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Title: **Diffusion Across Biological Membranes: A Simulation**

Authors: Dan Flerlage, Alternatives Community School, Ithaca, NY, 14850
Dick Ecklund, Cornell University, Ithaca, NY, 14853.
As revised by Glenn Simpson, Victor High School, Victor, NY, 14564.

Appropriate Level: Regents and Honors level, modifiable to AP level.

Abstract: This lab uses two different sizes of dialysis tubing to represent cellular and organelle membranes. Students design experiments in which they place solutions of iodine, starch, and glucose on different sides of a membrane. The movement of these materials is monitored with the use of indicator solutions.
Students are given a list of tasks, a description of supplies and equipment, and some instructions on how to get started. They are then asked to design a set of experiments that will allow them to accomplish as many of the tasks as they can in the allotted time.

Time Required: Day One- 45 minutes
Day Two- 25 minutes
Day Three - 45 minutes

Special Needs: Requires two sizes of dialysis tubing (see “Instructor's Materials” for sources).

Living Environment: **1-Inquiry, analysis, design**: 1-Purpose of scientific inquiry, 1.1a,1.3a; 2-Research plan, hypothesis, 2.1,2.3a-c,2.4; 3-Analysis of results, 3.1, 3.3, 3.4a; **4-Content**: 1-Living things, 1.2a,c,f-i; 5-Dynamic equilibrium, 5.2a,5.3a,b

Additional Teacher Information

Objectives:

Using dialysis tubing to simulate a biological membrane, this lab will demonstrate that:

1. Some molecules can pass through a membrane simply as the result of diffusion and that this same process can account for the movement of some molecules into or out of cells and organelles.

2. Membranes can have selectivity in the sense that not all molecules can freely pass through them.

3. The net diffusion of molecules proceeds from a region of higher concentration to a region of lower concentration.

4. Indicator solutions can be used to determine the presence of specific molecules.

Level of Lab:

This lab is appropriate for Regents level high school biology classes. It can be simplified for other groups in the following ways:

1. Omit the use of the organelle completely and offer only solutions of iodine and starch; or

2. Use only solutions of starch, iodine, and distilled H_2O. This simplifies the set-up and analysis, while still addressing all of the objectives.

The lab can be made more challenging by addressing the process of osmosis in addition to that of diffusion. This can be accomplished by determining the initial mass or volume of each of the solutions used, and then comparing them to the final masses or volumes.

Safety Tips:

A number of chemicals used in this lab are potentially harmful if used improperly. Follow all label precautions. Wear safety glasses and gloves when handling toxic or corrosive chemicals. Good laboratory practice should be followed at all times including, no food, eating or drinking in the laboratory.

Time Requirement:

This lab requires student involvement on three different days:

Day 1: one full 45 minute period
Introduction, presentation of materials, demonstration of diffusion across a membrane, use

of indicators, group discussion, and experimental design. Students hand in their experimental design.

Day 2: one half period
Return student's experimental designs, give additional help as needed. Students work in groups to set up their experiments. It will take 1 - 2 days for the experiments to come to equilibrium.

Day 3: one full 45 minute period
Data collection, analysis through the use of indicators, completion of written reports.

Information which students should be familiar:

1. The cell is the structural unit of living things.
2. All cells are surrounded by membranes.
3. Organelles are membrane-bound compartments inside eukaryotic cells, which perform specialized functions.
4. In order to stay alive, cells need to take in nutrients and rid themselves of wastes. These materials must pass across the membrane.
5. Diffusion is a random process in which molecules move from areas of higher concentration into areas of lower concentration.
6. Diffusion doesn't stop when the concentration of molecules on both sides of a membrane are equal. Rather, molecules continue to diffuse equally in both directions. In this equilibrium situation, molecules are said to be in dynamic equilibrium.
7. Diffusion is a passive process. It requires no input of energy from the cell.

Optional for advanced students:

8. The more observant student may notice a change in the volume of the dialysis bag. This is an obvious lead-in for a discussion of osmosis.
9. Normally, at equilibrium, the concentration of a given substance is the same on both sides of a membrane. In some circumstances, however, equilibrium is not achieved because molecules on one side of a membrane become trapped by binding to macromolecules or by conversion to some other molecule. In such cases, concentration gradients are formed. Cells use the strategy of "trapping" or changing specific molecules on one side of a membrane in order to establish a concentration gradient across that membrane.
10. Concentration gradients across biological membranes, a fundamental aspect of all living cells, are required for the proper function of cells.

