Cell Transport Mechanisms and Permeability

exercise 1

Objectives

1. To understand the selective permeability function of the plasma membrane
2. To be able to describe the various mechanisms by which molecules may passively cross the plasma membrane
3. To be able to describe the various mechanisms by which molecules are actively transported across the plasma membrane
4. To understand the differences between how membrane transporters work with and without the expenditure of cellular metabolic energy
5. To define **passive transport, active transport, simple diffusion, facilitated diffusion, osmosis, solute pump, hypotonic, isotonic,** and **hypertonic**

Each cell in your body is surrounded by a plasma membrane that separates the cell from interstitial fluid. The major function of the plasma membrane is to selectively permit the exchange of molecules between the cell and the interstitial fluid, so that the cell is able to take in substances it needs while expelling the ones it does not. These substances include gases, such as oxygen and carbon dioxide; ions; and larger molecules such as glucose, amino acids, fatty acids, and vitamins.

Molecules move across the plasma membrane either *passively* or *actively*. In **active transport,** molecules move across the plasma membrane with the expenditure of cellular energy (ATP). In **passive transport,** molecules pass through the plasma membrane without the expenditure of any cellular energy. Examples of passive transport are **simple diffusion, osmosis,** and **facilitated diffusion. Simple diffusion** is the spontaneous movement of molecules across a biological membrane's lipid bilayer from an area of higher concentration to an area of lower concentration. **Osmosis** is the diffusion of water across a semipermeable membrane. **Facilitated diffusion** is the movement of molecules across a selectively permeable membrane with the aid of specialized transport proteins embedded within the membrane.

In this lab, we will be simulating each of these cell transport mechanisms. We will begin by examining simple diffusion.

Simple Diffusion

All molecules, whether solid, liquid, or gas, are in continuous motion or vibration. If there is an increase in temperature, the molecules will move faster. The moving molecules bump into each other, causing each other to alter direction. Thus, the movement of molecules is said to be "random." If one were to release a drop of liquid food coloring into a large beaker of water, the food coloring molecules would randomly move until their concentration was equal throughout the beaker. The molecules would reach equilibrium through this process of *diffusion*. We define diffusion as the movement of molecules from one location to another as a result of their random thermal motion. **Simple diffusion** is diffusion across a biological membrane's lipid bilayer.

The speed at which a molecule moves across a membrane depends in part on the mass, or molecular weight, of the molecule. The higher the mass, the slower the molecule will diffuse. Normally, the rate at which a substance diffuses across the membrane can be determined by measuring the rate at which the concentration of the substance on one side of the membrane approaches the concentration of the substance on the other side of the membrane. The magnitude of the net movement across the membrane, or flux (F), is proportional to the concentration difference between the two sides of the membrane ($C_o - C_i$), the surface area of the membrane (A), and the membrane permeability constant (k_p):

$$F = k_pA(C_o - C_i)$$

Nonpolar substances will diffuse across a membrane fairly rapidly. The reason is that nonpolar substances will dissolve in the nonpolar regions of the membrane—regions that are occupied by the fatty acid chains of the membrane phospholipids. Gases such as oxygen and carbon dioxide, steroids, and fatty acids are prime nonpolar molecules that will diffuse through a membrane rapidly.

In contrast, polar substances have a much lower solubility in the membrane phospholipids. Certain compounds that are intermediates of metabolism are not usually allowed through the membrane, as they are often ionized and contain groups such as phosphate. Thus, once produced in a cell, they cannot leave even if their concentrations are higher inside the cell than they are outside the cell. From this we can see that it is the lipid bilayer portion of the plasma membrane that is responsible for the membrane's selectivity in what it allows through.

Ions, such as Na^+ and Cl^-, tend to diffuse across a membrane rather rapidly. This suggests that a protein component of the membrane is involved—and in fact, proteins do form channels that allow these ions to pass from one side of the membrane to the other. Remember that the channels are selective. Channels that allow sodium through will not usually allow other ions, such as calcium, through.

