#6800 ORGANELLES
Teacher and Student Instructions

This kit provides an introduction to subcellular components (organelles) and internal cell membranes suitable for secondary biology or life science courses. The materials provided will allow 15 students or groups of students to first observe organelles in plant cells, then to simulate the function of an organelle and organelle membrane, and finally, with the help of Janus Green B stain, to observe an organelle (mitochondrion) in action. Observations require access to microscopes of 3OOX capability, or better. Students with no background in biology can benefit from these activities, but it is useful if the students have some exposure to the concept of cells, cell structure, cell components, and enzyme activity prior to the use of the kit.

Contents:

- Microscope Slides (72)
- Cover Slips (100)
- Forceps (6)
- Razor Blades (15)
- Plastic Cups (15)
- Janus Green B Stain (1 - 30 ml bottle)
- Dialysis Tubing (4 meters)
- Hydrogen Peroxide Solution(1 - 240 ml bottle)
- Twist Ties (60)

- Teacher Manual (1)
- Student Instruction Master (1)

Additional Required Materials:

- Fresh Celery (4 to 5 stalks)
- Potato
- Microscopes (300X or higher)
- Funnels

ORGANELLES #6800 Teacher Manual

Introduction

Since the development of the microscope in the 17th century, much of biological study has focused on the cell. In recent years, the techniques of electron microscopy and radiography have led to new understandings of the structure, composition, and workings of the cell. Electron microscopy has revealed much information about the subcellular components of eukaryotic cells, the organelles. Organelles accomplish a "division of labor" within the cell, each responsible for specific functions of the cell's wide requirements, such as the breakdown of food for energy, synthesis of new materials, maintenance of genetic information, storage, packaging, regulation, etc.

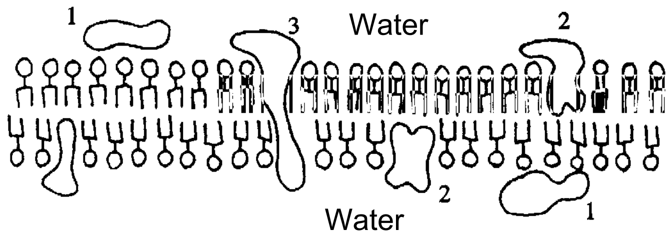
Background Information

Organelles are at the center of the study of modern biology. The organelles form an interdependent system of individual pieces that come together to form a living cell. Some examples of organelles are the mitochondria, plastids, ribosomes, endoplasmic reticulum, lysosome, and vacuoles. The mitochondria is the powerhouse of the cell, chemically breaking down food to form the energy used by the other organelles in the cell. *Plastids,* specifically *chloroplasts,* are the organelles containing photosynthetic pigments such as chlorophyll that enable plants to manufacture their own food. *Ribosomes* manufacture proteins for the cell, and are often found on the surface of the *endoplasmic reticulum,* a network of membranes that runs throughout the inside of a cell. *Lysosome* store the potentially harmful enzymes which are used in the digestion of food and old organelles, and vacuoles store food for digestion. The mitochondria and lysosome will be described in greater detail later in the text. The Science Source Kit #1700 on Cell Structure may be useful for further information and study of the organelles and cell structure.

Organelle Membranes

Organelles are processing stations that absorb raw materials, process them, and release a final product. Each organelle is an island of biochemical interactions, separated from the rest of the cell by a membrane. This common feature among the organelles, the membrane, has been the source of much study. It has been discovered that the organelle membrane has an amazing ability to control which substances stay inside or outside, and which ones travel into or out of the organelle.

The study of membrane composition, with the help of the electron microscope, has revealed that the organelles membranes, as well as the plasma membrane surrounding the entire cell, are composed of lipids and proteins. These lipids are molecules which have one hydrophobic ("water hating") area and one hydrophilic ("water loving") area. This means that, given the chance, these molecules will align themselves so that only the hydrophilic areas are exposed to water. Since cells live in a water environment, and have a watery cell and organelles cytoplasm, the molecules arrange themselves in the following manner. This structure reveals as few hydrophobic areas to the watery environs as possible:

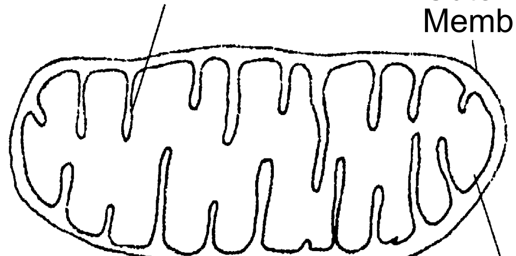


In this structure there are two layers of lipids facing each other, with their hydrophilic "heads pointing out into the cell cytoplasm or organelles cytoplasm, and the hydrophobic "tails" facing in toward each other. The large globular structures shown in the diagram represent proteins, which can be on the surface of the membrane (1), partially embedded within the membrane (2), or entirely embedded within the membrane so that parts of the protein are revealed on either side (3).

Membrane Control

Working together, these two parts of the organelles membrane, the lipids and proteins, achieve selective permeability; only the molecules that the organelle "wants" to go through will go through. The permeability of a molecule depends on its size, shape, polarity (distribution of partial charges), and charge (degree of ionization). The lipids will allow many small molecules such as water to pass through the membrane through passive diffusion. Diffusion is the process through which molecules move "down the concentration gradient," from areas of high concentrations to areas of low concentration. If, for example, there are many more molecules of water per cubic centimeter of fluid outside the organelle than there are inside the organelle, then the water will tend to flow into the organelle, and vice versa.

