General Biology (2)

Lab Topic 2

Microscopes and Cells

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify the parts of compound and stereoscopic microscopes and be proficient in their correct use in biological studies.
2. Describe procedures used in preparing materials for electron microscopy and compare these with procedures used in light microscopy.
3. Identify cell structures and organelles from electron micrographs and state the functions of each.
4. Describe features of specific cells and determine characteristics shared by all cells studied.
5. Distinguish between eukaryotic and prokaryotic cells.
6. Discuss the evolutionary significance of increasing complexity from unicellular to multicellular organization and provide examples from the lab.

Sajeda Abdullah ♡

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Introduction

According to cell theory, the *cell* is the fundamental biological unit, the smallest and simplest biological structure possessing all the characteristics of the living condition. All living organisms are composed of one or more cells, and every activity taking place in a living organism is ultimately related to metabolic activities in cells. Thus, understanding the processes of life necessitates an understanding of the structure and function of the cell.

The earliest known cells found in fossilized sediments 3.5 billion years old (called prokaryotic cells) lack nuclei and membrane-bound organelles. Cells with a membrane-bound nucleus and organelles (eukaryotic cells) do not appear in the fossil record for another 2 billion years. But the eventual evolution of the eukaryotic cell and its internal compartmentalization led to enormous biological diversity in single cells. The evolution of loose aggregates of cells ultimately to colonies of connected cells provided for specialization, so that groups of cells had specific and different functions. This early division of labor included cells whose primary function was locomotion or reproduction. The evolution of multicellularity appears to have originated more than once in eukaryotes and provided an opportunity for extensive adaptive radiation as organisms specialized and diversified, eventually giving rise to fungi, plants, and animals. This general trend in increasing complexity and specialization seen in the history of life will be illustrated in Lab Topic 2.

Given the fundamental role played by cells in the organization of life, one can readily understand why the study of cells is essential to the study of life.

Cells, however, are below the limit of resolution of the human eye. We cannot study them without using a microscope. The microscope has probably contributed more than any other instrument to the development of biology as a science and continues today to be the principal tool used in medical and biological research. There are four types of microscopes commonly used by biologists. You will learn how to use two of these microscopes, the compound microscope and the stereoscopic microscope, in today's laboratory. Both of these microscopes use visible light as the source of illumination and are called light microscopes. Two other microscopes, the scanning electron microscope and the transmission electron microscope, use electrons as the source of illumination. Electron microscopes are able to view objects much smaller than those seen in a light microscope. Although these microscopes are not used in this laboratory, you will be given the opportunity to learn more about them in Exercise 2.4.

Microscopes are used by biologists in numerous subdisciplines: genetics, molecular biology, neurobiology, cell biology, evolution, and ecology. The knowledge and skills you develop today will be used and enhanced throughout this course and throughout your career in biology. It is important, therefore, that you take the time to master these exercises thoroughly.

EXERCISE 2.1
The Compound Light Microscope

Materials

compound microscope

Introduction

The microscope is designed to make objects visible that are too difficult or too small to see with the unaided eye. There are many variations of light microscopes, including phase-contrast, darkfield, polarizing, and UV. These differ primarily in the source and manner in which light is passed through the specimen to be viewed.

The microscopes in biology lab are usually compound binocular or monocular light microscopes, some of which may have phase-contrast attachments. Compound means that the scopes have a minimum of two magnifying lenses (the ocular and the objective lenses). Binocular microscopes have two eyepieces, monoculars have only one eyepiece and light refers to the type of illumination used, that is, visible light from a lamp.

Your success in and enjoyment of a large portion of the laboratory work in introductory biology will depend on how proficient you become in the use of the microscope. When used and maintained correctly, these precision instruments are capable of producing images of the highest quality.

Although there are many variations in the features of microscopes, they are all constructed on a similar plan (Figure 2.1). In this exercise you will be introduced to the common variations found in different models of compound microscopes and asked to identify those features found on your microscope.

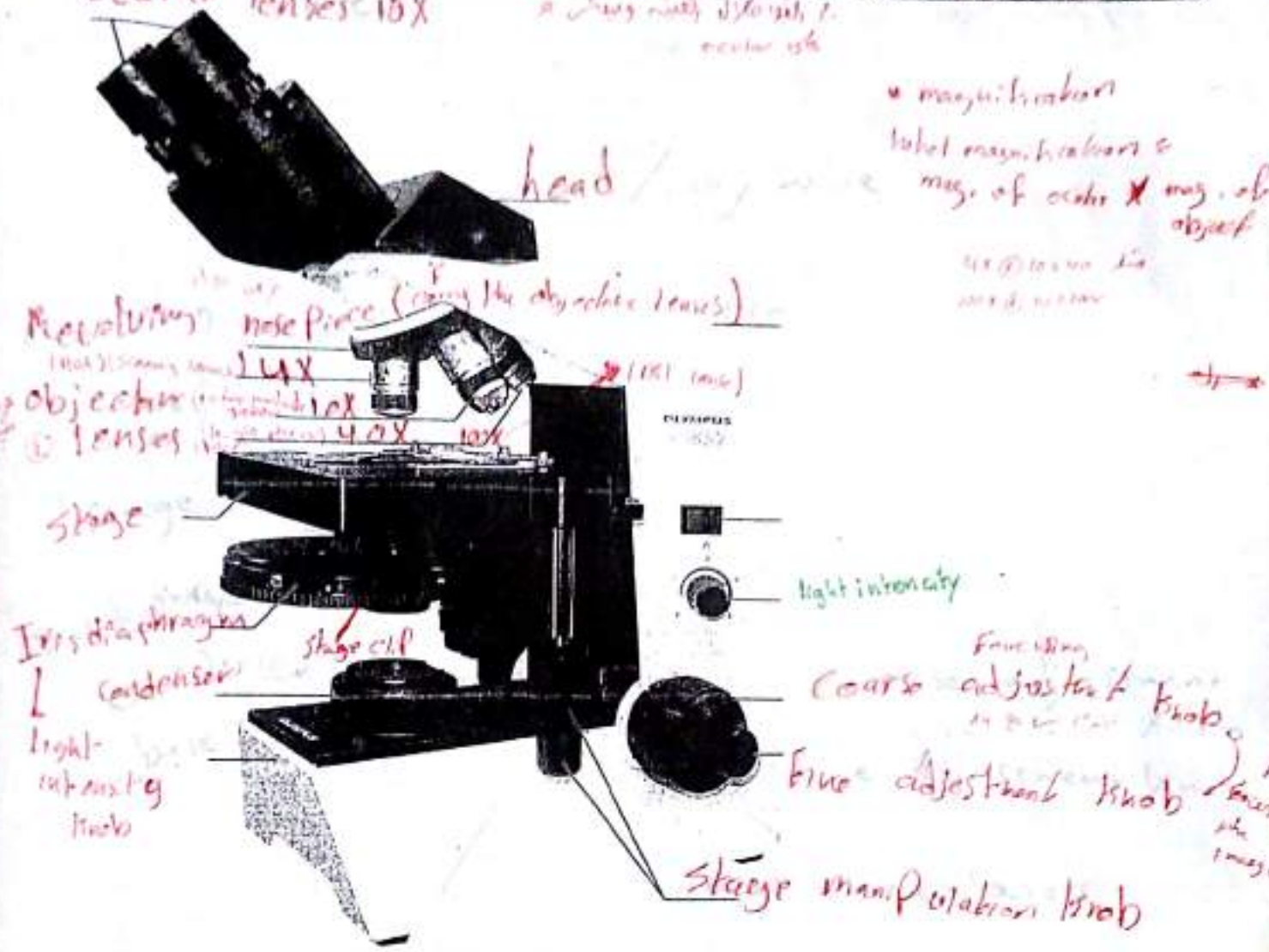


Figure 2.1a.
The compound binocular light microscope. Locate the parts of your microscope described in Exercise 2.1 and label this photograph. Indicate in the margin of your lab manual any features unique to your microscope.

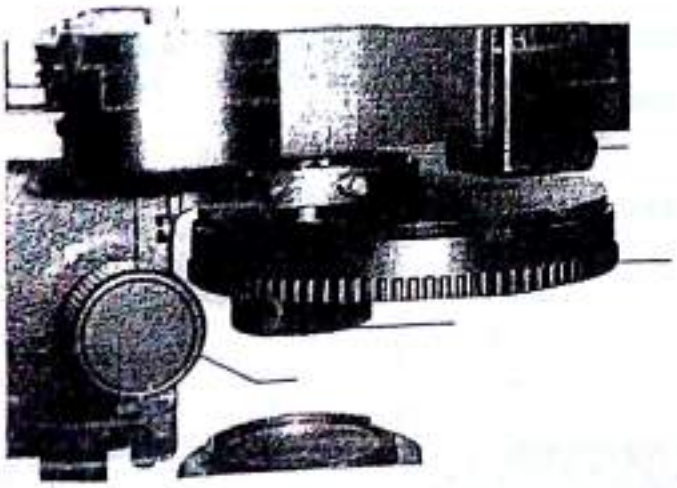
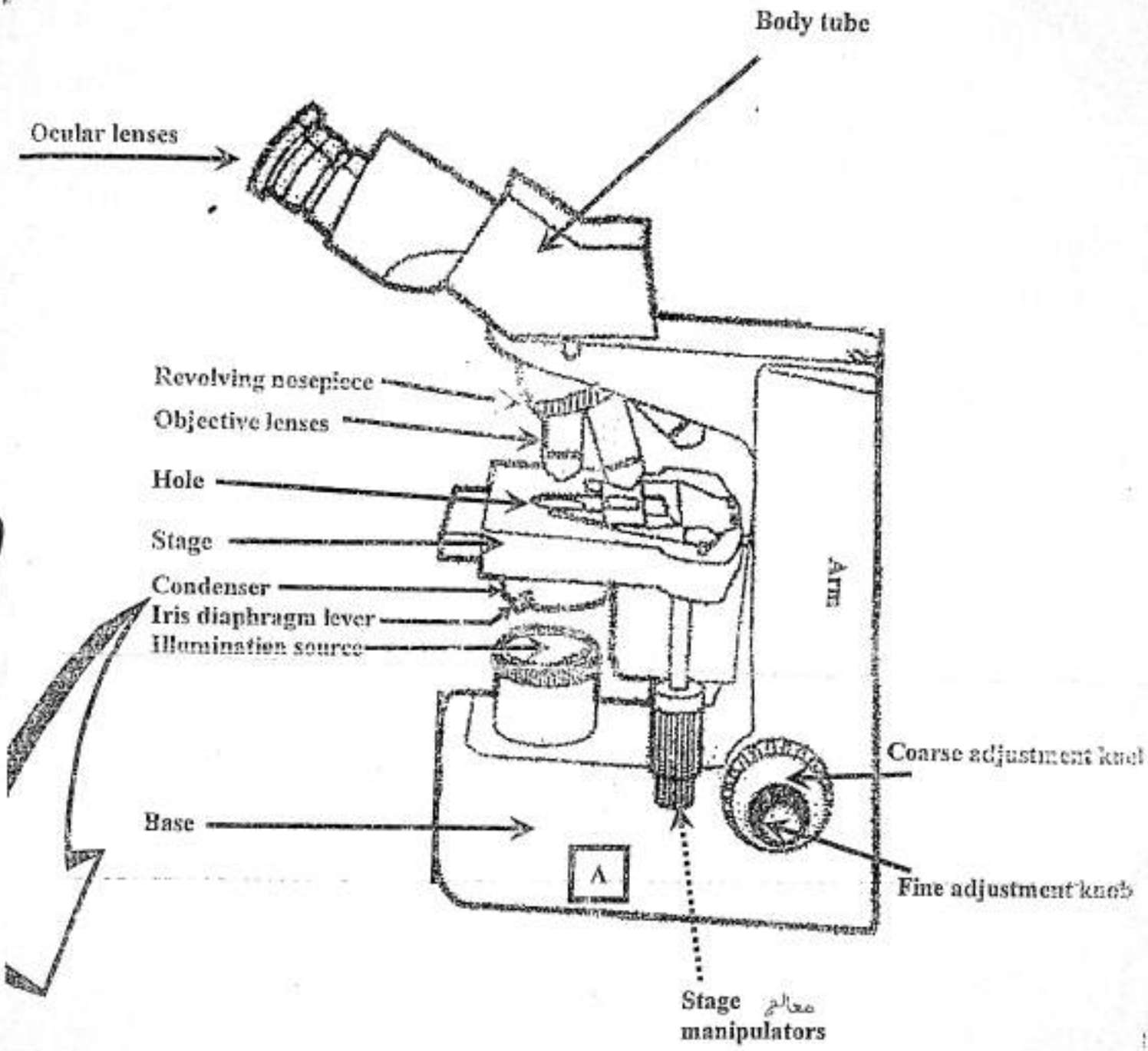
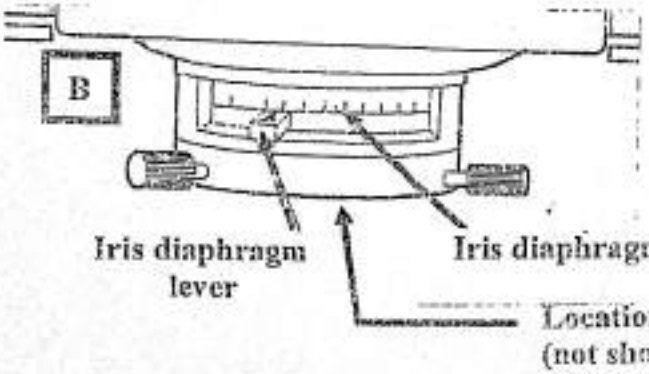


Figure 2.1b.
Enlarged photo of compound light microscope as viewed from under the stage. This microscope is equipped with phase-contrast optics. Locate the condenser, condenser adjustment knob, phase-contrast revolving turret, and iris diaphragm on your microscope (if present) and label them on the diagram.





⚠ Please treat these microscopes with the greatest care!

Procedure

1. Obtain a compound light microscope, following directions from your instructor. To carry the microscope correctly, hold the arm with one hand, and support the base with your other hand. Remove the cover, but do not plug in the microscope.
2. Locate the parts of your microscope, and label Figure 2.1. Refer to the following description of a typical microscope. In the spaces provided, indicate the specific features related to your microscope.

   a. The head supports the two sets of magnifying lenses. The ocular is the lens in the eyepiece, which typically has a magnification of 10×. If your microscope is binocular, the distance between the eyepieces (interpupillary distance) can be adjusted to suit your eyes. Move the eyepieces apart, and look for the scale used to indicate the distance between the eyepieces. Do not adjust the eyepieces at this time. A pointer has been placed in the eyepiece and is used to point to an object in the field of view, the circle of light that one sees in the microscope.

   Is your microscope monocular (one eyepiece) or binocular (two eyepieces)?

   What is the magnification of your ocular(s)? 10 x

✳ Although the eyepiece may be removable, it should not be removed from the microscope.

   b. Objectives are the three lenses on the revolving nosepiece. The shortest lens is typically 4× and is called the scanning lens. The intermediate lens is 10×, and the longest, the high-power lens, is 40× (the fourth position on the nosepiece is empty). It is important to clean both the objective and ocular lenses before each use. Dirty lenses will cause a blurring or fogging of the image. Always use lens paper for cleaning! Any other material (including Kimwipes®) may scratch the lenses.

   What is the magnification of each of your objectives? List them in order of increasing magnification.

   c. The arm supports the stage and condenser lens. The condenser lens is used to focus the light from the lamp through the specimen to be viewed. The height of the condenser can be adjusted by an adjustment knob. The iris diaphragm controls the width of the circle of light and, therefore, the amount of light passing through the specimen.

If your microscope has phase-contrast optics, the condenser may be housed in a revolving turret. When the turret is set on 0, the normal optical arrangement is in place. This condition is called brightfield microscopy. Other positions of the turret set phase-contrast optics in place. To use phase-contrast, the turret setting must correspond to the magnifying power of the objective being used.

Is your microscope equipped with phase-contrast optics?

The stage supports the specimen to be viewed. A mechanical stage can be moved right and left and back and forth by two stage adjustment knobs. With a stationary stage, the slide is secured under stage clips and moved slightly by hand while viewing the slide. The distance between the stage and the objective can be adjusted with the coarse and fine focus adjustment knobs.

Does your microscope have a mechanical or stationary stage?

d. The base acts as a stand for the microscope and houses the lamp. In some microscopes, the intensity of the light that passes through the specimen can be adjusted with the light intensity lever. Generally, more light is needed when using high magnification than when using low magnification. Describe the light system for your microscope.

EXERCISE 2.2

Basic Microscope Techniques

شرح تجربة حرف e

Materials

clear ruler
coverslips
prepared slides: letter and crossed thread
lens paper
blank slides
Kimwipes®
dropper bottle with distilled water

Introduction

In this exercise, you will learn to use the microscope to examine a recognizable object, a slide of the letter *e*. Recall that microscopes vary, so you may have to omit steps that refer to features not available on your microscope. Practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for the best contrast.

Procedure

1. Clean microscope lenses.

   Each time you use the microscope, you should begin by cleaning the lenses. Using lens paper moistened with a drop of distilled water, wipe the ocular, objective, and condenser lenses. Wipe them again with a piece of dry lens paper.

Use only lens paper on microscope lenses. Do not use Kimwipes®, tissues, or other papers.

2. Adjust the focus on your microscope.
   a. Plug your microscope into the outlet.
   b. Turn on the light. Adjust the light intensity to mid-range if your microscope has that feature.
   c. Rotate the 4× objective into position using the revolving nosepiece ring, not the objective itself.
   d. Take the letter slide and wipe it with a Kimwipe® tissue. Each time you study a prepared slide, you should first wipe it clean. Place the letter slide on the stage, and center it over the stage opening.

Slides should be placed on and removed from the stage only when the 4× objective is in place. Removing a slide when the higher objectives are in position may scratch the lenses.

   e. Look through the ocular and bring the letter into rough focus by slowly focusing upward using the coarse adjustment.
   f. For binocular microscopes, looking through the oculars, move the oculars until you see only one image of the letter *e*. In this position, the oculars should be aligned with your pupils. In the margin of your lab manual, make a note of the **interpupillary distance** on the scale between the oculars. Each new lab day, before you begin to use the microscope, set this distance.
   g. Raise the condenser to its highest position, and fully close the iris diaphragm.
   h. Looking through the ocular, slowly lower the condenser just until the graininess disappears. Slowly open the iris diaphragm just until the entire field of view is illuminated. This is the correct position for both the condenser and the iris diaphragm.
   i. Rotate the 10× objective into position.
   j. Look through the ocular and slowly focus upward with the coarse adjustment knob until the image is in rough focus. Sharpen the focus using the fine adjustment knob.

Do not turn the fine adjustment knob more than two revolutions in either direction. If the image does not come into focus, return to 10× and refocus using the coarse adjustment.

k. For binocular microscopes, cover your left eye and use the fine adjustment knob to focus the fixed (right) ocular until the letter *e* is in maximum focus. Now cover the right eye and, using the diopter ring on the left ocular, bring the image into focus. The letter *e* should now be in focus for both of your eyes. Each new lab day, as you begin to study your first slide, repeat this procedure.

l. You can increase or decrease the contrast by adjusting the iris diaphragm opening. Note that the maximum amount of light provides little contrast. Adjust the aperture until the image is sharp.

m. Move the slide slowly to the right. In what direction does the image in the ocular move?

n. Is the image in the ocular inverted relative to the specimen on the stage?

o. Center the specimen in the field of view; then rotate the 40× objective into position while watching from the side. *If it appears that the objective will hit the slide, stop and ask for assistance.*

Most of the microscopes have **parfocal** lenses, which means that little refocusing is required when moving from one lens to another. If your scope is *not* parfocal, ask your instructor for assistance.

p. After the 40× objective is in place, focus using the fine adjustment knob.

Never focus with the coarse adjustment knob when you are using the high-power objective.

q. The distance between the specimen and the objective lens is called the working distance. Is this distance greater with the 40× or the 10× objective?

3. Compute the total magnification of the specimen being viewed. To do so, multiply the magnification of the ocular lens by that of the objective lens.

a. What is the total magnification of the letter as the microscope is now set?

b. What would be the total magnification if the ocular were 20× and the objective were 100× (oil immersion)? This is the magnification achieved by the best light microscopes.

4. Measure the diameter of the field of view. Once you determine the size of the field of view for any combination of ocular and objective lenses, you can determine the size of any structure within that field.
   a. Rotate the 4× objective into position and remove the letter slide.
   b. Place a clear ruler on the stage, and focus on its edge.
   c. The distance between two lines on the ruler is 1 mm. What is the diameter (mm) of the field of view?

   d. Convert this measurement to micrometers (μm), a more commonly used unit of measurement in microscopy (1 mm = 1,000 μm).
   e. Measure the diameters of the field of view for the 10× and 40× objectives, and enter all three in the spaces below to be used for future reference.

      4× = _____ 10× = _____ 40× = _____

   f. What is the relationship between the size of the field of view and magnification?

5. Determine spatial relationships. The depth of field is the thickness of the specimen that may be seen in focus at one time. Because the depth of focus is very short in the compound microscope, focus up and down to clearly view all planes of a specimen.
   a. Rotate the 4× objective into position and remove the ruler. Take a slide of crossed threads, wipe it with a Kimwipe®, and place the slide on the stage. Center the slide so that the region where the two threads cross is in the center of the stage opening.
   b. Focus on the region where the threads cross. Are both threads in focus at the same time?

   c. Rotate the 10× objective into position and focus on the cross. Are both threads in focus at the same time?

   Does the 4× or the 10× objective have a shorter depth of field?

d. Focus upward (move the stage up) with the coarse adjustment until both threads are just out of focus. Slowly focus down using the fine adjustment. Which thread comes into focus first? Is this thread lying under or over the other thread?

e. Rotate the 40× objective into position and slowly focus up and down, using the fine adjustment only. Does the 10× or the 40× objective have a shorter depth of field?

6. At the end of your microscope session, use the following procedures to store your microscope.
   a. Rotate the 4× objective into position.
   b. Remove the slide from the stage.
   c. Return the phase-contrast condenser to the 0 setting if you have used phase-contrast.
   d. Set the light intensity to its lowest setting and turn off the power.
   e. Unplug the cord and wrap it around the base of the microscope.
   f. Replace the dust cover.
   g. Return the microscope to the cabinet using two hands; one hand should hold the arm, and the other should support the base.

These steps should be followed every time you store your microscope.

EXERCISE 2.3

The Stereoscopic Microscope

Materials

stereoscopic microscope
dissecting needles
living *Elodea*
microscope slides
droppers of water
coverslips

Introduction

The stereoscopic (dissecting) microscope has relatively low magnification, 7× to 30×, and is used for viewing and manipulating relatively large objects. The binocular feature creates the stereoscopic effect. The stereoscopic microscope is similar to the compound microscope except in the following ways: (1) The depth of field is much greater than with the compound microscope, so objects are seen in three dimensions, and (2) the light source can be directed down onto as well as up through an object, which permits the viewing of objects too thick to transmit light. Light directed down on the object is called **reflected** or **incident light**. Light passing through the

object is called transmitted light. Refer to the Websites section at the end of this lab topic for a video of stereomicroscope use.

Procedure

1. Remove your stereoscopic microscope from the cabinet and locate the parts labeled in Figure 2.2. Locate the switches for both incident and transmitted light. In the margin of your lab manual, note any features of your microscope that are not shown in the figure. What is the range of magnification for your microscope?
2. Observe an object of your choice at increasing magnification. Select an object that fits easily on the stage (e.g., ring, coin, fingertip, pen, ruler).
   a. Place the object on the stage and adjust the interpupillary distance (distance between the oculars) by gently pushing or pulling the oculars until you can see the object as a single image.
   b. Change the magnification and note the three-dimensional characteristics of your object.
   c. Adjust the lights, both reflected and transmitted. Which light gives you the best view of your object?

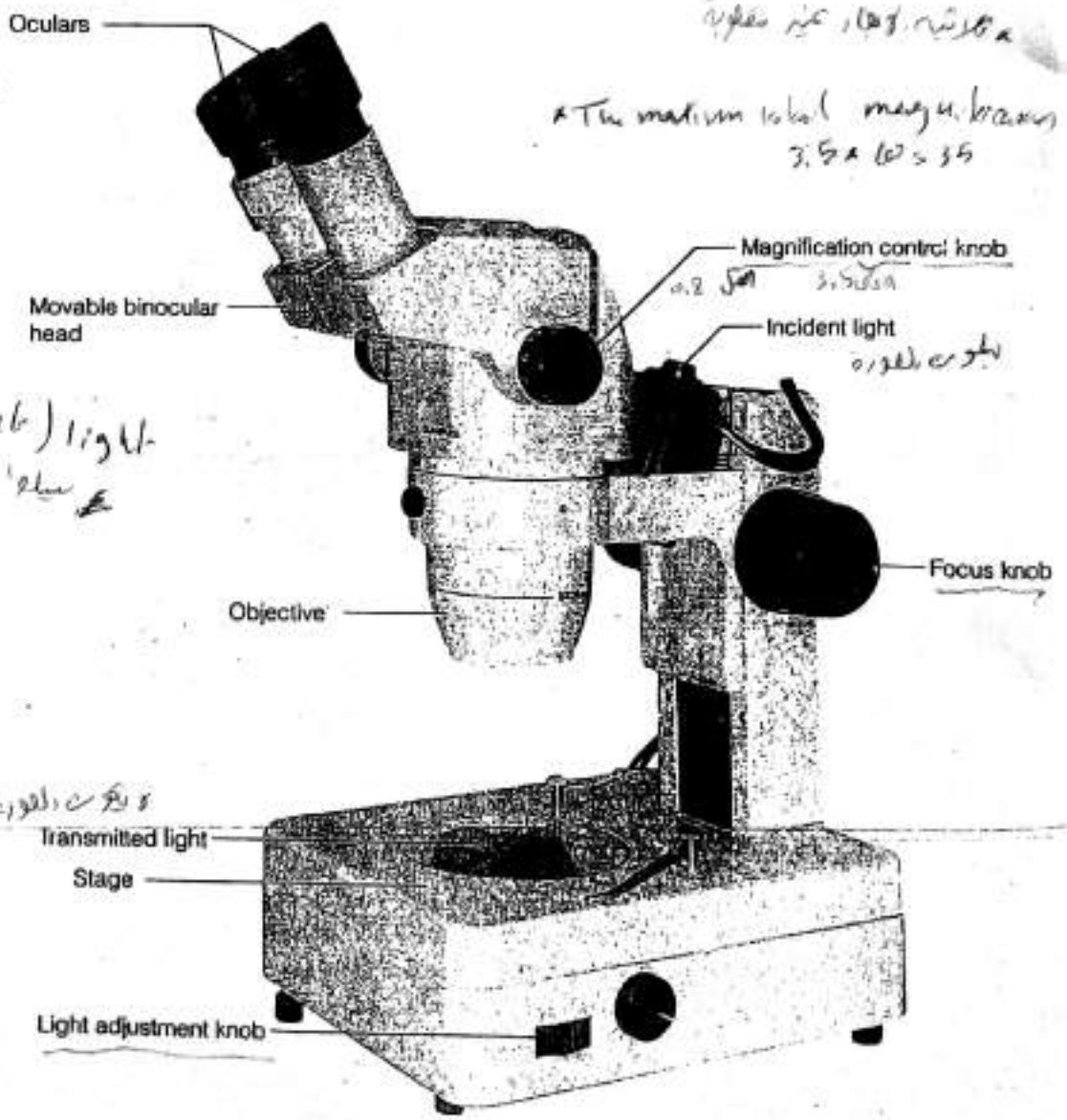


Figure 2.2.
The stereoscopic (dissecting) microscope. Locate the parts of your microscope by referring to this photograph. Note in the margin any features of your microscope that are not shown in the photograph.