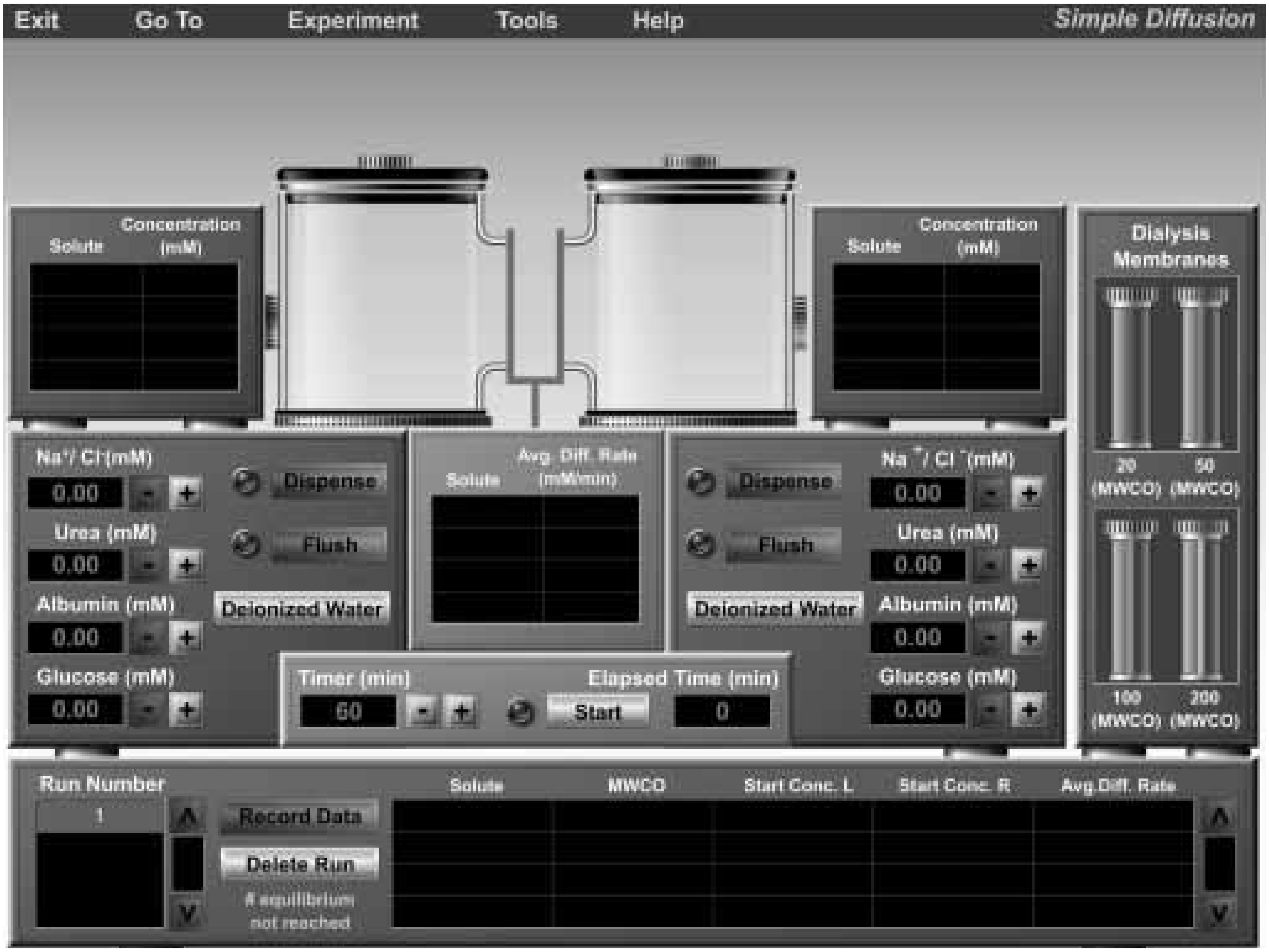

Figure 1.1 Opening screen of the Simple Diffusion experiment.

Diffusion will lead to a state in which the concentration of the diffusing solutes is constant in space and time. Diffusion across a membrane tends to equilibrate so that there are equal solute concentrations on both sides of the membrane. The rate of diffusion is proportional to both the area of the membrane and the difference in concentration of the solute on both sides of the membrane. **Fick's first law of diffusion** states

$$J = -DA\, \Delta_c/\Delta_x$$

where

J = net rate of diffusion (gms or mols/unit time)
D = diffusion coefficient for the diffusing solute
A = area of the membrane
Δ_c = concentration difference across the membrane
Δ_x = thickness of the membrane

Activity 1: Simulating Simple Diffusion

Follow the instructions in the "Getting Started" section at the front of this manual for starting PhysioEx 3.0. From the Main Menu, select the first lab: **Cell Transport Mechanisms and Permeability.** You will see the opening screen for the "Simple Diffusion" activity, shown in Figure 1.1.

In this activity we will be simulating the process of diffusion across the plasma membrane. Notice the two glass beakers at the top of the screen. You will be filling each beaker with fluid. Imagine that the right beaker represents the inside of a cell, while the left beaker represents the extracellular (interstitial) fluid. Between the two beakers is a membrane holder into which you will place one of four dialysis membranes found on the right side of the screen. Each of these membranes has a different "MWCO," which stands for "molecular weight cut off." Molecules with a molecular weight of less than this value may pass through the membrane, while molecules with higher molecular weight values cannot. To move a membrane to the membrane holder, click on the membrane, drag it to the membrane holder, and let go of your mouse button—the membrane will lock into place between the two beakers.

Below each of the two beakers is a solutions dispenser. You may set how many millimoles (mM) of different solutes (Na^+/Cl^-, urea, albumin, or glucose) you want to dispense into each beaker by clicking on the (+) or (−) buttons beneath each solute name. You may also dispense deionized water into either beaker by clicking the **Deionized Water** button under the beaker you wish to fill. Clicking the **Dispense** buttons under each beaker will then cause the beakers to fill with fluid. Clicking the **Flush** buttons under each beaker will empty the beakers.

At the bottom of the screen is a data recording box. After each experimental run, you may record your data by clicking the **Record Data** button. If you wish to delete the data for any given run, simply highlight the line of data you wish to delete and then click **Delete Run.** You may also print out your data by clicking Tools (at the top of the screen) and then selecting **Print Data.**

1. Using the mouse, click on the dialysis membrane with the MWCO of 20 and drag it into the membrane holder.
2. Adjust the mM concentration of Na^+/Cl^- for the left beaker to 9 mM by clicking the (+) button. Then click the **Dispense** button under the left beaker to fill the beaker.
3. Click the **Deionized Water** button under the right beaker and click **Dispense** under the right beaker to fill the beaker.
4. Set the Timer for 60 minutes by clicking the (+) button next to the Timer display (which will be compressed into 60 seconds.)
5. Click on the **Start** button to start the experimental run. Note that the membrane container descends into the equipment. Also note that the **Start** button is now a **Pause** button, which you may click to pause any run.
6. As the Elapsed Time display reaches 60, note the concentration readings for each beaker in the displays on each side of the two beakers.
7. Once the Elapsed Time display has reached 60, you will see a dialogue box pop up telling you whether or not equilibrium was reached.
8. Click **Record Data** to save the data from this run.
9. Click the **Flush** buttons on both the left and right sides to empty the beakers.
10. Return the dialysis membrane to its starting place by clicking and dragging it back to the membrane chamber.
11. Now, repeat steps 1–10 with each of the remaining dialysis membranes. Be sure to record the data for each run. After each run, flush both vessels and return the dialysis membrane.

Turn to the Periodic Table of Elements on p. 4 of this booklet.

What is the molecular weight of Na^+? ________

What is the molecular weight of Cl^-? ________

Which MWCO dialysis membranes allowed both of these ions through? ________

12. Repeat this experiment using each of the remaining solutes (urea, albumin, and glucose) in the left beaker and deionized water in the right beaker. Be sure to click **Record Data,** flush both beakers, and replace the dialysis membrane after each run. Click **Tools** → **Print Data** to print your data.

13. Fill in the chart below with your results.

Chart 1 Did Diffusion Take Place?

	Membrane (MWCO)			
Solute	20	50	100	200
NaCl				
Urea				
Albumin				
Glucose				

Which materials diffused from the left beaker to the right beaker?

____

Which did not?

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Why?

