blood cells, and predict the outcome of exposing the cells to each solution. State your hypothesis here:

Observe and record the shape of the cells and indicate whether the solution used is

Slide 1: Cell shape: _____ Solution: _____

Slide 2: Cell shape: _____ Solution: _____

Slide 3: Cell shape: _____ Solution: _____

Exercise 2.5: Filtration

Filtration is the separation of particles in a solution when pressure is applied on one side of a membrane, resulting in the passage of water and small particles through the membrane while larger particles unable to penetrate are left behind. The resulting solution that is formed by the process of filtration is referred to as the **filtrate**. Like diffusion and osmosis, filtration is a passive process. The amount of filtrate (fluids and solute) formed depends almost entirely on the pressure gradient (difference in pressure on the two sides of the membrane) and on the size of the membrane pores. When the pressure is exerted by water flow, it is called *hydrostatic pressure*. In the body, filtration occurs due to *blood pressure*. When blood passes through capillaries, the pressure exerted on the thin capillary walls forces fluid (plasma) and small solutes into the surrounding interstitial fluid. Filtration also occurs in the kidneys, where the pressure exerted by blood flow through kidney capillaries pushes water, small particles and wastes out of the bloodstream to form urine. Larger particles, including cells and large proteins, remain in the blood. The speed at which substances fil-

ter through a membrane is called the **filtration rate**. In this exercise, you will observe the process of filtration using a filter paper to represent the capillary wall (membrane). A solution comprised of various sized molecules will represent substances in the blood. You will observe how a pressure gradient will affect the filtration rate, and how the size of particles will affect their ability to be filtered across a semi-permeable membrane.

Materials

- o Ring stand and ring with clamp
- o Funnel
- o Filter paper
- o 500-ml graduated cylinder
- o 250-ml graduated cylinder
- o 500-ml bottle containing a solution of distilled water, 5% powdered charcoal, 1% copper sulfate, and 1% starch
- o Test tube and test tube rack
- o Lugol's solution

PROCEDURE

1. Your group will be assigned to complete one condition of this experiment using either 100 ml ("group A") or 400 ml ("group B") of the distilled water, charcoal, copper sulfate and starch solution.
2. Fold the circular filter paper in half twice and open it to form a cone. Place the cone-shaped filter paper into the funnel, which is held by the ring and ring stand. Place the **500 ml** graduated cylinder under the funnel.
3. Shake the solution of distilled water, charcoal, copper sulfate and starch.
4. **Group A** – Using a 250-ml graduated cylinder, measure out **100 ml** of the solution and keep for your group.
5. **Group B** – Take the remaining solution in the bottle (which is your **400 ml**).
6. Both groups – Noting the exact time, pour the entire amount of your solution into the funnel all at once.
7. Determine the amount of filtrate (in ml) that collects in the graduated cylinder *during the first 15 seconds* and record this below, under *Data Collection*.
8. Allow the remainder of the fluid to run through the filter into the graduated cylinder.
9. You must now determine which substances passed through the filter paper and record this below, under *Data Collection*. Note: charcoal is black, copper sulfate in

solution is turquoise-blue, starch is clear. You must test for the presence of starch in the filtrate using Lugol's solution. Pour a small amount of filtrate into a test tube and add 3 drops of Lugol's solution. Note any color change.

Data Collection

Amount of solution assigned to your group: _____

Amount of fluid collected during the first 15 seconds: _____

Which substances passed through the filter paper? _____

10. After you have collected the data on your experimental condition, write your data in the table on the board. After all class data has been reported for the experimental conditions, add the class data to Table 2.5 in your lab book. You will use this data to answer the lab report questions.

Table 2.5		
	Amount of fluid collected during the first 15 seconds?	Substance passed through filter paper
100 ml groups		
400 ml groups		

ACTIVE PROCESSES OF MEMBRANE TRANSPORT

Exercise 2.6: Observation of an Amoeba

Recall from Table 2.1 that active processes of movement require an input of energy in the form of ATP to drive the movement across the plasma membrane. The energy is needed to transport larger substances across the plasma membrane, and move substances against a concentration gradient; that is, from a region of low concentration to a region of high concentration. The processes include **active transport**, **endocytosis**, and **exocytosis**. Active processes are difficult to observe in a laboratory setting, so in this section, you will view a brief video which shows a single-celled organism known as an Amoeba. In order for an Amoeba to move in a given direction, it must extend its plasma membrane into pseudopodia ("false feet"). In order for it to eat, it must engulf its food by the process of phagocytosis. These activities require the cell to expend energy. The movement of Amoeba is similar to that of white blood cells in the human body.

LAB REPORT #2

NAME _____

Lab Day _____

PART A—DIFFUSION

1. Diffusion, osmosis and filtration are types of _____ processes of movement.
2. In exercise 2.1, the water in the flat-bottomed bowl is meant to represent what part of your body?

3. In exercise 2.2, the agar is meant to represent what part of your body?

4. In exercise 2.2, did the potassium permanganate and the methylene blue diffuse through the agar at the same rate? _____
If not, which molecule diffused faster? _____
5. What is the molecular weight of:
Potassium permanganate _____
Methylene blue _____
6. Based on results of Exercise 2.2 (diffusion of dye through agar), describe the relationship between the molecular size (weight) of molecules and the rate of diffusion (molecular movement)?

7. Did the potassium permanganate diffuse at the same rate in Exercise 2.1 (water) versus Exercise 2.2 (agar)? _____

How far (in millimeters) did the potassium permanganate diffuse

- a. in the water at the end of 15 minutes? _____
b. in the agar at the end of 15 minutes? _____

8. Using your observations and the results obtained from Exercise 2.1 and 2.2, would you expect diffusion of molecules to occur at a faster rate through the interstitial fluid or within the cytosol of your cells?

Explain how you came to this conclusion. _____

PART B—OSMOSIS

Using the results obtained in Exercise 2.3 and recorded in Table 2.4, answer the following questions:

1. The solution in beaker #1 is considered _____ to the solution in the dialysis bag submerged within.

2. Did the dialysis bag in beaker #1 lose or gain weight? _____
Explain the reason for this weight change.

3. The solution in Beaker #2 is considered _____ to the solution in the dialysis bag which was submerged within.

4. The solution in beaker #2 is considered _____ to the solution in the dialysis bag that was submerged within.

5. Did the dialysis bag in Beaker #3 lose or gain weight? _____
Explain why this weight change occurred. _____

6. a. What was the result of the Diastix test on the water in beaker #1?

- b. What was the result of the albutix test on the water in beaker #4?

- c. Based upon these results, which molecule could you conclude is larger, glucose or albumin? _____

Explain how you came to this conclusion.

7. Based upon your knowledge of osmosis in living cells and on your observations in this activity, why would you not want to transfuse distilled water intravenously into a dehydrated patient?

PART C—FILTRATION

1. Based upon the results of Exercise 2.5, which substances passed through the filter paper?

2. Which substances did not pass through the filter paper? _____

Explain why. _____

3. When the fluid was first poured into the funnels, which funnels had the greater hydrostatic pressure, those of group A (100 ml bottle) or group B (400 ml bottle)?

4. Which groups collected more fluid in their graduated cylinders within the first 15 seconds?

5. Based on your observations and results, explain the relationship between the hydrostatic pressure of the fluid in the funnel and the rate of filtration.

6. Urine is produced by the process of filtration, as blood plasma is filtered by the nephrons within the kidneys. Based on your observations in this activity, what changes in urine production would you expect to observe in a patient with hypertension (high blood pressure)?