3. Prepare a **wet mount** of *Elodea*. Living material is often prepared for observation using a wet mount. (The material is either in water or covered with water prior to adding a coverslip.) You will use this technique to view living material under the dissecting and compound microscopes (Figure 2.3).
   a. Place a drop of water in the center of a clean microscope slide.
   b. Remove a single leaf of *Elodea*, and place it in the drop of water.
   c. Using a dissecting needle, place a coverslip at a 45° angle above the slide with one edge of the coverslip in contact with the edge of the water droplet, as shown.
   d. Lower the coverslip slowly onto the slide, being careful not to trap air bubbles in the droplet. The function of the coverslip is threefold: (1) to flatten the preparation, (2) to keep the preparation from drying out, and (3) to protect the objective lenses. Over long periods of time, the preparation may dry out, at which point water can be added to one edge of the coverslip.

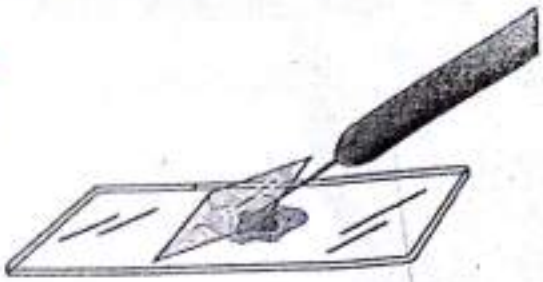


Figure 2.3.
Preparation of a wet mount. Place a drop of water and your specimen on the slide. Using a dissecting needle, slowly lower a coverslip onto the slide, being careful not to trap air bubbles in the droplet.

Specimens can be viewed without a coverslip using the stereoscopic microscope, but a coverslip must always be used with the compound microscope.

4. Observe the structure of the *Elodea* leaf at increasing magnification.
   a. Place the leaf slide on the stage and adjust the focus. Change the magnification and note the characteristics of the leaf at increased magnification.
   b. Sketch the leaf in the margin of your lab manual and list, in the space below, the structures that are visible at low and high magnification.

   Low:

   High:

   Is it possible to see cells in the leaf using the stereoscopic microscope?

   Organelles?

   c. Save your slide for later study. In Exercise 2.5, Lab Study C, you will be asked to compare these observations of *Elodea* with those made while using the compound microscope.

EXERCISE 2.4

The Transmission Electron Microscope

Materials

demonstration resources for the electron microscope
electron micrographs

Introduction

The transmission electron microscope (TEM) magnifies objects approximately 1,000× larger than a light microscope can (up to 1,000,000×). This difference depends on the **resolving power** of the electron microscope, which allows the viewer to see two objects of comparable size that are close together and still be able to recognize that they are two objects rather than one. Resolving power, in turn, depends on the wavelength of light passed through the specimen: the shorter the wavelength, the greater the resolution. Because electron microscopes use electrons as a source of illumination and electrons have a much shorter wavelength than does visible light, the resolving power of electron microscopes is much greater than that of light microscopes. Both the electron and light microscopes can be equipped with lenses that allow for tremendous magnification, but only the electron microscope has sufficient resolving power to make these lenses useful.

Procedure

1. Compare the features of the light and electron microscopes (Figure 2.4).
   a. Name three structures found in both microscopes.

   b. What is the energy source for the electron microscope?

      For the compound microscope?

   c. Describe how the lenses differ for the two microscopes.

2. Using the resources provided by your instructor, review the procedures and materials for preparing a specimen for electron microscopy. Websites that describe electron microscopy are listed at the end of this lab topic
3. Define the following terms on separate paper or in the margin of your lab manual.

fixation
embedding
ultramicrotome
boat on diamond or glass knife
staining with heavy metals
electromagnetic lenses
fluorescent screen
vacuum

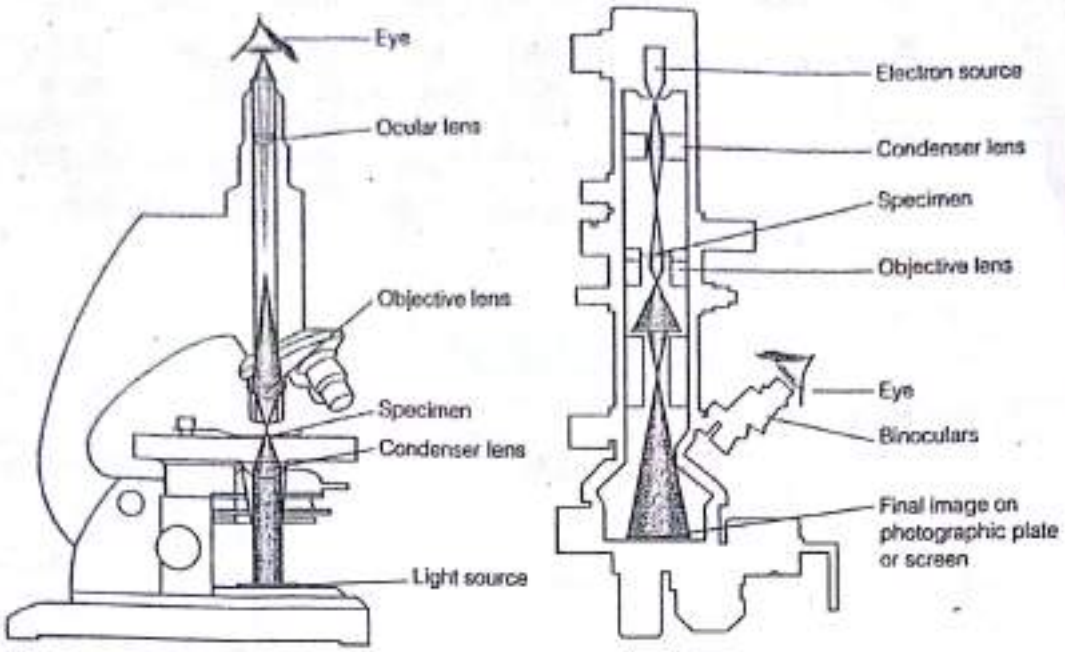


Figure 2.4.
Comparison of light microscope and electron microscope. The source of illumination is light for the light microscope and electrons for the electron microscope. The image is magnified by glass objectives in light microscopy and by electromagnets in electron microscopy.

4. When an electron microscope is used, cells are usually studied using electron micrographs, photographs taken of the image seen on the fluorescent screen. Observe electron micrographs on demonstration in the laboratory, in your textbook, or on websites listed at the end of this lab topic. For the following list of cellular organelles, *underline* those organelles and structures that you predict may be seen *only* with the electron microscope.

plasma membrane, cell wall, nucleus, chloroplast,
mitochondria, vacuole, Golgi apparatus,
peroxisome, lysosome, endoplasmic reticulum,
ribosome, flagella, cilia

EXERCISE 2.5
The Organization of Cells

In this exercise, you will examine the features common to all eukaryotic cells that are indicative of their common ancestry. However, you will observe that all cells are not the same. Some organisms are **unicellular** (single-celled), with all living functions (respiration, digestion, reproduction, and excretion) handled by that one cell. Others form random, temporary **aggregates**, or clusters, of cells. Clusters composed of a consistent and predictable number of cells are called **colonies**. Simple colonies are clusters

of cells of similar types with a predictable structure, but the cells have no physiological connections. More complex colonies have cells of different types. In some colonial algae the cells are called *somatic cells* (cells that are not reproductive) and cells that specialize in reproduction. In these colonies, if either type of cell is isolated from the colony, they may be reproductive, dividing and producing new colonies.

Other algae may contain both cell types, somatic and reproductive, but their somatic cells *never* become reproductive, even when isolated, and reproductive cells cannot persist independently, but must be associated with somatic cells to live. These organisms are described as **multicellular** because they have two or more types of cells with specialized structure and function, and these cell types, when isolated, are not capable of prepetuating the species in the wild. In more complex algae, fungi, plants, and animals, specialized cells may be organized into tissues that perform particular functions for the organism. Tissues, in turn, may combine to form organs, and tissues and organs combine to form a coordinated single organism.

In this exercise, you will examine selected unicellular, aggregate, colonial, and multicellular organisms. (See Color Plates 1–7.)

Lab Study A. Unicellular Organisms

Materials

microscope slides
culture of *Amoeba*
living termites
forceps
coverslips
dissecting needles
insect Ringers

Introduction

Unicellular eukaryotic organisms may be **autotrophic** (photosynthetic) or **heterotrophic** (deriving food from other organisms or their by-products). These diverse organisms, called protists, will be studied in detail in Lab Topic 14.

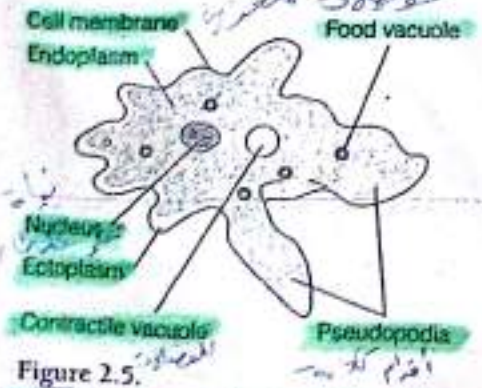


Figure 2.5.
Amoeba. An amoeba moves using pseudopodia. Observe the living organisms using the compound microscope (Color Plate 1).

Procedure

1. Examine a living *Amoeba* (Figure 2.5) under the compound microscope. Amoebas are aquatic organisms commonly found in ponds. To transfer a specimen to your slide, follow these procedures:
   a. Place the culture dish containing the amoeba under the dissecting microscope, and focus on the bottom of the dish. The amoeba will appear as a whitish, irregularly shaped organism attached to the bottom.
   b. Using a clean pipette (it is important not to interchange pipettes between culture dishes), transfer a drop with several amoebas to your microscope slide. To do this, squeeze the pipette bulb *before* you place the tip under the surface of the water. Disturbing the culture as little as possible, pipette a drop of water with debris from the *bottom* of the culture dish. You may use your stereoscopic microscope to scan the slide to locate amoebas before continuing.

c. Cover your preparation with a clean coverslip.

d. Under low power on the compound scope, scan the slide to locate an amoeba. Center the specimen in your field of view; then switch to higher powers.

e. Identify the following structures in the amoeba:

Cell membrane is the boundary that separates the organism from its surroundings.

Ectoplasm is the thin, transparent layer of cytoplasm directly beneath the cell membrane.

Endoplasm is the granular cytoplasm containing the cell organelles.

The **nucleus** is the grayish, football-shaped body that is somewhat granular in appearance. This organelle, which directs the cellular activities, will often be seen moving within the endoplasm.

Contractile vacuoles are clear, spherical vesicles of varying sizes that gradually enlarge as they fill with excess water. Once you've located a vacuole, watch it fill and then empty its contents into the surrounding environment. These vacuoles serve an excretory function for the amoeba.

Food vacuoles are small, dark, irregularly shaped vesicles within the endoplasm. They contain undigested food particles.

Pseudopodia ("false feet") are fingerlike projections of the cytoplasm. They are used for locomotion as well as for trapping and engulfing food in a process called **phagocytosis**.

Student Media Videos—Ch. 28: Amoeba; Amoeba Pseudopodia

2. Examine *Trichonympha* under a compound microscope. You will first have to separate the *Trichonympha* (Figure 2.6) from the termite with which it lives in a symbiotic relationship. *Trichonympha* and other organisms occupy the gut of the termites, where they digest wood particles eaten by the insect. Termites lack the enzymes necessary to digest wood and are dependent on *Trichonympha* to make the nutrients in the wood available to them. *Trichonympha* has become so well adapted to the environment of the termite's gut that it cannot survive outside of it.

To obtain a specimen:

a. Place a couple of drops of **insect Ringers** (a saline solution that is isotonic to the internal environment of insects) on a clean microscope slide.

b. Using forceps or your fingers, transfer a termite into the drop of Ringers.

c. Place the slide under the dissecting microscope.

d. Place the tips of dissecting needles at either end of the termite and pull in opposite directions.

e. Locate the long tube that is the termite's intestine. Remove all the larger parts of the insect from the slide.

f. Using a dissecting needle, mash the intestine to release the *Trichonympha* and other protozoa and bacteria.

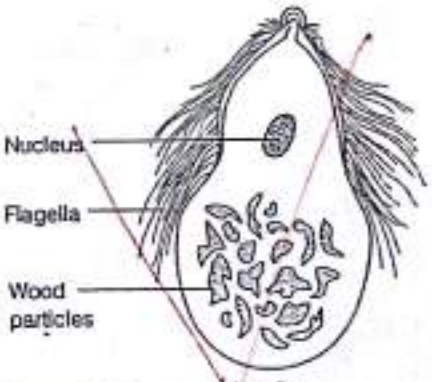


Figure 2.6. *Trichonympha*. A community of microorganisms, including *Trichonympha*, inhabits the intestine of the termite. Following the procedure in Exercise 2.5, Lab Study A, disperse the microorganisms and locate the cellular structures in *Trichonympha* (Color Plate 2).

g. Cover your preparation with a clean coverslip.

h. Transfer your slide to the compound microscope and scan the slide under low power. Center several *Trichonympha* in the field of view and switch to higher powers.

Several types of protozoans and bacteria will be present in the termite gut.

i. Locate the following structures under highest power.

Flagella are the long, hairlike structures on the outside of the organism. The function of the flagella is not fully understood. Within the gut of the termite, the organisms live in such high density that movement by flagellar action seems unlikely and perhaps impossible.

The **nucleus** is a somewhat spherical organelle near the middle of the organism.

Wood particles may be located in the posterior region of the organism.

Lab Study B. Aggregate and Colonial Organisms

Materials

microscope slides
dissecting needles
forceps
coverslips
cultures of *Protococcus* and *Scenedesmus*

Introduction

Unlike unicellular organisms, which live independently of each other, colonial organisms are cells that live in groups and are to some degree dependent on one another. The following organisms show an increasing degree of interaction among cells.

Procedure

1. Examine *Protococcus* under the compound microscope. *Protococcus* (Figure 2.7) is a terrestrial green alga that grows on the north sides of trees and is often referred to as "moss."

   a. To obtain a specimen, use a dissecting needle to brush off a small amount of the green growth on the piece of tree bark provided into a drop of water on a clean microscope slide. Avoid scraping bark onto the slide. Cover the preparation with a clean coverslip.

   b. Observe at highest power that these cells are **aggregates**: The size of the cell groupings is random, and there are no permanent connections between cells. Each cell is surrounded by a cell membrane and an outer **cell wall**.

   c. Observe several small cell groupings and avoid large clumps of cells. Cellular detail may be obscure.

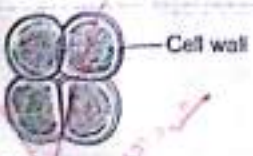


Figure 2.7.
Protococcus. *Protococcus* is a terrestrial green alga that forms loose aggregates on the bark of trees (Color Plate 3).

2. Examine living *Scenedesmus* under the compound microscope. *Scenedesmus* (Figure 2.8) is an aquatic green alga that is common in aquaria and polluted water.
   a. To obtain a specimen, place a drop from the culture dish (using a clean pipette) onto a clean microscope slide, and cover it with a clean coverslip
   b. Observe that the cells of this organism form a **simple colony**: The cells always occur in groups of from four to eight cells, and they are permanently united.
   c. Identify the following structures.

   The **nucleus** is the spherical organelle in the approximate middle of each cell.

   Vacuoles are the transparent spheres that tend to occur at either end of the cells.

   Spines are the transparent projections that occur on the two end cells.

   Cell walls surround each cell.

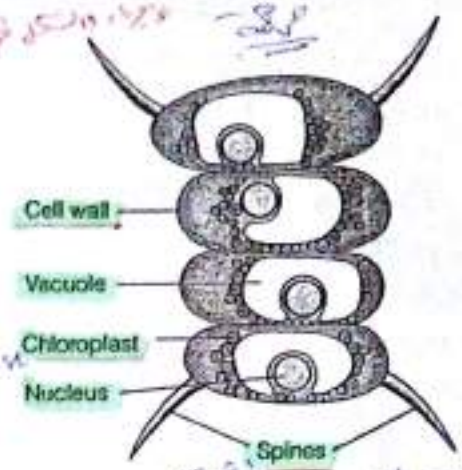


Figure 2.8. *Scenedesmus.* *Scenedesmus* is an aquatic alga that usually occurs in simple colonies of four cells connected by an outer cell wall (Color Plate 4).

Lab Study C. Multicellular Organisms

Materials

microscope slides
dropper bottles of water
toothpicks
coverslips
Elodea
methylene blue
finger bowl with disinfectant
broken glass chips
Volvox cultures

Introduction

Review the criteria for characterizing an organism as multicellular in the introduction of Exercise 2.5 on pp. 41–42. In multicellular organisms, there are two or more cell types with specialized structure and function that cannot persist when isolated from other cells in the organism. If these cells are isolated, they are not capable of perpetuating the species. In this lab study, you will examine an example of a green alga, a plant, and an animal to investigate the criteria for multicellularity and observe cells that compose basic tissue types.

Procedure

Volvox

Volvox (Figure 2.9) is an aquatic green alga that is common in aquaria, ponds, and lakes. In older literature this organism was described as colonial and was not considered to be multicellular. Today, however, scientists have concluded that it is more accurate to call *Volvox* multicellular. In this activity you will look for evidence that supports this conclusion.

1. Examine living *Volvox* under the compound microscope. To obtain a specimen, prepare a wet mount as you did for *Scenedesmus* with the following addition: Before placing a drop of the culture on your slide, place several glass chips on the slide. This will keep the coverslip from crushing these spherical organisms.

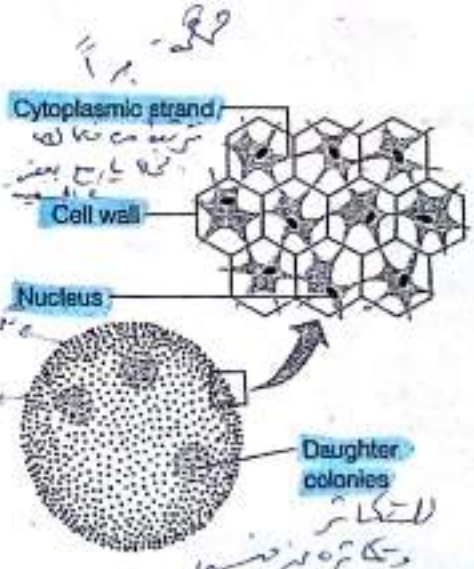


Figure 2.9. *Volvox.* In this organism, the individual cells are interconnected by cytoplasmic strands to form a sphere. Small clusters of cells, called daughter colonies, are specialized for reproduction (Color Plate 5).

2. Observe that the cells of this organism lie in a transparent matrix forming a large hollow sphere. The approximately 500 to 50,000 (depending on the species) nonreproductive somatic cells are permanently united by cytoplasmic connections. These cells have chloroplasts for photosynthesis and flagella that beat in a coordinated motion to move the colony like a ball. During asexual reproduction, certain cells in the sphere (reproductive cells) enlarge and migrate inward to become daughter colonies.
3. Identify the following structures: **somatic cells with cytoplasmic connections** and **flagella**. Depending on the magnification of your microscope, you may be able to distinguish **cell walls** and **nuclei** in the cells. **Daughter colonies** are smaller spheres within the larger colony. These are released when the parent colony disintegrates.

Student Media Video—Ch. 28: *Volvox* Colony

Figure 2.10.
Elodea. *Elodea* is an aquatic plant commonly grown in freshwater aquaria. The cell structures may be difficult to see because of the three-dimensional cell shape and the presence of a large central vacuole (Color Plate 6).

Plant Cells

1. The major characteristics of a typical plant cell are readily seen in the leaf cells of *Elodea*, a common aquatic plant (Figure 2.10). Prepare a wet mount and examine one of the youngest (smallest) leaves from a sprig of *Elodea* under the compound microscope.
2. Identify the following structures.

The cell wall is the rigid outer framework surrounding the cell. This structure gives the cell a definite shape and support. It is not found in animal cells.

Protoplasm is the organized contents of the cell, exclusive of the cell wall.

Cytoplasm is the protoplasm of the cell, exclusive of the nucleus.

The central vacuole is a membrane-bound sac within the cytoplasm that is filled with water and dissolved substances. This structure serves to store metabolic wastes and gives the cell support by means of turgor pressure. Animal cells also have vacuoles, but they are not as large and conspicuous as those found in plants.

Chloroplasts are the green, spherical organelles often seen moving within the cytoplasm. These organelles carry the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the cells, cytoplasm and chloroplasts may begin to move around the central vacuole in a process called *cytoplasmic streaming*, or *cyclosis*.

The nucleus is the usually spherical, transparent organelle within the cytoplasm. This structure controls cell metabolism and division.

3. What three structures observed in *Elodea* are unique to plants?

4. Compare your observations of *Elodea* using the compound scope with those made in Exercise 2.3 using the stereoscopic scope. List the structures seen with each.

   Stereoscopic:

   Compound:

Student Media Video—Ch. 6: Cytoplasmic Streaming

Animal Cells

1. Animals are multicellular heterotrophic organisms that ingest organic matter. They are composed of cells that can be categorized into four major tissue groups: epithelial, connective, muscle, and nervous tissue. In this lab study, you will examine epithelial cells. Similar to the epidermal cells of plants, epithelial cells occur on the outside of animals and serve to protect the animals from water loss, mechanical injury, and foreign invaders. In addition, epithelial cells line interior cavities and ducts in animals. Examine the epithelial cells (Figure 2.11) that form the lining of your inner cheek. To obtain a specimen, follow this procedure:
   a. With a clean toothpick, gently scrape the inside of your cheek several times.
   b. Roll the scraping into a drop of water on a clean microscope slide, add a small drop of methylene blue, and cover with a coverslip. Discard the used toothpick in disinfectant.
   c. Using the compound microscope, view the cells under higher powers.
2. Observe that these cells are extremely flat and so may be folded over on themselves. Attempt to locate several cells that are not badly folded, and study their detail.
3. Identify the following structures.

   The cell membrane is the boundary that separates the cell from its surroundings.

   The nucleus is the large, circular organelle near the middle of the cell.

   Cytoplasm is the granular contents of the cell, exclusive of the nucleus.

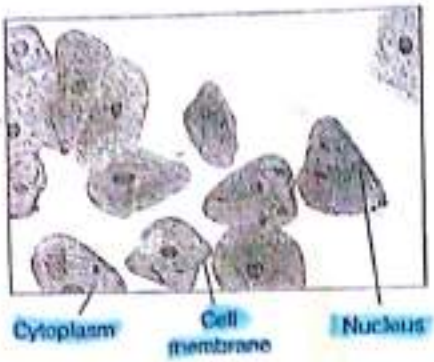


Figure 2.11.
Human epithelial cells. The epithelial cells that line your cheek are thin, flat cells that you can remove easily from your cheek by scraping it with a toothpick (Color Plate 7).

Lab Study D. Unknowns

Materials

microscope slides
coverslips
pond water or culture of unknowns

Introduction

Use this lab study to see if you have met the objectives of this lab topic. As you carry out this lab study, (1) think carefully about using correct microscopic techniques; (2) distinguish organisms with different cellular organization or configuration (unicellular, colonial, etc.); (3) note how the different organisms are similar yet different; and (4) note cell differences.

Lab Topic 3

Diffusion and Osmosis

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe the mechanism of diffusion at the molecular level.
2. List several factors that influence the rate of diffusion.
3. Describe a selectively permeable membrane, and explain its role in osmosis.
4. Define *hypotonic*, *hypertonic*, and *isotonic* in terms of relative concentrations of osmotically active substances.
5. Discuss the influence of the cell wall on osmotic behavior in cells.
6. Explain how incubating plant tissues in a series of dilutions of sucrose can give an approximate measurement of osmolarity of tissue cells.
7. Explain why diffusion and osmosis are important to cells.
8. Apply principles of osmotic activity to medical, domestic, and environmental activities.
9. Discuss the scientific process, propose questions and hypotheses, and make predictions based on experiments to test hypotheses.
10. Practice scientific persuasion and communication by constructing and interpreting graphs.

Introduction

Maintaining the steady state of a cell is achieved only through regulated movement of materials through cytoplasm, across organelle membranes, and across the plasma membrane. This regulated movement facilitates communication within the cell and between cytoplasm and the external environment. The cytoplasm and extracellular environment of the cell are aqueous solutions. They are composed of water, which is the **solvent**, or dissolving agent, and numerous organic and inorganic molecules, which are the **solutes**, or dissolved substances. Organelle membranes and the plasma membrane are **selectively permeable**, allowing water to freely pass through but regulating the movement of solutes.

The cell actively moves some dissolved substances across membranes, expending adenosine triphosphate (ATP) (biological energy) to accomplish the movement. Other substances move passively, without expenditure of ATP from the cell, but only if the cell membrane is permeable to those substances. Water and selected solutes move passively through the cell and cell

membranes by **diffusion**, a physical process in which molecules move from an area where they are in high concentration to one where their concentration is lower. The energy driving diffusion comes only from the intrinsic kinetic energy (energy of motion) in all atoms and molecules. If nothing hinders the movement, a solute will diffuse until it reaches equilibrium.