Chemicals, Materials, and Equipment:

Chemicals:

- Starch (corn or any other starch)
- Glucose
- Sodium bicarbonate*
- Bromthymol blue
- Acetic acid
- Benedict's Solution
- Iodine
- Sodium citrate*
- Copper sulfate*
- Sodium hydroxide
- Distilled water

*ingredients for Benedict's Sol.

Special materials:

- Dialysis tubing (Carolina Biological Supply, 2700 York Road, Burlington, NC 27215, 1-800-334-5551):
  1 inch flat width -- 10 foot roll, Catalog # 68-4212, approx. $4
  3 inch flat width -- 50 foot roll, Catalog # 68-4234, approx. $50
- Dental floss
- Test strips for diabetes - optional. Can be purchased at most drug stores.

Equipment for preparing solutions and for demonstration:

- Filter funnel and filters
- Graduated cylinders (100 and 1000 ml)
- Beakers (100, 250, and 1000 ml)
- Wash bottle with distilled water

Equipment and supplies for students:

- Water baths (one for 3-4 groups)
- Test tube rack (1 per group)
- Marking tape
- Test tubes (3-4 per group)
- 10 ml grad. cylinder (1 per group)
- 2 ml pipettes (1 per group)

Preparation of Solutions:

Starch Solution - (approx. 1 liter)

Add 10 g of starch (corn starch will do) to 50 ml of cold, distilled H_2O. Stir to form a thin paste. Add to 1000 ml of boiling distilled H_2O. Stir for 2 minutes then allow it to cool.

Iodine Solution - (approx. 1 liter)
Add 1.5 g of potassium iodide and 0.3 g of iodine to 1000 ml of distilled H_2O.

Glucose Solution - (approx. 1 liter)
Add 125 g of glucose (dextrose) to 1000 ml of distilled H_2O.

Benedict's Solution - (approx. 1 liter)
Dissolve 173 g of sodium citrate and 100 g of sodium bicarbonate in 700 ml of distilled H_2O. (Use heat as required.) Filter. Dissolve 17.3 g of copper sulfate in 100 ml of distilled H_2O. Slowly, add copper sulfate solution to the sodium citrate solution. Add H_2O to bring the total volume to 1 liter. Benedict's reagent can alternately be purchased from Connecticut Valley Biological Supply (1-800-628-7748, Catalog # BR-640A (1 pint). *NOTE: Instead of using Benedict's solution to test for glucose, you can use test strips for diabetes. They are available in most local drug stores.*

Demonstration Solutions:

Bromthymol blue stock solution (0.1%)
Add 0.5 g of bromthymol blue to 500 ml of distilled H_2O. (**Caution**: Bromthymol blue is toxic if ingested!)

1 M Sodium Hydroxide Solution
Dissolve 40 g of NaOH in 200 ml of distilled H_2O. Dilute with H_2O to make 1 liter of solution. (**Caution:** NaOH is very caustic)

20% Acetic Acid

Preparation of dialysis tubing
Pre-soak the tubing in warm distilled H_2O for at least an hour before lab; overnight soaking is fine. The soaked tubing can be stored in the refrigerator under water.

Lab Format:

This lab requires approx. 2 ½ periods. The lab handout should be given to the students well in advance and students should read it in its entirety before class. Begin the first day with a discussion of the purpose of the lab and a clarification of what the students are being asked to accomplish. Explain that they will have the opportunity to make decisions regarding the design of the lab, and that this will affect the information they obtain. Go over, in general terms, the use of indicators as a tool in biology. Have the students read through their section entitled “Getting Started.” Clarify this section as necessary and then have the students work in groups to complete this section of their lab.