Larger molecules, and ions that carry charges, cannot diffuse so easily through the lipid layer. Trans- membrane proteins form ways for these molecules to pass into the organelle. These proteins can form open channels that allow selective transport; they will allow ion "A" into the organelle, but not ion "B"; will allow ion "C" out of the organelle, but not ion "D". This type of transport is also dependent on diffusion. What if the organelle wishes to have more of a certain molecule, but the concentration of the molecule is already higher inside the organelle than outside? Again the proteins that run through the membrane solve the problem. Through a process called "active transport", the membrane proteins pump the necessary molecule into the cell. Unlike the other methods of transport, active transport requires the use of energy, in the form of ATP (adenosine triphosphate).



The protein and lipid composition of the membrane is also capable of enzymatic activity, which is the helping (catalysis) of specific chemical reactions to occur. This topic will come up again in the discussion of the mitochondria.

Examples of Organelles

Two examples of organelles that rely heavily on the organelles membrane are the *mitochondria*

and the *lysosome*. Found in all aerobic (oxygen using) cells, the mitochondrion is a double membrane sack that looks rather like a sausage. Mitochondria are responsible for the oxidative metabolism of energy sources. Simply put, the mitochondria "burns" food in a very controlled fashion, and changes the energy thus released into chemical energy, ATP, that the
cell can store and use as needed. The inner membrane in this double membrane structure is highly convoluted into folds called *cristae* and the outer membrane is smooth.

The proteins of the mitochondrial membranes are specialized to perform the various tasks involved in oxidative metabolism. This energy producing cycle is divided into three separate processes: *glycolysis, The Krebs cycle*, and the *electron transport system*. Glycolysis is a simple series of steps which manipulate and break down molecules of glucose in such a way to produce ATP. This process can occur anywhere in the cytoplasm of the cell. *The Kreb's cycle*, also called the *citric acid cycle*, takes place within the mitochondria, and is a more complex system than glycolysis. Through a series of chemical manipulations, the Kreb's cycle also produces energy in the form of ATP. The last process, the *electron transport system*, is an even more complex chain of chemical processes that takes place on the cristae of the mitochondria. Glycolysis, the Kreb's cycle, and the electron transport system are linked together, each system producing the necessary molecules for the next energy producing process. Through a complex series of reactions, the electron transport chain releases much more energy than the other two processes combined.

The lysosome is a membrane bound sack of digestive enzymes found in the cytoplasm of a cell. These enzymes break down foreign material and dead or malfunctioning parts of the cell. The enzymes contained by the lysosome are segregated from the rest of the cell, which is a necessity considering the fact that, if free within the cell, these enzymes are capable of digesting any cellular material. This concentration of enzymes also provides a more efficient approach to the digestion of cellular and food particles. A lysosome will fuse with food vacuoles, and the enzymes will act on the ingested material. A lysosome will also form around a damaged cell part and release their digestive enzymes. Mitochondria and lysosomes are two examples of the wide variety of shapes and functions that organelles may assume. They are also an example of some functions of organelle membranes. In the case of the mitochondria, the membrane serves to localize energy producing reactions, as well as provides enzymes (proteins) which catalyze such reactions. The membrane of the lysosome serves to localize destructive digestive enzymes within the cell, and to provide a concentration of such enzymes which allows an efficient attack on the materials to be digested.

Side Reactions

As organelles process materials, they may undergo reactions which are not directly involved in active metabolism. These by-products may not have the same permeability as the raw products, and may build up inside the organelle. Various membrane and enzyme materials within the organelle have a limited lifetime, regardless of the presence of potentially harmful by-products, and might not be replaced. Either way, the organelle will eventually stop functioning and must be replaced. Mitochondria are known to undergo fission reproduction, and might also be synthesized from small vesicles present within the cell. Organelles such as vacuoles, lysosome, and endoplasmic reticulum are formed from existing membranes within the cell.

The Origin of Organelles

The origin of organelles is a question that has arisen in the study of cellular life. Prokaryotic cells, such as bacteria, do not have any internal membranes or membrane bound organelles, whereas eukaryotic cells, such as animal cells, do. How did organelles arise within a cell? One theory suggests that a primitive heterotrophic (cannot make its own food) cell developed organelles from "out pouches" of the cell membrane. Another theory is that organelles were originally free living cells that were engulfed by, and then developed a symbiotic relationship with, other larger cells.

A substantial amount of evidence supports this second theory. Mitochondria and chloroplasts contain their own set of nucleic acid material, and their own complete ribosomes. These ribosomes are not the same as those produced by the rest of the cell, and are more similar to those found in bacteria and blue-green algae. Mitochondria and chloroplasts are both capable of fission independent of the rest of the cell. In addition, the membranes of the mitochondrion and chloroplast are highly specialized, and are distinctly different in composition from those membranes found in the rest of the cell.