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1 H Hydrogen 1.00794	Atomic number Symbol Element Atomic weight

IA 1	IIA 2	IIIB 3	IVB 4	VB 5	VIB 6	VIIB 7	VIIIB 8	VIIIB 9	VIIIB 10	IB 11	IIB 12	IIIA 13	IVA 14	VA 15	VIA 16	VIIA 17	VIIIA 18
1 **H** Hydrogen 1.008																	2 **He** Helium 4.003
3 **Li** Lithium 6.941	4 **Be** Beryllium 9.012											5 **B** Boron 10.81	6 **C** Carbon 12.01	7 **N** Nitrogen 14.01	8 **O** Oxygen 16.00	9 **F** Fluorine 19.00	10 **Ne** Neon 20.18
11 **Na** Sodium 22.99	12 **Mg** Magnesium 24.31											13 **Al** Aluminum 26.98	14 **Si** Silicon 28.09	15 **P** Phosphorus 30.97	16 **S** Sulfur 32.06	17 **Cl** Chlorine 35.45	18 **Ar** Argon 39.95
19 **K** Potassium 39.10	20 **Ca** Calcium 40.08	21 **Sc** Scandium 44.96	22 **Ti** Titanium 47.88	23 **V** Vanadium 50.94	24 **Cr** Chromium 52.00	25 **Mn** Manganese 54.94	26 **Fe** Iron 55.85	27 **Co** Cobalt 58.93	28 **Ni** Nickel 58.70	29 **Cu** Copper 63.55	30 **Zn** Zinc 65.38	31 **Ga** Gallium 69.72	32 **Ge** Germanium 72.59	33 **As** Arsenic 74.92	34 **Se** Selenium 78.96	35 **Br** Bromine 79.90	36 **Kr** Krypton 83.80
37 **Rb** Rubidium 85.47	38 **Sr** Strontium 87.62	39 **Y** Yttrium 88.91	40 **Zr** Zirconium 91.22	41 **Nb** Niobium 92.91	42 **Mo** Molybdenum 95.94	43 **Tc** Technetium (98)	44 **Ru** Ruthenium 101.1	45 **Rh** Rhodium 102.9	46 **Pd** Palladium 106.4	47 **Ag** Silver 107.9	48 **Cd** Cadmium 112.4	49 **In** Indium 114.8	50 **Sn** Tin 118.7	51 **Sb** Antimony 121.8	52 **Te** Tellurium 127.6	53 **I** Iodine 126.9	54 **Xe** Xenon 131.3
55 **Cs** Cesium 132.9	56 **Ba** Barium 137.3	57 **La*** Lanthanum 138.9	72 **Hf** Hafnium 178.5	73 **Ta** Tantalum 180.9	74 **W** Tungsten 183.9	75 **Re** Rhenium 186.2	76 **Os** Osmium 190.2	77 **Ir** Iridium 192.2	78 **Pt** Platinum 195.1	79 **Au** Gold 197.0	80 **Hg** Mercury 200.6	81 **Tl** Thallium 204.4	82 **Pb** Lead 207.2	83 **Bi** Bismuth 209.0	84 **Po** Polonium (209)	85 **At** Astatine (210)	86 **Rn** Radon (222)
87 **Fr** Francium (223)	88 **Ra** Radium (226.0)	89 **Ac**** Actinium (227)	104 **Rf** Ruther-fordium (261)	105 **Ha** Hahnium (262)	106 **Unh** Unnil-hexium (263)	107 **Uns** Unnil-septium (262)	108 **Uno** Unnil-octium (265)	109 **Une** Unnil-ennium (266)									

*Lanthanides	58 **Ce** Cerium 140.1	59 **Pr** Praseo-dymium 140.9	60 **Nd** Neo-dymium 144.2	61 **Pm** Promethium (145)	62 **Sm** Samarium 150.4	63 **Eu** Europium 152.0	64 **Gd** Gadolinium 157.3	65 **Tb** Terbium 158.9	66 **Dy** Dysprosium 162.5	67 **Ho** Holmium 164.9	68 **Er** Erbium 167.3	69 **Tm** Thulium 168.9	70 **Yb** Ytterbium 173.0	71 **Lu** Lutetium 175.0
**Actinides	90 **Th** Thorium 232.0	91 **Pa** Pro-tactinium (231)	92 **U** Uranium 238.0	93 **Np** Neptunium (237)	94 **Pu** Plutonium (244)	95 **Am** Americium (243)	96 **Cm** Curium (247)	97 **Bk** Berkelium (247)	98 **Cf** Californium (251)	99 **Es** Einsteinium (252)	100 **Fm** Fermium (257)	101 **Md** Mende-levium (258)	102 **No** Nobelium (259)	103 **Lr** Lawren-cium (260)

Periodic Table of Elements

Activity 2: Simulating Dialysis

Now, let's set up a mock dialysis machine experiment. These machines are used on patients who have lost kidney function. Urea, a breakdown product of amino acids, must be removed from the patient's blood or it will become toxic to the body and cause death. Dialysis machines take a patient's blood and pass it through a selectively permeable membrane in order to remove urea from the blood. On one side of the membrane is the patient's blood; on the other side are fluids carefully selected to mimic the concentrations found in the body of substances such as Na^+, K^+, Ca^{++} and HCO_3^-. To simulate this process:

1. Place the dialysis membrane of 200 MWCO into the membrane holder.
2. Set up the left beaker with 10 mM of each of the four solutes and dispense. This beaker will represent the dialysis patient's blood.
3. Set up the right beaker the same way, except set the urea concentration at 0 mM—in other words, the right beaker will contain no urea.
4. Set the Timer for 60 minutes, then click **Start** and wait for the experimental run to complete.

What happens to the urea concentration in the left beaker (the patient)?

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Why does this occur?

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Normally, dialysis machines are set to run so that the blood is subjected to diffusion twice, and urea is reduced by 75% rather than 50%. In addition, excess water is drawn from the patient, who has no other way to dispose of excess fluid. Dialysis patients need to have routine lab tests done to ensure that ion concentrations are maintained at normal levels.