Osmosis is a type of diffusion; in cells it is the diffusion of water through a selectively permeable membrane from a region where it is highly concentrated to a region where its concentration is lower. The difference in concentration of water occurs if there is an unequal distribution of at least one dissolved substance on either side of a membrane and the membrane is impermeable to that substance. For example, if a membrane that is impermeable to sucrose separates a solution of sucrose from distilled water, water will move from the distilled water, where it is in higher concentration, through the membrane into the sucrose solution, where it is in lower concentration.

Three terms, **hypertonic, hypotonic, and isotonic**, are used when referring to two solutions separated by a selectively permeable membrane (Figure 3.1). The hypertonic solution (Figure 3.1a) has a greater concentration of solutes that cannot cross the membrance (nonpenetrating solutes) than the solution on the other side of the membrane. It is described, therefore, as having a greater osmolarity (solute concentration expressed as molarity). The hypotonic solution (Figure 3.1b) has a lower concentration of nonpenetrating solutes, or a lower osmolarity, than the solution on the other side of the membrane. When the two solutions are in equilibrium, the concentration of nonpenetrating solutes being equal on both sides of the membrane, the osmolarities are equal and the solutions are said to be isotonic (Figure 3.1c). The *net flow* of water is from the hypotonic to the hypertonic solution. When the solutions are isotonic, there is no net flow of water across the membrane.

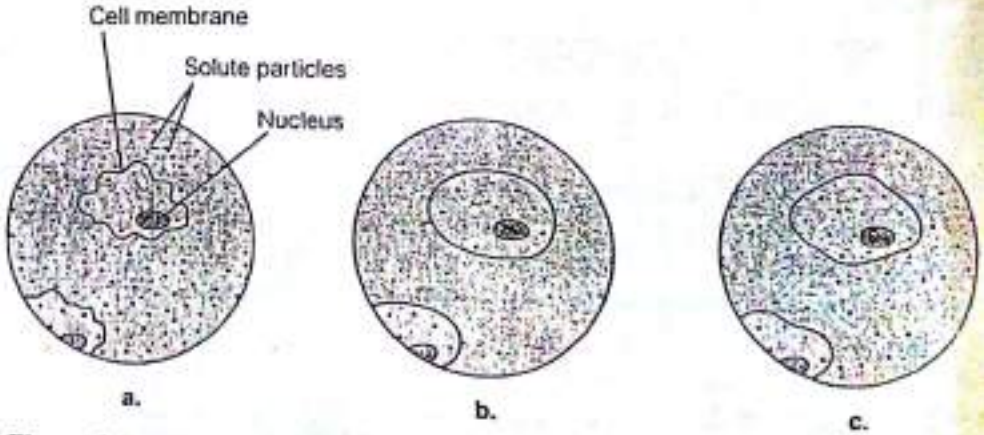


Figure 3.1.
Diagrammatic representation of cells in (a) hypertonic, (b) hypotonic, and (c) isotonic solutions. The hypertonic solution has a greater concentration of nonpenetrating solutes than the solution on the other side of the membrane; the hypotonic solution has a lower concentration of nonpenetrating solutes than the solution on the other side of the membrane, and the concentration of nonpenetrating solutes is equal on both sides of the membrane in isotonic solutions.

EXERCISE 3.1
Diffusion of Molecules

In this exercise you will investigate characteristics of molecules that facilitate diffusion, factors that influence diffusion rates, and diffusion of solutes through a selectively permeable membrane.

Experiment A. Kinetic Energy of Molecules

Materials

dropper bottle of water
carmine powder
dissecting needle
slide and coverslip
compound microscope

Introduction

Molecules of a liquid or gas are constantly in motion because of the intrinsic kinetic energy in all atoms and molecules. In 1827, Robert Brown, a Scottish botanist, noticed that pollen grains suspended in water on a slide appeared to move by a force that he was unable to explain. In 1905, Albert Einstein, searching for evidence that would prove the existence of atoms and molecules, predicted that the motion observed by Brown must exist, although he did not realize that it had been studied for many years. Only after the kinetic energy of molecules was understood did scientists ask if the motion observed by Brown and predicted by Einstein could be the result of molecular kinetic energy being passed to larger particles. We now know that intrinsic molecular kinetic energy is the driving force of diffusion. In this experiment, you will observe large particles suspended in water in motion similar to that observed by Brown, traditionally called **Brownian movement**. You will relate the motion observed to the forces that bring about diffusion.

Procedure

Work in pairs. One person should set up the microscope while the other person makes a slide as follows:

1. Place a drop of water on the slide.
2. Touch the tip of a dissecting needle to the drop of water and then into the dry carmine.
3. Add the carmine on the needle to the drop of water on the slide, mix, cover with a coverslip, and observe under the compound microscope.
4. Observe on low power and then high power. Focus as much as possible on one particle of carmine.
5. Record your findings in the Results section, and draw conclusions based on your results in the Discussion section.

Results

Describe the movement of single carmine particles.

1. Is the movement random or directional?
2. Does the movement ever stop?

3. Do smaller particles move more rapidly than larger particles? Other observations?

Discussion

1. Are you actually observing molecular movement? Explain.

2. How can molecular movement bring about diffusion?

3. Speculate about the importance of diffusion in cell metabolism.

Experiment B. Diffusion of Molecules through a Selectively Permeable Membrane

Materials

string or rubber band	500-mL beaker one-third filled with water
wax pencil	handheld test tube holder
30% glucose solution	3 standard test tubes
starch solution	disposable transfer pipettes
I_2KI solution	2 400-mL beakers to hold dialysis bag
Benedict's reagent	30-cm strip of moist dialysis tubing
hot plate	

Introduction

Dialysis tubing is a membrane made of regenerated cellulose fibers formed into a flat tube. If two solutions containing dissolved substances of different molecular weights are separated by this membrane, some substances may readily pass through the pores of the membrane, but others may be excluded.

Working in teams of four students, you will investigate the selective permeability of dialysis tubing. You will test the permeability of the tubing to the reducing sugar, glucose (molecular weight 180), starch (a variable-length polymer of glucose), and iodine potassium iodide (I_2KI). You will place a solution of glucose and starch into a dialysis tubing bag and then place this bag into a solution of I_2KI. Sketch and label the design of your experiment in the margin of your lab manual to help you develop your hypotheses.

You will use two tests in your experiment:

1. *I_2KI test for presence of starch.*

   When I_2KI is added to the unknown solution, the solution turns purple or black if starch is present. If no starch is present, the solution remains a pale yellow-amber color.

2. *Benedict's test for reducing sugar.*

   When Benedict's reagent is added to the unknown solution and the solution is heated, the solution turns green, orange, or orange-red if a reducing sugar is present (the color indicates the sugar concentration). If no reducing sugar is present, the solution remains the color of Benedict's reagent (blue).

Question

Remember that every experiment begins with a question. Review the design of this experiment in the Introduction above. Formulate a question about the permeability of dialysis tubing. The question may be broad, but it must propose an idea that has measurable and controllable elements.

Hypothesis

Hypothesize about the selective permeability of dialysis tubing to the substances being tested.

Prediction

Predict the results of the I_2KI and Benedict's tests based on your hypothesis (if/then).

Procedure

1. Prepare the dialysis bag with the initial solutions.
   a. Fold over 3 cm at the end of a 25- to 30-cm piece of dialysis tubing that has been soaking in water for a few minutes, pleat the folded end "accordion style," and close the end of the tube with the string or a rubber band, forming a bag. This procedure must secure the end of the bag so that no solution can seep through.
   b. Roll the opposite end of the bag between your fingers until it opens, and add 4 pipettesful of 30% glucose into the bag. Then add 4 pipettesful of starch solution to the glucose in the bag.
   c. Hold the bag closed and mix its contents. Record its color in Table 3.1 in the Results section. Carefully rinse the outside of the bag in tap water.
   d. Add 300 mL of water to a 400- to 500-mL beaker. Add several drop- ... until it is visibly yellow-amber.

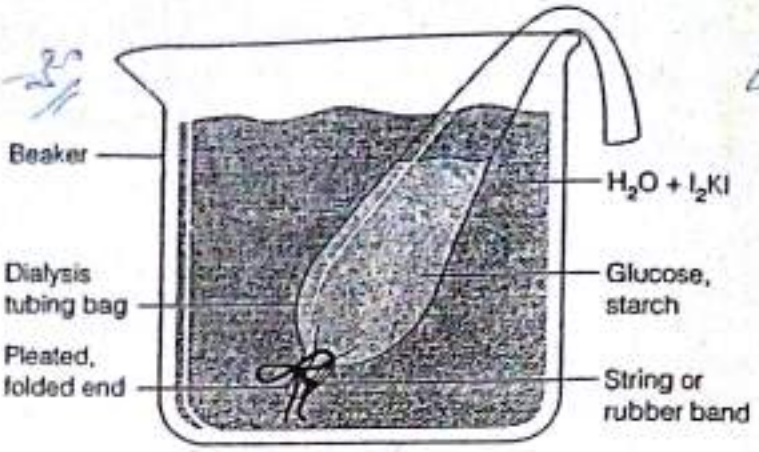


Figure 3.2.
Setup for Exercise 3.1, Experiment B. The dialysis tubing bag, securely closed at one end, is placed in the beaker of water and I_2KI. The open end of the bag should drape over the edge of the beaker.

e. Place the bag in the beaker so that the untied end of the bag hangs over the edge of the beaker (Figure 3.2). *Do not allow the liquid to spill out of the bag!* If the bag is too full, remove some of the liquid and rinse the outside of the bag again. If needed, place a rubber band around the beaker, holding the bag securely in place. If some of the liquid spills into the beaker, dispose of the beaker water, rinse, and fill again.

2. Leave the bag in the beaker for about 30 minutes. (You should go to another lab activity and then return to check your setup periodically.)
3. After 30 minutes, carefully remove the bag and stand it in a dry beaker.
4. Record in Table 3.1 the final color of the solution in the bag and the final color of the solution in the beaker.
5. Perform the Benedict's test for the presence of sugar in the solutions.
   a. Label three clean test tubes: control, bag, and beaker.
   b. Put 2 pipettesful of water in the control tube.
   c. Put 2 pipettesful of the bag solution in the bag tube.
   d. Put 2 pipettesful of the beaker solution in the beaker tube.
   e. Add 1 dropperful of Benedict's reagent to each tube.
   f. Heat the test tubes in a boiling water bath for about 3 minutes.
   g. Record your results in Table 3.1.
6. Review your results in Table 3.1 and draw your conclusions in the Discussion section.

Results

Complete Table 3.1 as you observe the results of Experiment B.

Table 3.1
Results of Experiment Investigating the Permeability of Dialysis Tubing to Glucose, I_2KI, and Starch

Solution Source	Original Contents	Original Color	Final Color	Color after Benedict's Test
Bag				

Experiment A. Osmotic Behavior of Animal Cells

Materials

On demonstration:
- test tube rack
- 3 test tubes with screw caps, each containing one of the three solutions of unknown osmolarity
- ox blood
- newspaper or other printed page

For microscopic observations:
- 4 clean microscope slides and coverslips
- wax pencil
- dropper bottle of ox blood
- dropper bottles with three solutions of unknown osmolarity

Introduction

Mature red blood cells (erythrocytes) are little more than packages of hemoglobin bound by a plasma membrane permeable to small molecules such as oxygen and carbon dioxide, but impermeable to larger molecules such as proteins, sodium chloride, and sucrose. In mammals these cells even lack nuclei when mature, and as they float in isotonic blood plasma their shape is flattened and pinched inward into a biconcave disk. Oxygen and carbon dioxide diffuse across the membrane, allowing the cell to carry out its primary function, gas transport, which is enhanced by the increased surface area created by the shape of the cell. Scientists questioning what happens to red blood cells in different molar solutions observed that the cells respond dramatically if they are not in an isotonic environment. When water moves into red blood cells placed in a hypotonic solution, the cells swell and the membranes burst, or undergo lysis. When water moves out of red blood cells placed in a hypertonic solution the cells shrivel and appear bumpy, or crenate. In this experiment, you will investigate the behavior of red blood cells when the osmolarity of the environment changes from isotonic to hypertonic or hypotonic. (See Color Plate 8.)

Hypothesis

Hypothesize about the behavior of red blood cells when they are placed in hypertonic or hypotonic environments.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Observe the three test tubes containing unknown solutions and blood on demonstration. These tubes have been prepared in the following way:

   Test tube 1: 15 mL of unknown solution A

   Test tube 2: 15 mL of unknown solution B

   Test tube 3: 15 mL of unknown solution C

   Your instructor has added 5 drops of ox blood to each test tube.

   Observe the appearance of each test tube. Is it opaque? Is it translucent? Describe your observations in Table 3.2 in the Results section.
2. Be sure each test tube cap is securely tightened, then hold each test tube flat against the printed newspaper article or page of text.
3. Attempt to read the print. Describe in Table 3.2 in the Results section.

   Continue your investigation of osmotic behavior of animal cells by performing microscopic observations of cells in the three unknown solutions.

Have your microscope ready, and observe slides immediately after you have prepared them. Do one slide at a time.

4. Label four clean microscope slides A, B, C, and D.
5. Place a drop of blood on slide D, cover with a coverslip, and observe the shape of red blood cells with no treatment. Record your observations in Table 3.2 in the Results section.
6. Put a drop of solution A on slide A and add a coverslip. Place the slide on the microscope stage and carefully add a small drop of blood to the edge of the coverslip. The blood cells will be drawn under the coverslip by capillary action.
7. As you view through the microscope, carefully watch the cells as they come into contact with solution A; record your observations in Table 3.2.
8. Repeat steps 3 and 4 with solutions B and C.
9. Record your observations in Table 3.2. Draw your conclusions in the Discussion section.

Results

1. Record your observations of the demonstration test tubes in Table 3.2.

Table 3.2
Appearance of Unknown Solutions A, B, and C

	Appearance of the Solution	Can You Read the Print?
Test tube 1 (unknown A)		
Test tube 2 (unknown B)		
Test tube 3 (unknown C)		

2. Record your microscopic observations of red blood cell behavior in Table 3.3.

Table 3.3
Appearance of Red Blood Cells in Test Solutions

Solution	Appearance/Condition of Cell
D (blood only)	
A	
B	
C	

Discussion

Explain your results in terms of your hypothesis.

1. Explain the appearance of the three test tubes on demonstration.

2. Based on the demonstration and your microscopic investigation, which of the three solutions is hypotonic to the red blood cells?

Hypertonic?

Isotonic?

Verify your conclusions with the laboratory instructor.

3. What conditions might lead to results other than those expected?

Experiment B. Osmotic Behavior in Cells with a Cell Wall

Materials

On demonstration: 2 compound microscopes labeled A and B
1 slide of *Elodea* in a hypertonic salt solution
1 slide of *Elodea* in distilled water

Introduction

In their natural environment, cells of freshwater plants and algae are bathed in water containing only dilute concentrations of solvents. The net flow of water is from the surrounding medium into the cells. To understand this process, review the structure of *Elodea* cells from Lab Topic 2.

The presence of a cell wall and a large fluid-filled central vacuole in a plant or algal cell will affect the cell's response to solutions of differing molarities. When a plant cell is placed in a hypertonic solution, water moves out of the cell; the protoplast shrinks and may pull away from the cell wall. This process is called **plasmolysis**, and the cell is described as **plasmolyzed** (Figure 3.3). In a hypotonic solution, as water moves into the cell and ultimately into the cell's central vacuole, the cell's **protoplast** (the plant cell exclusive of the cell wall—the cytoplasm enclosed by plasma membrane) expands. The cell wall, however, restricts the expansion, resulting in **turgor pressure** (pressure of the protoplast on the cell wall owing to uptake of water). A high turgor pressure will prevent further movement of water into the cell. This process is a good example of the interaction between pressure and osmolarity in determining the direction of the net movement of water. The hypertonic condition in the cell draws water into the cell until the membrane-enclosed cytoplasm presses against the cell wall. Turgor pressure begins to force water through the membrane and out of the cell, changing the direction of net flow of water (Figure 3.4).

Scientists call the combined force created by solute concentration and physical pressure **water potential**. For a detailed explanation of water potential, see a discussion of plant transport mechanisms in your text (e.g., Chapter 36 in *Campbell Biology*, 9th ed.). In contrast to an animal cell, the

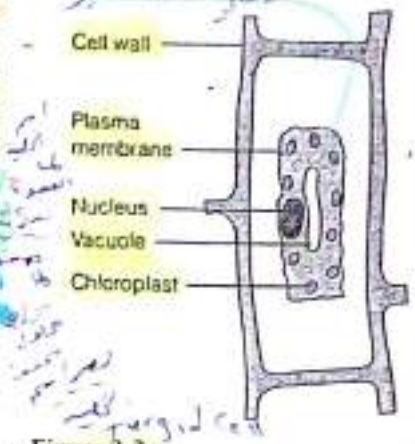


Figure 3.3.
Plant cell placed in a hypertonic solution. Water leaves the central vacuole and the cytoplasm shrinks, a process called plasmolysis.

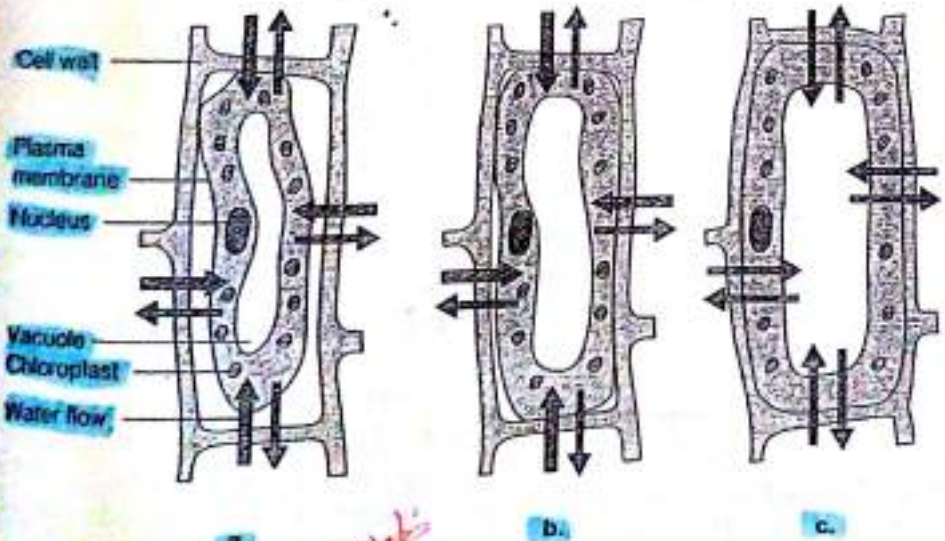


Figure 3.4.
The effect of turgor pressure on the cell wall and the direction of net flow of water in a plant cell. A plant cell undergoes changes in a hypotonic solution. (a) Low turgor pressure. The net flow of water comes into the cell from the surrounding hypotonic medium. (b) Turgor pressure increases. The protoplast begins to press on the cell wall. (c) Greatest turgor pressure. The tendency to take up water is ultimately restricted by the cell wall, creating a back pressure on the protoplast. Water enters and leaves the cell at the same rate.

ideal state for a plant cell is turgidity. When a plant cell is turgid, it is isotonic with its surroundings but is hypertonic, having a higher solute concentration than its surroundings. In this state, the plant cell presses on the cell wall. The pressure of the protoplast on the cell wall is important force in plant activity. For example, it may cause young cells "grow" as the elastic cell wall expands.

For this experiment, two slides have been set up on demonstration scopes. On each slide, *Elodea* has been placed in a different molar solution is hypotonic (distilled water) and one is hypertonic (concentrated salt

Question

Propose a question about the movement of water in *Elodea* leaves placed different molar solutions.

Hypothesis

Hypothesize about the movement of water in cells with a cell wall wh they are placed in hypertonic or hypotonic environments.

Prediction

Predict the appearance of *Elodea* cells placed in the two solutions (if/the

Procedure

1. Observe the two demonstration microscopes with *Elodea* in solutio A and B.
2. Record your observations in Table 3.4 in the Results section, and do your conclusions in the Discussion section.

Results

Describe the appearance of the *Elodea* cells in Table 3.4.

Table 3.4
Appearance of *Elodea* Cells in Unknown Solutions A and B

Solution	Appearance/Condition of Cells
A	
B	

Discussion

1. Based on your predictions and observations, which solution is hypertonic?

   Hypotonic?

2. Which solution has the greatest osmolarity?

3. Would you expect pond water to be isotonic, hypertonic, or hypotonic to *Elodea* cells? Explain.

4. Verify your conclusions with your laboratory instructor.

Student Media Videos—Ch. 7: Turgid *Elodea;* Plasmolysis

EXERCISE 3.3

Investigating Osmolarity of Plant Cells

Knowing the solute concentration of cells has both medical and agricultural applications. In plants, scientists know that for normal activities to take place, the amount of water relative to solute concentration in cells must be maintained within a reasonable range. If plant cells have a reduced water content, all vital functions slow down.

In the following experiments, you will estimate the osmolarity (solute concentration) of potato tuber cells using two methods, change in weight and change in volume. You will incubate pieces of potato tuber in sucrose solutions of known molarity. The object is to find the molarity at which weight or volume of the potato tuber tissue does not change, indicating that there has been no net loss or gain of water. This molarity is an indirect measure of the solute concentration of the potato tuber. This measure is indirect because water movement in plant cells is also affected by the presence of cell walls (see Figure 3.4c).

Work in teams of four. Each team will measure either weight change volume change. Time will be available near the end of the laboratory riod for each team to present its results to the class for discussion conclusions.

Experiment A. Estimating Osmolarity by Change in Weight

Materials

1 large potato tuber
7 250-mL beakers (disposable cups may be substituted)
wax marking pencil
forceps
balance that weighs to the nearest 0.01 g
aluminum foil
petri dish
sucrose solutions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 molar (*M*)
razor blade
cork borer
deionized (DI) water (0 molar)
paper towels
metric ruler
calculator

Introduction

In this experiment, you will determine the weight of several potato tub cylinders and incubate them in a series of sucrose solutions. After the cyli ders have incubated, you will weigh them and determine if they ha gained or lost weight. This information will enable you to estimate the molarity of the potato tuber tissue.

Question

What question is being investigated in this experiment?

Hypothesis

Hypothesize about the osmolarity of potato tuber tissue in relation to sucrose solutions.

Prediction

Predict the results of the experiment based on your hypothesis (if/then)

Cork borers and razor blades can cut! Use them with extreme care! To use the cork borer, hold the potato in such a way that the borer will not push through the potato into your hand.

2. Use a sharp cork borer to obtain seven cylinders of potato. Push the borer through the length of the potato, twisting it back and forth. When the borer is filled, remove from the potato and push the potato cylinder out of the borer. You must have seven complete, undamaged cylinders at least 5 cm long.
3. Line up the potato cylinders and, using a sharp razor blade, cut all cylinders to a uniform length, about 5 cm, removing the peel from the ends.
4. Place all seven potato samples in a petri dish, and keep them covered to prevent their drying out.

In subsequent steps, treat each sample individually. Work quickly. To provide consistency, each person should do one task to all cylinders (one person wipe, another weigh, another slice, another record data).

5. Remove a cylinder from the petri dish, and place it between the folds of a paper towel to blot sides and ends.
6. Weigh it to the nearest 0.01 g on the aluminum sheet on the balance. Record the weight in Table 3.5 in the Results section.
7. Immediately cut the cylinder lengthwise into two long halves.
8. Transfer potato pieces to the water beaker.
9. Note what time the potato pieces are placed in the water beaker. Time: _____.
10. Repeat steps 5 to 8 with each cylinder, placing potato pieces in the appropriate incubating solution from 0.1 to 0.6 *M*.

Be sure that the initial weight of the cylinder placed in each test solution is accurately recorded.

11. Incubate 1.5 to 2 hours. (As this takes place, you will be performing other lab activities.)
12. Swirl each beaker every 10 to 15 minutes as the potato pieces incubate.
13. At the end of the incubation period, record the time when the potato pieces are removed. Time: _____.
    Calculate the approximate incubation time in Table 3.5.
14. Remove the potato pieces from the first sample. Blot the pieces on a paper towel, removing excess solution only.
15. Weigh the potato pieces and record the final weight in Table 3.5.

16. Repeat this procedure until all samples have been weighed in the chronological order in which they were initially placed in the test solutions.
17. Record your data in the Results section, and complete the questions in the Discussion section.

Results

1. Complete Table 3.5. To calculate percentage change in weight, use this formula:

$$\text{Percentage change in weight} = \frac{\text{weight change}}{\text{initial weight}} \times 100$$

If the sample gained in weight, the value should be positive. If it lost in weight, the value should be negative.

Table 3.5
Data for Experiment Estimating Osmolarity by Change in Weight

Approximate time in solutions	Sucrose Molarity						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Final weight (g)	2.11	2.41	2.39	2.20	2.05	2.15	2.16
Initial weight (g)	1.93	2.34	2.39	2.24	2.17	2.33	2.41
Weight change (g)	0.18	0.07	0.0	-0.04	-0.12	-0.18	-0.25
% change in weight							

2. Plot percentage change in weight as a function of the sucrose molarity in Figure 3.5.
   a. Place a 0 in the middle of the y axis. Choose appropriate scales.
   b. Label the axes of the graph: Determine dependent and independent variables, and place each on the appropriate axis (see Lab Topic 1 for assistance in graphing).
   c. Graph your results. Weight increase (positive values) should be above the zero change line on the "percentage change in weight" axis. Weight decrease should be below the zero change line.
   d. Construct a curve that best fits the data points. How would you estimate the

Experiment B. Estimating Osmolarity by Change in Volume

Materials

1 large potato tuber
vernier caliper
7 250-mL beakers (disposable cups may be substituted)
wax marking pencil
forceps
petri dish
razor blade
cork borer (0.5-cm diameter)
sucrose solutions: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 M
DI water (0 M)
metric ruler
paper towels
calculator

Introduction

In this experiment, you will determine the volume of several potato tu cylinders by measuring the length and diameter of each. You will then cubate them in a series of sucrose solutions. After the cylinders have b bated, you will again measure their length and diameter and determi they have increased or decreased in size. This information will enable to estimate the osmolarity (solute concentration) of the potato tuber ti

Question

What question is being investigated in this experiment?

Hypothesis

Hypothesize about the osmolarity of potato tuber tissue.