Using The Kit In The Classroom

Objectives

The kit is intended primarily for use by high school and junior high school students of the life sciences. Prior to using these activities, the student should have some exposure to the concept of cells as a basic unit of life, capable of complex chemical activity. In addition, some background of enzymes is useful, although not essential. Upon completion of the activities in this kit, the student shall:

- Be familiar with a model for cellular organelles, and be able to relate the following features of the model to the life processes of an organelle: selective membrane permeability, localization of enzyme activity, chemical processing of raw materials, production and storage of material, and side reactions affecting the lifetime of an organelle.

- Develop the idea of organelle importance in terms of specialization of function and efficiency of activity .

- Observe the presence of organelles in living tissue, and evidence of their chemical activity. The student shall be able to relate these results to the concept of enzymatic activity within the organelle.

- Be able to interpret observable changes in biological systems in terms of enzymatic activity, and shall be able to relate the action of these enzymes to everyday situations.

- Develop and utilize laboratory technique appropriate to the handling of living material for microscopic examination.

Preparing the Materials

The activities in this lab require the use of 300X or better microscopes. If the class is not familiar with the use of microscopes, you may wish to have the students practice focusing filter paper or leaves before beginning the lab.
The first section, a general observation of the organelles in a plant cell, requires the use of celery. In all, about 4-5 stalks should be provided for a class of 30. Directions are given in the next section and in the student instructions for preparation of a slide from fresh celery.

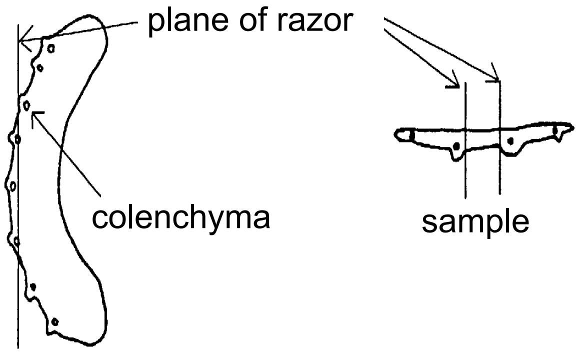
The materials provided for the model of organelles activity require some preparation. A sink is needed for disposal of the materials, and some funnels, enough to be shared throughout the class. To perform the activity, the students need a source of fresh potato. *It is highly recommended that the teacher or one of the students prepares the potato in advance.* This requires a blender or food processor of some sort. Cut five small to medium potatoes (for a class of 30 students) into small chunks and put them in the blender or food processor with a small amount of crushed ice. Blend the potatoes for about a minute, or until a slushy mixture is formed. Make sure this mixture is fairly liquid (add more water if necessary). Store the mixture in the refrigerator until needed.

The last section again uses fresh celery, and Janus Green B stain, the slide preparation procedure is given in the student manual.

Guide to Student Activities

The student instructions begin with an introduction to the definition and function of organelles. The two "typical" cells provide a schematic reference for later cell observations.

The first activity uses a microscope slide of living plant tissue for a general observation of organelles within cells. The preparation of the sample for viewing requires some patience. The sample should be from a fresh piece of celery, and should be as thin as possible. The diagram pictured at left should help to illustrate the correct technique.



The nuclei of the cells, as well as the chloroplasts, which are a bright green color, and several clear, elliptical bodies, the mitochondria, should be clearly visible under high power. Many organelles are too small to be seen with a light microscope, or are clear and therefore hard to spot. For this reason you may wish to show the students pictures of light and electron microscope views of cells in order to familiarize them with cell and organelle structure.

The second activity sets up a model to simulate organelle activity. The dialysis tubing, which allows small molecules to pass through, will simulate an organelle membrane. The blended potato provides a source of two enzymes such as would be present within an organelle. The first enzyme provided by the potato is *catalase,* which catalyzes the following reaction:

$$2H_2O_2 \; 6 \; O_2 \; 8 \; + \; 2H_2O$$

The hydrogen peroxide, then, simulates the raw materials which an organelle would acquire. The enzyme catalase, within the semipermeable membrane of dialysis tubing, processes this raw material, and the final products are water and oxygen. The water can easily pass through the dialysis tubing, but not the oxygen. This collects on the inside of the sack of tubing, causing it to swell.

The second enzyme is *catacholase*, which, in the presence of oxygen, catalyzes the formation of the pigment melanin, which has a characteristic brown or pinkish brown color. As the first enzyme catalyzed reaction releases oxygen, this second enzyme begins to work, tuning the potato extract within the sack a pinkish brown color within a few minutes. This secondary reaction represents a side reaction within an organelle, producing a by-product that may be detrimental to the organelle.

Caution the students to be careful with the cellophane tubing; any rip or tear in the tubing will destroy the experiment. The twist ties are preferable to tying a knot in the tubing, but the students should be careful to avoid poking a hole in the tubing.

The hydrogen peroxide should be handled with the customary care given all chemical agents. One-half ounce of the material should be sufficient for each student pair; this is approximately 1/3 of an inch in the cup.

After the students have observed the setup for about 5 minutes, they should check to be sure that the pressure inside the cellophane has not risen too high. The oxygen will eventually diffuse out of the tubing, but it may cause the bag to burst if allowed to build up. The cellophane tubing, if it does not have any holes, may be reused. If this is desired, wash the tubing gently in warm water, and allow it to soak in warm tap water for a while.