Facilitated Diffusion

Simple diffusion accounts for the transmembrane transport of some ions, but not all of them. Some molecules that are too polar to diffuse still manage to get through the plasma membrane's lipid bilayer. Similarly, some molecules that are too large to pass through protein channels still manage to cross the membrane. How? The passage of such molecules and the nondiffusional movement of ions through a membrane is mediated by integral proteins known as **transporters.** Transporters are embedded within the plasma membrane and work by undergoing a conformational change that allows transport to occur. A molecule first binds to a receptor site on a transporter. When bound, the transporter changes shape so that the binding site moves from one side of the membrane to the other side. The molecule then dissociates from the transporter and is released on the other side of the membrane. This type of transport is called **facilitated diffusion.** It is considered a form of passive transport because no cellular energy is expended in the process.

The term *facilitated diffusion* is a bit misleading since the process does not really involve diffusion (which, you will recall, is the movement of molecules from one location to another along a concentration gradient, as a result of random thermal motion). In facilitated diffusion, molecules are still moving from one location to another along a concentration gradient, but it is transport proteins that result in this movement—not random thermal motion. The end results of diffusion and facilitated diffusion are the same. The net flux proceeds from an area of high concentration to an area of low concentration until the concentrations are equal on both sides of the membrane.

Among the most important facilitated-diffusion systems in the body are those that move glucose across the membrane. Without transporters, the relatively large, polar glucose molecule would never be able to pass into a cell. However, the number of transport proteins in a given cell membrane is finite, so only a certain amount of glucose can be transported per unit of time. Transport of glucose into the cell is especially interesting in that the glucose is converted to glucose-6-phosphate as soon as it enters the cell, so that there is always a low concentration of glucose inside the cell, which favors transport into the cell. ■

Activity 3: Facilitated Diffusion

Using the mouse, click on **Experiment** at the top of the screen. A drop-down menu will appear. Select **Facilitated Diffusion.** A new screen will appear (see Figure 1.2). You will notice two key changes from the first screen. First, in place of the dialysis membranes on the right side of the screen, there is now a "membrane builder." This will be used to "make" membranes that will transport molecules from one beaker to the other. The second change is that in this experiment, we will be working with glucose and Na^+/Cl^- solutes only.

1. Note that the **Glucose Carriers** display is currently set at 500. Click on **Build Membrane** in order to create a membrane with 500 glucose carriers.
2. Click and drag this membrane to the membrane holder between the two beakers.

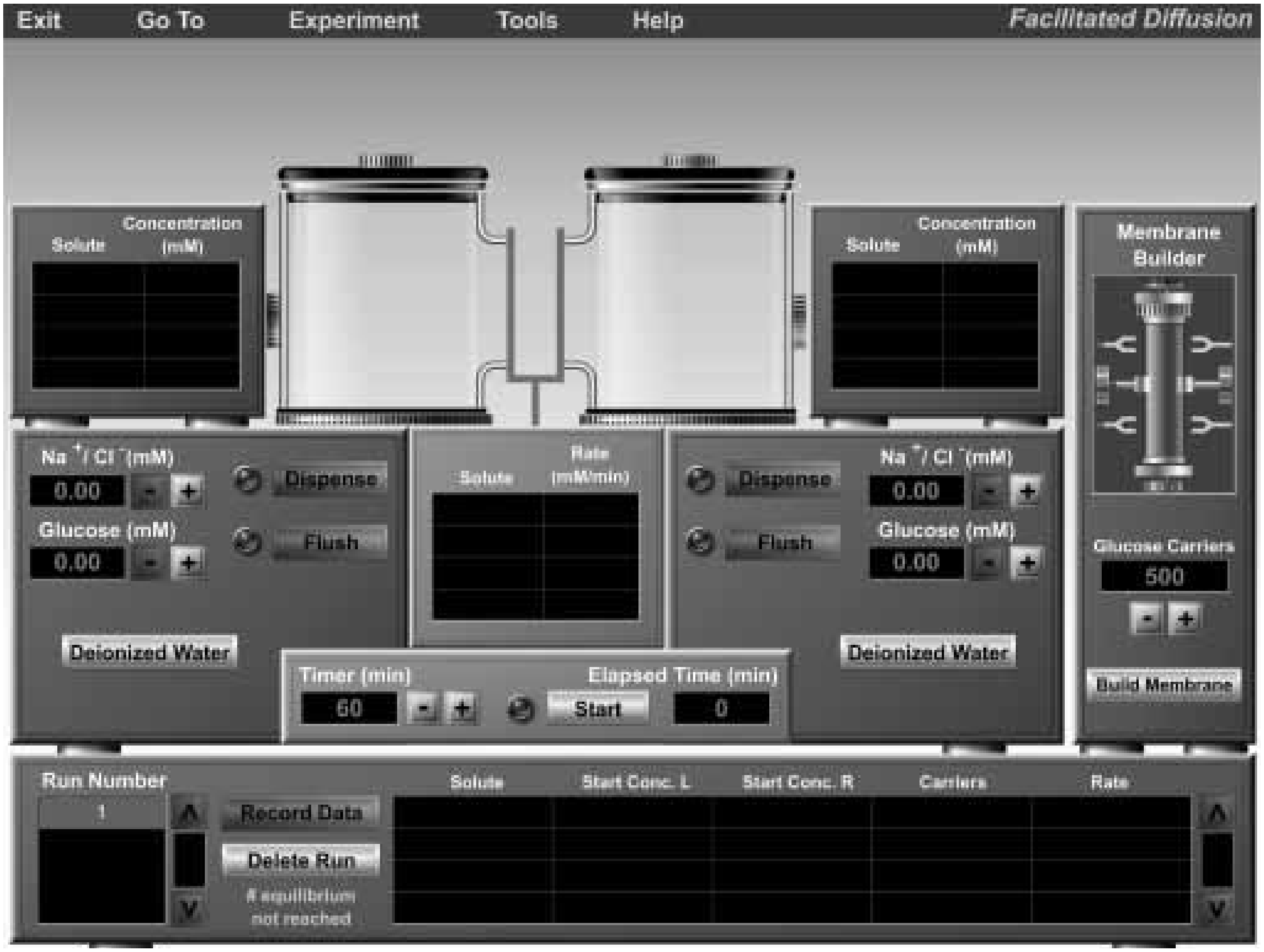

Figure 1.2 Opening screen of the Facilitated Diffusion experiment.