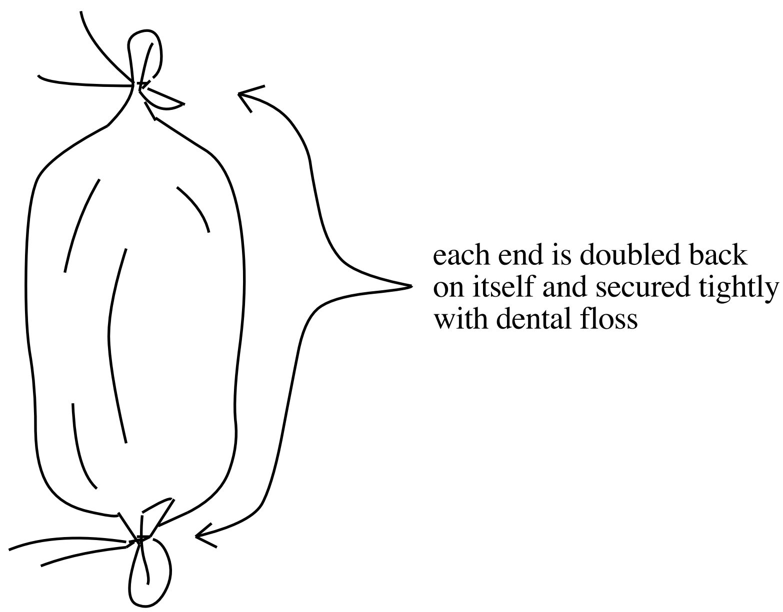
When the students reach the section entitled “Membrane Simulation” perform the following demonstration of diffusion. The demonstration will help students to visualize a set-up they might use in their experiments.

1. Place enough 0.1% bromthymol blue solution into a 1 inch wide dialysis tubing to create a "bag" approximately 5-10 cm in length. (Secure each end of the tubing with dental floss, being sure to double the tubing back on itself before tying.) Bromthymol blue is an

indicator which has a range of pH 6.0 - pH 7.6. It exhibits a blue color in an alkaline solution, and a yellow color in an acidic solution.

2. Prepare two 250 ml beakers. In one, place 150 ml of a 1M sodium hydroxide solution. In the other, place 150 ml of 20% acetic acid. Set the beakers side by side. First gently lower the bromthymol bag (by a string) into the beaker containing the sodium hydroxide. Within two minutes, the bromthymol solution will turn dark blue. The hydroxide ions in the sodium hydroxide solution diffuse into the bromthymol solution, raising the pH and turning the indicator blue. Stress the importance of rinsing the outside of each bag before use to ensure that the solution is present only within the membrane. Then place the bromthymol bag into the acid. In the presence of the acid, enough hydrogen ions diffuse into the bag to lower the pH, resulting in a change back to the original color.

After the demonstration, the students will break up into groups to discuss how best to address the purpose of their experiments. Encourage each group to think through a number of experimental designs. In each case, have them write down any predictions they feel they can make, given their set-up. Ultimately, have each group choose the design that they feel will best address their "Purpose." Have the students hand in an illustration of their experimental design, a description of the tests that they will perform after equilibrium has been established, and a list of the possible outcomes of their experiment. Provide the students with written feedback on their designs at the beginning of the next period (Day 2). Work with the groups that still need help finalizing their designs. Before students start assembling their equipment, review the use of dialysis tubing, with special emphasis given to the closure technique illustrated below:



Allow at least 24 hours for diffusion before analysis of results. Begin Day 3 with a review of the use of indicators to determine whether glucose or starch, for example, has moved across a membrane. Emphasize that only small volumes of the solution (2 ml) need to be removed in order to determine the presence or absence of these molecules, and that they can remove samples that they wish to test from any chamber. Finally, go over the data chart included in their lab handout; review how to use it as an aid to keeping track of information.

Expected Results (from the student handout, "Purpose"):

A and B. Both iodine and glucose should diffuse across the dialysis tubing. They should move in both directions across the membranes.

C. Starch should not cross any membrane (the starch molecules are too large to fit through the pores in the dialysis tubing).

D. Iodine binds strongly to starch and, in doing so, turns purple. With time, more and more iodine molecules bind. Because of this, iodine will not reach a dynamic equilibrium in these simulations. The process can be highlighted by setting up a demonstration in which a membrane containing only starch solution is suspended in a beaker containing only iodine solution, and leaving it in plain view for several days.

Final Class Discussion:

Because students have the freedom to choose where they place various solutions, some designs will not provide information pertinent to each of the tasks assigned. This can be dealt with in a follow up discussion. Each student group should make a presentation to the class showing their "Design" and their results. (Here simple line diagrams on the board or a poster showing where each substance started is helpful.) Students will find it interesting to hear what results were gained from designs other than their own. It should be stressed that completing all potential tasks is less important than fully analyzing their results. It should also be pointed out that in order for the students to answer the questions under "Related Materials/Extending the Concepts," they may have to include information gained during class discussion as well as information found in their texts.