In discussing the results of the experiment, the students should focus on the fact that this model applies to real situations. Some of the key features of the model include:

- The semipermeable cellophane will permit one-way flow of materials, thus simulating both the intake of raw materials by the organelle and the retention of enzyme within an organelle.
- The enzyme in the "organelle" processes the raw material and produces a definite product.
- The product of enzymatic activity is stored within the organelle for some period of time. Point out to the students that the oxygen will eventually be released by diffusion through the tubing, as are the products of real organelles.
- As the organelle is acting on the raw material, a side reaction occurs which alters the organelle (alters the color, in this case). The production of melanin simulates the accumulation of waste products and demonstrates one reason why real organelles have a fixed lifetime, and must eventually be replaced.

The last experiment involves the observation of organelles at work in living cells. The organelle which the students will observe is the mitochondrion, since it is possible to stain this organelle in such a way as to demonstrate chemical activity. The stain that this activity depends on is Janus Green B. This dye is greenish blue under normal conditions. Removal of a pair of hydrogen atoms from its structure, however, causes a loss of color. During the normal activity of the mitochondria, dehydrogenase enzymes catalyze the removal of hydrogen atoms from substrate molecules. By staining the cells with Janus Green B, it is possible to demonstrate the activity of these dehydrogenase enzymes in the mitochondria.

The preparation of the slide in this section is done in the same manner as in section A. As the Janus Green B is added to the sample, the mitochondria will absorb the stain, and appear bluish in color. As the mitochondria metabolizes the stain, it will lose its color, and the mitochondria will again appear clear. This process takes from one to five minutes.

Areas For Further Discussion

After completing the initial activities in the kit, you may wish to pursue some of the following topics at length:

1) Identify the various organelles found within the cell with both light and electron microscopy. The Science Source Kit #1700, **Cell Structure**, provides materials for organelle study with a light microscope.

2) Investigate the relationship between the function of cells as a whole and the distribution of organelles within the cell. Cells such as liver cells, which tend to perform a wide variety of activities, show a broad distribution of organelle types. On the other hand, cardiac muscle cells are highly specialized, and show a preponderance of mitochondria related to their high energy requirements.

3) Investigate some of the properties of semipermeable membranes. Introduce the subjects of diffusion, passive transport, active transport, etc. Dialysis tubing can be

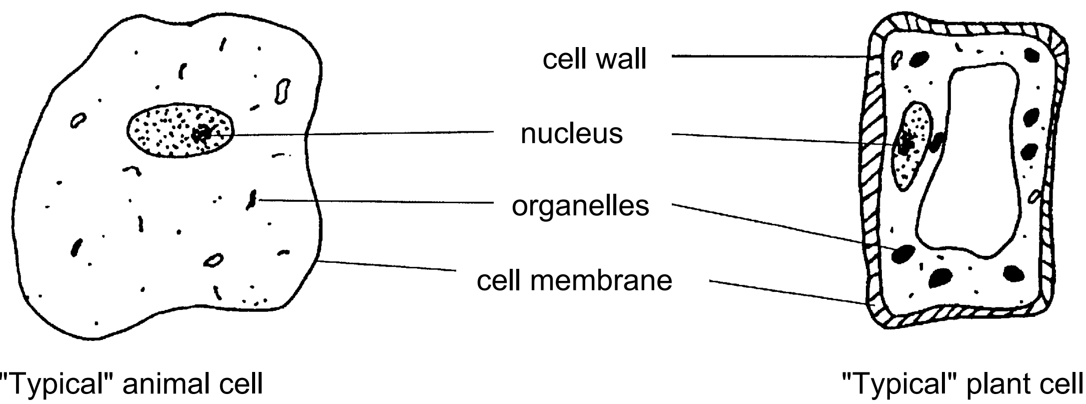
purchased from most science supply companies. Set up dialysis bags with one end wrapped around a piece of thin bore glass tubing. Fill bags with solutions of different materials which vary in molecular size and polarity, salt, sugar, urea, glycerine, etc. Immerse the bags in beakers of water, and observe the levels of liquid within the tube over the course of a day or two.

4) Investigate the nature of enzymes, and their importance to organelles. Prepare experiments to investigate the effects of heat, poisons, pH, etc. on the activity of enzymes. The Science Source Kit #1000, **How Poisons Work**, may be of interest to this topic.

5) Investigate the activity of the mitochondrion in detail. Discuss the relationships among the various parts of the energy cycle, and contrast the activity of the mitochondrion with that of the chloroplast. As part of this study, you may wish to present electron micrographs of the mitochondrion, and discuss the specialization of function within the organelle itself.

ORGANELLES #6800 Student Instructions (Annotated)

All life is made up of different types of cells, each performing a specific purpose. Muscle cells are obviously different from fat cells, and both are different from nerve cells. Yet these cells which seem so different from each other, each serving their own

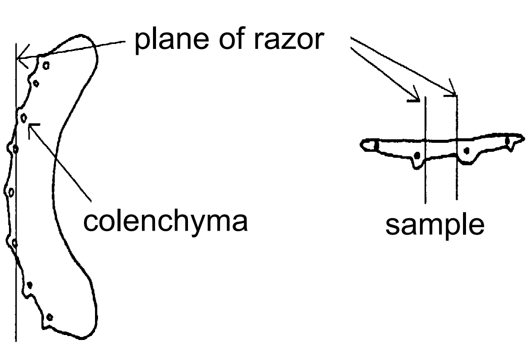


specific purpose, are in fact very similar. The important thing is to look for similarities, rather than differences.