3. For the left beaker, set Na^+/Cl^- to 9 mM and glucose to 9 mM by clicking on the corresponding (+) buttons. Then click **Dispense** to fill up the left beaker.

4. For the right beaker, click on the **Deionized Water** button below the beaker and then click **Dispense.**

5. Set the timer for 60 minutes and click **Start.**

6. Allow the run to complete. When the Elapsed Timer reaches 60, click on **Record Data** to record your data. Also record your data in Chart 2.

7. Click the **Flush** button under each beaker to empty the beakers, and return the membrane to the membrane builder.

8. Build a new membrane with 300 glucose carriers and repeat this experiment. Be sure to record your results, flush the beakers, and replace the membrane after each run.

9. Build a membrane with 700 glucose carriers and repeat the experiment.

10. Build a membrane with 900 glucose carriers and repeat the experiment.

11. For comparison, lower the glucose concentration to 3 mM and repeat steps 1–10 of the experiment. Record your results after each run by clicking **Record Data** and by filling in Chart 2 below.

Chart 2 Facilitated Diffusion Results

Glucose Concentration (mM)	No. of glucose carrier proteins 300	500	700	900
3				
9				

12. Click **Tools** → **Print Data** to print your data.

At a given glucose concentration, how does the amount of time it takes to reach equilibrium change with the number of carriers used to "build" the membrane?

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Does the diffusion rate of Na^+/Cl^- change with the number of receptors?

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What is the mechanism of the Na^+/Cl^- transport?

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If you put the same amount of glucose in the right beaker as in the left, would you be able to observe any diffusion?

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Does being unable to observe diffusion necessarily mean that diffusion is not taking place?

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Osmosis

A *semipermeable membrane* is a membrane that is permeable to water but not to solutes. **Osmosis** is defined as the flow of water across a semipermeable membrane from an area of higher water concentration (lower solute concentration) to an area of lower water concentration (higher solute concentration). The greater the solute concentration, the lower the water concentration. Osmosis is further defined as a "colligative property" because it depends on solute concentration rather than solute chemical properties. Water is a small, polar molecule that diffuses across cell membranes very rapidly. Because of its polar nature, one might expect that water would not penetrate the nonpolar lipid regions of the cell membrane. Membrane proteins, called *aquaporins,* form channels through which water can diffuse. The concentration of these aquaporins varies with tissue type.

It is essential to understand that the degree to which water concentration is decreased by addition of solute depends on the number of solute particles added. For example, 1 mol of glucose decreases the water concentration approximately the same as a 1 mol solution of amino acid or 1 mol of urea. A molecule that ionizes decreases the water concentration in proportion to the number of ions formed. Therefore, a 1 mol solution of Na^+/Cl^- produces a 1 mol solution of Na^+ plus a 1 mol solution of Cl^-. Therefore, it is basically a 2 mol solution.

Two beakers separated by a dialysis membrane (such as the ones we have been working with) are not infinitely expandable. The transfer of water from one compartment to the other will increase the amount of water in the second compartment. If the limits of the beaker cannot expand, pressure within the second beaker will increase, eventually preventing further water entry. The amount of pressure that needs to be supplied to the second beaker in order to prevent further water entry from the first beaker is called *osmotic pressure.* Osmotic pressure is another characteristic that depends on the solution's water concentration.

If the solutions in the beakers have the same concentration of nonpenetrating solutes on either side of the membrane, the two solutions are said to be **isotonic** (iso = same). Solutes that are "penetrating" do not contribute to the tonicity of a solution as they pass from one side of the membrane to the next with no problems. When two solutions are compared and one has a lower concentration of solutes, that solution is said to be **hypotonic** (hypo = less). The other solution, the one with the higher concentration, is said to be **hypertonic** (hyper = more). This is important when discussing cells. If a cell is hypertonic to its surrounding medium, water will flow into the cell to dilute the hypertonic solution. Often, so much water enters the cell that the cell bursts.

Activity 4: Osmosis

Click on **Experiment** at the top of the screen and then select **Osmosis.** A new screen will appear (Figure 1.3). The screen is similar to the one we saw for the **Simple Diffusion** experiment. The main change is that on top of each beaker is a pressure indicator, which we will be watching during experimental runs.

1. Drag the 20 MWCO membrane and place it between the two beakers.
2. Set the Na^+/Cl^- concentration for the left beaker at 9 mM and click **Dispense.**
3. Fill the right beaker with **Deionized Water** and click Dispense.
4. Set the Timer for 60 minutes.
5. Click on **Start** and allow the experiment to run. Pay attention to the "Pressure" indicators on top of each beaker.
6. Once the Elapsed Time is up, click Record Data. Record the data in Chart 3 on p. 8 as well.
7. Click **Flush** under both beakers to empty them.
8. Return the membrane to its original place.
9. Repeat the experiment using the remaining three membranes. Be sure to record all of your data, flushing the beakers in between each run.

Did you observe any pressure changes during this experiment? If so, in which beaker(s), and with which membranes?

