CELL STRUCTURE #1700

This kit is designed to provide a practical laboratory experience in the preparation of cell mounts and the observation of cell components. It is designed for up to 30 junior or senior high school biology students. This kit provides materials for the following lab experiments:

- Observation and staining of animal cells (human cheek cells)
- Observation and staining of plant cells (potato)
- Observation of chloroplasts in Elodea
- Demonstration of membrane permeability to water (Elodea in hypertonic environment)
- Observation of mitochondria in both plant and animal cells

The lab will take approximately 2 hours, and can easily be split into 1 hour sessions.

Contents:

- Microscope Slides (72)
- Cover Slips (100)
- Petri Dishes, 100x15(10)
- Pipets (2)
- Forceps (6)
- Razor Blades (15)
- Toothpicks (150)
- Filter Paper (6 meters)
- Lugol's Solution (2 - 30ml bottles)
- Janus B Green Stain (2 - 30 ml bottle)
- Hypertonic Solution (2-30ml bottles)

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

Additional Required Materials:

- Celery (4 to 5 stalks)
- Potato
- Microscopes (300X or higher)
- Elodea or Anachias (or lettuce)

CELL STRUCTURE #1700 **Teacher Manual**

Introduction

The study of cellular biology has become a central theme of modern biology courses. Students of the life sciences spend their introductory work studying the types of cells, structures of cells, the chemical reactions in cells, and the relationships between cells within an organism. All the activities of "life": movement, reproduction, procurement of nutrients, elimination of waste, adaptation, and communication, occur within each cell to one degree or another. Students can gain a basic understanding of many of the major themes of biology by studying the structures and activities of a cell.

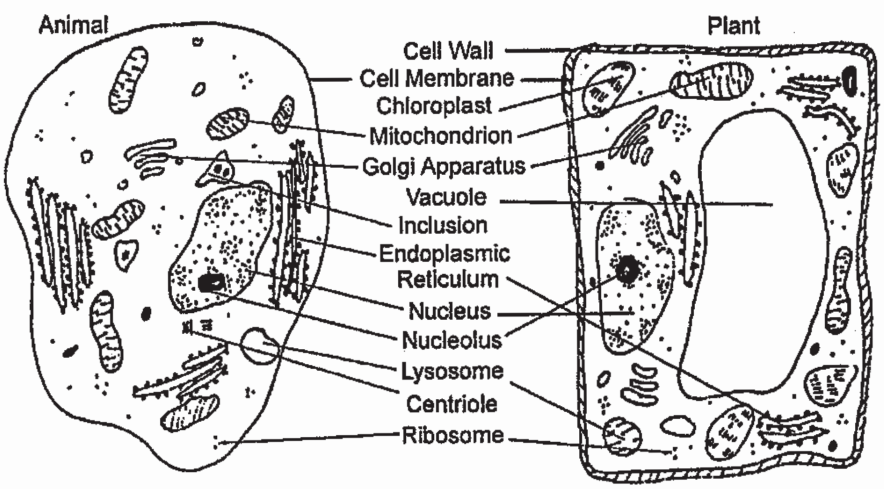
Students use samples of familiar organisms for the activities, including some of their own cells. The staining techniques used are quite simple and permit the students to observe most of the major components of cells within the allotted lab time (about two 45 minute class periods). Discussion material provides a basic introduction to cell biology to supplement text or class presentations. The kit may be used by students who have already discussed basic concepts of cellular structure.

Activities in the kit are most effectively presented using microscopes of 450X capability, but 300X microscopes are acceptable. The student instructions do not include directions for the use of microscopes. Before beginning the activities, present some introductory discussion and practical work in setting up and focusing the microscope.

Background Information

The study of cell biology is like looking through a zoom lens at the world around us. In 1665 Francis Hooke looked through a microscope at a piece of cork and identified the first "cells". These cells were actually the leftover framework of cell walls from dead plants. Early work centered on identifying the presence of cells in different types of living material. As microscopes increased in power and definition, cytologists began to distinguish the presence of subcellular components (organelles). As the study of cell biology shifted to the study of organelles, it became clear that all cells had certain common features. Biologists became aware that the structure of the cell was directly related to its function.

Since the invention and development of the electron microscope and advances in techniques such as centrifugation and radiography, exploration of the fine structure of cells and cell components has become possible. This section presents a brief description of the structure and function of the major cellular organelles. Refer to the diagrams of "typical" animal and plant cells for a better idea of the size, shape, and structure of the various organelles.