Prediction

Predict the results of the experiment based on your hypothesis (if/t

Procedure

1. Practice measuring with the vernier caliper (Figure 3.6a, b).
   a. Identify the following parts of the caliper and add these Figure 3.6a: *stationary arm*, *movable arm*, *ruler*, *vernier scal* that the numbers on the bottom ruler scale are centimet graduated line is 1 mm.
   b. Choose a small object (a coin will work) and place it bet two arms, adjusting the movable arm until both arms just object.
   c. Note the 0 mark on the vernier scale (Figure 3.6b). The

Table 3.6
Data for Experiment Estimating Osmolarity by Change in Volume

Approximate time in solutions	Sucrose Molarity						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Final diameter (mm)							
Final length (mm)							
Final volume (mm^3)							
Initial diameter (mm)							
Initial length (mm)							
Initial volume (mm^3)							
Change in volume (mm^3)							
% change in volume							

1. Plot percentage change in volume as a function of the sucrose molarity in Figure 3.7.
   a. Place a 0 in the middle of the y axis. Choose appropriate scales.
   b. Label the axes of the graph. Determine dependent and independent variables, and place each on the appropriate axis (see Lab Topic 1).
   c. Graph your results. Volume increase should be above the zero change line on the "percentage change in volume" axis. Volume decrease should be below the zero change line.

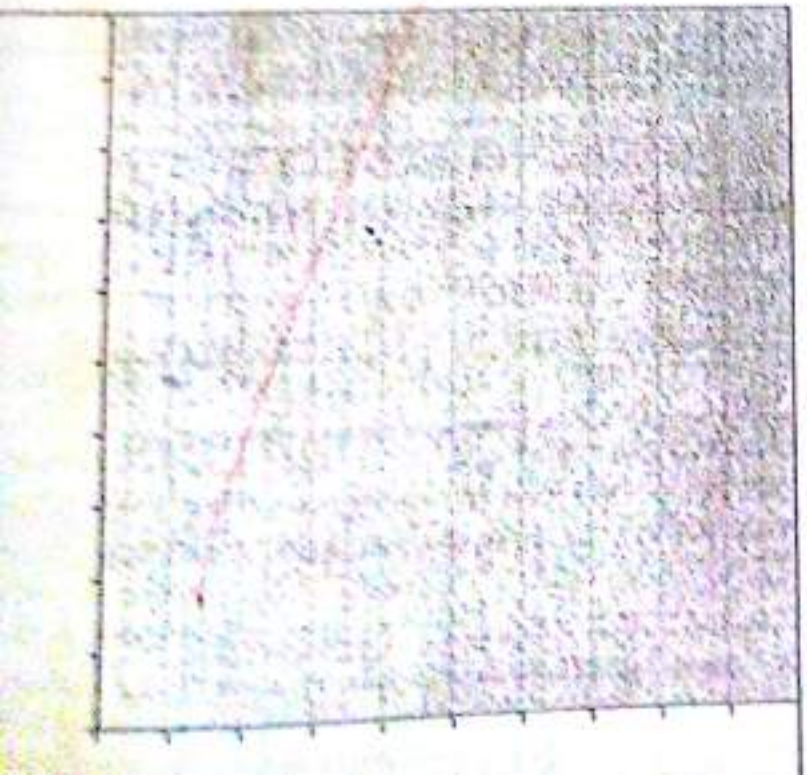


Figure 3.7.

d. Construct a curve that best fits the data points. Use this cu... estimate the osmolarity of the potato tuber.

e. Compose an appropriate figure title.

Discussion

1. At what sucrose molarity does the curve cross the zero change li... the graph?

2. Explain how this information can be used to determine the osmo... of the potato tuber tissue.

3. In more dilute concentrations of sucrose, the volume of the po... pieces _____ (increases/decreases) after incubation. W... forces other than solute concentration will have an impact on... amount of water taken up by the pieces?

4. Estimate the osmolarity of the potato tuber tissue.

Reviewing Your Knowledge

1. Once you complete this lab topic, you should be able to define an... the following terms. Provide examples if appropriate.

   selectively permeable, solvent, solute, diffusion, osmosis, nonpenet... solute, hypotonic, hypertonic, isotonic, turgor pressure, osmolarity, wat... tential, Brownian movement, lysis, crenate, plasmolysis, plasmolyzed,

2. Compare the response of plant and animal cells placed in hyper... isotonic, and hypotonic solutions.

Applying Your Knowledge

1. Unlike animals, plants never absorb enough water that their cells ... but they frequently lose enough water to wilt. Describe plant wilti... terms of turgor pressure. What is the optimum environment for gr... plants?

2. The pond water samples you observed in Lab Topic 2, Lab Study D probably contained a variety of multicellular, colonial, and single-celled organisms. Some of these may have had cell walls, but others were lacking cell walls. What adaptations for osmoregulation are found in single-celled organisms, such as the *Amoeba*, and multicellular organisms that lack cell walls but live in a hypotonic environment?

3. Shrimp fishing off the coast of Georgia was closed in 2001, due to a drastic reduction in the shrimp population. Harvest of blue crab plummeted in 2002 and 2003 following 5 years of drought. In 2008 and 2009, however, rainfall amounts reached near normal and the shrimp population returned to predrought numbers. Blue crab harvest has more than doubled compared with 2002 totals. Rainfall amounts in upland regions of the state have a significant effect on the salinity of coastal estuaries, regions where open ocean and fresh water from underground aquifers and rivers mix. These estuaries are the "nurseries" for many marine animals. Speculate about possible causes for the decline of shrimp in 2001 and blue crab in 2002 and 2003, and the recovery of these species in 2009.

4. Each year in the U.S., millions of people become ill and thousands die from eating food contaminated with bacteria. Controlling the growth of microorganisms on food is a particular challenge and over the course of many centuries various methods have been developed for controlling microorganisms in food. Some of these include preservation by irradiation, drying, freezing, canning, and salting. Preservation by salting is based on the principle of osmosis. One example of this is the preservation of ham by applying large amounts of salt ("salt-cured") or sugar ("sugar-cured") to the meat within 48 hours after slaughter. Using information you learned in this lab, speculate about how this process works to preserve the ham.

Investigative Extensions

1. Organisms that live in marine environments are described as being **euryhaline** (able to live in waters of a wide range of salinity) or **stenohaline** (unable to withstand wide variation in salinity of the surrounding water). This characteristic often determines the range of habi-

the salinity changes as the tide floods and ebbs is more euryhaline than an organism living in the open ocean.

Design an experiment to test the range of tolerance of two or more marine invertebrates (for example, barnacles, sea squirts, small crabs, marine mussels, periwinkles) available from biological supply houses. Determine if they may be described as euryhaline or stenohaline organisms.

2. General science teachers have long known that white vinegar (or a 1% acetic acid solution) will dissolve the shell of a chicken egg, leaving intact the membranes surrounding the albumin and yolk.
   a. Design and perform an experiment to allow you to estimate the *osmolarity* of the chicken egg cell. Hint: It will take 24 to 36 hours to completely remove the shell. If the process goes too slowly, move the eggs to a fresh acid solution after about 12 hours.
   b. Design and perform an experiment to answer the question, "Does the *concentration* of the solute have an effect on the *rate of movement* of the solvent (water) across the membrane of a chicken egg?"
   c. Design and perform an experiment to answer the question, "Does temperature have an effect on the *rate* of osmosis?"

Student Media: BioFlix, Activities, Investigations, and Videos

www.masteringbiology.com (select Study Area)

BioFlix—Ch. 7: Membrane Transport
Activities—Ch. 1: Graph It! An Introduction to Graphing
Ch. 7: Membrane Structure; Selective Permeability of Membranes; Diffusion; Osmosis and Water Balance in Cells
Investigations—Ch. 7: How Do Salt Concentrations Affect Cells?
Videos—Ch. 7: Turgid *Elodea*; Plasmolysis

References

Exercise 4.1, Experiment A, was adapted from D. R. Helms and S. B. Miller, *Principles of Biology: A Laboratory Manual for Biology 110*. Apex, NC: Contemporary Publishing, 1978. Used by permission.

Lang, F., and S. Waldegger. "Regulating Cell Volume." *American Scientist*, 1997, vol. 85, pp. 456–463.

Reece, J., et al. *Campbell Biology*, 9th ed. San Francisco, CA: Pearson Education, 2011.

Websites

A discussion of osmosis and diffusion in kidney dialysis:
http://www.rockwellmed.com/hemodefn.htm

A discussion of reverse osmosis and its applications:
http://en.wikipedia.org/wiki/Reverse_osmosis

Includes sections entitled "Real-Life Applications" and Osmosis and Medicine:
http://www.answers.com/topic/osmosis

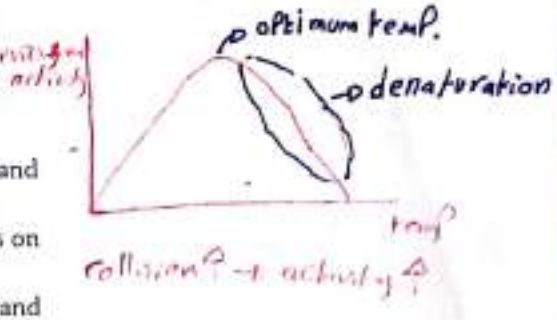
[redacted]

Lab Topic 4

Enzymes

Enzymes: catalyst; ↑ rxn rate; not used up or altered; ↓ activation; substrate + E ⇌ Product + E; active site

* Factors affect enzyme Activity
- temp - inhibitors
- [ ] - activators
- pH - cofactor, coenzyme



collision ↑ → activity ↑

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Define *enzyme* and describe the activity of enzymes in cells.
2. Differentiate competitive and noncompetitive inhibition.
3. Discuss the effects of varying environmental conditions such as pH and temperature on the rate of enzyme activity.
4. Discuss the effects of varying enzyme and substrate concentrations on the rate of enzyme activity.
5. Discuss the scientific process, propose questions and hypotheses, and make predictions based on hypotheses and experimental design.
6. Practice scientific thinking and communication by constructing and interpreting graphs of enzyme activity.

Introduction

Living cells perform a multitude of chemical reactions very rapidly because of the participation of enzymes. Enzymes are biological catalysts, compounds that speed up a chemical reaction without being used up or altered in the reaction. The material with which the catalyst reacts, called the substrate, is modified during the reaction to form a new product (see Figure 4.1). But because the enzyme itself emerges from the reaction unchanged and ready to bind with another substrate molecule, a small amount of enzyme can alter a relatively enormous amount of substrate.

The **active site** of an enzyme will bind with the substrate, forming the **enzyme-substrate complex**. It is here that catalysis takes place, and when it is complete, the complex dissociates into enzyme and product or products.

Enzymes are, in part or in whole, proteins and are highly specific in function. Because enzymes lower the energy of activation needed for reactions to take place, they accelerate the rate of reactions. They do not, however, determine the direction in which a reaction will go or its final equilibrium.

Enzyme activity is influenced by many factors. Varying environmental conditions, such as pH or temperature, may change the three-dimensional shape of an enzyme and alter its rate of activity. Specific chemicals may also bind to an enzyme and modify its shape. Chemicals that must bind for the enzyme to be active are called activators. Cofactors are nonprotein substances that usually bind to the active site on the enzyme and are essential for the enzyme to work.

inhibitors =>

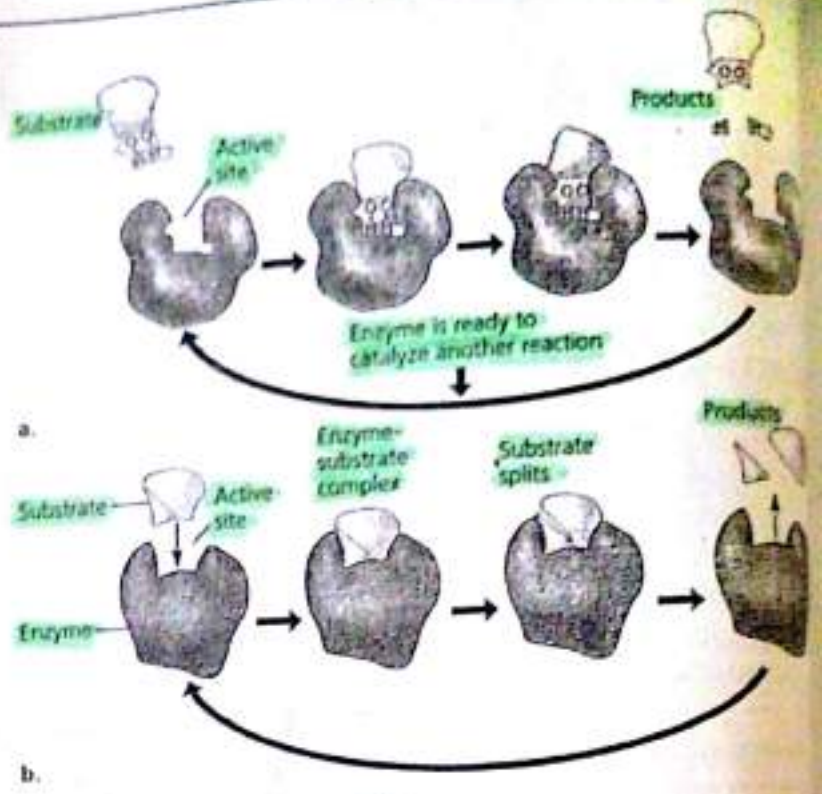


Figure 4.1
Enzyme activity A substrate or substrates bind to the active site of the enzyme, forming the enzyme-substrate complex, which then dissociates into enzyme and product(s). The enzyme may catalyze the addition or removal of a molecule or a portion of a molecule from the substrate to produce the product (a), or the enzyme may catalyze the splitting of a substrate into its component subunits (b).

Organic cofactors are called coenzymes, but other cofactors may be metal ions. Chemicals that shut off enzyme activity are called **inhibitors**; their action can be classified as **competitive or noncompetitive inhibition**.

Review Figure 4.1, illustrating enzyme activity. There are two ways to measure enzyme activity: (1) Determine the rate of disappearance of the substrate, and (2) determine the rate of appearance of the product.

In this laboratory, you will use both methods to investigate the activity of two enzymes, **catechol oxidase** and **amylase**. You will use an inhibitor to influence the activity of catechol oxidase and determine if it is a competitive or noncompetitive inhibitor. Additionally, you will investigate the effect of changing environmental conditions on the rate of amylase activity.

EXERCISE 4.1
Experimental Method and the Action of Catechol Oxidase

Materials

test-tube rack
3 small test tubes
small Parafilm™ squares
calibrated 5-mL pipette
3 calibrated 1-mL pipettes
disposable pasteur pipettes
pipette filler
pipette bulb
distilled or deionized (DI) water
potato extract
catechol
disposable gloves (optional)

Introduction

This exercise will investigate the result of catechol oxidase activity. In the presence of oxygen, catechol oxidase catalyzes the removal of electrons and hydrogens from **catechol**, a phenolic compound found in plant cells. Catechol is converted to benzoquinone, a pigment product. The hydrogens combine with oxygen, forming water (Figure 4.2). The pigment products are responsible for the darkening of fruits and vegetables, such as apples and potatoes, after exposure to air.

In this exercise you will use an extract of potato tuber to test for the presence of catechol oxidase and to establish the appearance of the products when the reaction takes place.

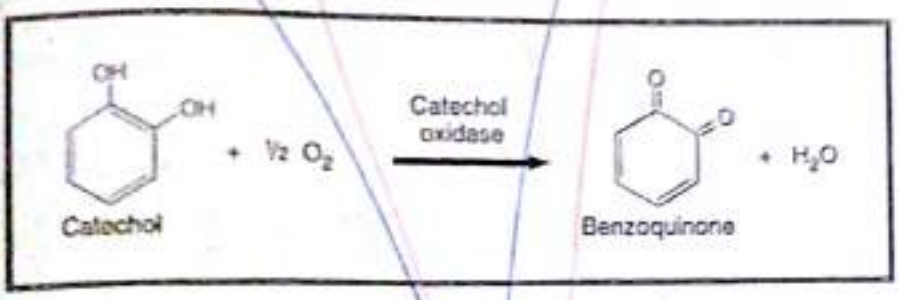


Figure 4.2.
The oxidation of catechol. In the presence of catechol oxidase, catechol is converted to benzoquinone. Hydrogens removed from catechol combine with oxygen to form water.

Question

Remember that every experiment begins with a question. Review the information given above about the activity of catechol oxidase. You will be performing an experiment using potato extract.

Formulate a question about catechol oxidase and potato extract. The question may be broad, but it must propose an idea that has measurable and controllable elements.

Hypothesis

Construct a hypothesis for the presence or absence of catechol oxidase in potato extract. Remember, the hypothesis must be testable. It is possible for you to propose one or more hypotheses, but all must be testable.

Prediction

Predict the result of the experiment based on your hypothesis. To test for the presence or absence of catechol oxidase in potato extract, your prediction would be what you expect to observe as the result of this experiment (if/then).

Catechol is a poison! Avoid contact with all solutions. Do not pipette any solutions by mouth. Wash hands thoroughly after each experiment. If a spill occurs, notify the instructor. If the instructor is unavailable, wear disposable gloves and use dry paper towels to wipe up the spill. Follow dry towels with towels soaked in soap and water. Dispose of all towels in the trash.

Procedure

1. Using Table 4.1, prepare the three experimental tubes. Note that all tubes should contain the same total amount of solution. Do not cross-contaminate pipettes! After each tube is prepared, use your finger to hold a Parafilm™ square securely over the tube mouth and then invert the tube to mix the contents thoroughly. Use a fresh square for each tube.

Table 4.1
Contents of the Three Experimental Tubes

Tube	Distilled Water	Catechol	Distilled Water	Enzyme
1	5 mL	0.5 mL (10 drops)	0.5 mL (10 drops)	—
2	5 mL	0.5 mL (10 drops)	—	0.5 mL (10 drops)
3	5 mL	—	0.5 mL (10 drops)	0.5 mL (10 drops)

2. Explain the experimental design: What is the purpose of each of the test tubes? Which is the control tube? Is more than one control tube necessary? Explain. Which is the experimental tube? Why is an additional 0.5 mL of distilled water added to tubes 1 and 3, but not tube 2?

3. Observe the reactions in the tubes, and record your observations in the Results section below. Explain your conclusions in the Discussion section.

Results

Design a simple table to record results (Table 4.2).

Discussion

Explain your results in terms of your hypothesis.

Table 4.2
Results of Catechol Oxidation Experiment

EXERCISE 4.2
Inhibiting the Action of Catechol Oxidase

Materials

test-tube rack
3 small test tubes
small Parafilm™ squares
calibrated 5-mL pipette
4 calibrated 1-mL pipettes
disposable pasteur pipettes
pipette bulb
distilled water
potato extract
catechol
phenylthiourea (PTU)
disposable gloves (optional)

Introduction

This exercise will investigate the inhibition of enzyme activity by specific chemicals called inhibitors. The specific inhibitor used will be **phenylthiourea (PTU)**. To be active, catechol oxidase requires copper as a cofactor. PTU is known to combine with the copper in catechol oxidase and inhibit its enzymatic activity.

An inhibitor molecule affects an enzyme in one of two ways. **Competitive inhibition** takes place when a molecule that is structurally similar to the substrate for a particular reaction competes for a position at the active site on the enzyme. This ties up the enzyme so that it is not available to the substrate. Competitive inhibition can be reversed if the concentration of substrate is raised to sufficiently high levels while the concentration of the inhibitor is held constant (Figure 4.3).

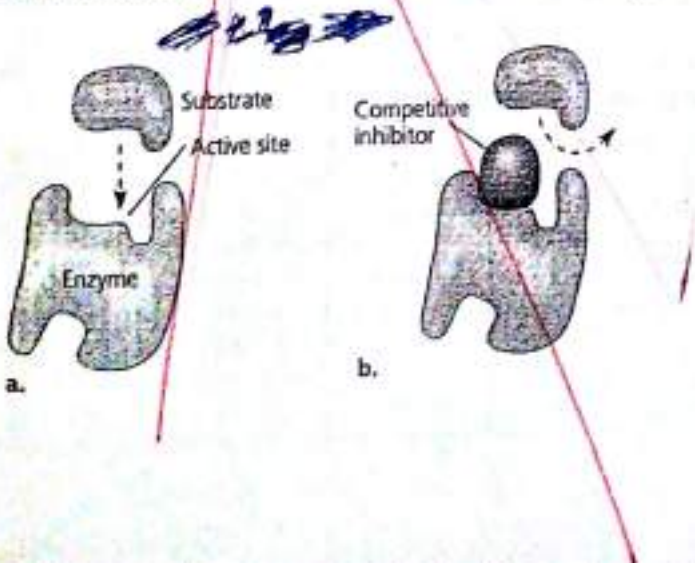


Figure 4.3.
Action of a competitive inhibitor. (a) Substrate normally can bind to the active site of an enzyme. (b) A competitive inhibitor mimics the substrate and competes for the position at the active site on the enzyme.

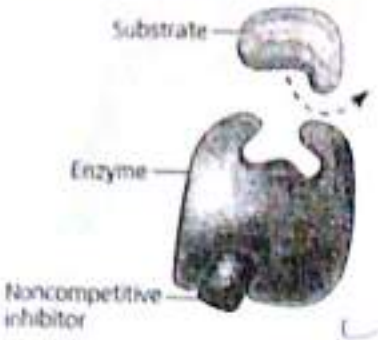


Figure 4.4.
Action of a noncompetitive inhibitor. The noncompetitive inhibitor binds to the enzyme at a location away from the active site, either blocking access to the active site or changing the conformation of the enzyme, rendering it inactive.

In **noncompetitive inhibition**, the inhibitor binds to a part of the enzyme that is *not* the active site. In so doing, it changes the nature of the enzyme so that its catalytic properties are lost. This can happen in two ways. The noncompetitive inhibitor itself physically blocks the access to the active site, or it causes a conformational change in the protein, thus inactivating the active site. In noncompetitive inhibition the inhibitor can become unbound, reversing the inhibition. However, unlike competitive inhibition, additional substrate will not reverse the inhibition (Figure 4.4).

In the following experiment, you will determine if PTU is a competitive or noncompetitive inhibitor.

Question

Pose a question about the activity of PTU.

Hypothesis

Hypothesize about the nature of inhibition by PTU.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

PTU and catechol are poisons! Avoid contact with solutions; do not pipette any solutions by mouth. Wash hands thoroughly after the experiment. If a spill occurs, notify the instructor. If instructor is unavailable, wear disposable gloves and use disposable paper towels to wipe up the spill. Follow dry towels with towels soaked in soap and water. Dispose of all towels in the trash.

1. Using Table 4.3, prepare three experimental tubes. Be sure to add solutions in the sequence given in the table (water first, potato extract, PTU next, etc.). Cover each tube with a fresh Parafilm™ square and

Table 4.3
Contents of the Three Experimental Tubes

Tube	Distilled Water	Potato Extract	PTU	Distilled Water	Catech
1	5 mL	0.5 mL	0.5 mL	0.5 mL	0.5
2	5 mL	0.5 mL	0.5 mL	—	1 m
3	5 mL	0.5 mL	—	1 mL	0.5

2. Which test tube is the control?

3. Why was the concentration of catechol increased in test tube 2?

4. Why should the catechol be added to the test tubes last?

5. Record your observations in the Results section, and explain your results in the Discussion section.

Results

Design a table to record your results (Table 4.4).

Table 4.4
Results of Inhibition Experiment

Discussion

1. Explain your results in terms of your hypothesis.

2. One member of your team is not convinced that you have adequately tested your hypothesis. How could you expand this experiment to provide additional evidence to strengthen your conclusion?

EXERCISE 4.3

Influence of Concentration, pH, and Temperature on the Activity of Amyla

Introduction

In the following exercise, you will investigate the influence of en concentration, pH, and temperature on the activity of the en amylase. Amylase is found in the saliva of many animals, inc humans, that utilize starch as a source of food. Starch, the pri reserve carbohydrate stores of plants, is a polysaccharide composed large number of glucose monomers joined together. Amylase is respo ble for the preliminary digestion of starch. In short, amylase breaks t chains of glucose molecules in starch into maltose, a two-glucose compound. Further digestion of this disaccharide requires other enz present in pancreatic and intestinal secretions. To help us follow digestion of starch into maltose by salivary amylase, we will take ad tage of the fact that starch, but not maltose, turns a dark purple when treated with a solution of I_2KI (this solution is normally yel amber in color). Draw equations to help you remember these reactio the margin of your lab manual.

In the following experiments, the **rate of disappearance** of starch in di ent amylase concentrations allows a quantitative measurement of rea rate. Recall that the rate of appearance of the product (in this case, mal would give the same information, but the starch test is simpler.

You will be assigned to a team with three or four students. Each team carry out only one of the experiments. However, each student is respo ble for understanding all experiments and results. Be prepared to pre your results to the entire class. Your instructor may require you to wr component of a scientific paper. (See Appendix A.)

Experiment A. The Influence of Enzyme Concentration on the Rate of Starch Digestion

Materials

test-tube rack
10 standard test tubes
wax pencil
test plate
flask of distilled or DI water
beaker of distilled or DI rinse water
5-mL graduated cylinder
1 calibrated 1-mL pipette
2 calibrated 5-mL pipettes
disposable pasteur pipettes
pipette bulb
buffer solution (pH = 6.8)
I_2KI solution
1% starch solution
1% amylase solution

Introduction

In this experiment you will vary the concentration of the enzyme amy to determine what effect the variation will have on the rate of the rea You will make serial dilutions of the amylase resulting in a range of en

concentrations. For serial dilutions, you will take an aliquot (sample) of the original enzyme and dilute it with an equal amount of water for a 1 : 1 dilution (50% of the original concentration). You will then take an aliquot of the resulting 1 : 1 solution and add an equal amount of water for a 1 : 3 dilution of the original concentration. You will continue this series of dilutions until you have four different amylase concentrations.

Question

Pose a question about enzyme concentration and reaction rate.