Finally, a class discussion format is an excellent way to highlight one of the more subtle but interesting aspects of the lab . . . that iodine, when used in conjunction with a starch solution, fails to reach a dynamic equilibrium (due to becoming "trapped" by the large starch molecules). Here it would be instructive to brainstorm examples within living cells where a strategy such as this is used to the cells' advantage (see student's lab "Related Material/Extending the Concepts" #4).

Diffusion Across Biological Membranes: A Simulation

New York State Learning Standards

Standard 1: Inquiry Analysis and Design

Key Idea 1: The purpose of scientific inquiry is to develop explanations of natural phenomena in a continuing and creative process.

1.1- Elaborate on basic scientific and personal explanations of natural phenomena and develop extended visual models and mathematical formulations to represent one's thinking.

1.3- Work towards reconciling competing explanations; clarify points of agreement and disagreement.

Key Idea 2: Scientific inquiry involves the testing of proposed explanations involving the use of conventional techniques and procedures and usually requiring considerable ingenuity.

2.1- Devise ways of making observations to test proposed explanations.

2.3- Develop and present proposals including formal hypotheses to test explanations; i.e., predict what should be observed under specific conditions of the explanation is true.

2.4- Carry out a research plan for testing the explanations, including selecting and developing techniques, acquiring and building apparatus, and recording observations as necessary.

Key Idea 3: The observations made while testing proposed explanations, when analyzed using conventional and invented methods, provide new insights into natural phenomena.

3.1- Use various methods of representing and organizing observations (i.e., diagrams, tables, charts, graphs, equations, matrices) and insightfully interpret the organized data.

3.3- Assess correspondence between the predicted result contained in the hypothesis and actual result, and reach a conclusion as to whether the explanation on which the prediction was based is supported.

3.4- Based on the results of the test and through public discussion, revise the explanation and contemplate additional research.

Standard 4: Content

Key Idea 1: Living things are both similar and to and different from each other and from nonliving things.

1.1- Describe and explain the structures and functions of the human body at different organizational levels

Key Idea 5: Organisms maintain a dynamic equilibrium that sustains life.

5.2- Explain disease as a failure of homeostasis

5.3- Relate processes at the system level to the cellular level in order to explain dynamic equilibrium in multi-celled organisms.

Key Idea 6: Plants and animals depend on each other and their physical environment

6.1- Explain factors that limit growth of individuals and populations.

Diffusion Across Biological Membranes: A Simulation

CIBT
Student Laboratory Exercise

Background Concepts:

Living things ,whether they are huge and multicellular (a blue whale), or tiny and unicellular (an amoeba or bacterium) are composed of cells that are enclosed by a membrane. Staying alive requires a flow of materials into and out of cells, and these materials must of necessity pass through the membrane. Nutrients to build new cellular parts or to fuel cellular processes must continuously enter the cell, whereas waste materials as well as other products are continuously being expelled. Much of the movement of molecules into and out of cells occurs by means of **diffusion**, which is ***the movement of molecules from a region of higher concentration to a region of lower concentration***. Diffusion occurs spontaneously and doesn't cost the cell anything in terms of expenditure of energy…it's basically "free."

Purpose:

Together with other members of your lab group, you will design, conduct, and analyze the results of one or more simulations. In these exercises, dialysis tubing will be used to simulate the outer membrane of a cell and the membrane surrounding organelles within cells. Dialysis tubing, which is made from a kind of plastic, has one of the important characteristics of biological membranes: it will allow some kinds of molecules to move across and not others. In this way the membrane is **selectively permeable**. In addition to dialysis tubing, you will be provided with substances that you may use to represent different cellular components, together with reagents and procedures for "tracking" these substances.

Choose one or more of the simulations described below as your task:

1. Simulate a situation in which molecules move by diffusion across a membrane <u>into</u> a "cell."
2. Simulate a situation in which molecules move by diffusion across a membrane <u>out of</u> a "cell."
3. Simulate a situation in which molecules <u>cannot</u> move by diffusion across a membrane either into or out of a "cell."
4. Simulate a situation in which molecules move by diffusion across a membrane into or out of an "organelle."

