Exercise 5 - Cell Structure and Membrane Function

Introduction
The cell is the lowest level of biological organization performing all activities of life. Therefore, it is the fundamental unit of structure in living things. As such, the characteristics of cells are of monumental concern to the understanding of biology. The structure of cellular components reflects adaptation to accomplish those functions necessary for life. The collective functions of individual cells allow for the activity and behavior of the entire organism of which those cells are a part.

In this laboratory exercise, you will use a compound light microscope to examine cells and observe cellular activity. You will also conduct experiments illustrating some of the basic mechanisms of cellular transport.

Materials

**Equipment**
- compound light microscope
- microscope slides
- coverslips
- test tubes and racks
- beakers
- droppers
- dialysis tubing
- dental floss or string
- scissors
- triple beam balances
- 95°C water bath

**Biological Specimens**
- *Elodea*

**Reagents and Solutions**
- Benedict’s
- IKI
- 10.0% saline solution
- concentrated glucose
- concentrated starch

Part A: Cellular Transport
Cellular transport mechanisms are typically divided into two categories: **Passive Transport** and **Active Transport**. The basic differences between them is summarized in Table 5.1
Table 5.1 Basic Differences Between Passive and Active Transport

<table>
<thead>
<tr>
<th>Types and Direction of Transported Substances</th>
<th>Passive Transport</th>
<th>Active Transport</th>
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</thead>
<tbody>
<tr>
<td>Involves the movement of water or solute through a semi-permeable membrane down their concentration gradient (i.e., from regions of higher concentration of water or solutes to regions of lower concentration).</td>
<td></td>
<td>Involves the movement of solutes through a semi-permeable membrane against their concentration gradient (i.e., from regions of lower concentration of solutes to regions of higher concentration).</td>
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<tr>
<td>Cellular Energy</td>
<td>Does NOT require cellular energy in the form of ATP.</td>
<td>Requires cellular energy in the form of ATP.</td>
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<tr>
<td>Membrane Transport Proteins</td>
<td>Passive transport systems include two types of diffusion and osmosis:</td>
<td>Requires membrane transport proteins.</td>
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<tr>
<td>Simple diffusion – membrane transport proteins not required</td>
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<td></td>
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<tr>
<td>Facilitated diffusion – membrane transport proteins required</td>
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<tr>
<td>Osmosis – specific to the passive transport of water from an area of higher water concentration to an area of lower water concentration (lower to higher concentration of solutes). Water moves through protein channels known as aquaporins.</td>
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Solutions are often described using the terms hypotonic, hypertonic, and isotonic. Tonicity is a comparative term related to the concentration of solutes in a solution. It may be defined as the ability of a solution to cause a cell to gain or lose water. Hypotonic solutions contain less solute by % (i.e., more water) when compared to hypertonic solutions, which contain more solutes by % (i.e., less water). With a hypotonic solution that is separated from a hypertonic one by a selectively permeable membrane that allows water molecules to pass through but not solutes, the net movement of water molecules will be from a region of high water concentration (i.e., low solute – hypotonic) to a region of lower water concentration (i.e., high solute – hypertonic). Isotonic solutions are equal to one another in solute concentration; therefore, a concentration gradient does not exist and water moves in equal rate back and forth across the membrane.

This exercise will explore some of these basic principles of cellular transport.
Part A1: Passive Transport in a Model Cell

Procedure
1. Obtain a piece of dialysis tubing
2. Working quickly so the dialysis tubing won’t dry out, fold one end and tie off with floss or string
3. Open the other end of the tubing by sliding your fingers back and forth across the top
4. Place 10 ml of concentrated glucose and 10 ml of concentrated starch into the bag
5. Squeeze out the excess air from the bag before folding its other end and tying off
6. Rinse the bag gently under running water at the sink and blot dry with a paper towel. Make sure the bag is not leaking
7. Weigh the bag to the nearest 0.1 g and record as initial mass of the bag in Table 5.2
8. Fill a beaker with enough distilled water to completely submerge the bag
9. Add just enough IKI to the beaker water to turn it light yellow
10. Place the dialysis bag in the beaker. The bag should be fully submerged
11. Let your beaker sit no less than 30 minutes

This model cell system consists of four different molecules which could possibly move through the small holes in the dialysis bag. What are they?

1. _______________ 2. _______________ 3. _______________ 4. _______________

Based on the molecular size of these four molecules, develop a hypothesis to describe which molecules will move into the bag, which will move out and why. Record your hypothesis in Table 5.3

12. After your bag has soaked for the appropriate amount of time (no less than 30 minutes), remove it from the beaker and gently blot dry with a paper towel

   What color is the solution in the bag? _______________
   What color is the solution in the beaker? _______________

13. Weigh the bag again to the nearest 0.1 g and record in Table 5.2
14. Calculate the change in the mass of the bag by subtracting the initial mass from the final mass of the bag and record in Table 5.2
15. Calculate the % mass change of the bag using this formula and record in Table 5.2

$$\% \text{ mass change of bag} = \frac{\text{final bag mass} - \text{initial bag mass}}{\text{initial bag mass}} \times 100$$