Cell Membrane

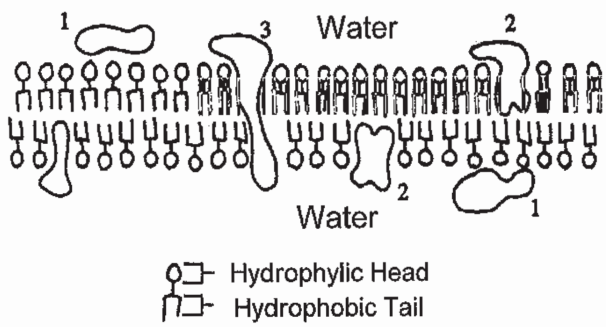
All cells are surrounded by a membrane that separates them from their environment. The cell membrane regulates the levels of materials, actively transports materials, forms food vacuoles, recognizes foreign materials, influences division and growth rates within a cell, and communicates between cells. The structure of the membrane was not clear until the development of the electron microscope. The cell membrane is made up of a double layer of proteins and lipids. The lipid molecules line up with their hydrophobic portions oriented toward each other and hydrophilic functional groups extending outwards. On the surfaces and running through the lipid layers are various types of protein molecules. Many of the protein molecules are involved in active transport of materials across the membrane. Some proteins form channels or gates through which molecules can passively diffuse. By coupling the flow of materials with the activity of these proteins, the membrane achieves fine control over the internal chemical composition and status of the cell. The membrane is visible as a thin line around the cell.

Cell Wall (plants only)

The cell wall is a mesh of cellulose and other carbohydrate and protein molecules surrounding a plant cell. Though actually outside the cell membrane, the cell wall is manufactured within the cell. The cell wall provides support and protection for the cell. Cell wall material occupies a large percentage of a plant body. The cell itself can die, leaving the cell wall behind as wood, cork, or plant fiber.

The cell wall consists of a randomly-oriented primary layer, and secondary layers of parallel fibers. Each of the secondary layers is oriented at an angle to the previous layer. This provides tremendous strength to the structure as a whole. The cell wall is usually visible without staining, but it may be enhanced with the use of various carbohydrate stains for contrast.

Vacuole (plants only) and Inclusions (animals only)



The distinction between a vacuole and an inclusion is one of terminology. Both are membrane-bound sacs of material within the cell. In plant cells, the vacuoles often form one large structure which may take up to 90% of the cell volume. There is some evidence that the membrane of the vacuole, called the tonoplast, is chemically different from other membranes within the cell. The vacuole usually contains the cell "sap", a water solution of salts and sugars kept at high concentration by the active transport of ions through the vacuole membrane. This high concentration causes the osmosis of water into the cell. As the vacuole fills with water, it pushes the cytoplasm of the cell against the cell wall, making the cell turgid. Turgor (water pressure within the cells) is the cause of rigidity in living plant tissue. Besides water, vacuoles may be filled with waste material or stored food.

Inclusion in animal cells are often formed as the cell engulfs some external material through the process of phagocytosis (engulfing a solid) and pinocytosis (engulfing a liquid). The inclusions may also result from the secretion process of wastes, stored food, enzymes, etc.... In some animal cells, such as the cells found in fatty tissue, the single inclusion occupies as much of the cell as the single vacuole does in many plant cells.

Lysosome

The lysosome is a specialized type of inclusion originating from the Golgi body. The lysosome is a double membrane-bound sac containing digestive enzymes responsible for breaking down dead, damaged, or foreign material with the cell.

The diagram of the animal cell on page 2 shows a small fragment of a mitochondrion within the lysosome. A lysosome will fuse with a food vacuole or inclusion containing material to be digested. Freed nutrients are transported across the lysosomal membrane to be used by the cell. The lysosome is known to be involved in the process of cell death, although its exact role is not understood. Cells which are responsible for "policing" the body, such as white blood cells, usually have large numbers of lysosomes.

Endoplasmic Reticulum

The interior of most cells contains a system of membrane-bound sacs, tubes, and vesicles referred to as the endoplasmic reticulum. The endoplasmic reticulum (ER) can be classified in two functionally distinct forms, the smooth endoplasmic reticulum (SER) and the rough endoplasmic reticulum (RER). The SER synthesizes the components of cell membranes. Liver cell SER contains a number of enzymes which breakdown carcinogens. The present of ribosomes on the surface of the RER causes its rough appearance. The RER often takes the form of flattened sacs called cisternae. These cisternae are associated with ribosomes in the synthesis and export of proteins. The RER is capable of changing its form from cisternal to vesicular SER as the cell changes its functional role. The three-dimensional nature of both types of endoplasmic reticulum are still being researched using the electron microscope.

Golgi Apparatus

The Golgi Apparatus is named for the Italian scientist who was able to stain the complex with a solution of silver salts. The structure of the Golgi consists of a stack of several flattened sacs, the edges of which may be connected to the ER. The Golgi is responsible for what is referred to as the "final packaging" of some glycoproteins. Autoradiography has demonstrated the time sequence in the secretion of proteins by a cell: first ER, then Golgi Apparatus, then secretory vesicles that bud from the Golgi, and finally export from the cell. Its detailed structure and interrelationships with the ER has been made clear only with the use of electron microscopy.

Nucleolus

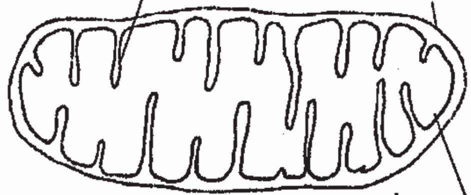
The nucleolus is a rounded, darkly staining object found in the nucleus of many interphase (between mitotic divisions) cells. It is not visible on occasion because of the plane of sectioning for preparing slides. The nucleolus stains very darkly with basic dyes that bind to nucleic acids. Disappearing from the cell during mitosis, the nucleolus reforms shortly after the nucleus has divided. The nucleolus is the site of RNA (ribonucleic acid) formation needed for the processes of protein synthesis, particularly the RNA which makes up ribosomes. It appears to be associated with a particular segment of DNA (deoxyribonucleic acid) called the nucleolar organizer. The nucleolus sets off the necessary cellular machinery for protein synthesis.