If you look at a sample of living material under a microscope, you will see the individual cells. In most cells the *nucleus* is very easy to pick out. The nucleus is the control center, organizing all the activities that occur within the cell. Around the nucleus there may be small dots or blobs. These are the *organelles.* There are many kinds of organelles, but almost all cells contain the same types. That means that a plant cell will contain almost all the same kinds of organelles as an animal cell. But what are these organelles? What do they do? In the following activities, you will observe organelles at work, and will set up an experimental model to help understand what an organelle is.

PART A General Observations and Introduction

In this experiment you will need a small piece of celery ½ - 1 inch long (cut crosswise), forceps, a razor blade, a microscope slide and cover slip, and a microscope. If you are not familiar with microscopes, make sure you have your teacher show you how to use one.



Take the piece of celery and stand it up on the slide. Carefully cut a piece of celery off by slicing with the razor straight down along the outer side. Try to get as thin a piece as possible. Lay this small piece of celery flat on the slide, with the inner side up, and carefully cut out the area between two celery threads. Leave this part of the celery on the slide, and discard the two edges.

The picture at left will illustrate the correct technique.

Add a drop of water to the thin sheet of cells, and drop a cover slip onto the specimen. Place the slide under the microscope, and observe under first low, and then high power. You should be able to see the *nucleus* of the cell, as well as the bright green *chloroplasts,* and the clear, blob-like *mitochondria.* Make a labeled drawing of what you see.

Each organelle performs a specific job for the cell. This is an example of "division of labor", a very efficient way of working that lets each part of a whole do one specific task over and over. People use the same system in business. Each member of a business has a specific job to do, such as making the product, keeping track of inventory, advertising, etc., all of which are controlled by management, or "The Boss". In the case of a cell, "The Boss" is the nucleus, which controls most of the actions of the organelles.

There are many types of organelles, each performing specific tasks, in each cell. Two examples of these organelles, the *chloroplasts* (plants only), which make food using sunlight and carbon dioxide, you have just observed in the celery cells and the *mitochondria,* which digest food in the presence of oxygen to release energy for the cell. Several other important organelles are:

Endoplasmic reticulum - a series of membranes stretching throughout a cell that provide a system of communication and transport within the cell.

Golgi apparatus - a group of sack like membranes that are involved in the manufacture and storage of an important group of molecules called the glycoproteins.

Lysosome - a sack of digestive *enzymes* which "clean" the cell by breaking down damaged cell parts and foreign materials such as bacteria

Many organelles are too small to see with a light microscope. You may want to look for electron microscope pictures of the cell in your textbook to see these other organelles.

Each organelle is surrounded by either one or two layers of a *membrane.* This membrane is special in that it can control what enters and leaves the organelle. The ability of a membrane to allow molecules to pass through it is called *permeability.* Organelle membranes are *semipermeable,* that is, they only allow certain molecules through, and can keep other molecules either in or out of the cell.

The organelles "import" the raw materials required for their specific task. Inside the organelle, these raw materials enter a series of chemical reactions. Each of these reactions is catalyzed, or helped, by the presence of specific *enzymes.* Enzymes are special protein molecules that are concentrated within organelles. Each enzyme helps one or two specific reactions to occur. The raw materials go through these chemical reactions and are changed into the final end-products that the organelle is supposed to be making. In the case of a chloroplast, the end product is food in the form of starch. This end-product can be stored within the organelle for some period of time, and is released when it is needed.

This entire process is not as neat as it might sound. As the organelle manufactures the end-product, chemical reactions which are not useful occur. In some cases these reactions produce substances that the organelle cannot get rid of, or are harmful to the organelle. Eventually the organelle will stop working and must be replaced.

PART B - Model Of An Organelle

In this lab section, you will prepare a simulation of an organelle. Obtain the following materials, and bring them to your lab station: a clear plastic cup, 2 twist-ties, 18-20cm of cellophane tubing, and a funnel. Pour some tap water into the plastic cup, and soak the cellophane tubing in the water for about two minutes.

While the tubing is soaking, locate the prepared potato mixture. This mixture is just raw potatoes and water blended together. Potatoes contain certain enzymes that are released when they are crushed.

After the two minutes, take the tubing from the water. It is rather fragile, so be careful! Be especially careful not to put any holes in it. Wrap a twist-tie around the tubing about 1" from the end, and twist it tightly. Open the other end of the tubing by gently rolling the wet tubing between your fingers. Continue down the tube until you reach the tied end. Place the funnel neck into the open end of the tubing, and pour the potato extract into the tube. If the extract will not go down the funnel, try adding a little more water to it. Fill the tube until it is about 3-4" full of potato. Squeeze the tube until the liquid level is about 1" from the end of the tube, and try to get any air out. Tie off the top with another twist-tie. The tube should be rather limp.

Describe the contents of the tube (including the color)

Cloudy liquid, pale grey or light pinkish brown in color, with bits of potato floating in it

Empty the plastic cup of the tap water and pour ½ of an ounce of hydrogen peroxide into it (about 1/3 of an inch in the bottom of the cup). Add enough tap water to the hydrogen peroxide to fill the cup ½ to 2/ 3 full. Describe the hydrogen peroxide and water mixture:

A clear, colorless liquid. A few small bubbles form on the side of the cup.