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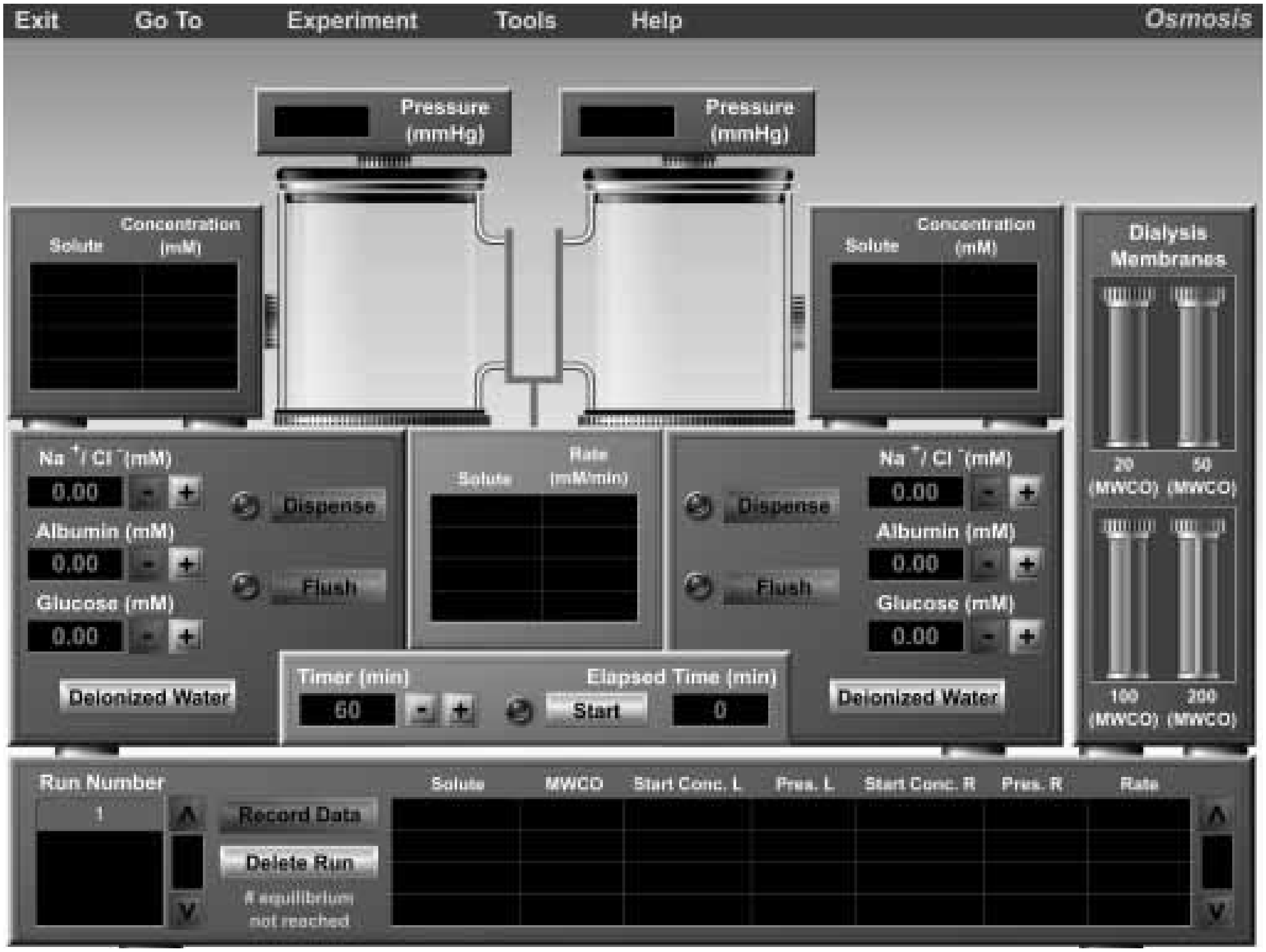

Figure 1.3 **Opening screen of the Osmosis experiment.**

Why?

Did the Na^+/Cl^- diffuse from the left beaker to the right beaker? If so, with which membrane(s)?

Why?

10. Repeat the experiment, first using 9 mM albumin in the left beaker, then 9mM glucose. Click **Record Data** after each run; also record your data in Chart 3.

Chart 3 Osmosis Results (pressure in mm Hg)

	Membrane (MWCO)			
Solute	20	50	100	200
Na^+/Cl^-				
Albumin				
Glucose				

11. Click **Tools** → **Print Data** to print your data.

Explain the relationship between solute concentration and osmotic pressure.

Does diffusion allow osmotic pressure to be generated?

Would pressure be generated if solute concentrations were equal on both sides of the membrane?

Why or why not?

Would pressure be generated if you had 9 mM glucose on one side of a 200 MWCO membrane and 9 mM NaCl on the other side? If so, which solution was generating the pressure?

Would pressure be generated if you had 9 mM albumin on one side of a 200 MWCO membrane and 9 mM NaCl on the other side? If so, which solution was generating the pressure?

Filtration

At the same time that diffusion is allowing cells to take in oxygen and nutrients while expelling carbon dioxide and metabolic wastes, another process is also taking place. This process occurs mainly in capillaries of the body (such as those in the kidneys) where fluid pressure of the blood—called *hydrostatic* pressure—forces materials across a capillary wall. Both blood and interstitial fluid contain dissolved solutes. Usually, the osmotic pressure of the interstitial fluid is not as great as the hydrostatic pressure of the blood, so there is a net movement of fluid and/or solutes out of capillaries—a process called **filtration.** What is filtered out depends solely on the molecular size of the solute and the size of the "pores" in the membrane. Filtration is considered a passive process, since it occurs without the expenditure of metabolic energy.

Activity 5: Filtration

Click on **Experiment** at the top of the screen and select **Filtration.** You will see an opening screen that looks noticeably different from the earlier activities (Figure 1.4). Note the two beakers situated on the left side of the screen, one on top of the other. Note also that the top beaker contains a pressure gauge. Unlike the Osmosis experiment, in which the pressure gauge detected pressure developed due to water movement, this pressure gauge measures the hydrostatic pressure that will filter fluid from the top beaker into the bottom beaker. Finally, note the "Membrane Residue Analysis" box. This will be used to detect if any solutes are left on a membrane after each experimental run.

1. Click and drag the 20 MWCO membrane into the membrane holder between the two beakers.
2. Set Na^+/Cl^- to 9 mM, urea and glucose to 5 mM, and powdered charcoal to 5 mg/ml by clicking the (**+**) button next to each solute. Then click **Dispense** to dispense into the upper beaker.
3. Leave the pressure at 50 mm Hg and the timer at 60 minutes, the default settings. Click on **Start.** You will see fluid being filtered into the bottom beaker.
4. Watch the Filtrate Analysis Unit (next to the bottom beaker) for any activity. This will tell you which solutes are passing through the membrane.
5. When the 60 minutes are up, drag the membrane to the Membrane Residue Analysis unit and let go of your mouse. The membrane will lock into place. Click on **Start Analysis.** In the data box below, you will see what solute(s) were detected on the membrane used for filtration.
6. Record your data by clicking **Record Data.**

What were the results of your initial membrane analysis?