Ribosome

Ribosomes are small globular objects composed of RNA and protein. They are responsible for the assemblage of protein for the cell, using messenger RNA formed inside the nucleus as a template. Specific amino acids line up on specific pieces of RNA and are joined by enzymes in the ribosome to become proteins. The ribosomes may be either free-floating in the cytoplasm or may be attached to the endoplasmic reticulum. Cells which are responsible for exporting protein of move sort, digestive enzymes, for example, have a large proportion of their volume given over tot the ER, and associated ribosomes. This ribosome-coated endoplasmic reticulum is referred to as rough ER, or RER. If the cell is manufacturing protein primarily for internal use, the ribosomes are usually free-floating in the cytoplasm. They may occur in groups called polyribosomes. Identification of the ribosome was possible only after the development of the electron microscope.

Mitochondrion

The mitochondrion (plural: mitochondria) is often called the powerhouse of the cell. The mitochondrion is a double membrane sack with a smooth sausage shaped outer membrane and a highly convoluted inner membrane. The mitochondrion is responsible for the major step in the production of usable chemical energy in the form of adenosine triphosphate (ATP) through the breakdown of food in a process called *The Krebs Cycle* or citric acid cycle. The mitochondrion itself also carries out oxidative metabolism, a very controlled form of burning hydrogen by oxygen. This type of energy release is, chemically, the transfer of an electron from each of two hydrogen atoms to oxygen. Hydrogen is therefore oxidized (electrons have been removed) and oxygen is reduced (electrons have been added). This reaction produces a great deal of free energy as heat. The mitochondrion controls the reaction of the folds on the inner membrane, called cristae, through a series of stepwise reactions called the electron transport system. This stepwise release of energy is coupled with an enzyme system to produce ATP. The ATP which is produced by the mitochondrion is ultimately exported to the rest of the cell, providing an energy source for cellular processes. Mitochondria can be seen as small rods or dots under the light microscope. Understanding their complex internal structure came only after the electron microscope was developed.



Chloroplasts (plant cells only)

The chloroplast is possibly the most important organelle for life on earth. The chloroplast is responsible for trapping light energy and using that energy to manufacture ATP, sugar, and starch (photosynthesis). The chloroplast, like the mitochondrion, has a double membrane structure. The inner membrane of the chloroplast is smooth. Inside this membrane is yet another membrane that is highly folded. These folds form sets of flattened, disk like sacs called thylakoids, and are similar to the cristae of the mitochondria. Attached to the membranes are enzymes and pigments such as chlorophyll involved in photosynthesis. In many plants, chloroplasts have formed so much starch and sugar that they become, in effect, storage granules. These can be distinguished from vacuoles or inclusions by their internal membrane structure. Chloroplasts are easy to observe under the light microscope, since they have their own natural stain (chlorophyll is a green pigment). In many plants, the chloroplasts demonstrate cytoplasmic streaming, a process which circulates the organelles within the body of the cell. The complex structure of the chloroplast was recognized only after the development of the electron microscope.

Centriole (animal cells only)

The centriole is involved in the formation of the mitotic spindle, an array of tubules necessary for the movement of the chromosomes during mitosis. The centrioles themselves are composed of a bundle of tubules, similar to those in cilia and flagella. The existence and structure of the centrioles were determined only after the electron microscope was developed. Although plant cells do not have centrioles, performance of the centrioles' function is achieved by other means during plant cell divisions.

From this discussion, you can see that the cell is, in fact, a complicated system (a mini-factory) of organelles functioning as a unit. Cytologists are particularly interested in cellular structures which reflect the functions of the cell within the organism. For example, unicellular plants and animals tend to have a balanced array of organelles, since a single cell must carry out all the functions of life. In multicellular organisms, cells are specialized into tissues, organs, and organ systems. A cell in the lining of the small intestine may be responsible for the secretion of enzymes. This cell tends to show large amounts of ER and ribosomes, Golgi, and mitochondria (protein synthesis requires a great deal of energy). A cell from the kidney, which is responsible for pumping waste materials out of the body, has a highly folded cell membrane to increase surface area for active transport and has large numbers of mitochondria to provide the ATP needed for active transport. The relationships between structure and function are extremely important in the study of cellular biology.

Using The Kit In The Classroom

The kit is intended for secondary level biology classes but is quite suitable for capable middle school students. Prior to using this kit or in conjunction with the first activity of the kit, the student should receive instruction in the use of the microscope as a laboratory tool. Proper utilization of this kit requires that pairs of students have access to a microscope of at least 300X, and preferably 450X, magnification.