Supplies and Equipment:

Day 1:
- distilled H_20
- starch solution
- iodine solution
- glucose solution
- test tubes (3-4/group)
- test tube rack
- 10 ml graduated cylinder
- marking tape
- Benedict's test solution or test strips
- wax pencil
- hot water bath (one for every 2-3 lab groups)

Day 2:
- distilled H_20
- starch solution
- iodine solution
- glucose solution
- dental floss
- beakers (1000 ml) or quart jars
- dialysis tubing: 1 inch flat width, pre-soaked
- dialysis tubing: 3 inch flat width, pre-soaked

Day 3:
- starch solution
- iodine solution
- Benedict's solution or test strips
- 10 ml graduated cylinder
- test tubes (3-4/group)
- test tube rack
- marking tape or wax pencil
- pipettes
- hot water bath (one for every 2-3 lab groups)

Getting Started:

A. Substances and How to Track Them:

Your lab group is provided with 4 different substances **which can be used to represent cellular components** : distilled water, starch, iodine, and glucose. Each substance may or may not be diffusible through your membrane. In order to accomplish your simulation you will need to be able to detect the presence of each substance in order to see whether they have indeed crossed a membrane. We have provided you with reagents that you can use to detect starch, iodine, and glucose.

Try This: Iodine is an indicator for a particular substance. It changes color when added to this one particular substance but not when added to other substances. Does iodine react with H_2O? With starch? With glucose? To answer this, do the following.

Using a marker, label three test tubes, H_2O, starch, and glucose.

Add 2 ml of distilled H_20 to one test tube (**use a rinsed graduated cylinder)** then add 2 ml of the starch solution to a second test tube (rinse the cylinder) and add 2 ml of the glucose solution to a third test tube. Add 2 ml of the iodine solution to each tube.

Record on your Data Sheet, in the appropriate space, any changes in color which occur.

Try This Also:

Benedict's solution is also an indicator for a particular substance. Determine which of the four substances below changes color when added to Benedict's solution and heated in a hot water bath for three minutes.

Add the following to 4 separate test tubes:
- 2 ml of water
- 2 ml of the starch solution
- 2 ml of the glucose solution
- 2 ml of the iodine solution

Add 2 ml of Benedict's test solution to each tube. **(CAUTION: Benedict's solution is toxic if swallowed.)**

Place the test tubes in a hot water bath for three minutes.

Record on your Data Sheet, in the appropriate space, any changes in color that occur.

B. Membrane Simulations:

Sections of dialysis tubing are used here to simulate cell membranes. Dialysis tubing is semi-permeable; that is, it is permeable to some molecules and impermeable to others, depending on the size of the molecules. Think about the relative sizes of the molecules you are testing. An iodine molecule is composed of two iodine atoms. The molecular formula for glucose is $C_6H_{12}O_6$ and a molecular formula for starch is $C_{300}H_{520}O_{260}$. Water has the molecular formula H_2O.

Predict whether the molecules you are given are too large to pass through dialysis tubing or small enough to pass through? Circle your answer on the Data Sheet.

Observe the demonstration given by your lab instructor at this time.

Note that a section of 3 inch wide dialysis tubing can act nicely as a cell membrane, whereas a section of the 1 inch size tubing can be used as an organelle.

Procedure:

<u>Day 1</u>: Now that you are more familiar with dialysis tubing, and how to track starch, glucose, and iodine, you are ready to design your "Cell Simulation." Go back and read the "Purpose" section. Also look over the information you generated while completing the section "Getting Started." Discuss with the other members of your group how you will design your "Cell simulation." You have four solutions with which to work and two different sizes of dialysis tubing with which you can simulate cell and organelle membranes. You can place any of the four solutions wherever you think best. Once your lab group has chosen a design, complete the "Cell Simulation Design Sheet" by **drawing an illustration** showing which solutions will be placed where. Write down the predictions your group feels comfortable making with regard to the behavior of the substances in your "Cell Simulation." Hand your Design Sheet in to your teacher for approval.

<u>Day 2</u>: Using the design you developed last period and any feedback from your instructor, you can now build your "cell." Watch as your teacher demonstrates the use of dialysis tubing. Be extremely careful to firmly secure each end of the tubing with dental floss.