Place the cellophane tube with the potato mixture into the cup of hydrogen peroxide and water, and observe for 5 to 10 minutes. Do you notice anything happening? Record your observations:

The inside of the bag appears foamy after a minute or so. Close inspection shows that many small bubbles are being formed inside the bag. These rise to the top of the bag. A few bubbles form on the outside of the bag. As the gas is produced, the bag begins to swell, and appears taut after a few minutes, while the bubbles are being produced, the contents of the bag appear to be changing color. A pinkish brown color appears within the bag, and it deepens as time goes by. Eventually the bubbles stop forming, and the color gets no deeper. The liquid outside the bag still appears clear (unless the bag has leaked).

Hydrogen peroxide breaks down into water and oxygen, but normally at a very slow rate. What do you think is happening here?

Something from the potato must be making the peroxide break down faster than normal

An enzyme from the potato, *catalase,* is speeding up the break down of hydrogen peroxide. How do you think that the hydrogen peroxide from outside the tubing and the enzyme from inside the tubing came together, and why do you think that there is no oxygen being produced outside of the tube?

The cellophane tubing must be letting something through it. Since the oxygen is only forming on the inside, the tubing must be letting the hydrogen peroxide in. For the results that are being observed, the tubing must also be keeping the oxygen inside, not letting it pass through.

In addition to the production of oxygen, you probably noticed a color change in the potato extract. Potatoes contain another enzyme called *catacholase* that causes the formation of a pigment called *melanin.* Melanin has a characteristic brown color. This reaction requires oxygen. Why do you think that the color change occurred so rapidly inside the "organelle"?

The oxygen produced by the first reaction is speeding up the second, is speeding up the formation of the pigment.

Why does a potato turn color when it is cut open?

Oxygen in the air causes the same reaction. (This also occurs in apples and pears)

After about 5 minutes the rate of bubbling will slow down. If the cellophane bag has become very full from oxygen bubbles, be careful in untiing the twist-tie. It is best to open the bag over a sink. Pour the liquid down the drain, and dispose of the cellophane tubing as directed by your teacher.

What parts of an organelle do you think the various ingredients and reactions of this lab represent?

The cellophane tubing represented the organelle membrane, and the potato extract represented the inside of the organelle, which is full of enzymes. The water in the cup is equivalent to the cytoplasm of the cell. Of the two reactions that you saw, the first one represents organelle function. The tubing allowed hydrogen peroxide, the raw material, inside, where there were enzymes that processed it. In this case, the end-product was oxygen. The second reaction is an undesired side reaction, using up the desired end product (oxygen) to produce a pigment.

PART C - Observation Of Organelles In Living Tissue

We have already mentioned that mitochondria are responsible for breaking down food in the presence of oxygen. The enzymes that the mitochondria use to do this are called *dehydrogenases,* which remove hydrogen atoms from food molecules. We can "fool" the mitochondria by giving it other molecules with hydrogen, and the mitochondrion's enzymes will react the same way. The kit contains a dye called *Janus Green B*. The molecules of this dye are greenish-blue in color, and will be transported to the mitochondria as if they were food. If hydrogen atoms are removed from the dye molecules, though, the dye looses its color. By observing the loss of color in the mitochondria, we can observe mitochondria at work.

Obtain a second piece of celery, the same size as the first, a razor blade, a microscope slide and a cover slip, a pair of forceps, and a microscope.

Cut a piece of celery in the same manner as in Part A, isolating a piece of thin tissue from in between two celery threads. Add a drop of water to the slice, and drop a cover slip on top of it. Place the slide under the microscope as before and focus it, first on low power and then on high power.

Obtain a bottle of Janus Green B stain. Tear off a piece of paper towel, and have it ready next to your lab station. Place a drop of the Janus Green B stain on the slide immediately next to the cover slip. Place the piece of paper towel along the opposite edge of the coverslip. The paper towel will absorb the water underneath the coverslip, and the drop of stain on the other side will be drawn into its place. Observe the cells as they are stained. The stain will congregate in the mitochondrial cells, and will turn them bluish green. Observe the slide for several minutes, alternating with your partner, until you notice a change in the color of the mitochondria. Record and explain your observations:

The bluish-green color disappears from the mitochondria, indicating that the mitochondria have undergone chemical activity.

Discussion Questions

Why do you think that it is important that enzymes be restricted to inside organelles?

If the enzymes were simply allowed to circulate around inside of the cell. the cell could not control their activity very well, and they would probably not be very efficient. Enzymes will also interfere with each other, one enzyme building some molecule while its neighbor tears that molecule apart. Also, there is a danger, if the digestive enzymes are free, that the enzymes might damage the cell parts.

What advantage do you think a cell has because of its organization into organelles?

The specialization of labor allows each organelle to be highly efficient in terms of its particular function. Because the organelle is located inside a cell, each organelle can depend on the cell and other organelles for its other needs. By changing the pattern of organelles, the cells of different tissues can modify their overall function capability.

Could the model for an organelle be used as a model for a cell? What are the differences between cells and organelles?