Preparing the Materials

Inspect the kit to become familiar with laboratory equipment which will be used by the students. Evaluate needed safety modifications or instructions and provide appropriate instructions, demonstrations, or warning to the students prior to any actual laboratory work. *Be sure that microscopes are in working order, including light sources. Have lens tissue available to clean eyepieces and mirrors.*

Have available samples of the following materials: Potato, celery, *Elodea* or Anachais (this aquarium plant looks like a large green pipe cleaner); if you do not have any available, it can be purchased inexpensively from an aquarium supply or pet shop. Lettuce may be used in place of *Elodea* of Anachais. A thin layer can be obtained from these by ripping the leaf and then peeling off the thin "skin" from the surface. Samples of algae from a polluted pond or other source can be used. These are excellent for observing vacuoles, cell walls, and chloroplasts. They show students diversity of cellular structure which can be found. Prepared slides of various subjects, including human tissue, if possible, anc be used to enhance activities. Textbooks and references with photographs of both light and electron microscopy can be used for discussion and comparison.

Guide to Student Activities

The introduction focuses student attention on the existence of cellular components and introduces the term organelles. As the students are working through the activities, encourage them to use the new terms which they are learning. Mention relevant text sections and useful reference material.

The purpose of the first activity (Part A) is to point out the importance of stains in the study of cellular structure and to involve students in making lab drawing. This activity involves the preparation of a cheek cell squash slide. Be sure that students are not hurting themselves trying to get a large chunk of cells for the slide. One gentle scrape along the inner surface of the cheek will collect enough cells for observation. Spend a few minutes explaining that good drawings rely heavily on outlining, rather than shading, areas of different color, density or texture.

Observation of the cheek cell also illustrates the existence of the nucleus and nucleolus. The nucleolus may not be clearly visible in all cells. Tell students to search the slide systematically for a cell which contains visible nucleoli. The discussion of the nucleus is brief, but provides an initial exposure to the topic of genetic control of protein synthesis. The ribosomes which are so important in this process are impossible to see with a light microscope, as are the details of the endoplasmic reticulum, or ER. Readings may be assigned that further cover these topics.

In observing cheek cells, students should notice that the cytoplasm is the cell appears to contain many small objects of different sizes and shapes. Each of these visible organelles is offered in detail during the course of the activities.

Activity #2 in part A involves observing a thin slice of fresh potato stained with Lugol's iodine solution, an indicator for starch. If the students are having trouble making thin slices, suggest that they make a thin wedge of material and observe the thinnest part of the wedge. Watch for students who are having trouble with the razor blades. If you are uneasy about having the students use razor blades, prepare several dozen slices ahead of time. Store them in cold water until they are needed.

Lugol's iodine solution turns deep blue or purple in the presence of starch. This makes both the cell walls and the starch storage granules visible. The nucleus and nucleolus may be hard to find since they may be hidden by the cell wall. In some cases, the students may be able to see the fibrous structure of the cell wall. Starch grains from ruptured cells will be floating freely in the field of view. The cells near the surface of the potato, particularly in greenish areas of the potato, may have visible chloroplasts. Tess the students not to worry if they don not see chloroplasts. Chloroplasts will be clearly visible in *Elodea* (Anachais or lettuce).

The third activity in part A involves *Elodea*. Chloroplasts are very obvious in this leaf. An *Elodea* cell may also demonstrate cytoplasmic streaming, where the chloroplasts and other organelles, which appear as small colorless blobs, will be streaming around the inside of the cell. The clear region in the center of the cell, which does not appear to contain any chloroplasts, is the vacuole. Diagrams such as the one at the beginning of the teacher guide, or in reference texts, may be helpful for the student.

The *Elodea* is also used in Activity #1 in part B to demonstrate the response of cells to a hypertonic saline solution. Water tends to move toward the area of greatest ion concentration. The hypertonic saline solution (salt water) contains many ions, therefore water passes through the cell membrane and out of the cell. As students add saline solution, the water in the vacuole leaves the cell by osmosis, and the cell membrane will shrink away from the cell wall. Discuss other aspects of cell membrane function, including active transport, pinocytosis and phagocytosis.

Activity #2 in part B uses Janus Green B stain to highlight the mitochondria in a cell. When students observe celery (if it is fresh), for several minutes, they will notice a loss of color by the mitochondria. This occurs as the mitochondria produce dehydrogenase enzymes which remove hydrogen atoms form the molecules of the stain. This causes the loss of color in the stained mitochondria.

Encourage students to make slides and drawings of other types of cells (end of part B). Samples of fresh meat teased apart are interesting, as are sections of plant roots and stems. Encourage the students to relate the structure of what they are seeing to a function in the life of the organism.

Other Areas for Study

After the students have completed the initial set of activities, pursue some or all of the following topics:

1 Discuss the biochemistry of the various organelles. Begin with a discussion of important cellular compounds (such as found in The Science Source kit #1800 Cellular Chemistry). Study the major metabolic pathways in the cell. Relate the fine structure of the organelles to their particular role in the life of the cell. Electron micrographs are very useful for this.

2 Investigate the activities of living cells. Obtain a sample of algae. Prepare an experiment using a respirometer to measure oxygen production by chloroplasts of a cell. Alternately, use a respirometer with potassium hydroxide as a CO2 absorber to measure oxygen consumption of plants in the dark. Investigate cellular digestion, and the role of enzymes in cellular process. Simple demonstrations of pH may be performed with aquatic plants.