7. Click **Flush** and return the membrane to its original location.
8. Drag the 50 MWCO membrane to the membrane holder between the beakers.
9. Leave the pressure at 50 and repeat the experiment. When the timer has reached 60 minutes, perform a membrane analysis and click **Record Data.**
10. Click **Flush** and return the membrane
11. Repeat steps 8–10 with the remaining two membranes. Be sure to record your data for each run.
12. Increase the pressure to 100 mm Hg and repeat the entire experiment. Again, record all experimental data.
13. Click **Tools** → **Print Data** to print your data.

Does the membrane MWCO affect filtration rate?

Does the amount of pressure applied affect the filtration rate?

Did all solutes pass through all the membranes?

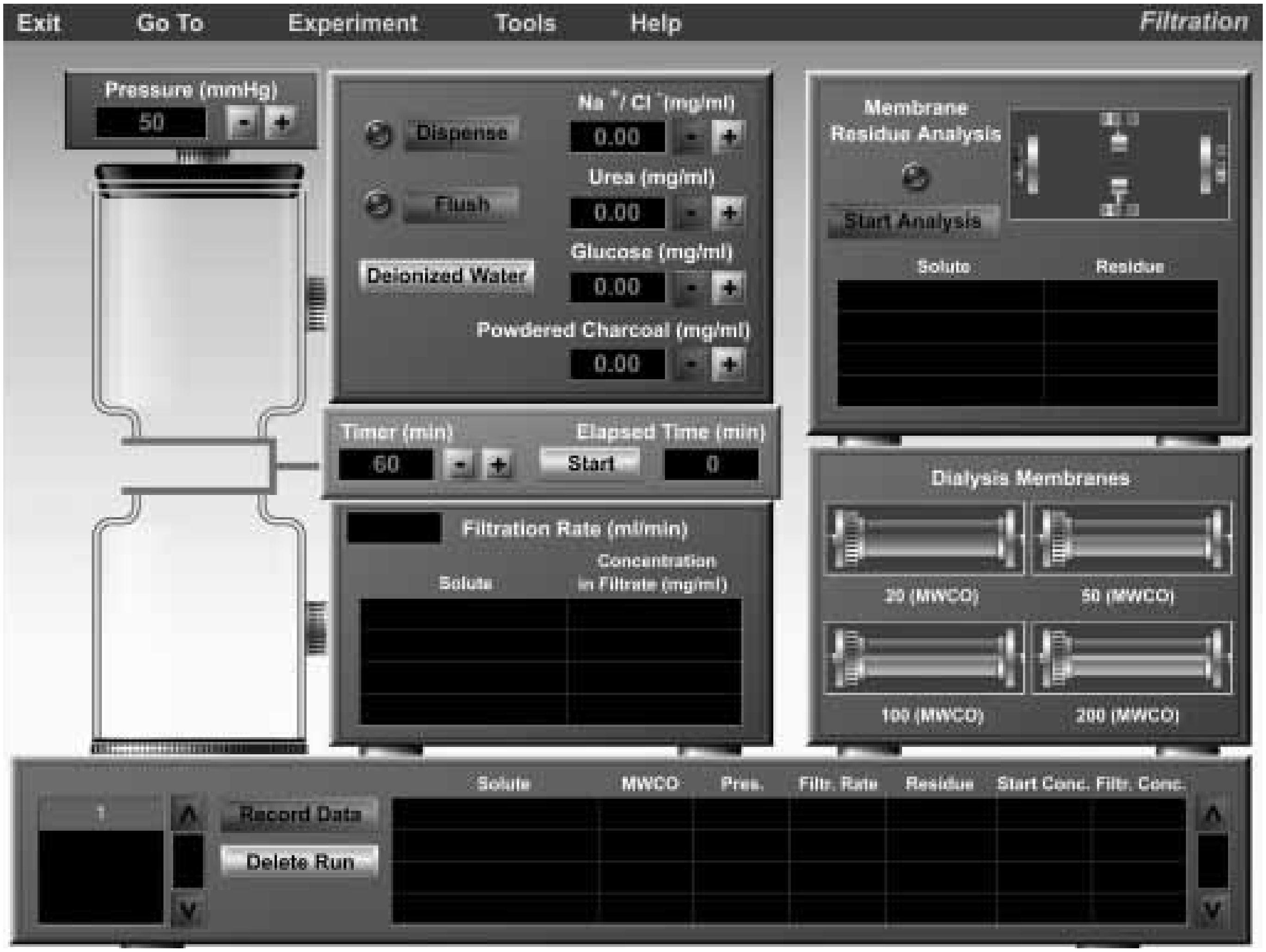

Figure 1.4 Opening screen of the Filtration experiment.

If not, which one(s) did not?

Why?

How can the body selectively increase the filtration rate of a given organ or organ system?

Active Transport

Active transport differs from passive transport in that energy derived from metabolism is used to move solutes across the membrane. It also differs in that solutes are moved from an area of low concentration to an area of high concentration—the opposite of facilitated diffusion. As with facilitated diffusion, binding of a substance to a transporter is required. Since the bound substance is moving "uphill" to an area of higher concentration, the transporters are often spoken of as **pumps.** The net movement from lower to higher concentration and the maintenance of a higher steady-state concentration on one side of a membrane can be achieved only by the continuous input of energy into the active-transport mechanism. The energy input can alter the affinity of the binding site on the transporter so that there is a higher affinity when facing one direction over the other, or the energy may alter the rates at which the transporter moves the binding site from one side of a membrane to the other. As with facilitated diffusion, the number of transport molecules per cell is finite.