Hypothesis

Hypothesize about the effect of changing enzyme concentration on the rate of reaction.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Prepare the amylase dilution (test-tube set 1):
   a. Number five standard test tubes 1 through 5.
   b. Using the 5-mL graduated pipette, add 5 mL distilled water to each test tube.
   c. Make serial dilutions as follows (use the graduated cylinder):

      Tube 1: Add 5 mL amylase and mix by rolling the tube between your hands. (Dilution: 1 : 1; 0.5% amylase)

      Tube 2: Add 5 mL amylase solution from tube 1 and mix. (Dilution: 1 : 3; 0.25% amylase)

      Tube 3: Add 5 mL amylase solution from tube 2 and mix. (Dilution: 1 : 7; 0.125% amylase)

      Tube 4: Add 5 mL amylase solution from tube 3 and mix. (Dilution: 1 : 15; 0.063% amylase)

      Tube 5: Add 5 mL amylase solution from tube 4 and mix. (Dilution: 1 : 31; 0.031% amylase)

      Rinse the graduated cylinder thoroughly.
2. Prepare the experimental test tubes (test-tube set 2):
   a. Number a second set of five standard test tubes 1 through 5.
   b. Beginning with tube 5 of the first set, transfer 2 mL of this dilution into tube 5 of the second set. Use a 5-mL pipette for the transfer. Rinse the pipette in distilled water, and repeat the procedure for tubes 4, 3, 2, and 1, transferring 2 mL of tube 4 (first set) into tube 4 (second set), etc. After these transfers have been carried out, test-tube set 1 will no longer be used.

c. Add 40 drops of pH 6.8 buffer solution to each of the tubes in the second set. Mix by rolling the tubes between your hands. Set these tubes aside.

d. Add 1 or 2 drops of I_2KI to each compartment of four rows of a test plate. You will use a separate row for each concentration of amylase.

e. Using the second set of tubes, proceed with the tests beginning with tube 5.

(1) Using a clean 1-mL pipette, add 1 mL of the 1% starch solution to tube 5 and mix by rolling the tube between your hands. One team member should immediately record the time. This is time 0.

(2) Quickly remove 1 drop of the mixture with a disposable pasteur pipette, and add it to a drop of I_2KI in the first compartment of the test plate (time 0).

Remember, when the enzyme and substrate are together, the reaction has begun!

(3) Sample the reaction mixture at 10-second intervals, *each time in a new compartment of the test plate*. Continue until a blue color is no longer produced and the I_2KI solution remains yellow-amber (indicating the digestion of all the starch). Record the time required for the digestion of the starch in Table 4.5.

(4) Repeat steps 1 through 4 for the other four concentrations (tubes 4, 3, 2, and 1 of set 2).

3. Finish recording your findings in the Results section, and state your conclusions in the Discussion section.

Results

1. Complete Table 4.5 as you determine rates of digestion (time of disappearance) in different enzyme concentrations.

Table 4.5
Time of Starch Disappearance in Different Concentrations of the Enzyme Amylase

Tube	% Amylase	Time of Starch Disappearance (in seconds)
1	0.50	
2	0.25	
3	0.125	

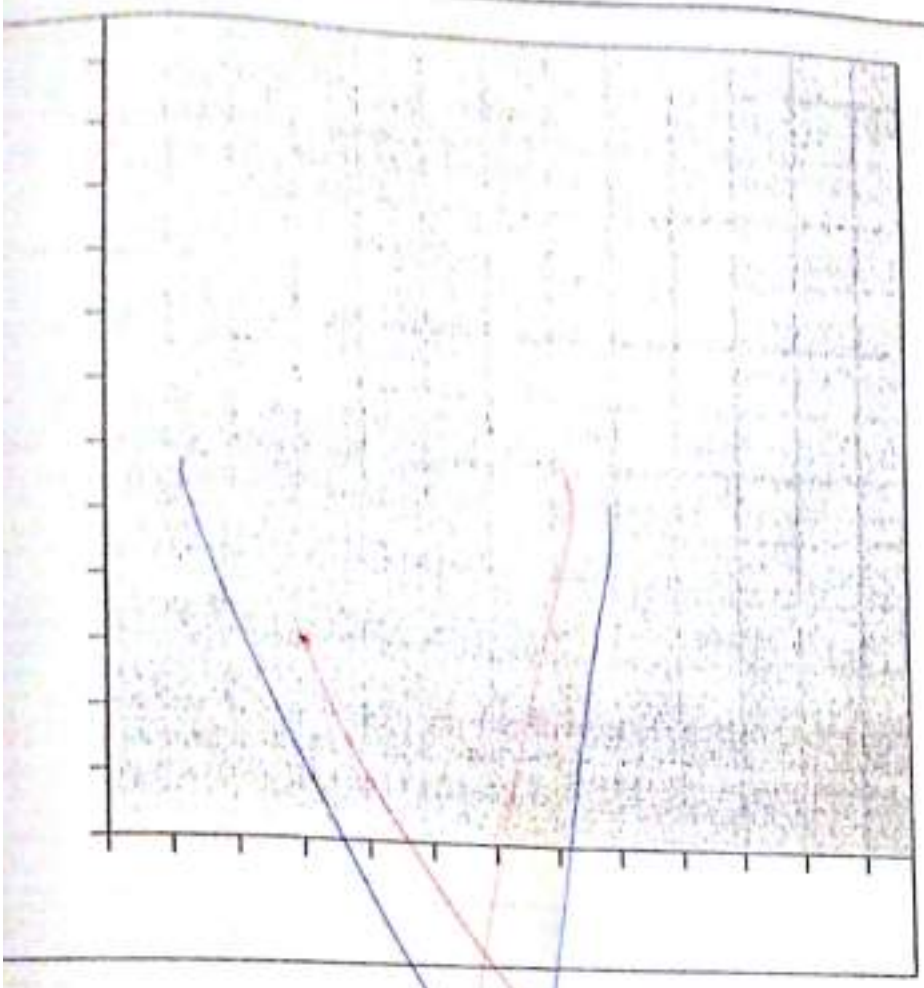


Figure 4.5.
Enzyme reaction rate (time of starch disappearance) for different concentrations of amylase.

2. Construct a graph (Figure 4.5) to illustrate your results. See Lab Topic 1 for assistance in graph construction.
   a. What is the independent variable? Which is the appropriate axis for this variable?

   b. What is the dependent variable? Which is the appropriate axis for this variable?

   c. Label the axes of the graph. Using Table 4.5, note the maximum number of seconds in your results, and choose an appropriate scale for the dependent variable. Reaction rate, the dependent variable, was measured as time of starch (product) disappearance. The data must, therefore, be graphed in reverse order because the highest values indicate the slowest reaction rate. You should place "0" at the end of the axis and write "fast" by your 0. Place your highest number near the origin (where the x and y axes cross). Write "slow" near the origin. Choose an appropriate scale for the independent variable (% amylase), and label this axis.

Discussion

1. Explain your results in terms of your hypothesis. Describe the shape of the reaction rate curve as substrate concentration increases. What factors are responsible for the change in reaction rate?

2. Speculate about the shape of a curve measuring reaction rate if you increased the concentration of enzyme, but held the concentration of substrate constant.

Experiment B. The Effect of pH on Amylase Activity

Materials

test-tube rack
6 standard test tubes
test plate
wax pencil
pipette bulb
3 5-mL calibrated pipettes
disposable pasteur pipettes

1% amylase solution
I_2KI solution
1% starch solution
beaker of distilled or DI rinse water
6 buffer solutions
(pH = 4, 5, 6, 7, 8, 9)
pH paper

Introduction

The environmental factor pH can influence the three-dimensional shape of an enzyme. Every enzyme has an optimum pH at which it is most active. In this experiment you will determine the optimum pH for the activity of amylase. What was the source of the amylase used in this experiment? (Check the Introduction to this exercise.)

Question

Pose a question about pH and reaction rate.

Hypothesis

Hypothesize about the rate

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Using a wax pencil, number six standard test tubes 1 through 6. Beginning with tube 1 and pH 4, mark one tube for each pH of buffer (4, 5, 6, 7, 8, 9). After you mark the test tubes, use a 5-mL graduated pipette to add 5 mL of the appropriate buffer to each test tube (5 mL buffer 4 to tube 1, 5 mL buffer 5 to tube 2, etc.). Rinse the pipette with distilled water after dispensing each buffer.

Buffers can burn skin! Avoid contact with all solutions. Do not pipette any solutions by mouth. Wash hands thoroughly after each experiment. If a spill occurs, notify the instructor. If the instructor is unavailable, wear disposable gloves and use dry paper towels to wipe up the spill. Follow dry towels with towels soaked in soap and water. Dispose of all towels in the trash.

2. Using a clean 5-mL graduated pipette, add 1.5 mL amylase solution to each tube and mix by rolling the tubes in your hands.
3. Introduce 1 or 2 drops of I_2KI into the compartments of several rows of the test plate.
4. Using only tube 1, add 2.5 mL of the 1% starch solution with a clean 5-mL pipette. Leave the pipette in the starch solution. Mix by rolling the tube in your hands. One team member should immediately record the time. This is time 0. Start testing immediately (next step).

Remember, when the enzyme and substrate are together, the reaction has begun!

5. Using a disposable pasteur pipette, remove a drop of the reaction mixture from tube 1. Add to a drop of I_2KI on the test plate.
6. Sample the reaction mixture at 10-second intervals, *each time using a new compartment of the test plate*. Continue until a blue color is no longer produced and the I_2KI solution remains yellow-amber (indicating the digestion of all the starch).
7. Record the time required for the digestion of the starch in Table 4.6. If after 7 minutes there is no color change, terminate the experiment with that reaction mixture.
8. Repeat steps 4 through 7 using the other five test tubes. Use separate rows on the test plate for each pH. Rinse the pipette between uses. Record results in Table 4.6.
9. Graph your observations in the Results section, and explain your results in the Discussion section.

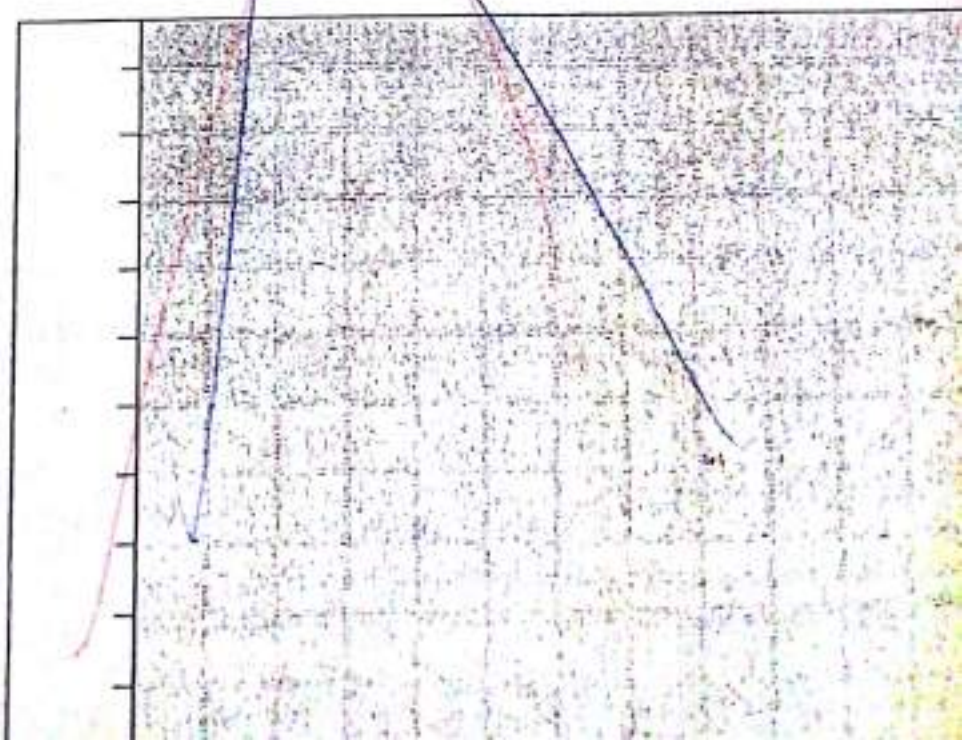
Results

1. Complete Table 4.6 as rates of digestion (time of starch disappe in different pHs are determined.

Table 4.6
Time of Starch Disappearance in Different pH Environments for the Enzyme Amylase

Tube	pH	Time of Starch Disappearance (in minutes)
1	4	
2	5	
3	6	
4	7	
5	8	
6	9	

2. Construct a graph using Figure 4.6 to illustrate your results. Topic 1 for assistance in graph construction.
   a. What is the independent variable? Which is the appropriate this variable?



b. What is the dependent variable? Which is the appropriate axis for this variable?

c. Label the axes of the graph. Using Table 4.6, note the maximum number of minutes in your results, and choose an appropriate scale for the dependent variable. Reaction rate, the dependent variable, was measured as time of starch (product) disappearance. The data must, therefore, be graphed in reverse order because the highest values indicate the slowest reaction rate. You should place "0" at the end of the axis and write "fast" by your 0. Place your highest number near the origin (where the x and y axes cross). Write "slow" near the origin. Choose an appropriate scale for the independent variable (pH) and label the axis.

Discussion

Explain your results in terms of your hypothesis. Describe the shape of the reaction rate curve obtained with change in pH. What factors are responsible for the shape of this curve?

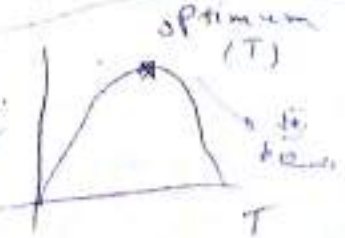
Experiment C. The Effect of Temperature on Amylase Activity

Materials

8 standard test tubes
test-tube rack
2 5-mL calibrated pipettes
2 1-mL calibrated pipettes
disposable 7.5-inch pasteur pipettes
pipette bulb
wax pencil
1% starch solution
I_2KI solution
buffer solution (pH = 6.8)
1% amylase solution
flask of DI water
On the demonstration table:
water bath at 80°C
water bath at 37°C
test-tube rack at room temperature
beaker of crushed ice for ice bath

Introduction

Chemical reactions accelerate as temperature rises, partly because increased temperatures speed up the motion of molecules. This means that substrates collide more frequently with enzyme active sites. Generally, a 10° rise in temperature results in a two- to threefold increase in the rate of a particular reaction. However, at high temperatures, the integrity of proteins can be irreversibly denatured. The activity of enzymes is dependent on the proper tertiary and quaternary structures; the optimum temperature for activity, therefore, may vary, depending on the structure of the enzyme.



What was the source of the amylase used in this experiment? (
Introduction to this exercise.)

(A)
1- add 1 ml Amylase
2- add 4 ml D.water + 1 ml buffer PH=7
+ 0.5 ml starch

(B) incubate for 10 min separated

(C) add 1 to 2 → take zero min

(D) incubate for 10 min → take
10 min sample

(E) Incubate for 20 min → take
20 min sample

Question

Pose a question about temperature and reaction rate.

Hypothesis

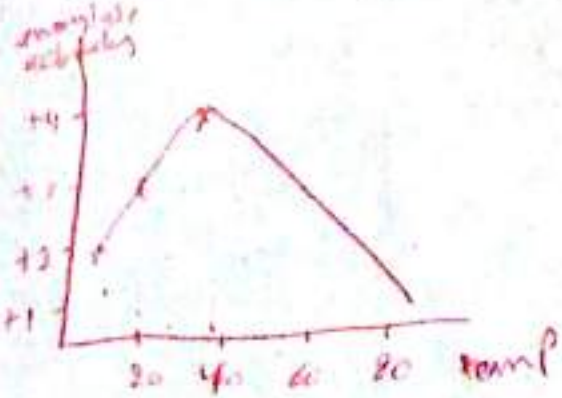
Hypothesize about the rate of activity of amylase at various ten

starch —amylase→ maltose
↓ ↓
disappearance of substrate — Appearance of Product

Prediction

Predict the results of the experiment based on your hypothesis

+4 highest activity
+3
+2
+1 lowest activity



Procedure

1. Number four standard test tubes 1 through 4.
2. Using the 5-mL calibrated pipette, add 2 mL of the 1% sta to each tube.
3. Using a clean 5-mL pipette, add 4 mL DI water to each tub
4. Add 1 mL of 6.8 buffer to each tube.
5. Place the test tubes as follows:
   Tube 1: 80°C water bath
   Tube 2: 37°C water bath
   Tube 3: test-tube rack (room temperature, or about 22°C)
   Tube 4: beaker of crushed ice (4°C)
6. Number and mark a second set of standard test tubes 1A Use the 1-mL calibrated pipette to add 1 mL amylase to ea place as follows (do not mix together the solutions in th tubes until instructed to do so):
   Tube 1A: 80°C water bath
   Tube 2A: 37°C water bath
   Tube 3A: test-tube rack (room temperature, or about 22°
   Tube 4A: beaker of crushed ice (4°C)

Lab Topic 6

Photosynthesis

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe the roles played by light and pigment in photosynthesis.
2. Name and describe pigments found in photosynthesizing tissues.
3. Explain the separation of pigments by paper chromatography, based on their molecular structure.
4. Demonstrate an understanding of the process of spectrophotometry and the procedure for using the spectrophotometer.

Introduction

Without photosynthesis, there could be no life on Earth as we know it. The Earth is an open system constantly requiring an input of energy to drive the processes of life. All energy entering the biosphere is channeled from the sun into organic molecules via the process of photosynthesis. As the sun's hydrogen is converted to helium, energy in the form of photons is produced. These photons pass to Earth's surface and those with certain wavelengths within the visible light portion of the electromagnetic spectrum are absorbed by pigments in the chloroplasts of plants, initiating the process of photosynthesis.

In photosynthesis, light energy is transformed into chemical energy. That chemical energy is used to synthesize organic compounds (glucose) from CO_2, and in the process water is used and O_2 is released. Glucose, a primary source of energy for all cells, may be converted to sucrose and transported or stored in the polymer starch. These organic molecules are building blocks for plant growth and development. Animals consume plants and convert the plant molecules into their own organic molecules and energy sources—the ultimate in recycling. Oxygen, also produced by photosynthesis, is necessary for aerobic respiration in the cells of plants, animals, and other organisms (Figure 6.1).

In this laboratory, you will investigate cellular and environmental components utilized in the process of photosynthesis. In several experiments, you will determine photosynthetic activity by testing for the production of starch, using iodine potassium iodide (I_2KI), which stains starch purple-black. A change from the yellow-amber color of the iodine solution to a purple-black solution is a positive test for the presence of starch. This is the same test for the presence of starch that you used to study starch digestion by amylase in Lab Topic 4 Enzymes.

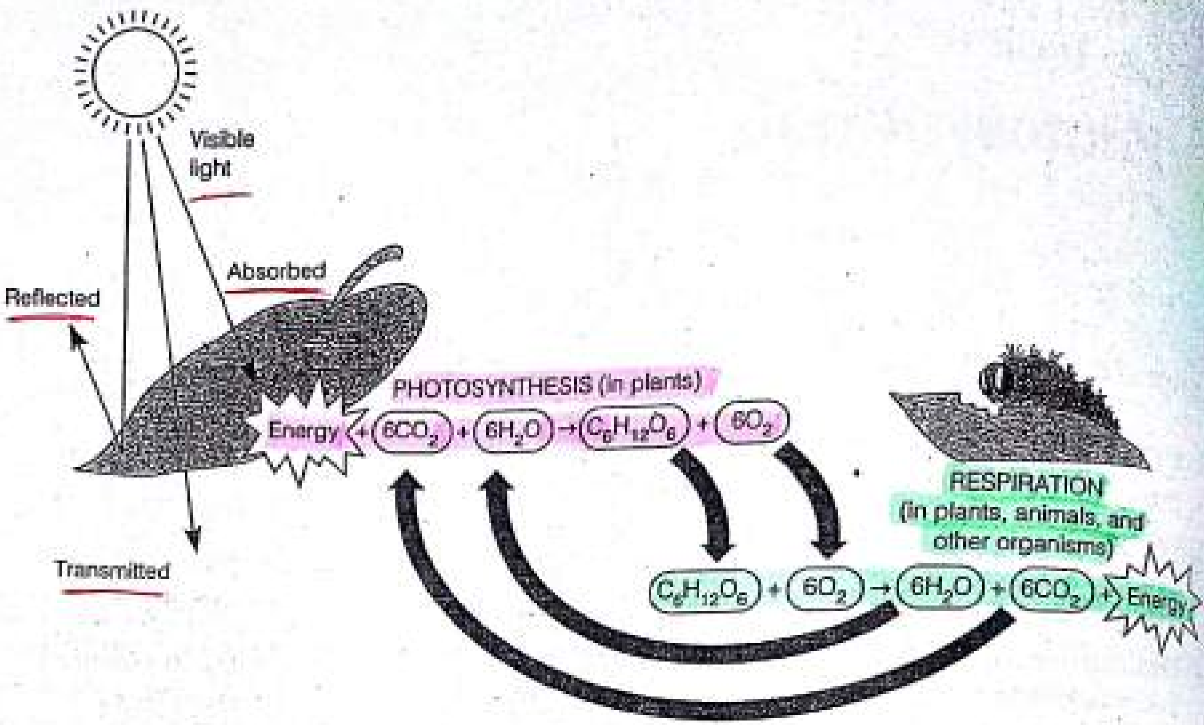
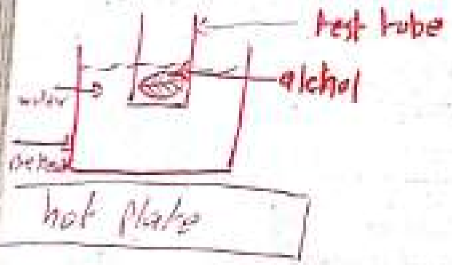


Figure 6.1.

Energy flow through photosynthesis and respiration. Energy flows from the sun into the biological systems of Earth, and visible light may be reflected, transmitted, or absorbed. Plants absorb light energy and convert it to chemical energy during photosynthesis. In this process, carbon dioxide and water are used to synthesize glucose (and ultimately other organic compounds) and to release oxygen. These organic molecules and the energy stored in them can be utilized by animals and other organisms that consume them. The energy in organic molecules is released to form ATP during cellular respiration in plants, animals, and other organisms.

Geranium
① kept in dark
② kept in light



boil → no more green color
→ analysis → add lugols

EXERCISE 6.1
The Wavelengths of Light for Photosynthesis

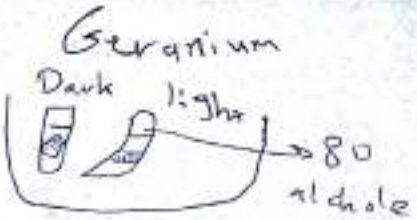
Materials

black construction paper
green, red, and blue plastic filters
paper clips
forceps
1 geranium plant with at least 4 good leaves per 8 students
1 1,000-mL beaker filled with 300 mL of water
1 400-mL beaker filled with 200 mL of 80% ethyl alcohol
dropper bottle with concentrated I_2KI solution
hot plate
petri dish
squirt bottle of water
scissors

Introduction

In this exercise, you will investigate the photosynthetic activity of different wavelengths of light. You will determine if products of photosynthesis are present in leaf tissue that has been exposed to different wavelengths of

light for several days. Working with other students in groups of eight, you will cover small portions of different leaves of a geranium plant with pieces of black paper and green, red, and blue plastic filters. Each pair of students will be responsible for one of the four treatments. Later you will determine photosynthetic activity by testing for the presence of starch under the paper or filters. Starch is synthesized from glucose molecules and can be easily tested as an indirect product of photosynthesis. Starch will turn dark in the presence of iodine (I_2KI).



Plastic filters used in this exercise are designed to reflect and transmit the appropriate wavelengths of light to correspond to the visible light spectrum. For example, green filters reflect and transmit green light. What wavelengths of light will be absorbed by the green filter? Refer to Color Plate 9 at the end of the lab manual, which shows the electromagnetic spectrum. What wavelength of light will be reflected and transmitted by the black paper and each of the colored filters? Note that the same wavelengths of light are reflected and transmitted by the filters.

Hypothesis

Hypothesize about photosynthetic activity in leaf cells covered with different colored filters as described.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Four to 5 days before the experiment is to be carried out, cut a piece from each color of plastic filter and one from the black construction paper. Each piece should be a rectangle approximately 2.5 cm by 5 cm. Double over the strip and slide the edge of a healthy geranium leaf, still attached to the plant, between the folded edges. Carefully slip a slightly sprung paper clip over the paper, securing the paper to the leaf. The paper should be on both sides of the leaf. Follow this procedure with the other colors and the black construction paper, using a different leaf for each strip. Return the plant with treated leaves to bright light. Your instructor may have already carried out this step for you.
2. On the day of the lab, carry the plant with leaves covered to your desk. You will have to be able to recognize each leaf after the paper is removed and the leaf is boiled. To facilitate this, with your teammates devise a way to distinguish each leaf, and write the distinction in the space provided. Differences in size or shape may distinguish different leaves, [illegible] distinguishing features, such as by

2. Sketch and label each leaf after staining to show the location of the stain.

Discussion

1. Which treatment allowed the greatest photosynthetic activity? (Explain your results in terms of your hypothesis.)

2. When the red filter is placed on a leaf, what wavelengths of light pass through and reach the leaf cells below? (Check wavelengths in Color Plate 9, which shows the electromagnetic spectrum.)

   Green filter?

   Blue filter?

3. Was starch present under the black construction paper? Explain this in light of the fact that black absorbs all wavelengths of light.

EXERCISE 6.2

Pigments in Photosynthesis

التجربة وخطواتها مش
مطلوبة .. بس المعلومات الموجودة
بال Introduction حفظ

Materials

Coleus plant with multicolored leaves
forceps
1 1,000-mL beaker filled with 300 mL of water
1 400-mL beaker filled with 200 mL of 80% ethyl alcohol
dropper bottle with concentrated I_2KI solution
hot plate
squirt bottle of water

Introduction

A variety of pigments are found in plants, as anyone who visits a botanical garden in spring or a deciduous forest in autumn well knows. A pigment is a substance that absorbs light. If a pigment absorbs all wavelengths of

visible light, it appears black. The black construction paper used in Exercise 6.1 is colored with such a pigment. Other pigments absorb some wavelengths and reflect others. Yellow pigments, for example, reflect light wavelengths in the yellow portion of the visible light spectrum, green reflects in the green portion, and so on.

Some colors are produced by only one pigment, but an even greater diversity of colors can be produced by the cumulative effects of different pigments in cells. Green colors in plants are produced by the presence of chlorophylls *a* and *b* located in the chloroplasts. Yellow, orange, and bright red colors are produced by carotenoids, also in chloroplasts. Blues, violets, purples, pinks, and dark reds are usually produced by a group of water-soluble pigments, the anthocyanins, that are located in cell vacuoles and do not contribute to photosynthesis. Additional colors may be produced by mixtures of these pigments in cells.