3 Investigate the nature and action of enzymes and their importance to organelles of the cell. Prepare experiments to investigate the effects of pH, heat, and poisons on enzyme activity. (The Science Source kit #1000 How Poisons Work is useful for this topic). Relate the complexity of enzymes to their specificity, and the narrow range of conditions which limit cell survival.

4 Set up materials to prepare permanent slides. Many reference books have instructions for the fixation, dehydration, embedding, and staining of tissue samples. Talk about the reasons for each step as you work through the procedure.

5 Compare light and electron microscopy, including the ways of preparing materials for viewing. Discuss the use of these techniques in the study of cells: electrophoresis, centrifugation, radioactive tracers, and autoradiography; and tissue culture experiments. Discuss the importance of using the results of all these techniques simultaneously to an accurate representation of cellular biology using several different perspectives.

THE STRUCTURE OF CELLS #1700 Student Instructions (Annotated)

Much of the study of biology is really the study of cells. All life as we know it is composed of cells. Most cells are too small to be seen with the naked eye, although some, like a frog's egg, are quite visible. For the study of most cells, a biologist needs a specialized tool like the microscope. The microscope has had a tremendous effect on our understanding of the world around us, possibly as much of an effect as any invention in recorded history.

This kit is designed to show you a sample of typical cells and to help you understand something about the structure and function of cells. Throughout the activity, you will be examining cells for various structures called *organelles.* A cell is made up of these specialized components. Each organelle has a particular role in the life of the cell.

Part A - Typical Cells

Obtain the following materials: microscope slides, cover slips, a toothpick, a razor blade (*BE CAREFUL*), and a plastic dish. Locate the bottles of Lugol 's Solution, Janus Green B stain, and hypertonic saline, and a microscope. Lugol's Solution is a mixture of iodine and potassium iodide in alcohol. *Be careful with the Lugol's Solution as it may stain your skin or clothing*. Ask your instructor how to use the microscope if you do not already know.

1 Using a toothpick, gently rub the inside of your cheek. Put the material on a glass slide. Gently drop a cover slip over the material and GENTLY squeeze the cover slip using the eraser end of your pencil.

Place the slide under the microscope and focus on the material using low power, then high power. Make drawings below of what you see:

Tear off a small piece of filter paper or toweling. Put a drop of Lugol's Solution stain on the slide next to the cover slip. Place the piece of filter paper next to and touching the cover slip on the side opposite the drop of stain, and draw the stain under the cover slip. Look at the slide under high power. Make a drawing of what you see.

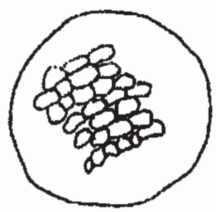
You probably noticed that you could see more detail after you stained the cells with the Lugol's Solution. This is the reason that scientists use stains in their study of cells.

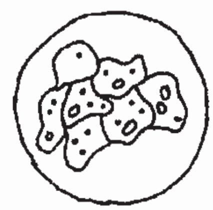
Inside each cell is a darkly stained object which is either round or oval in shape called the ***nucleus*** The nucleus is the control center of the cell. It contains a molecule called *DNA* which carries a chemical code for all of the activities of the cell. The code is transferred from the DNA into molecules of protein. Each protein performs a different task in the cell.

Look closely. Notice that inside the nucleus is a small area which is darker than the rest of the nucleus. The ***nucleolus*** makes small objects called ***ribosomes*** which then manufacture the proteins for the cell. Ribosomes are too small to be seen with a light microscope. Scientists can see them using an electron microscope. A picture of ribosomes may be available in your text or reference book.

The material outside of the nucleus is called the ***cytoplasm.*** Many small objects may be present in the cytoplasm. These objects are ***organelles*** ("little organs") of the cell. Cheek cells have several types of organelles: ***mitochondria, Golgi apparatus, inclusions, endoplasmic reticulum,*** and ***lysosomes.*** Each of these will be discussed later.

Get a piece of potato and place it in a plastic dish. *CAREFULLY,* using a razor blade, make as thin a slice as you can near the outer edge of the potato, including the skin. Try to get a piece which is nearly transparent. Carefully transfer the slice to a clean slide. Drop a cover slip on it. Examine the slide under low and high power. Make drawings of what you see:

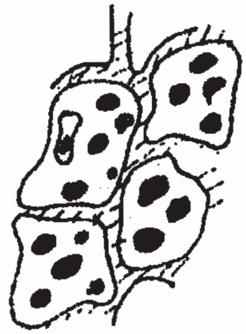




Using the same technique as with the cheek cells, add a drop of Lugol's Solution to the slide. What is visible, even before examining the slide under the microscope?

The potato turns purple (or dark blue)

Make a drawing of the field of view under high power. Label the nucleus and nucleolus if they are identifiable.