In some ways, a cell is simply a complex organelle. The cell takes in material, processes it chemically, accumulates some products of reactions, and may undergo breakdown because of various side reactions. A cell. however, is essentially self-contained and self-supporting, while an organelle must depend on the rest of the cell for its needs. Some cells are extremely specialized and are not really self-supporting, such as nerve cells. In these cases, the similarity between the cell and an organelle is increased.

The organelles of a cell have been compared to the parts of the body: each part of the body performs a specific function. In what ways do you think this analogy is correct?

Each organelle is specialized to perform a specific function. If all of the organelles perform their function correctly, then the cell can function well overall. If one of the organelles is not functioning correctly, the cell remains deficient in that organelle 's product.. The analogy breaks down when one considers that many organelles break down and are replaced without the cell as a whole dying. The parts of the body do not have this capability.

When you observed the living tissue of the celery, you may have noticed that the organelles were flowing around inside the cell. This process is called cytoplasmic streaming. Why do you think that this might be important to the cell?

This movement allows the various organelles to come in contact with new raw materials, and with each other for the purpose of exchanging materials. If the cell had to depend on diffusion, then organelles which were very far apart would not have an efficient exchange of materials.

ORGANELLES #6800 Student Instructions

All life is made up of different types of cells, each performing a specific purpose. Muscle cells are obviously different from fat cells, and both are different from nerve cells. Yet these cells which seem so different from each other, each serving their own specific purpose, are in fact very similar. The important thing is to look for similarities, rather than differences.

If you look at a sample of living material under a microscope, you will see the individual cells. In most cells the *nucleus* is very easy to pick out. The nucleus is the control center, organizing all the activities that occur within the cell. Around the nucleus there may be small dots or blobs. These are the *organelles.*

There are many kinds of organelles, but almost all cells contain the same types. That means that a plant cell will contain almost all the same kinds of organelles as an animal cell. But what are these organelles? What do they do? In the following activities, you will observe organelles at work, and will set up an experimental model to help understand what an organelle is.

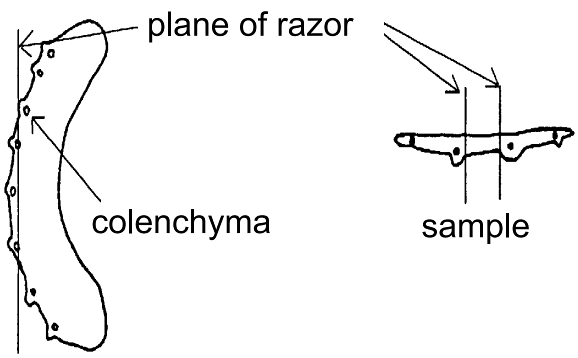
PART A General Observations and Introduction

In this experiment you will need:

- A small piece of celery ½ - 1 inch long (cut crosswise)
- Forceps
- Microscope slide and cover slip
- Razor blade

If you are not familiar with microscopes, make sure you have your teacher show you how to use one.

Take the piece of celery and stand it up on the slide. Carefully cut a piece of celery off by slicing with the razor straight down along the outer side. Try to get as thin a piece as possible. Lay this small piece of celery flat on the slide, with the inner side up, and carefully cut out the area between two celery threads. Leave this part of the celery on the slide, and discard the two edges. The following picture will illustrate the correct technique:



Add a drop of water to the thin sheet of cells, and drop a cover slip onto the specimen. Place the slide under the microscope, and observe under first low, and then high power. You should be able to see the *nucleus* of the cell, as well as the bright green *chloroplasts,* and the clear, blob-like *mitochondria.* Make a labeled drawing of what you see.

Each organelle performs a specific job for the cell. This is an example of "division of labor", a very efficient way of working that lets each part of a whole do one specific task over and over. People use the same system in business. Each member of a business has a specific job to do, such as making the product, keeping track of inventory, advertising, etc., all of which are controlled by management, or "The Boss". In the case of a cell "The Boss" is the nucleus, which controls most of the actions of the organelles.

There are many types of organelles, each performing specific tasks, in each cell. Two examples of these organelles, the *mitochondria,* which digest food in the presence of oxygen to release energy for the cell, and *chloroplasts* (plants only), which make food using sunlight and carbon dioxide, you have just observed in the celery cells.

Several other important organelles are:

Endoplasmic reticulum - a series of membranes stretching throughout a cell that provide a system of communication and transport within the cell.

Golgi apparatus - a group of sack like membranes that are involved in the manufacture and storage of an important group of molecules called the glycoproteins.

Lysosome - a sack of digestive *enzymes* which "clean" the cell by breaking down damaged cell parts and foreign materials such as bacteria

Many organelles are too small to see with a light microscope. You may want to look for electron microscope pictures of the cell in your textbook to see these other organelles.

Each organelle is surrounded by either one or two layers of a *membrane.* This membrane is special in that it can control what enters and leaves the organelle. The ability of a membrane to allow molecules to pass through it is called *permeability.* Organelle membranes are *semipermeable,* that is, they only allow certain molecules through, and can keep other molecules either in or out of the cell.