Working with one other student, you will use the I_2KI test for starch as in Exercise 6.1 to determine which pigment(s) in a *Coleus* leaf support photosynthesis. Before beginning the experiment, examine your *Coleus* leaf and hypothesize about the location of photosynthesis based on the leaf colors. (See Color Plate 10.)

التجربة وخطواتها
مش مطلوبة..
لكن المعلومات المكتوبة
حفظ

Hypothesis

Hypothesize about the location of photosynthesis based on the leaf colors.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Remove a multicolored leaf from a *Coleus* plant that has been in strong light for several hours.
2. In Table 6.1, list the colors of your leaf, predict the pigments present to create that color, and predict the results of the I_2KI starch test in each area of the leaf.
3. Sketch the leaf outline in the Results section, mapping the color distribution before the I_2KI test.
4. Extract the pigments as previously described in Exercise 6.1, and test the leaf for photosynthetic activity using I_2KI.

⚠ Ethyl alcohol is highly flammable! Do not place the beaker of alcohol directly on the hot plate. To bring it to a boil, raise the temperature of the hot plate until the alcohol just boils, and then reduce the temperature to maintain slow boiling. Do not leave boiling alcohol unattended.

Table 6.1
Predicted and Observed Results for the Presence of Starch in Colored Regions of the Coleus Leaf

Color	Pigments	Starch Present (predicted)	Starch Present (actual result)
Green			
Purple			
Pink			
White			
Other			

5. Sketch the leaf again in the Results section, outlining the areas showing a positive starch test.

Results

1. Record the results of the I_2KI test in Table 6.1.
2. Compare the sketches of the *Coleus* leaf before and after the I_2KI test.

Before I_2KI Test: **After I_2KI Test:**

3. Which pigments supported photosynthesis? Record your results in Table 6.1.

Discussion

Describe and explain your results based on your hypothesis.

EXERCISE 6.3

Separation and Identification of Plant Pigments by Paper Chromatography

Materials

capillary tube
beakers
extractions of leaf pigments in acetone
chromatography paper stapled into a cylinder marked with a pencil line about 1 cm from one end
quart jar with lid, containing solvent of petroleum ether and acetone
forceps
scissors
acetone

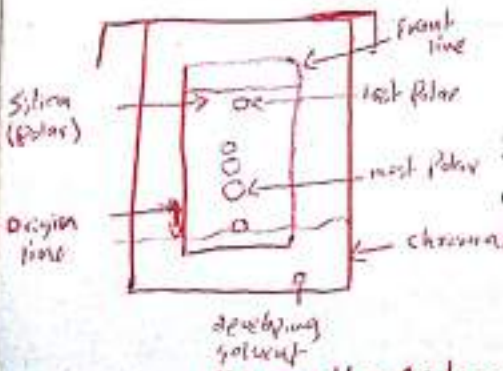
Introduction

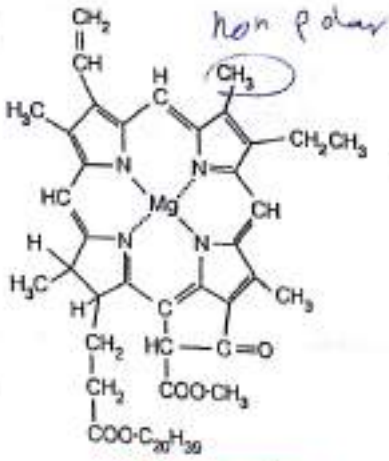
Your instructor has prepared an extract of chloroplast pigments from fresh green grass or fresh spinach. A blender was used to rupture the cells, and the pigments were then extracted with acetone, an organic solvent. Working with one other student, begin this exercise by separating the pigments extracted using paper chromatography. To do this, you will apply the pigment extract to a cylinder of chromatographic paper. You will then place the cylinder in a jar with the organic solvents petroleum ether and acetone. The solvents will move up the paper and carry the pigments along; the pigments will move at different rates, depending on their different solubilities in the solvents used and the degree of attraction to the paper. The leading edge of the solvent is called the **front**. Discrete pigment bands will be formed from the front back to the point where pigments were added to the paper.

The following information will be helpful to you as you make predictions and interpret results:

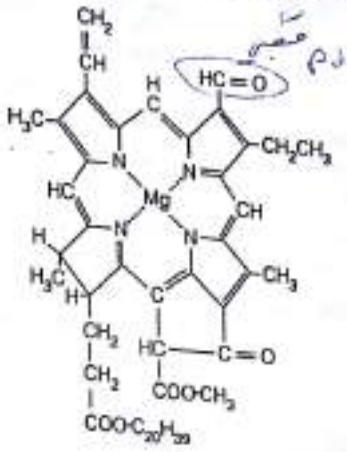
1. **Polar molecules** or substances dissolve (or are attracted to) polar molecules.
2. **Nonpolar molecules** are attracted to nonpolar molecules to varying degrees.
3. Chromatography paper (cellulose) is a polar (charged) substance.
4. The solvent, made of petroleum ether and acetone, is relatively nonpolar.
5. The *most nonpolar* substance will dissolve in the nonpolar solvent *first*.
6. The *most polar* substance will be attracted to the polar chromatography paper; therefore, it will move *last*.

Use this information and the molecular structure of major leaf pigments to predict the relative solubilities and separation patterns for the pigments and to identify the pigment bands. Study the molecular structure of the four common plant pigments in Figure 6.2. As you study these diagrams, rank the pigments according to polarity in the space provided. To determine polarity, count the number of polar oxygens present in each molecule.

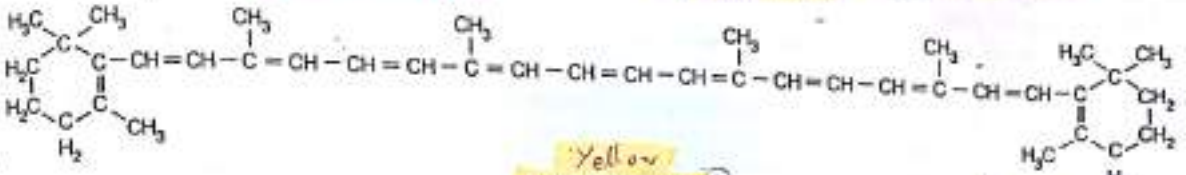




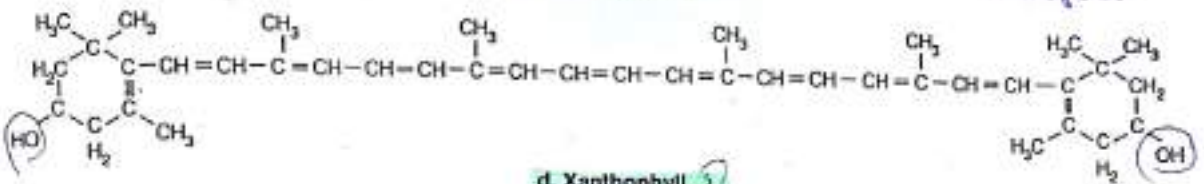
a. Chlorophyll *a*



b. Chlorophyll *b*



c. Beta carotene



d. Xanthophyll

Figure 6.2.
Molecular structure of major leaf pigments. The molecular structure of chlorophyll (*a*), chlorophyll (*b*), carotene (*c*), and xanthophyll (*d*). To determine polarity, count the number of polar oxygens present in each molecule.

Most polar:

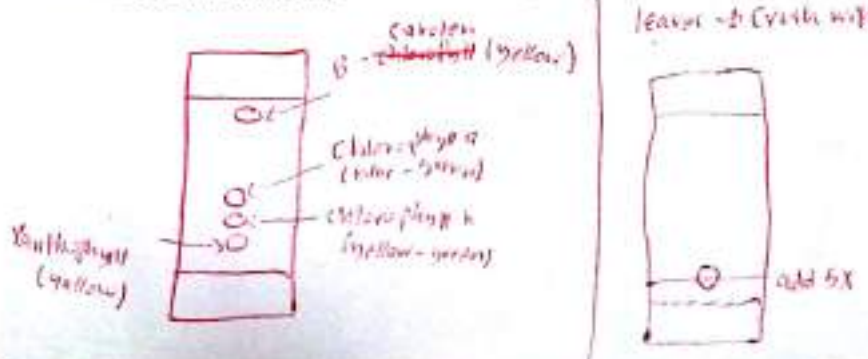
Least polar:

Hypothesis

State a hypothesis relating polarities and solubilities of pigments.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).



Lab Topic 7

Mitosis and Meiosis

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe the activities of chromosomes, centrioles, and microtubules in the cell cycle, including all phases of mitosis and meiosis.
2. Recognize human chromosomes in leukocytes.
3. Identify the phases of mitosis in root tip and whitefish blastula cells.
4. Describe differences in mitosis and cytokinesis in plant and animal cells.
5. Describe differences in mitosis and meiosis.
6. Explain crossing over, and describe how this can bring about particular arrangements of ascospores in the fungus *Sordaria*.

Introduction

The nuclei in cells of eukaryotic organisms contain chromosomes with clusters of genes, discrete units of hereditary information consisting of duplicated deoxyribonucleic acid (DNA). Structural proteins in the chromosomes organize the DNA and participate in DNA folding and condensation. When cells divide, chromosomes and genes are duplicated and passed on to daughter cells. Single-celled organisms divide for reproduction. Multicellular organisms have reproductive cells (eggs or sperm), but they also have somatic (body) cells that divide for growth or replacement.

In somatic cells and single-celled organisms, the nucleus divides by **mitosis** into two daughter nuclei, which have the same number of chromosomes and the same genes as the parent cell. For example, the epidermis or outer layer of skin tissue is continuously being replaced through cell reproduction involving mitosis. All of these new skin cells are genetically identical. Yeast and amoeba are both single-celled organisms that can reproduce asexually through mitotic divisions to form additional organisms—genetically identical clones.

Cancerous cells are characterized by uncontrolled mitotic and cell division, and therefore, the study of mitosis and its regulation is key to developing new cancer treatments. In 2009, three scientists studying chromosomes and the regulation of mitosis were awarded the Nobel Prize in medicine for their discovery of **telomeres**. Telomeres are DNA sequences on the ends of chromosomes that become shorter during every mitotic cycle of somatic cells. Without telomeres protecting the chromosome ends, important genes located at the ends of chromosomes might be lost in mitosis. The loss of telomeres in each division of somatic cells may be one of many regulatory mechanisms

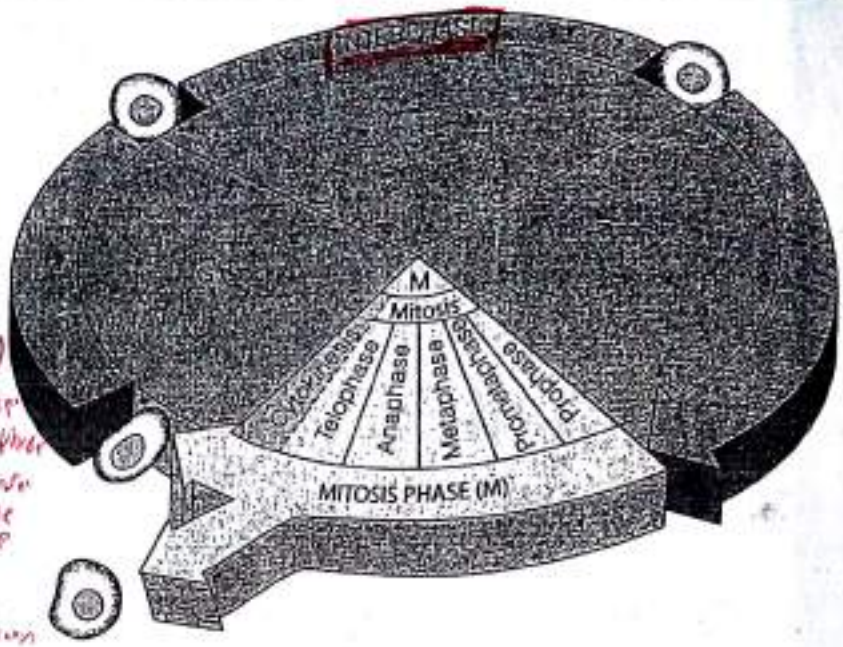


Figure 7.1.
The cell cycle. In interphase (G_1, S, G_2), DNA replication and most of the cell's growth and biochemical activity take place. In the M phase, the nucleus divides in mitosis, and the cytoplasm divides in cytokinesis.

that limit the number of mitotic cycles that cells can undergo. If telomeres fail to shorten, cells may continue to divide, as in cancer cells.

In multicellular organisms, in preparation for sexual reproduction, a type of nuclear division called **meiosis** takes place. In meiosis, certain cells in ovaries or testes (or sporangia in plants) divide twice, but the chromosomes only replicate once. This process results in the four daughter nuclei with new combinations of chromosomes. Eggs or sperm (or spores in plants) are eventually formed. In contrast to mitosis, the process of meiosis contributes to the genetic variation that is important in sexual reproduction. Generally in both mitosis and meiosis, after nuclear division the cytoplasm divides, a process called **cytokinesis**.

Events from the beginning of one cell division to the beginning of the next are collectively called the **cell cycle**. The cell cycle is divided into two major phases: interphase and mitotic phase (M). The M phase represents the division of the nucleus and cytoplasm (Figure 7.1).

EXERCISE 7.1
Modeling the Cell Cycle and Mitosis in an Animal Cell

Materials

60 pop beads of one color
60 pop beads of another color
4 magnetic centromeres
4 centrioles

Introduction

Scientists use models to represent natural structures and processes that are too small, too large, or too complex to investigate directly. Scientists develop their models from observations and experimental data, usually accumulated from a variety of sources. Building a model can represent the culmination of a body of scientific work, but most models represent a well-developed hypothesis that can then be tested against the natural system and modified.

Linus Pauling's novel and successful technique of building a physical model of hemoglobin was based on available chemical data. This technique was later adopted by Francis Crick and James Watson to elucidate the nature of the hereditary material, DNA. Watson and Crick built a wire model utilizing evidence collected by many scientists. They presented their conclusions about the structure of the DNA helix in the journal *Nature* in April 1953 and were awarded the Nobel Prize for their discovery in 1962.

Today in lab you will work with a partner to build models of cell division: mitosis and meiosis. Using these models will enhance your understanding of the behavior of chromosomes; centrioles, membranes, and microtubules during the cell cycle. After completing your model, you will consider ways in which it is and is not an appropriate model for the cell cycle. You and your partner should discuss activities in each stage of the cell cycle as you build your model. After going through the exercise once together, you will demonstrate the model to each other to reinforce your understanding.

In the model of mitosis that you will build, your cell will be a **diploid** cell ($2n$) with four chromosomes. This means that you will have two homologous pairs of chromosomes. In diploid cells, **homologous chromosomes** are the same length, have the same centromere position, and contain genes for the same characters. One pair will be long chromosomes, the other pair, short chromosomes. (**Haploid** cells have only one of each homologous pair of chromosomes, denoted n.)

Lab Study A. Interphase

During interphase, a cell performs its specific functions: Liver cells produce bile; intestinal cells absorb nutrients; pancreatic cells secrete enzymes; skin cells produce keratin. Interphase consists of three subphases, G_1, S, and G_2, which begin as a cell division ends. As interphase begins, there is approximately half as much cytoplasm in each cell as there was before division. Each new cell has a nucleus that is surrounded by a **nuclear envelope** and that contains chromosomes in an uncoiled, or decondensed, state. In this uncoiled state, the mass of DNA and protein is called **chromatin.** Located outside the nucleus is the **centrosome,** a granular region that contains a pair of **centrioles** in animal cells. The centrosome is the organizing center for microtubules (Figure 7.2).

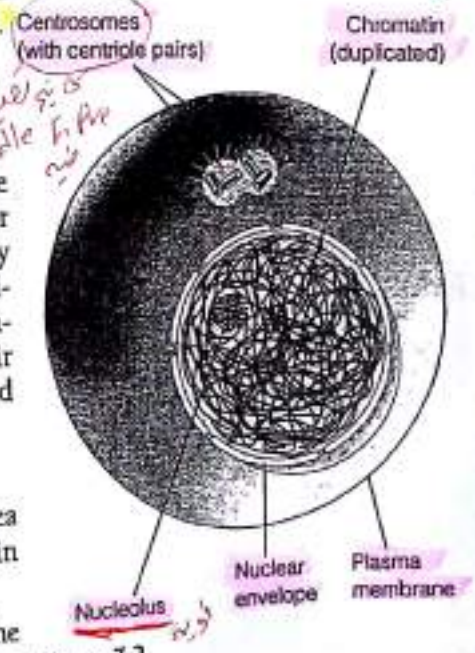


Figure 7.2. **Interphase.**

Procedure

1. Build a homologous pair of single chromosomes using 10 beads of one color for one member of the long pair and 10 beads of the other color for the other member of the pair. Place the magnetic centromere at any position in the chromosome, but note that it must be in the same position on homologous chromosomes. The centromere appears as a constricted region when chromosomes are condensed. Build the short pair with the same two different colors, but use fewer beads. You should have enough beads left over to duplicate each chromosome later.
2. Model **interphase** of the cell cycle:
   a. Pile all the assembled chromosomes in the center of your work area to represent the decondensed chromosomes as a mass of chromatin in G_1 **(gap 1).**
   b. Position two centrioles as a pair just outside your nucleus. Have the two members of the centriole pair at right angles to each other. (Recall, however, that most plant cells do not have centrioles.)

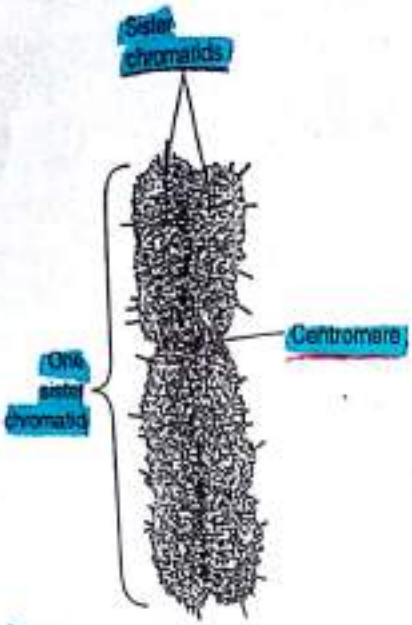


Figure 7.3.
Duplicated chromosome composed of two sister chromatids held together at the centromere; condensed as in prometaphase.

In the G_1 phase, the cytoplasmic mass increases and will continue to do so throughout interphase. Proteins are synthesized, new organelles are formed, and some organelles such as mitochondria and chloroplasts grow and divide in two. Throughout interphase one or more dark, round bodies, called **nucleoli** (singular, **nucleolus**), are visible in the nucleus.

c. Duplicate the centrioles: Add a second pair of centrioles to your model; again, have the two centrioles at right angles to each other. **Centriole duplication** begins in late G_1 or early S phase.

d. Duplicate the chromosomes in your model cell to represent DNA replication in the **S (synthesis) phase:** Make a second strand that is identical to the first strand of each chromosome. In duplicating chromosomes, you will use two magnets to form the new centromere. In the living cell, the centromere in a cell is a single unit until it splits in metaphase. In your model, consider the pair of magnets to be the single centromere.

Unique activities taking place during the S phase of the cell cycle are the replication of chromosomal DNA and the synthesis of chromosomal proteins. DNA synthesis continues until chromosomes have been duplicated. Each strand of a duplicated chromosome is called a **sister chromatid**. Sister chromatids are identical to each other and are held together tightly at the centromere (Figure 7.3).

e. Do not disturb the chromosomes to represent G_2 (gap 2).

During the G_2 phase, in addition to continuing cell activities, cells prepare for mitosis. Enzymes and other proteins necessary for cell division are synthesized during this phase.

f. Separate your centriole pairs, moving them toward opposite poles of the nucleus to represent that the G_2 phase is coming to an end and mitosis is about to begin.

How many pairs of homologous chromosomes are present in your cell during this stage of the cell cycle?

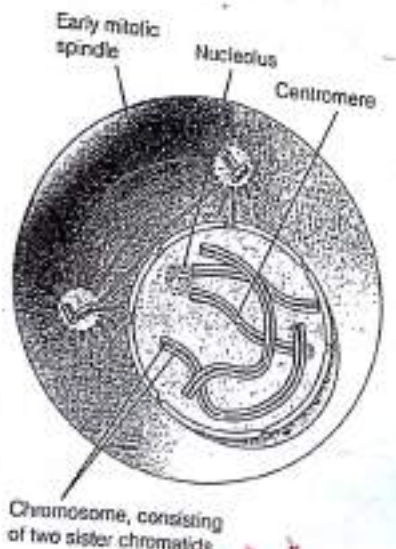


Figure 7.4.
Prophase mitosis.

Lab Study B. M Phase (Mitosis and Cytokinesis)

In the M phase, the nucleus and cytoplasm divide. Nuclear division is called *mitosis*. Cytoplasmic division is called *cytokinesis*. Mitosis is divided into five subphases: prophase, prometaphase, metaphase, anaphase, and telophase.

Procedure

1. To represent **prophase**, leave the chromosomes piled in the center of the work area.

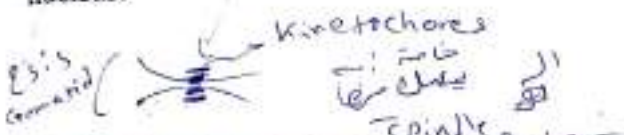
Prophase begins when chromosomes begin to coil and condense. At this time they become visible in the light microscope. Centrioles continue to move to opposite poles of the nucleus, and as they do so, a fibrous, rounded structure tapering toward each end, called a **spindle**, begins to form between them. Nucleoli begin to disappear (Figure 7.4).

What structures make up the fibers of the spindle? (Check text if necessary.)

2. At prometaphase, the centrioles are at the poles of the cell. To represent the actions in prometaphase, move the centromeres of your chromosomes to lie on an imaginary plane (the equator) midway between the two poles established by the centrioles.

During prometaphase chromosomes continue to condense (Figure 7.3). The nuclear envelope breaks down as the spindle continues to form. Some spindle fibers become associated with chromosomes at protein structures called **kinetochores**. Each sister chromatid has a kinetochore at the centromere. These spindle microtubules now extend from the chromosomes to the centrosomes at the poles. The push and pull of spindle fibers on the chromosomes ultimately leads to their movement to the equator. When the centromeres lie on the equator, prometaphase ends and the next phase begins (Figure 7.5).

How many duplicated chromosomes are present in your prometaphase nucleus?



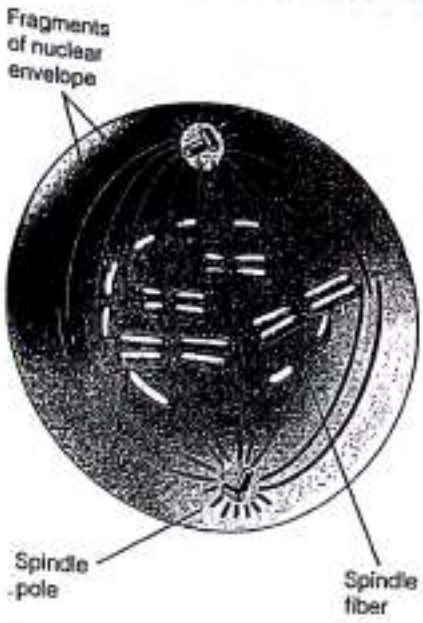


Figure 7.5.
Prometaphase mitosis.

Students often find it confusing to distinguish between chromosome number and chromatid number. To simplify this problem, count the number of centromeres. The number of centromeres represents the number of chromosomes. In your model, the pair of joined magnets represents one centromere.

3. To represent ~~metaphase, a relatively static phase, leave the chromosomes with centromeres lying on the equator.~~

In metaphase, duplicated chromosomes lie on the equator (also called the metaphase plate). The two sister chromatids are held together at the centromere. Metaphase ends as the centromere splits.

Label Figure 7.6 with *chromosome, spindle fibers, centrosome, centrioles, kinetochore, equator.*

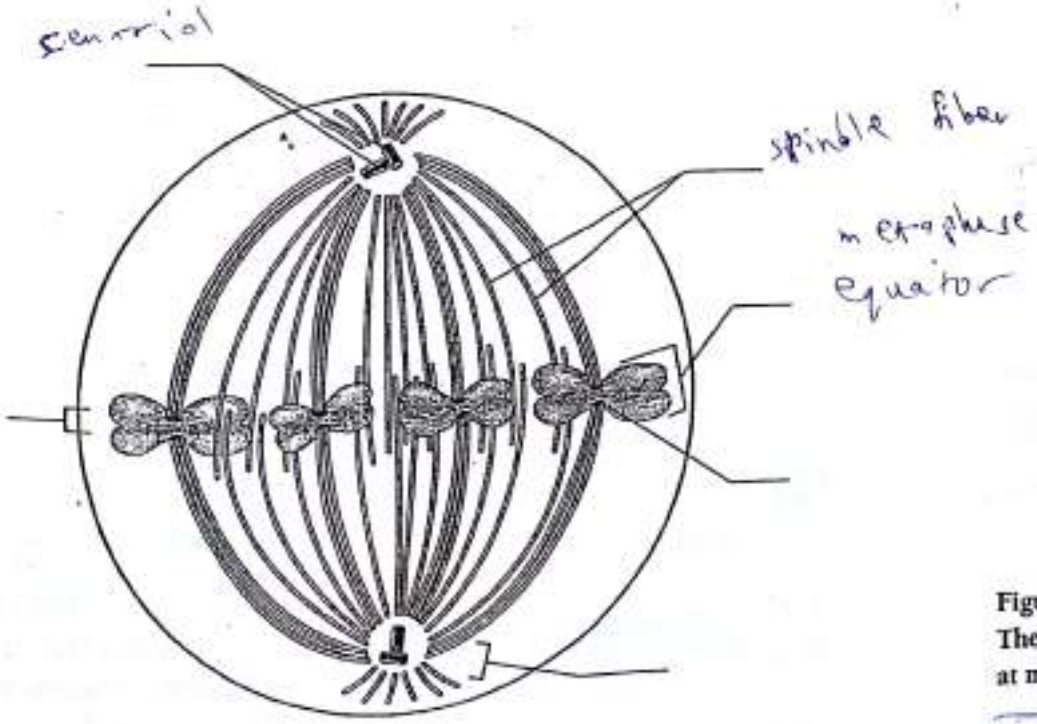


Figure 7.6.
The mitotic spindle at metaphase.