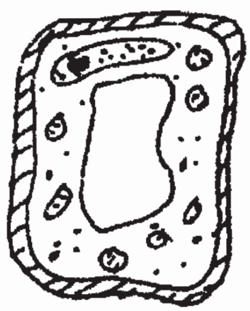


Besides the nucleus and nucleolus, several new structures are present. Notice that each cell is surrounded by a band of material. This is the *cell wall.* It is found only in plant cells. Inside each cell, several rounded or oval objects which are bluish in color should be visible. These are *starch grains* which store the starch the plant has made. They are similar to inclusions in animal cells.

If the potato is very fresh, some small green objects in the cells near the skin of the potato may be visible. These are ***chloroplasts.*** They allow the plant to make food using the sun's energy. These may not be visible since potatoes spend most of their time underground and they do not need many chloroplasts!

To see the chloroplasts better, locate a piece of the aquarium plant *Elodea* (you may be using lettuce instead). This plant grows in long strands with many short leaves sticking out in all directions - it looks something like a big green pipe cleaner. Tear off one of the leaves. Put the leaf on a glass slide. Add a drop of aquarium water and a cover slip. Look at the slide under medium or high power. What do you notice about the cells?

The inside of the cell has small green dots which seem to flow around.



Inspect the above cell. Label the nucleus, chloroplasts, cell wall, other organelles, and other large open region in the center of the cell called the ***vacuole.*** The vacuole is like an inclusion, except that it stores mostly water.

Material inside the cells may have been flowing around the vacuole. This is called ***cytoplasmic streaming.*** It helps organelles to come in contact with light energy and get nutrients they need. Save this slide for the next activity.

Part B - Membranes

A very important part of the cell which cannot be seen with a light microscope is the *cell membrane.* Although many people think of it as being like a plastic bag around the cell, it is much more complex and important than that. The membrane controls the flow of material into and out of the cell. It allows water to pass through freely. Water always tends to move toward areas that have a greater concentration of nonwater molecules (this is called *osmosis).* We will use the Elodea leaf to show how the cell responds to its environment.

1 Obtain the bottle of hypertonic saline solution. This is a fairly concentrated solution of salt in water. Since the Elodea lives usually in fresh water, it does not do very well in salt water. Let's see what happens. Put a drop or two of hypertonic saline next to the cover slip of your previously prepared slide. Draw it under the slip with a piece of filter paper or paper towel. Describe what happens to the flow of chloroplasts within the cell as you add the saline solution.

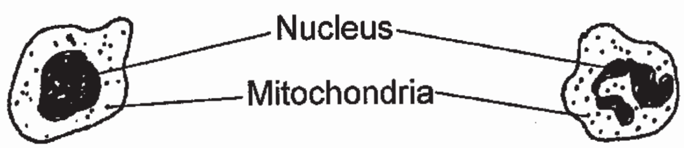
The cytoplasm collects in the middle of the cell and the streaming slows down. All of the chloroplasts are bunched in the center of the cell.

Since the hypertonic solution has salt dissolved in it, the water inside the cell will try to move toward this area of greater concentration. The cell membrane allows water within the cell to flow out of the cell. As this happens, the cell actually shrinks away from the cell wall.

2 a Cut a section about ½ inch long from a piece of fresh celery. Stand this section on end on a clean glass slide. Make a slice through the celery as close to the outer edge as possible. Put this thin sheet, inner side up, on the slide. Cut out the region from between two of the strands of the celery. Add a drop of Janus Green B stain to the piece of celery and add a cover slip. Look at the cells under high power and make a drawing of one typical cell:

Some of the organelles in the cytoplasm have stained a pale bluish-green color. These are the mitochondria. They are responsible for breaking down the food and providing the cells with chemical energy.

Make a slide of your cheek cells and stain them with the Janus Green B. Make a drawing of what you see. Label any organelles you see, such as the nucleus and mitochondria/



We said before that many cells contain a Golgi ***apparatus*** and ***endoplasmic reticulum.*** These are both difficult to see with a light microscope. Books may have pictures of the organelles. The endoplasmic reticulum, along with the ribosomes, is responsible for the manufacture of proteins. The endoplasmic reticulum and the Golgi package and store the proteins for further use.

Make slides of other types of cells from available materials using the Lugol 's and Janus Green B stains. Each time an observation is made, make and label a drawing of what you see. Be sure to clean up after each slide is finished.

Questions for Discussions

1. Why do you think that organelles are important to the cell?

   Since they are specialized to perform particular functions, the organelles can be more efficient than if the functions were distributed throughout the cell. Organelles also allow the cell to control the various activities more closely.

2. What are the differences between the structures of plants and animal cells?

   Plant cells have cell walls and chloroplasts, but animal cells do not. Plant cells have a single large vacuole, while animal cells usually have several small inclusions. Animal cells are usually more random in shape than plant cells.

3. There are many hundreds of different stains and staining techniques. Why would biologists use such a wide variety of techniques?

   Each stain points out a different type of organelle. It should be possible to use stains which react with different types of chemicals found in the different organelles.

4. Heart muscle cells need a great deal of energy for their activity. The cells of fat tissue are responsible for storing excess food in the body. What organelles would you expect to find in large numbers in these two types of cells?