The organelles "import" the raw materials required for their specific task. Inside the organelle, these raw materials enter a series of chemical reactions. Each of these reactions is catalyzed, or helped, by the presence of specific *enzymes.* Enzymes are special protein molecules that are concentrated within organelles. Each enzyme helps one or two specific reactions to occur. The raw materials go through these chemical reactions and are changed into the final end-products that the organelle is supposed to be making. In the case of a chloroplast, the end product is food in the form of starch. This end-product can be stored within the organelle for some period of time, and is released when it is needed.

This entire process is not as neat as it might sound. As the organelle manufactures the end-product, chemical reactions which are not useful occur. In some cases these reactions produce substances that the organelle cannot get rid of, or are harmful to the organelle. Eventually the organelle will stop working and must be replaced.

PART B - Model Of An Organelle

In this lab section, you will prepare a simulation of an organelle. Obtain the following materials, and bring them to your lab station:

a clear plastic cup	2 twist-ties
18-20 cm of cellophane tubing	funnel

Pour some tap water into the plastic cup, and soak the cellophane tubing in the water for about two minutes.

While the tubing is soaking, locate the prepared potato mixture. This mixture is just raw potatoes and water blended together. Potatoes contain certain enzymes that are released when they are crushed.

After the two minutes, take the tubing from the water. It is rather fragile, so be careful! Be especially careful not to put any holes in it. Wrap a twist-tie around the tubing about 1" from the end, and twist it tightly. Open the other end of the tubing by gently rolling the wet tubing between your fingers. Continue down the tube until you reach the tied end. Place the funnel neck into the open end of the tubing, and pour the potato extract into the tube. If the extract will not go down the funnel, try adding a little more water to it. Fill the tube until it is about 3-4" full of potato. Squeeze the tube until the liquid level is about 1" from the end of the tube, and try to get any air out. Tie off the top with another twist-tie. The tube should be rather limp.

Describe the contents of the tube (including the color):

Empty the plastic cup of the tap water and pour ½ of an ounce of hydrogen peroxide into it (about 1/3 of an inch in the bottom of the cup). Add enough tap water to the hydrogen peroxide to fill the cup ½ to 2/ 3 full. Describe the hydrogen peroxide and water mixture:

Place the cellophane tube with the potato mixture into the cup of hydrogen peroxide and water, and observe for 5 to 10 minutes. Do you notice anything happening? Record your observations:

Hydrogen peroxide breaks down into water and oxygen, but normally at a very slow rate. What do you think is happening here?

An enzyme from the potato, *catalase,* is speeding up the break down of hydrogen peroxide. How do you think that the hydrogen peroxide from outside the tubing and the enzyme from inside the tubing came together, and why do you think that there is no oxygen being produced outside of the tube?

In addition to the production of oxygen, you probably noticed a color change in the potato extract. Potatoes contain another enzyme called *catacholase* that causes the formation of a pigment called *melanin.* Melanin has a characteristic brown color. This reaction requires oxygen. Why do you think that the color change occurred so rapidly inside the "organelle"?

Why does a potato turn color when it is cut open?

After about 5 minutes the rate of bubbling will slow down. If the cellophane bag has become very full from oxygen bubbles, be careful in untiing the twist-tie. It is best to open the bag over a sink. Pour the liquid down the drain, and dispose of the cellophane tubing as directed by your teacher.

What parts of an organelle do you think the various ingredients and reactions of this lab represent?

PART C - Observation Of Organelles In Living Tissue

We have already mentioned that mitochondria are responsible for breaking down food in the presence of oxygen. The enzymes that the mitochondria use to do this are called *dehydrogenases,* which remove hydrogen atoms from food molecules. We can "fool" the mitochondria by giving it other molecules with hydrogen, and the mitochondrion's enzymes will react the same way. The kit contains a dye called *Janus Green B*. The molecules of this dye are greenish-blue in color, and will be transported to the mitochondria as if they were food. If hydrogen atoms are removed from the dye molecules, though, the dye looses its color. By observing the loss of color in the mitochondria, we can observe mitochondria at work.

Obtain a second piece of celery, the same size as the first, a razor blade, a microscope slide and a cover slip, a pair of forceps, and a microscope.

Cut a piece of celery in the same manner as in Part A, isolating a piece of thin tissue from in between two celery threads. Add a drop of water to the slice, and drop a cover slip on top of it. Place the slide under the microscope as before and focus it, first on low power and then on high power.

Obtain a bottle of Janus Green B stain. Tear off a piece of paper towel, and have it ready next to your lab station. Place a drop of the Janus Green B stain on the slide immediately next to the cover slip. Place the piece of paper towel along the opposite edge of the coverslip. The paper towel will absorb the water underneath the coverslip, and the drop of stain on the other side will be drawn into its place. Observe the cells as they are stained. The stain will congregate in the mitochondrial cells, and will turn them bluish green. Observe the slide for several minutes, alternating with your partner, until you notice a change in the color of the mitochondria. Record and explain your observations:

Discussion Questions

Why do you think that it is important that enzymes be restricted to inside organelles?

What advantage do you think a cell has because of its organization into organelles?

Could the model for an organelle be used as a model for a cell? What are the differences between cells and organelles?

The organelles of a cell have been compared to the parts of the body: each part of the body performs a specific function. In what ways do you think this analogy is correct?

When you observed the living tissue of the celery, you may have noticed that the organelles were flowing around inside the cell. This process is called cytoplasmic streaming. Why do you think that this might be important to the cell?