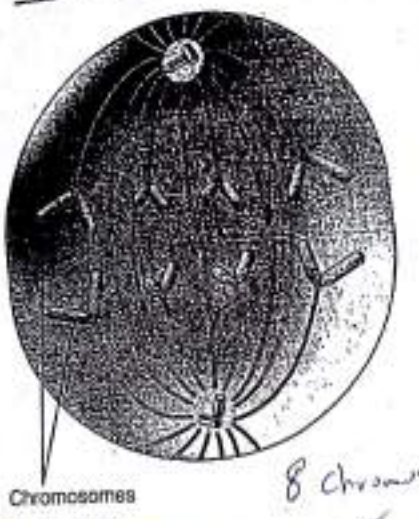


Figure 7.7.
Anaphase mitosis.

4. Holding on to the centromeres, pull the magnetic centromeres apart and move them toward opposite poles. This action represents **anaphase**. After the centromere splits, sister chromatids separate and begin to move toward opposite poles. Chromatids are now called **chromosomes**. Anaphase ends as the chromosomes reach the poles (Figure 7.7).

Describe the movement of the chromosome arms as you move the centromeres to the poles.

Certain biologists are currently investigating the role played by spindle fibers in chromosome movement toward the poles. Check your text for a discussion of one hypothesis, and briefly summarize it here.

5. Pile your chromosomes at the poles to represent **telophase**. As chromosomes reach the poles, anaphase ends and telophase begins. The spindle begins to break down. Chromosomes begin to uncoil, and nucleoli reappear. A nuclear envelope forms around each new cluster of chromosomes. Telophase ends when the nuclear envelopes are complete (Figure 7.8).

How many chromosomes are in each new nucleus?

How many chromosomes were present in the nucleus when the process began?

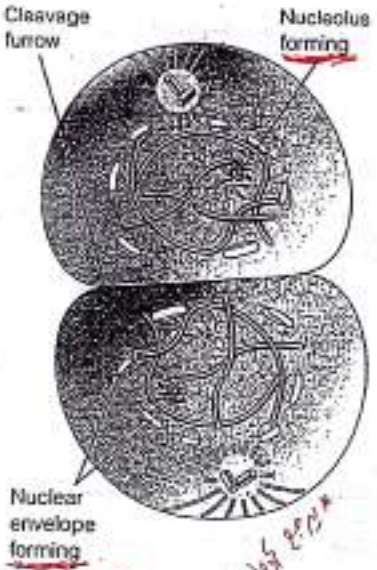


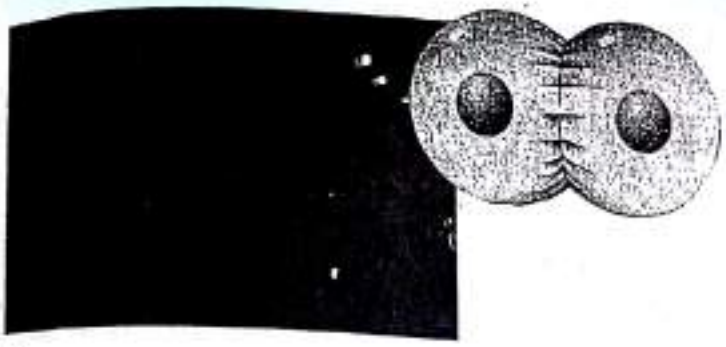
Figure 7.8.
Telophase mitosis.

How would the condition of telomeres have changed from a previous cell cycle? (See the Introduction to this lab topic.)

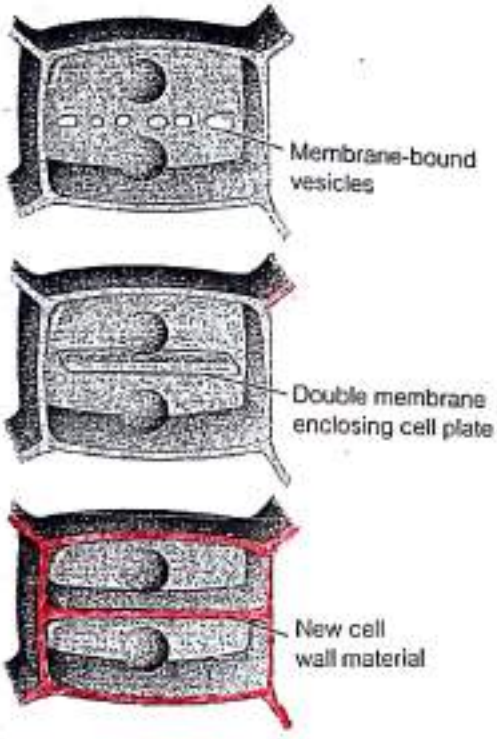
6. To represent cytokinesis, leave the two new chromosome masses at the poles.

The end of telophase marks the end of nuclear division, or mitosis. Sometime during telophase, the division of the cytoplasm, or cytokinesis, results in the formation of two separate cells. In cytokinesis in cells of animals, fungi, and slime molds, a **cleavage furrow** forms at the equator and eventually pinches the parent cell cytoplasm in two (Figure 7.9a). Actin and myosin, the same molecules found in muscle cells, contribute to the formation of the cleavage furrow. In plant cells, membrane-bound vesicles migrate to the center of the equatorial plane and fuse to form the **cell plate**. This eventually extends across the cell

dividing the cytoplasm in two. Cell wall materials in the vesicles are released into the space between the membranes of the cell plate forming the new cell wall (Figure 7.9b, c).



a.



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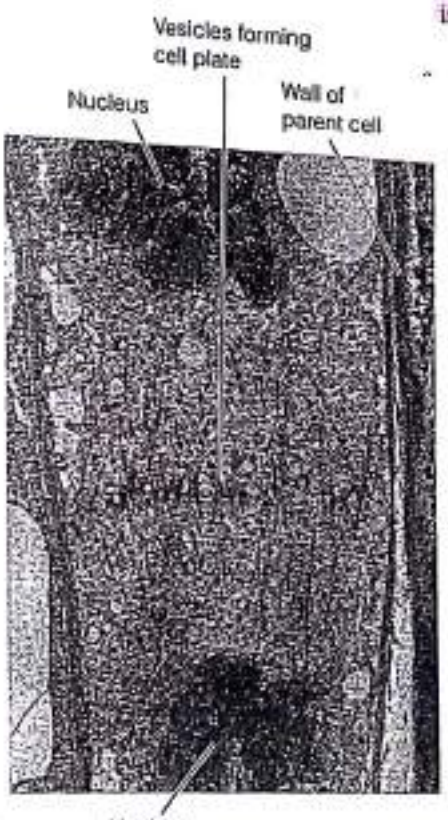


c.

Figure 7.9.
Cytokinesis in animal and plant cells. (a) In animal cells, a cleavage furrow forms at the equator and pinches the cytoplasm in two. (b) In plants, a cell plate forms in the center of the cell and grows until it divides the cytoplasm in two. (c) Photomicrograph of cytokinesis in a plant cell.

EXERCISE 7.2

Observing Mitosis and Cytokinesis in Plant Cells

Materials

prepared slide of onion root tip
compound microscope

Introduction

The behavior of chromosomes during the cell cycle is similar in animal and plant cells. However, differences in cell division do exist. Plant cells have no centrioles, yet they have bundles of microtubules that converge toward the poles at the ends of a spindle. Cell walls in plant cells dictate differences in cytokinesis. In this exercise, you will observe dividing cells in the zone of cell division of a root tip (Color Plate 11).

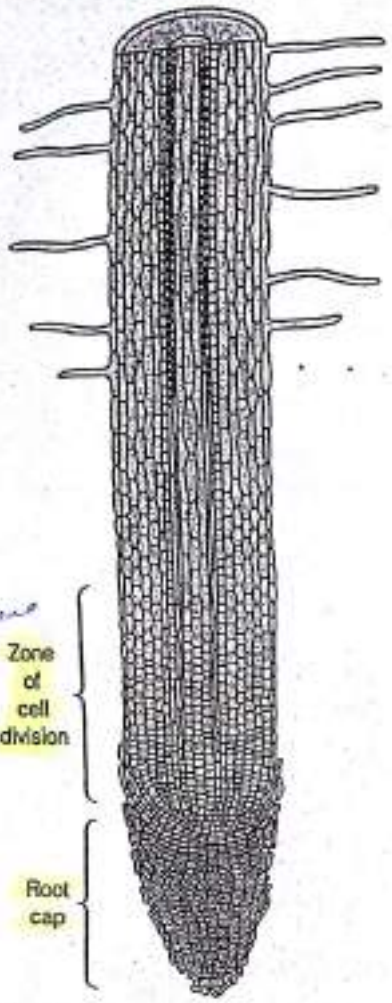


Figure 7.10.
Longitudinal section through a root tip. Cells are dividing in the zone of cell division just behind the root cap.

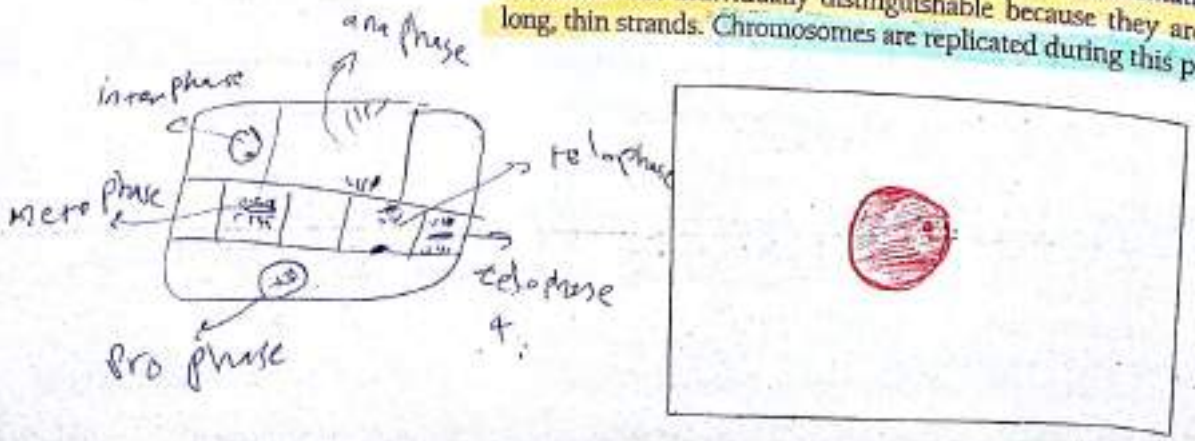
Procedure

1. Examine a prepared slide of a longitudinal section through an onion root tip using low power on the compound microscope.
2. Locate the region most likely to have dividing cells, just behind the root cap (Figure 7.10).

   At the tip of the root is a root cap that protects the tender root tip as it grows through the soil. Just behind the root cap is the **zone of cell division**. Notice that rows of cells extend upward from this zone. As cells divide in the zone of cell division, the root tip is pushed farther into the soil. Cells produced by division begin to mature, elongating and differentiating into specialized cells, such as those that conduct water and nutrients throughout the plant.
3. Focus on the zone of cell division. Then switch to the intermediate power, focus, and switch to high power.
4. Survey the zone of cell division and locate stages of the cell cycle: interphase, prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis.
5. As you find a dividing cell, speculate about its stage of division, read the following descriptions given of each stage to verify that your guess is correct, and, if necessary, confirm your conclusion with the instructor.
6. Draw the cell in the appropriate boxes provided. Label nucleus, nucleolus, chromosome, chromatin, mitotic spindle, and cell plate when appropriate.

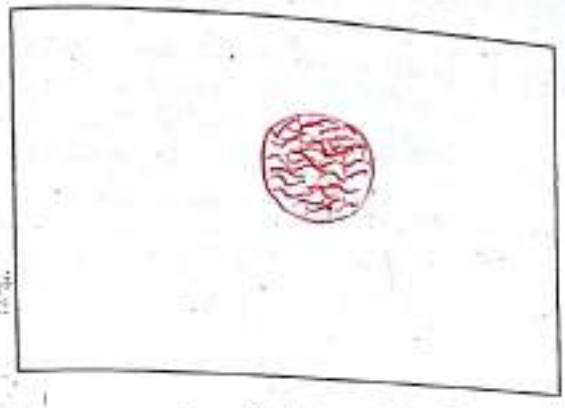
Interphase (G_1, S, G_2)

Nuclear material is surrounded by a nuclear envelope. Dark-staining bodies, nucleoli, are visible. Chromosomes appear only as dark granules within the nucleus. Collectively, the chromosome mass is called *chromatin*. The chromosomes are not individually distinguishable because they are uncoiled into long, thin strands. Chromosomes are replicated during this phase.



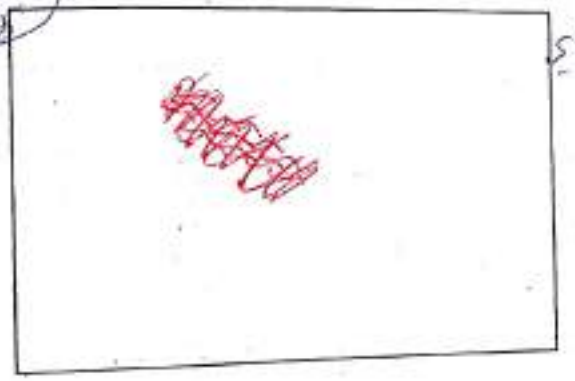
Prophase

Chromosomes begin to coil and become distinguishable thin, threadlike structures, widely dispersed in the nucleus during prophase. Although there are no centrioles in plant cells, a spindle begins to form. Nucleoli begin to disappear. The nuclear envelope is still intact.



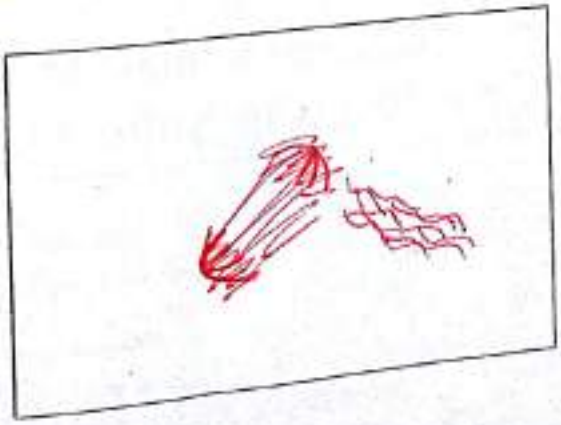
Prometaphase

By prometaphase, the chromosomes are thick and short. Each chromosome is duplicated consisting of two chromatids held together by a centromere. The nuclear membrane and nucleoli break down in prometaphase. Chromosomes move toward the equator.



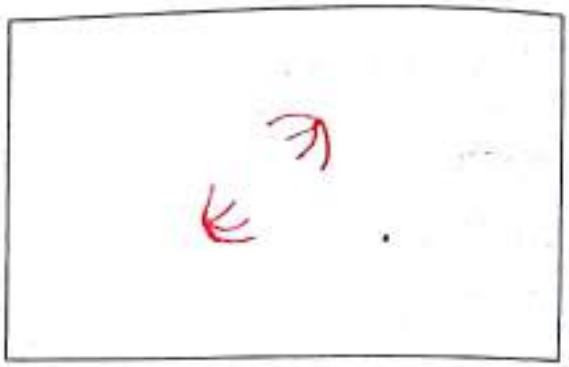
Metaphase

Metaphase begins when the centromeres of the chromosomes lie on the equator of the cell. The arms of the chromatids extend randomly in all directions. A spindle may be apparent. Spindle fibers are attached to kinetochores at the centromere region and extend to the poles of the cell. As metaphase ends and anaphase begins, the centromeres split.



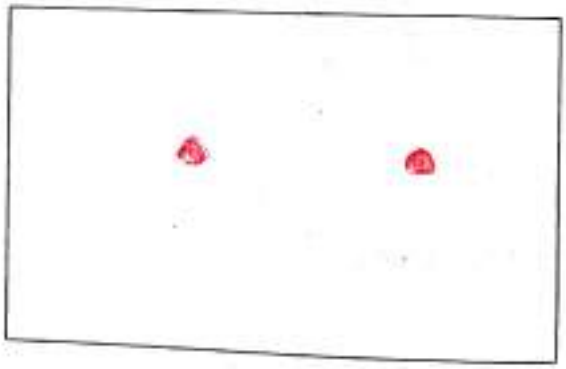
Anaphase

The splitting of centromeres marks the beginning of anaphase. Each former chromatid is now a new single chromosome. These chromosomes are drawn apart toward opposite poles of the cell. Anaphase ends when the migrating chromosomes reach their respective poles.



Telophase and Cytokinesis

Chromosomes have now reached the poles. The nuclear envelope re-forms around each compact mass of chromosomes. Nucleoli reappear. Chromosomes begin to uncoil and become indistinct. Cytokinesis is accomplished by the formation of a cell plate that begins in the center of the equatorial plane and grows outward to the cell wall.



EXERCISE 7.3
Observing Chromosomes, Mitosis, and Cytokinesis in Animal Cells

In this exercise, you will look at the general shape and form of human chromosomes and observe chromosomes and the stages of mitotic division in the whitefish. You will also compare these chromosomes with the plant chromosomes studied in Exercise 7.2. Chromosome structure in animals and plants is basically the same in that both have centromeres and arms. However, plant chromosomes are generally larger than animal chromosomes.

Lab Study A. Mitosis in Whitefish Blastula Cells

Materials

prepared slide of sections of whitefish blastulas
compound microscope

Introduction

The most convenient source of actively dividing cells in animals is the early embryo, where cells are large and divide rapidly with a short interphase. In blastulas (an early embryonic stage), a large percentage of cells will be dividing at any given time. By examining cross sections of whitefish blastulas, you should be able to locate many dividing cells in various stages of mitosis and cytokinesis (Color Plate 12).

Procedure

1. Examine a prepared slide of whitefish blastula cross sections. Find a blastula section on the lowest power, focus, switch to intermediate power, focus, and switch to high power.
2. As you locate a dividing cell, identify the stage of mitosis. *Be able to recognize all stages of mitosis in these cells.*
3. Identify the following in several cells:

   nucleus, nuclear envelope, and **nucleolus**

   chromosomes

   mitotic spindle

   asters—an array of microtubules surrounding each centriole pair at the poles of the spindle

   centrioles—small dots seen at the poles around which the microtubules of the spindle and asters appear to radiate

   cleavage furrow

Results

1. List several major differences you have observed between mitosis in animal cells and mitosis in plant cells:

2. Locate, draw, and label in the space provided a blastula cell in metaphase and a cell in telophase/cytokinesis to illustrate these differences.

Telophase/Cytokinesis

Student Media BioFlix and Videos—Animal Mitosis (time-lapse); Sea Urchin Embryonic Development (time-lapse)

Lab Study B. Human Chromosomes in Dividing Leukocytes

Materials

slides of human leukocytes (white blood cells) on demonstration with compound microscopes

Introduction

Cytogeneticists examining dividing cells of humans can frequently detect chromosome abnormalities that lead to severe mental retardation. To examine human chromosomes, leukocytes are isolated from a small sample of the patient's blood and cultured in a medium that inhibits spindle formation during mitosis. As cells begin mitosis, chromosomes condense and become distinct, but in the absence of a spindle they cannot move to the poles in anaphase. You will observe a slide in which many cells have chromosomes condensed as in prometaphase or metaphase, but they are not aligned on a spindle equator (see Color Plate 13).

Procedure

1. Attempt to count the chromosomes in one cell in the field of view. Normally, humans have 46 chromosomes. Persons with trisomy 21 (three copies of chromosome 21), or Down syndrome, have 47 chromosomes. Are the cells on this slide from a person with a normal chromosome number?

2. Notice that each chromosome is duplicated, being made up of two sister chromatids held together by a single centromere. In very high magnifications, bands can be seen on the chromosomes. Abnormalities in banding patterns can also be an indication of severe mental retardation.

EXERCISE 7.4
Modeling Meiosis

Materials

60 pop beads of one color
60 pop beads of another color
8 magnetic centromeres
4 centrioles
letters *B*, *D*, *b*, and *d* printed on

Introduction

Meiosis takes place in all organisms that reproduce sexually. In animals, meiosis occurs in special cells of the gonads; in plants, in special cells of the sporangia. Meiosis consists of two nuclear divisions, **meiosis I and II**, with an atypical interphase between the divisions during which cells do not grow and synthesis of DNA does not take place. This means that meiosis I and II result in four cells from each parent cell, each containing half the number of chromosomes, one from each homologous pair. Recall that cells with only one of each homologous pair of chromosomes are haploid (n) cells. The parent cells, with pairs of homologous chromosomes, are diploid ($2n$). The haploid cells become sperm (in males), eggs (in females), or spores (in plants). One advantage of meiosis in sexually reproducing organisms is that it prevents the chromosome number from doubling with every generation when fertilization occurs.

What would be the consequences in successive generations of offspring if the chromosome number were not reduced during meiosis?

Meiosis involves the very precise movement and sorting of chromosomes (in the case of humans, 23 homologous pairs, 46 chromosomes, or 92 chromatids). This complicated process is not always perfect. Sometimes the homologous pairs do not separate properly or the sister chromatids fail to separate. When this happens the result is that the final nuclei may have either one too many chromosomes or one too few. As you observed in Exercise 7.3 Lab Study B, individuals with Down syndrome have 47 chromosomes as a result of an error in meiosis, in which a gamete from one of the parents had two copies of chromosome 21.

Lab Study A. Interphase

Working with another student, you will build a model of the nucleus of a cell in interphase before meiosis. Nuclear and chromosome activities are similar to those in mitosis. You and your partner should discuss activities in the nucleus and chromosomes in each stage. Go through the exercise once together, and then demonstrate the model to each other to reinforce your understanding. Compare activities in meiosis with those in mitosis as you build your model.

Procedure

1. Build the premeiotic interphase nucleus much as you did the mitotic interphase nucleus. Have two pairs of chromosomes ($2n = 4$) of distinctly different sizes and different centromere positions. Have one member of each pair of homologues be one color, the other, a different color.
2. To represent G_1 (gap 1), pile your four chromosomes in the center of your work area. The chromosomes are decondensed.

   Cell activities in G_1 are similar to those activities in G_1 of the interphase before mitosis.

   In G_1, are chromosomes single or duplicated?

meiosis I بين mitosis و interphase في

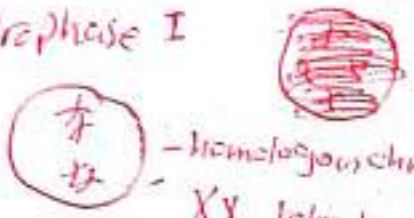
meiosis II و meiosis I بين interphase في

3. Duplicate the chromosomes to represent DNA duplication in the S (synthesis) phase. Recall that in living cells, the centromeres remain single, but in your model you must use two magnets. What color should the sister chromatids be for each pair?

4. Duplicate the centriole pair.
5. Leave the chromosomes piled in the center of the work area to represent G_2 (gap 2).

   As in mitosis, in G_2 the cell prepares for meiosis by synthesizing proteins and enzymes necessary for nuclear division.

Prophase I



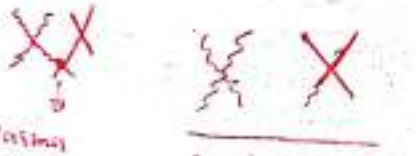
- homologous chromosomes

XX tetrad

4 non sister chromatid

- synapsis : pairing of homologous chromosomes

* crossing over



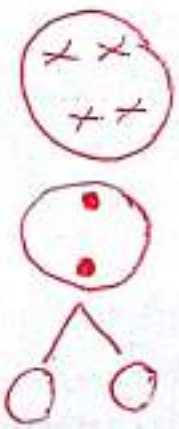
chiasma

genetic variation

metaphase I

anaphase I

telophase I



Lab Study B. Meiosis I

Meiosis consists of two consecutive nuclear divisions, called *meiosis I* and *meiosis II*. As the first division begins, the chromosomes coil and condense, as in mitosis. Meiosis I is radically different from mitosis, however, and the differences immediately become apparent. In your modeling, as you detect the differences, make notes in the margin of your lab manual.

Procedure

1. Meiosis I begins with the chromosomes piled in the center of your work area.

   As chromosomes begin to coil and condense, prophase I begins. Each chromosome is duplicated, made up of two sister chromatids. Two pairs of centrioles are located outside the nucleus.
2. Separate the two centriole pairs and move them to opposite poles of the nucleus.

   The nuclear envelope breaks down and the spindle begins to form as in mitosis.
3. Move each homologous chromosome to pair with its partner. You should have four strands together.

   Early in prophase I, each chromosome finds its homologue and pairs in a tight association. The process of pairing is called **synapsis**. Because the chromosomes are duplicated, this means that each paired duplicated chromosome complex is made of four strands. This complex is called a **tetrad**.

   How many tetrad complexes do you have in your cell, which is $2n = 4$?

4. Represent the phenomenon of **crossing over** by detaching and exchanging identical segments of any two nonsister chromatids in a tetrad. Crossing over takes place between nonsister chromatids in the tetrad. In this process, a segment from one chromatid will break and exchange with the exact same segment on a nonsister chromatid in the tetrad. The crossover site forms a **chiasma** (plural, **chiasmata**).
5. Return the exchanged segments of chromosome to their original chromosomes before performing the crossing-over activity in the next step.

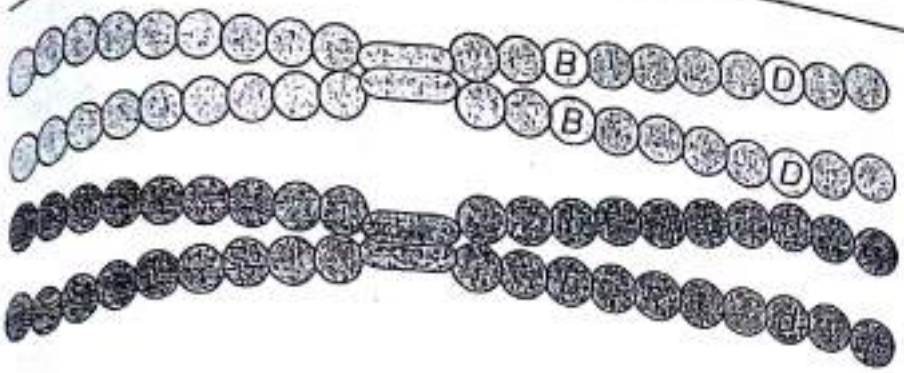


Figure 7.11.
Arrangement of alleles *B*, *b*, *D*, and *d* on chromosome models. One duplicated homologous chromosome has B alleles and D alleles on each chromatid. The other has *b* and *d* alleles on each chromatid.