> **NOTE:** When setting up your lab be sure to **rinse the outside of each "bag"** after it is filled and tied off. This will ensure that the material placed inside is only present within the membrane.
>
> With these precautions in mind, build your "cell" and set it aside for 1-2 days.
>
> **Record** your chosen location of the solutions on the chart provided on your Data Sheet.

<u>Day 3</u>: Your substances have now had approximately 24 hours for diffusion to occur. Today you will determine whether these substances have in fact diffused across the membranes in your simulation. For instance, you will need to test the solution you originally placed in the organelle to see whether any of the substances from other compartments in your simulation have moved across the membrane. Use the indicators available to determine the current location of each substance. You may want to review the use of indicators under the section entitled "Getting Started." Remove only a small amount (about 2 ml) of solution from each compartment of your simulation for a given test. Add this to an equal amount of indicator solution and record your findings in the chart provided on your Data Sheet in section 6 .

Putting the Information Together:

Now go back to the section labeled "Purpose." Which of the four tasks in that section relates to your simulation? Summarize the design and results of your simulation(s). For those tasks that you did not address, how would you design simulations for those situations?

Related Material / Extending the Concepts:

After completion of the lab and class discussion, please answer the following questions **in complete sentences**.

1. What molecules (found outside of a cell, but used inside a cell) might enter a living cell by passing through its cell membrane by diffusion?

2. What molecules (generated inside a cell, but dangerous to the cell in high concentrations) might leave a living cell by passing through its cell membrane by diffusion?

3. What molecules (inside or outside of a cell) might the cellular membrane prove impermeable to? How might this impermeability help or hinder the living cell?

4. Choose one of the following and explain how it was simulated in these exercises:
   - O_2 continuously being provided to mitochondria within a cell.
   - CO_2 continuously being provided to chloroplasts within a cell.
   - Amino acids continuously being provided to the cytoplasm within cells.

"CELL SIMULATION" DESIGN SHEET

Draw the design of your "Cell Simulation." Include in your drawing the cell membrane, organelle membrane, and the location of each substance.

DATA SHEET

Predictions:

Fill in the following chart showing where each substance was originally placed and whether diffusion across the membrane will occur:

Substance	Original Location	Where the Substance Has Moved
Starch		
Glucose		
Iodine		

A. SUBSTANCES AND HOW TO TRACK THEM:

Original Solution	Added	Observed Results
Distilled H_2O	Iodine	
	Benedict's	
Starch	Iodine	
	Benedict's	
Glucose	Iodine	
	Benedict's	
Iodine	Benedict's	

Please answer all of the following questions in complete sentences.

1. What does Iodine test for ?

2. What does Benedict's reagent test for?

3. Why did you use water in one of the tubes?

4. Draw a conclusion from the above activity.

5. For each of these molecules, make a guess whether the molecule is too large to pass through dialysis tubing or small enough to pass through? Circle your answer.

Iodine: I_2
too large / small enough

Water: H_2O
too large / small enough

Glucose: $C_6H_{12}O_6$
too large / small enough

Starch: $C_{300}H_{520}O_{260}$
too large / small enough

6. **Chart of Substance Movement after 24 hrs.** Fill in from your Day 3 observations.

Solution Location:	Originally Contained:	After 24 hrs. Indicators Show:		
		Starch	Iodine	Glucose
Outside of Cell		Yes or No	Yes or No	Yes or No
Inside of Cell		Yes or No	Yes or No	Yes or No
Within Organelle		Yes or No	Yes or No	Yes or No

Putting the Information Together

1. Which of the four tasks relates to your simulation?

2. Summarize the design and results of your cell simulation(s).

3. Design simulations for those tasks (A, B, C, or D) that you did not address.

Related Material / Extending the Concepts:

1. What molecules (found outside of a cell, but used inside a cell) might enter a living cell by passing through its cell membrane by diffusion?

2. What molecules (generated inside a cell, but dangerous to the cell in high concentrations) might leave a living cell by passing through its cell membrane by diffusion?

3. What molecules (inside or outside of a cell) might the cellular membrane prove impermeable to? How might this impermeability help or hinder the living cell?

4. Choose one of the following and explain how it was simulated in these exercises:
    a. O_2 continuously being provided to mitochondria within a cell.
    b. CO_2 continuously being provided to chloroplasts within a cell.
    c. Amino acids continuously being provided to the cytoplasm within cells.