The heart muscle cells would tend to have many mitochondria, since they provide the cell with energy. Fat cells might have many storage inclusions to store the excess fat. Both cells would have a nucleus and other organelles as needed.

5. Would there be more chloroplasts on the top or the bottom side of a leaf, and why?

There would probably be more chloroplasts on the top of a leaf than the bottom. The chloroplasts are responsible for trapping light, and more light hits the upper surface than the lower. It would be more efficient for the plant to have most of the chloroplasts near the light energy.

SAFETY INSTRUCTIONS: IMPORTANT

NOTICE TO TEACHERS REGARDING LABORATORY REAGENTS

Perhaps the best general rule regarding the safe handling of laboratory chemicals is to treat all of them as being potentially dangerous. This means that none of them should be taken internally, and that any external contact should be washed thoroughly. In fact, most of the chemicals provided in The Science Source kits are diluted enough that they are not hazardous. The following lists indicate appropriate antidotes for the hazardous chemicals. Check this list for the chemicals provided in the kit:

I. **Concentrated Acids & Bases - Do not** induce vomiting, dilute with water, then milk or egg white, call a physician immediately.

1. 25 % Acetic Acid
2. 3M Hydrochloric Acid
3. Concentrated Sulfuric Acid

II **Dilute Acids & Bases - Dilute** with water, then milk.

1. 1 M, 0.5M, 0.1 M Hydrochloric Acid
2. Oxalic Acid
3. Sodium Hydroxide
4. Ammonium Hydroxide

III **Miscellaneous Chemicals** - Dilute immediately with water. Induce vomiting with warm salt water, or warm baking soda solution.

1. Ammonium Chloride
2. Ammonium Oxalate
3. Barium Chloride
4. Biuret Reagent
5. Bromthymol Blue
6. Calcium Chloride
7. Ethanol (Denatured Alcohol)
8. Ferric Ammonium Sulfate
9. Hydrogen Peroxide
10. Janus Green B
11. Lead Nitrate
12. Lugol's Solution
13. Magnesium Reagent (Titan Yellow, Alcohol)
14. Mercuric Nitrate
15. Methylene Blue
16. Ninhydrin
17. Phosphorus Reagent (Ammonium Molybdate, Nitric Acid)
18. Potassium Ferricyanide
19. Potassium Permanganate
20. Silver Nitrate
21. Sodium Carbonate
22. Sodium Thiosulfate
23. Sudan IV

IV. Organic Solvents - Do not induce vomiting. Dilute with water and milk. Call a physician immediately.

Isopropyl Alcohol

CELL STRUCTURE #1700 Student Instructions

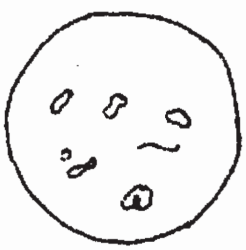
Much of the study of biology is really the study of cells. All life as we know it is composed of cells. Most cells are too small to be seen with the naked eye, although some, like a frog's egg, are quite visible. For the study of most cells, a biologist needs a specialized tool like the microscope. The microscope has had a tremendous effect on our understanding of the world around us, possibly as much of an effect as any invention in recorded history.

This kit is designed to show you a sample of typical cells and to help you understand something about the structure and function of cells. Throughout the activity, you will be examining cells for various structures called *organelles.* A cell is made up of these specialized components. Each organelle has a particular role in the life of the cell.

Part A - Typical Cells

Obtain the following materials: microscope slides, cover slips, a toothpick, a razor blade (BE CAREFUL), and a plastic dish. Locate the bottles of Lugol 's Solution, Janus Green B stain, and hypertonic saline, and a microscope. Lugol's Solution is a mixture of iodine and potassium iodide in alcohol.*Be careful with the Lugol's Solution as it may stain your skin or clothing.* Ask your instructor how to use the microscope if you do not already know.

1 Using a toothpick, gently rub the inside of your cheek. Put the material on a glass slide. Gently drop a cover slip over the material and GENTLY squeeze the cover slip using the eraser end of your pencil.



Place the slide under the microscope and focus on the material using low power, then high power. Make drawings below of what you see:

Tear off a small piece of filter paper or toweling. Put a drop of Lugol's Solution stain on the slide next to the cover slip. Place the piece of filter paper next to and touching the cover slip on the side opposite the drop of stain, and draw the stain under the cover slip. Look at the slide under high power. Make a drawing of what you see.

You probably noticed that you could see more detail after you stained the cells with the Lugol's Solution. This is the reason that biologists use stains in their study of cells.

Inside each cell is a darkly stained object which is either round or oval in shape called the ***nucleus*** The nucleus is the control center of the cell. It contains a molecule called *DNA* which carries a chemical code for all of the activities of the cell. The code is transferred from the DNA into molecules of protein. Each protein performs a different task in the cell.

Look closely. Notice that inside the nucleus is a small area which is darker than the rest of the nucleus. The ***nucleolus*** makes small objects called ***ribosomes*** which then manufacture the proteins for the cell. Ribosomes are too small to be seen with a light microscope. Scientists can see them using an electron microscope. A picture of ribosomes may be available in your test or reference book.