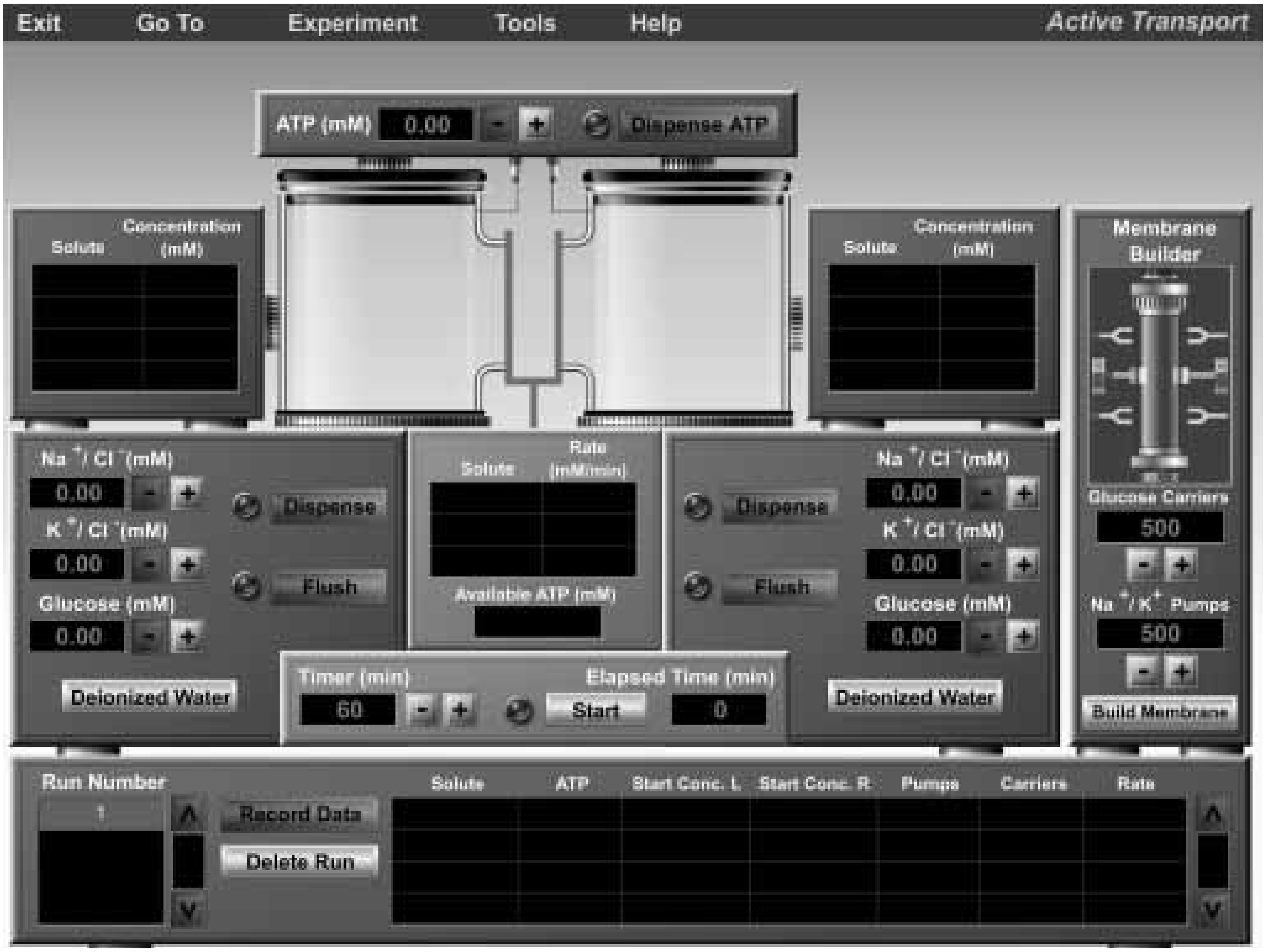

Figure 1.5 **Opening screen of the Active Transport experiment.**

Energy for active transport is derived from cellular metabolism. Inhibition of ATP blocks the active-transport mechanism. In order for solutes to be moved from an area of lower concentration to an area of higher concentration, the transport must be coupled with the flow of energy from a higher energy level to a lower energy level. If ATP is used directly in the transport, the transport mechanism is known as *primary active transport.*

Energy is derived from hydrolysis of ATP by a transporter which is an ATPase that catalyzes the breakdown of ATP and phosphorylates itself. This phosphorylation of the transporter will either alter the affinity of the binding site or the rate of conformational change. Four primary active transport proteins have been identified. In all plasma membranes, there is the sodium-potassium ATPase, responsible for the outward flow of sodium and inward flow of potassium. Sodium is the primary ion found in the extracellular fluid, while potassium is the ion found, for the most part, inside cells. Other transport proteins are involved with calcium transport, hydrogen transport, and hydrogen-potassium transport.

Activity 6:
Active Transport

Click on **Experiment** at the top of the screen and select **Active Transport.** A new screen will appear that resembles the screen from facilitated diffusion (Figure 1.5). The key change is the addition of an ATP dispenser on top of the beakers. Remember, since ATP is needed for the system to run, it must be added for each run.

1. In the membrane builder, be sure that the number of glucose carriers is set at 500 and that the number of Na^+/Cl^- pumps is also set at 500.
2. Click on **Build Membrane.**
3. Drag the "built" membrane to the membrane holder between the two beakers.
4. For the left beaker, set Na^+/Cl^- to 9 mM by clicking the (+) button and click **Dispense.**
5. For the right beaker, click **Deionized Water** and then click **Dispense.**

6. Set ATP to 1 mM and then click **Dispense ATP.**

7. Be sure the Timer is set at 60 minutes, and then click **Start.**

At the end of this experimental run, did the Na^+/Cl^- move from the left vessel to the right vessel?

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Why?

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8. Click **Flush** under both beakers.

9. Add 9 mM Na^+/Cl^- to the left beaker and 9 mM KCl to the right beaker. Click **Dispense**.

10. Set ATP to 1 mM, click **Dispense ATP** and click **Start.**

11. At the end of the run, click **Record Data.**

As the run progresses, the concentrations of the solutes will change in the windows next to the two beakers. The rate will slow down markedly, then stop before completed. Why?

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Now that you have performed the basic experiment, let's conduct two variations.

12. Repeat the experiment, except increase the amount of ATP added to the system.

Does the amount of NaCl/KCl transported change?

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13. Repeat the experiment, except change the number of carriers and pumps when you build the membrane.

Does the amount of solute transported across the membrane change with an increase in carriers or pumps?

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Is one solute more affected than the other?

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Does the membrane you "built" allow simple diffusion?

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If you placed 9 mM NaCl on one side of the membrane and 15 mM on the other side, would there be movement of the NaCl?

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Why?

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Does the amount of ATP added make any difference?

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14. Click **Tools** → **Print Data** to print your recorded data. ■