<table>
<thead>
<tr>
<th>Table 5.2 Mass and Time of Dialysis Bag Experiment</th>
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<tbody>
<tr>
<td>Mass</td>
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<tr>
<td>------</td>
</tr>
<tr>
<td>Final</td>
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<tr>
<td>Initial</td>
</tr>
<tr>
<td>Change in Mass of Bag</td>
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<tr>
<td>% Mass Change of Bag</td>
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</tbody>
</table>

16. Pour about 1 ml of the contents of the bag into a test tube
17. Test the bag contents with Benedict’s reagent
18. Pour about 1 ml of the contents of the beaker into a test tube
19. Test the beaker contents with Benedict’s
20. Fill in Table 5.3

Table 5.3 Results of Dialysis Bag Experiment

<table>
<thead>
<tr>
<th>Molecular Component of the Dialysis Bag System</th>
<th>Net Movement of Molecules Across the Dialysis Bag (In / Out / None)</th>
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<tbody>
<tr>
<td></td>
<td>Hypothesis</td>
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Part A2: Osmosis in *Elodea*

*Elodea* is a common aquatic plant related to *Hydrilla*. It has leaves of only two layers of thickness.

In this exercise, the thin leaves of *Elodea* will be useful in exploring some of the principles of osmosis. As seen under the compound microscope, the movement of cytoplasm with the *Elodea* leaf cells along the perimeter of the cell called cyclosis or cytoplasmic streaming will be observed.

Procedure

1. Using forceps, remove one leaf from an *Elodea* plant
2. Prepare a wet mount of the leaf using distilled water
3. Observe the leaf at high power under the microscope
4. Identify the parts of an *Elodea* leaf (Fig. 5.1)
   - Where are the chloroplasts located? _______________
   - Do you see cyclosis (cytoplasmic streaming)? _______________
5. Draw your *Elodea* cell and label the visible parts

![Diagram of Elodea cell]

6. Using the replacement staining technique, replace the distilled water under your coverslip with the saline solution
7. After **5-10 minutes**, observe the cells again and make note of any changes that have occurred
8. Draw the cell again

![Diagram of Elodea cell after replacement]

Where are the chloroplasts located now?

What cellular structure (not visible previously) has receded from the cell wall?

What happened to the volume of the central vacuole to cause this change?

In what type (hypotonic, hypertonic, isotonic) of environment is the *Elodea* cell in?
Part A3: Osmoregulation in Protists

Some single-celled organisms live in a fresh water environment that is hypotonic to their cellular fluid which means they are continually taking on water through osmosis. They stay alive because they possess abilities to regulate internal water pressure using contractile vacuoles. These contractile vacuoles remove excess water from the cell. Contractile vacuoles typically appear a “fluid-filled bubbles” in the cytoplasm that slowly get large and then suddenly disappear.

Procedure

1. Using the web, find and view pictures and video clips of contractile vacuole function in Paramecium. Your instructor may also make some clips available online or have you view them in class.
2. Draw a *Paramecium* and label the contractile vacuole

What is its function?

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**Part B: Structure and Motility in Protists**

Most groups of **protists** are capable of movement. This motility is made possible by one of three types of structures. Organisms like **Amoeba** move by means of **pseudopodia** ("false foot") which are extensions of the cytoplasm. **Paramecium** and similar organisms move using **cilia**, fine hair-like structures covering the cell membrane. Organisms typically have many, many cilia. Other protists, such as **Euglena**, move using **flagella**, which are whipped back and forth. Organisms usually have one or just a few flagella. Finally, some protists lack the ability to move at all.

**Procedure**

1. Using the web, find and view pictures and video clips of protist structure and movement. Your instructor may also make some clips available online or have you view them in class.
2. Using online resources and the text book, draw and label the following parts for **Amoeba**, **Paramecium**, and **Euglena**

- cell membrane
- cytoplasm
- pseudopod (**Amoeba**)
- cilia (**Paramecium**)
- flagella (**Euglena**)
- contractile vacuole
- nucleus
- chloroplast (**Euglena**)
- food vacuole
Practice Problems and Review Questions

1. If the initial mass of a dialysis bag was 8.2 g and final mass was 10.9 g, what is the % mass change of the bag?

2. If the initial mass of a dialysis bag was 10.6 g and final mass was 11.1 g, what is the % mass change of the bag?

3. If the initial mass of a dialysis bag was 9.9 g and final mass was 8.8 g, what is the % mass change of the bag?

4. A pre-weighed dialysis bag which contained a solution of 10% glucose was placed in a beaker containing a solution of 20% glucose. After one hour, the bag was weighed again. Calculate the % mass change of this dialysis bag from the following information:
   - Mass of bag before experiment: 15.3 g
   - Mass of bag after experiment: 12.7 g

5. Was the beaker solution in question 4 hypertonic, hypotonic, or isotonic to the dialysis bag contents?

6. What are the major differences between the following pairs of cells?
   - prokaryotic and eukaryotic
7. How was the dialysis bag in your experiment an example of a semi-permeable membrane?

8. Define these terms:
   - hypertonic
   - hypotonic
   - isotonic

9. Complete the following sentence: When two aqueous solutions are separated by a semi-permeable membrane, the net water movement is always from a _______tonic to a _______tonic solution.