The material outside of the nucleus is called the ***cytoplasm.*** Many small objects may be present in the cytoplasm. These objects are ***organelles*** ("little organs") of the cell. Cheek cells have several types of organelles: ***mitochondria, Golgi apparatus, inclusions, endoplasmic reticulum,*** and ***lysosomes.*** Each of these will be discussed later.

Get a piece of potato and place it in a plastic dish. *CAREFULLY,* using a razor blade, make as thin a slice as you can near the outer edge of the potato, including the skin. Try to get a piece which is nearly transparent. Carefully transfer the slice to a clean slide. Drop a cover slip on it. Examine the slide under low and high power. Make drawings of what you see:

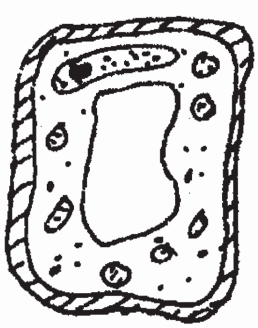
Using the same technique as with the cheek cells, add a drop of Lugol's Solution to the slide. What is visible, even before examining the slide under the microscope?

Make a drawing of the field of view under high power. Label the nucleus and nucleolus if they are identifiable.

Besides the nucleus and nucleolus, several new structures are present. Notice that each cell is surrounded by a band of material. This is the *cell wall.* It is found only in plant cells. Inside each cell, several rounded or oval objects which are bluish in color should be visible. These are *starch grains* which store the starch the plant has made. They are similar to inclusions in animal cells.

If the potato is very fresh, some small green objects in the cells near the skin of the potato may be visible. These are ***chloroplasts.*** They allow the plant to make food using the sun's energy. These may not be visible since potatoes spend most of their time underground and they do not need many chloroplasts!

To see the chloroplasts better, locate a piece of the aquarium plant *Elodea* (you may be using lettuce instead). This plant grows in long strands with many short leaves sticking out in all directions - it looks something like a big green pipecleaner. Tear off one of the leaves. Put the leaf on a glass slide. Add a drop of aquarium water and a cover slip. Look at the slide under medium or high power. What do you notice about the cells?



Inspect the above cell. Label the nucleus, chloroplasts, cell wall, other organelles, and other large open region in the center of the cell called the ***vacuole.*** The vacuole is like an inclusion, except that it stores mostly water.

Material inside the cells may have been flowing around the vacuole. This is called ***cytoplasmic streaming.*** It helps organelles to come in contact with light energy and get nutrients they need. Save this slide for the next activity.

Part B - Membranes

A very important part of the cell which cannot be seen with a light microscope is the *cell membrane.* Although many people think of it as being like a plastic bag around the cell, it is much more complex and important than that. The membrane controls the flow of material into and out of the cell. It allows water to pass through freely. Water always tends to move toward areas that have a greater concentration of nonwater molecules (this is called *osmosis).* We will use the Elodea leaf to show how the cell responds to its environment.

1 Obtain the bottle of hypertonic saline solution. This is a fairly concentrated solution of salt in water. Since the Elodea lives usually in fresh water, it does not do very well in salt water. Let's see what happens. Put a drop or two of hypertonic saline next to the cover slip of your previously prepared slide. Draw it under the slip with a piece of filter paper or paper towel. Describe what happens to the flow of chloroplasts within the cell as you add the saline solution.

Since the hypertonic solution has salt dissolved in it, the water inside the cell will try to move toward this area of greater concentration. The cell membrane allows water within the cell to flow out of the cell. As this happens, the cell actually shrinks away from the cell wall.

2 a Cut a section about 1/2 inch long from a piece of fresh celery. Stand this section on end on a clean glass slide. Make a slice through the celery as close to the outer edge as possible. Put this thin sheet, inner side up, on the slide. Cut out the region from between two of the strands of the celery. Add a drop of Janus Green B stain to the piece of celery and add a cover slip. Look at the cells under high power and make a drawing of one typical cell:

Some of the organelles in the cytoplasm have stained a pale bluish-green color. These are the mitochondria. They are responsible for breaking down the food and providing the cells with chemical energy.

Make a slide of your cheek cells and stain them with the Janus Green B. Make a drawing of what you see. Label any organelles you see, such as the nucleus and mitochondria.

We said before that many cells contain a Golgi ***apparatus*** and ***endoplasmic reticulum.*** These are both difficult to see with a light microscope. Books may have pictures of the organelles. The endoplasmic reticulum, along with the ribosomes, is responsible for the manufacture of proteins. The endoplasmic reticulum and the Golgi package and store the proteins for further use.

Make slides of other types of cells from available materials using the Lugol 's and Janus Green B stains. Each time an observation is made, make and label a drawing of what you see. Be sure to clean up after each slide is finished.

Questions for Discussions

1. Why do you think that organelles are important to the cell?

2. What are the differences between the structures of plants and animal cells?

3. There are many hundreds of different stains and staining techniques. Why would biologists use such a wide variety of techniques?

4. Heart muscle cells need a great deal of energy for their activity. The cells of fat tissue are responsible for storing excess food in the body. What organelles would you expect to find in large numbers in these two types of cells?

5. Would there be more chloroplasts on the top or the bottom side of a leaf, and why?