Genes (traits) are often expressed in different forms. For example, when the gene for seed color is expressed in pea plants, the seed may be green or yellow. Alternative forms of genes are called alleles. Green and yellow are alleles of the seed-color gene. It is significant that crossing over produces new allelic combinations among genes along a chromatid. To see how new allelic combinations are produced, proceed to step 6.

6. Using the letters printed on mailing labels, label one bead (gene locus) on each chromatid of one chromosome *B* for brown hair color. Label the beads in the same position on the two chromatids of the other member of the homologous pair *b* for blond hair color.

   The *B* and *b* represent alleles, or alternate forms of the gene for hair color.

   On the chromatids with the *B* allele, label another gene *D* for dark eye color. On the other member of the homologous pair of chromosomes, label the same gene *d* for pale eyes. In other words, one chromosome will have *BD*, the other chromosome, *bd* (Figure 7.11).

7. Have a single crossover take place involving only two of the four chromatids between the loci for hair color and eye color. Remember, the crossover must take place between nonsister chromatids.

   What combinations of alleles are now present on the chromatids?

8. Confirm your results with your laboratory instructor.

If you are having difficulty envisioning the activities of chromosomes in prophase I and understanding their significance, discuss these events with your lab partner and, if needed, ask questions of your lab instructor before proceeding to the next stage of meiosis I.

9. Move your tetrads to the equator, midway between the two poles.

   Late in prophase I, tetrads move to the equator.

10. To represent metaphase I, leave the tetrads lying at the equator.

    During this phase, tetrads lie on the equatorial plane. *Centromeres do not split as they do in mitosis.*

11. To represent anaphase I, separate each duplicated chromosome from its homologue, and move one homologue toward each pole. In our model, the two magnets in sister chromatids represent one centromere holding together the two sister chromatids of the chromosome.

How does the structure of chromosomes in anaphase I differ from that in anaphase in mitosis?

12. To represent telophase I, place the chromosomes at the poles. You should have one long and one short chromosome at each pole, representing a homologue from each pair.

Two nuclei now form, followed by cytokinesis. How many chromosomes are in each nucleus?

The number of chromosomes is equal to the number of centromeres. In this model, two magnets represent one centromere in duplicated chromosomes.

Would you describe the new nuclei as being diploid ($2n$) or haploid (n)?

How has crossing over changed the combination of alleles in the new nuclei?

Are both chromosomes of the same color in the same nucleus? Compare your results with others.

13. To represent meiotic interphase, leave the chromosomes in the two piles formed at the end of meiosis I.

The interphase between meiosis I and meiosis II is usually short. There is little cell growth and no synthesis of DNA. All the machinery for a second nuclear division is synthesized, however.

14. Duplicate the centriole pairs.

Lab Study C. Meiosis II

The events that take place in meiosis II are similar to the events of mitosis. Meiosis I results in two nuclei with half the number of chromosomes as the parent cell, but the chromosomes are duplicated (made of two chromatids).

just as they are at the beginning of mitosis. The events in meiosis II must change duplicated chromosomes into single chromosomes. As meiosis II begins, two new spindles begin to form, establishing the axes for the dispersal of chromosomes to each new nucleus.

Procedure

1. To represent prophase II, separate the centrioles and set up the axes of the two new spindles. Pile the chromosomes in the center of each spindle.

   The events that take place in each of the nuclei in prophase II are similar to those of a mitosis prophase. In each new cell the centrioles move to the poles, nucleoli break down, the nuclear envelope breaks down, and a new spindle forms. The new spindle forms at a right angle to the axis of the spindle in meiosis I.

2. Align the chromosomes at the equator of their respective spindles.

   As the chromosomes reach the equator, prophase II ends and metaphase II begins.

3. Leave the chromosomes on the equator to represent metaphase II.

4. Pull the two magnets of each duplicated chromosome apart.

   As metaphase II ends, the centromeres finally split and anaphase II begins.

5. Separate sister chromatids (now chromosomes) and move them to opposite poles.

   In anaphase II, single chromosomes move to the poles.

6. Pile the chromosomes at the poles.

   As telophase II begins, chromosomes arrive at the poles. Spindles break down. Nucleoli reappear. Nuclear envelopes form around each bunch of chromosomes as the chromosomes uncoil. Cytokinesis follows meiosis II.

   a. What is the total number of nuclei and cells now present?

   b. How many chromosomes are in each?

   c. How many cells were present when the entire process began?

   d. How many chromosomes were present per cell when the entire process began?

   e. How many of the cells formed by the meiotic division just modeled are genetically identical? (Assume that alternate forms of genesexist on homologues.)

Prophase II

metaphase II

Anaphase II

Telophase II

* Difference between Mitosis and Meiosis:

Mitosis	Meiosis
- growth - repair - asexual reproduction	sexual reproduction
Somatic cell	sexual (reproductive) cells gonads
1 cell (2n) → 2 cell (2n) diploid	1 cell (2n) → 4 cell (n) haploid
X	✓

EXC 7.1 page 152 ✓

EXC 7.2 page 152 ✓

EXC 7.3 page 160 X

EXC 7.4 page 162 ✓

EXC 7.5 page 163 X

f. Explain your results in terms of independent assortment and crossing over. (Refer to your textbook.)

Results

Summarize the major differences between mitosis and meiosis in Table 7.1.

Table 7.1
Comparing Nuclear and Chromosomal Activities in Mitosis and Meiosis

	Mitosis	Meiosis
Synapsis		
Crossing over		
Centromeres split		
Chromosome structure and movement during anaphase		
No. of divisions		
No. of cells resulting		
No. of chromosomes in daughter cells		
Genetic similarity of daughter cells to parent cells		

EXERCISE 7.5
Meiosis in *Sordaria fimicola*: A Study of Crossing Over

Materials

petri dish containing mycelia resulting from a cross between *Sordaria* with black and tan spores
slides and coverslips
dropper bottles of water
matches
wire bacterial transfer loop
alcohol lamp

Introduction

In the study of meiosis, you demonstrated that genetic recombination may occur as a result of the exchange of genetic material between homologous chromosomes in the process of crossing over. Crossing over occurs during prophase I, when homologous chromosomes synapse. While they are joined in this complex, nonsister chromatids may break at corresponding points and exchange parts. A point at which they appear temporarily joined as a result of this exchange is called a **chiasma** (Figure 7.12).

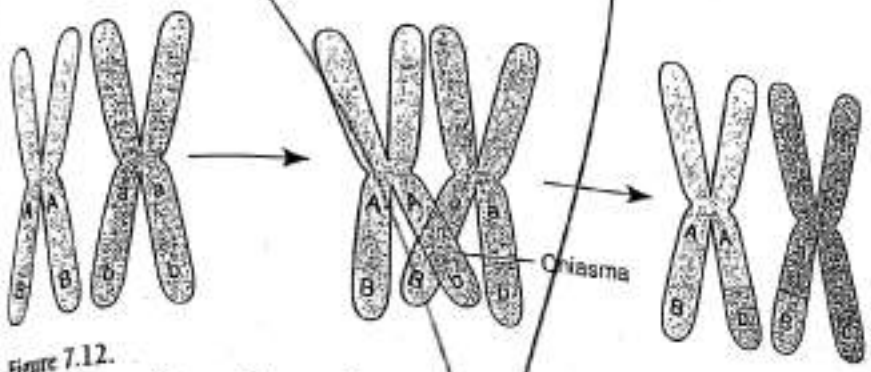
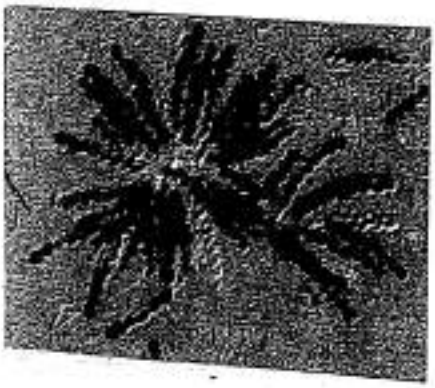


Figure 7.12.
Crossing over. Chromatid arms break and rejoin with a nonsister member of the tetrad, forming a chiasma between nonsister chromatids. This process results in the exchange of genetic material.

Sordaria fimicola is a fungus that spends most of its life as a haploid **mycelium**, a mass of cells arranged in filaments. When conditions are favorable, cells of filaments from two different mating types fuse (see Figures 7.13a and b); ultimately, the nuclei fuse (Figure 7.13c) and $2n$ zygotes are produced, each inside a structure called an **ascus** (plural, asci) (Figure 7.13d). Asci are protected within a **perithecium**. Each $2n$ zygote undergoes meiosis, and the resulting cells (ascospores) remain aligned, the position of an ascospore within the ascus depending on the orientation of separating chromosomes on the equatorial plane of meiosis I. After meiosis, each resulting ascospore divides once by mitosis (Figure 7.13e), resulting in eight ascospores per ascus (Figure 7.13f). This unique sequence of events means that it is easy to detect the occurrence of crossing over involving chromatids carrying alleles that encode for color of spores and mycelia.

If two mating types of *Sordaria*, one with black spores and the other with tan spores, are grown on the same petri dish (Color Plate 14), mycelia from the two may grow together, and certain cells may fuse. Nuclei from two fused cells then fuse, and the resulting zygote contains one chromosome carrying the allele for black spores and another carrying the allele for tan spores. After meiosis takes place, one mitosis follows, and the result is eight ascospores in one ascus: four black spores and four tan spores. If no crossing over has taken place, the arrangement of spores will appear as in Figure 7.14.

Figure 7.13.
Abbreviated diagram of the life cycle of Sordaria fimicola.
(a) Cells from filaments of two different mating types fuse. (b) One cell with two nuclei is formed. (c) The two nuclei fuse, forming a $2n$ zygote. (d) The zygote nucleus begins meiosis, and an ascus begins to form in a perithecium. (e) Meiosis continues, followed by mitosis. (f) The mature ascus contains eight ascospores. (g) Micrograph of crushed perithecium with asci containing ascospores.



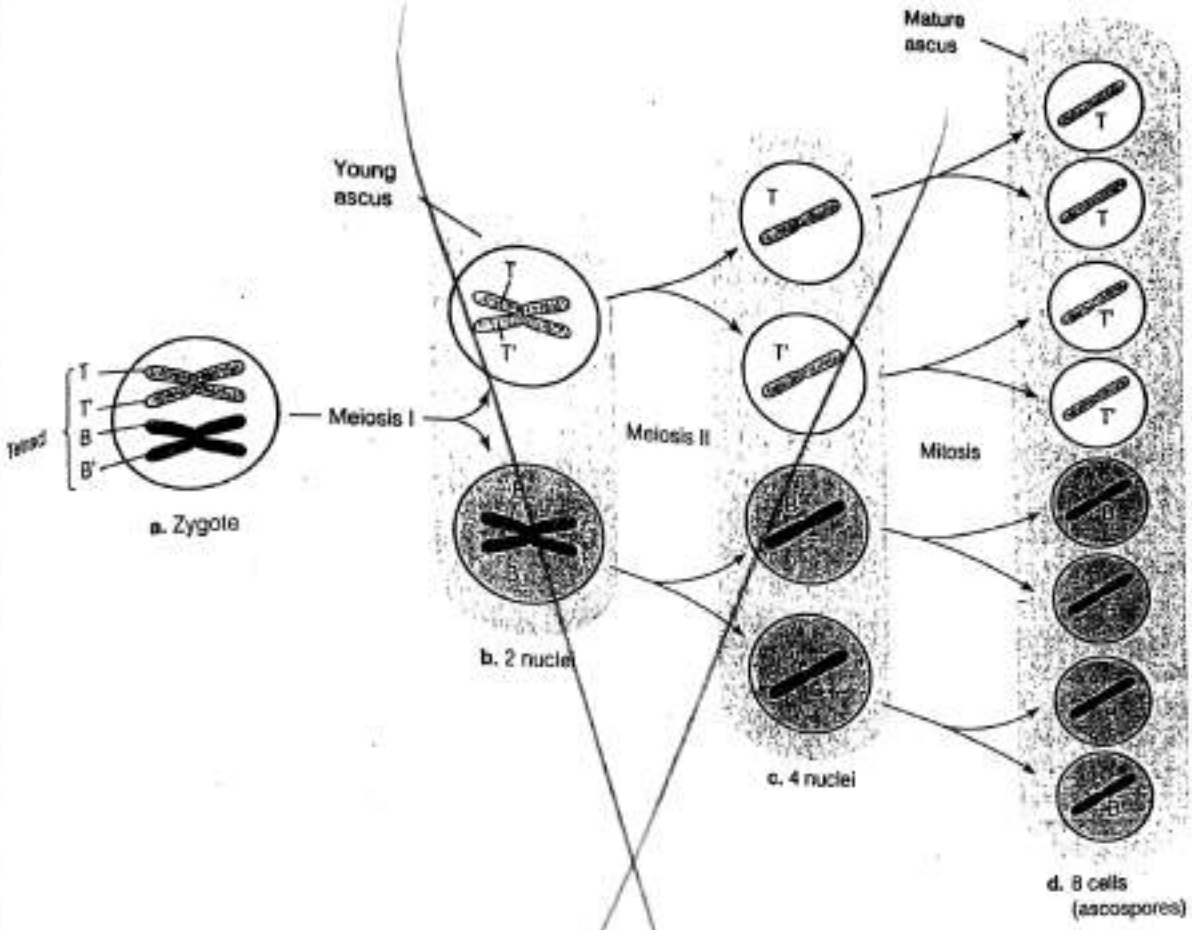
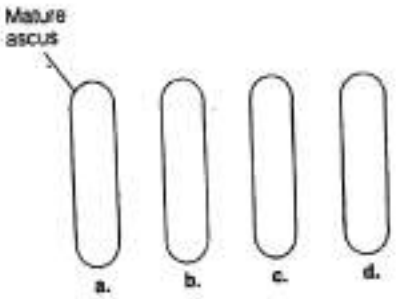


Figure 7.14.
Arrangement of spores in asci resulting from a cross between fungi with black spores and fungi with tan spores when no crossing over takes place. (a) In the zygote nucleus, the light homologous chromosome has chromatids labeled T and T′. Each chromatid has identical tan alleles for spore color. The dark homologous chromosome (chromatids labeled B and B′) has black alleles. (b) During meiosis I, the two homologous chromosomes separate into two different nuclei retained in one developing ascus. (c) Meiosis II produces four nuclei, two containing a chromosome with the tan allele and two containing a chromosome with the black allele, still within the one ascus. (d) Now each nucleus divides by mitosis, followed by cytokinesis, resulting in eight cells, called ascospores. The ascus now contains eight ascospores. Four of the spores have the tan allele in their nuclei and appear tan. Four ascospores have the black allele and appear black.

If crossing over does take place, the arrangement of spores will differ. In the spaces provided, using Figure 7.14 as a reference, draw diagrams that illustrate the *predicted* arrangement of spores in the ascus when crossing over takes place between the following chromatids and the alleles for color are exchanged: (a) T and B, (b) T and B′, (c) T′ and B, and (d) T′ and B′.



In lab today, you will observe living cultures of crosses between blackand tan *Sordaria*. You will look for asci with spores arranged as in yourpredictions.

Procedure

1. Place a drop of water on a clean slide, and carry it and a coverslip to the demonstration table.
2. Light the alcohol lamp and flame a transfer loop.

Lab Topic 15

Plant Diversity I: Nonvascular Plants (Bryophytes) and Seedless Vascular Plants

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe the distinguishing characteristics of nonvascular plants and seedless vascular plants.
2. Discuss the ancestral and derived features of nonvascular plants and seedless vascular plants relative to their adaptations to the land environment.
3. Recognize and identify representative members of each phylum of nonvascular plants and seedless vascular plants.
4. Describe the general life cycle and alternation of generations in the nonvascular plants and the seedless vascular plants, and discuss the differences between the life cycles of the two groups of plants using examples.
5. Identify fossil members and their extant counterparts in the seedless vascular plants.
6. Describe homospory and heterospory, including the differences in spores and gametophytes.
7. Discuss the ecological role and economic importance of these groups of plants.

Introduction

In the history of life on Earth, one of the most revolutionary events was the colonization of land, first by plants, then by animals. Evidence from comparisons of extant land plants and phyla of algae suggests that the first land plants shared a common ancestor with green algae, particularly the charophytes. These first colonists are thought to be most similar to the living, branched, multicellular green alga *Chara* (studied in Lab Topic 14 Protists and Fungi). Once these simple ancestral plants arrived on land over 475 million years ago, they faced new and extreme challenges in their physical environment. Only individuals that were able to survive the variations in temperature, moisture, gravitational forces, and substrate would thrive. Out of this enormous selective regime would come new and different adaptations and new and different life forms: the land plants.

Land plants generally have complex, multicellular plant bodies that are specialized for a variety of functions. Land plants in the Kingdom Plantae produce embryos and have evolved specialized structures for protection of the vulnerable stages of sexual reproduction. The plant body is often covered →

algae أول شكل للنبات · Kingdom Plantae المملكة النباتية

نباتات لا وعائية — non-Vascular Plants (Bryophytes)

ما عندها أوعية ناقلة / تعيش في أماكن رطبة / قريبة للماء

3 Philum / Philap — مفرد / جمع — طائفة / قبيلة

- Bryophyta — eg: Moss حزازيات
- hepatophyta
- anthocerophyta

نباتات وعائية عندها أوعية ناقلة — Vascular Plants

- لابذرية — seedless (تنتج spores)
  - lycophyta → eg: lyco podium
  - pterophyta → eg: horse tail — ferns سرخسيات → Equisotum
- بذرية — seeds
  - Gymnosperms معراة البذور → Coniferophyta → eg: pinus صنوبر (البذور معرّاة)
  - Angiosperms → Anthophyta (فيها ثمار بداخلها البذور) — Antho = flower (أي فيها أزهار) — mono / di

* كل النباتات عندها مبدأ تعاقب أجيال ← جيل 1 ← جيل 2 (alternation of generation) → يكون عندي نبات الطور السائد والهادي ونبات الثاني ← يعيش بقل فترة أطول — domenent stage

- sporophyte — 2n — spores
- gametophyte — يلي ينتج جاميتات — 1n — Female / male
- zygote

النباتات البسيطة (اللاوعائية) الطور السائد فيها gameto — أما الوعائي السائد هو sporo أما الجمي يختفي بسرعة

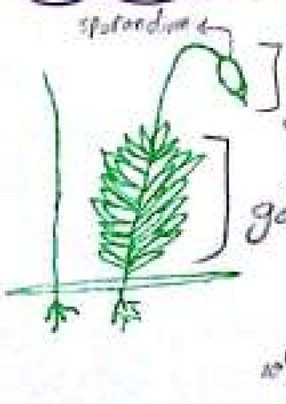
phyta → phylum
ae → Kingdome
e → stage

diploid → 2n → sporophyte / zygot
haploid → 1n → spores / gametes

antheridium: ذكر
archegonium: أنثى

spores → mioses
gametes < sperm, egg > → mitoses → على الحيوانات تنتج الجاميتات من مشيمي لأن فيها أعضاء

non-vascular (Bryophytes) → Bryophyta — Moss → most common non-vascular plant



small, non vascular, lacking vascular tissue → specialized cell for transport material — water conducting tube

* gametophyte → فباتو بيتغلف مع كل اللاوعائي

thallus — أسهل صورة — ما تطور لدرجة جذور وساقه وأوراقه

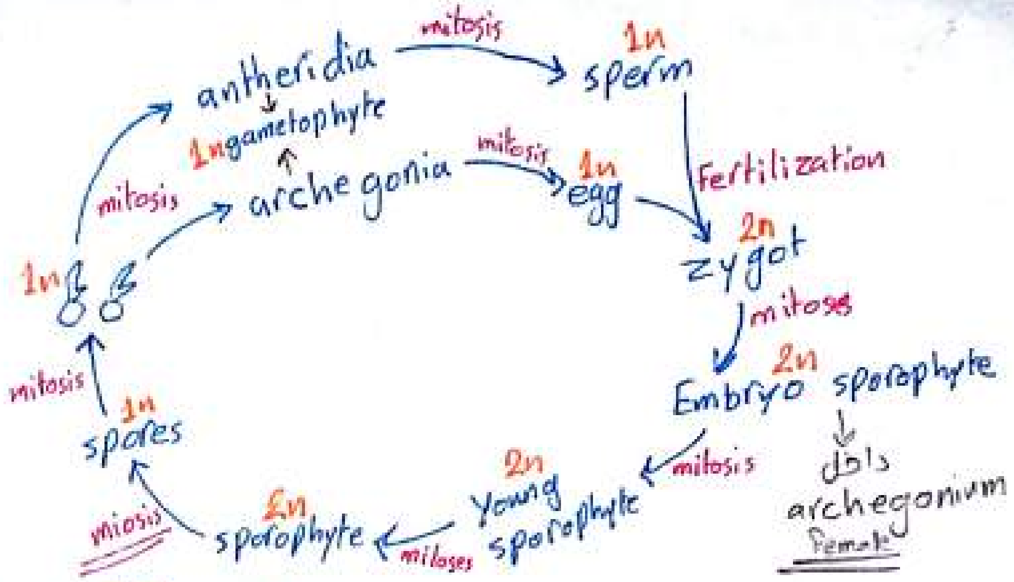
* moist habitats — remain closed to the ground

* have cuticle → lack stomata

تابع → Moss

* the leafy moss is gametophyte and sporophyte is depented on it.

* deriving its water and nutrients from the body of gametophyte



* gametophyte جواه → gametangiam →(mitosis) gametes
→ antheridia → antheridum → sperm
جواه → archegonium → has very long neck and round base
→ archegonia
فيه egg

sperm تنتقل خلال الماء

* sporophyte → sporangium → spores
محفظة بوغة
production of spores ← capsol
وظيفته
هي أشياء أوراقه بس بتحول بالمثلوثي
فيه الذكري زي كيس بس عليها sperm لما ينفجر ويتنقل المياه أو الرطوبة توذي

---

seedless

* vast swampy area تعيش في

* vascular plant have vascular tissue for conducting water, nuterients photosynthitic product

بتعتمد على الماء في عملية fertlization

freeliving unprotected
* الجنين غير مكون

داخلها sporophyte
هو domenant

* فيها تعاقب الأجيال بس ما يكون نوعين ...

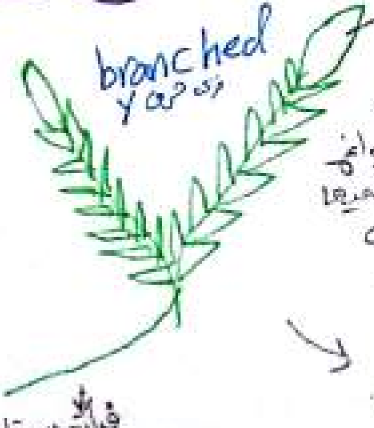
* well developed leaves, root, stomata, structure support tissue.

the most common group of seedless is ferns → على seedless petophyta lycophyta

lycophyta → licopodam (قريب للأوراقية كتير لكنه seedless وعائي)

بيرتبط الشكل مع Moss

*miost hapitat



sporophyte

* بتكون على شكل cluster of sporangia

* بتكون dormat ميتة خلال low rainfull بحيث بتطلع when it rains الحياة

* أحد الخصائص انها seedless بتعطي نوعين من الجاميتات يسمى بالهيتيرو heterospory
megaspor female
microspore male
الفرع هو نمو الفرع طويل هون بعد الآخر (ماهو ماي بتفصل)
فيها سيقان عرضية وسيقان طولية

homospory ← (seedless و non vascular) ما بتعطي نوع واحد

Table 15.1 Classification of Land Plants

Division	Common Name	Illustration
Nonvascular Plants (Bryophytes)		
Phylum Bryophyta	Mosses	
Phylum Hepatophyta	Liverworts	
Phylum Anthocerophyta	Hornworts	
Vascular Plants		
Seedless Plants		
Phylum Lycophyta	Club mosses	
Phylum Pterophyta	Ferns, horsetails, whisk ferns	
Seed Plants		
Gymnosperms		
Phylum Coniferophyta	Conifers	
Phylum Cycadophyta	Cycads	
Phylum Ginkgophyta	Ginkgo	
Phylum Gnetophyta	Mormon tea	
Angiosperms		
Phylum Anthophyta	Flowering plants	

with a waxy cuticle that prevents desiccation. However, the waxy covering also prevents gas exchange, a problem solved by the presence of openings called **stomata** (sing., **stoma**). Some land plants have developed vascular tissue for efficient movement of materials throughout these complex bodies, which are no longer bathed in water. As described in the following section, the reproductive cycles and reproductive structures of these plants are also adapted to the land environment.

In the two plant diversity labs, you will be investigating the diversity of land plants (Table 15.1 and Figure 15.1), some of which will be familiar to you (flowering plants, pine trees, and ferns) and some of which you may never have seen before (whisk ferns, horsetails, and liverworts). You will study the nonvascular plants and seedless vascular plants in this lab topic, Plant Diversity I, and seed plants in Lab Topic 16 Plant Diversity II.

Figure 15.1
Evolution of land plants. The nonvascular plants and vascular plants probably evolved from ancestral green algae over 475 million years ago. Seedless vascular plants dominated Earth 300 million years ago, and representatives of two phyla have survived until the present. Seed plants replaced the seedless plants as the dominant land plants, and today flowering plants are the most diverse and successful group in an amazing variety of habitats. The representatives studied in Plant Diversity I and II are indicated.

To maintain your perspective in the face of all this diversity—and to reinforce the major themes of these labs—bear in mind the following questions.

1. What are the special adaptations of these plants to the land environment?
2. How are specialized plant structures related to functions in the land environment?
3. What are the major trends in the plant kingdom as plant life evolved over the past 500 million years?
4. In particular, how has the fundamental reproductive cycle of alternation of generations been modified in successive groups of plants?

Plant Life Cycles

All land plants have a common sexual reproductive life cycle called **alternation of generations**, in which plants alternate between a haploid **gametophyte** generation and a diploid **sporophyte** generation (Figure 15.2). In living land plants, these two generations differ in their morphology, but they are still the same species. In all land plants except the bryophytes (mosses and liverworts), the diploid sporophyte generation is the dominant (more conspicuous) generation.

The essential features in the alternation of generations life cycle beginning with the sporophyte are:

- The diploid sporophyte undergoes meiosis to produce haploid spores in a protective, nonreproductive jacket of cells called the **sporangium**.
- Dividing by mitosis the spores germinate to produce the haploid gametophyte.

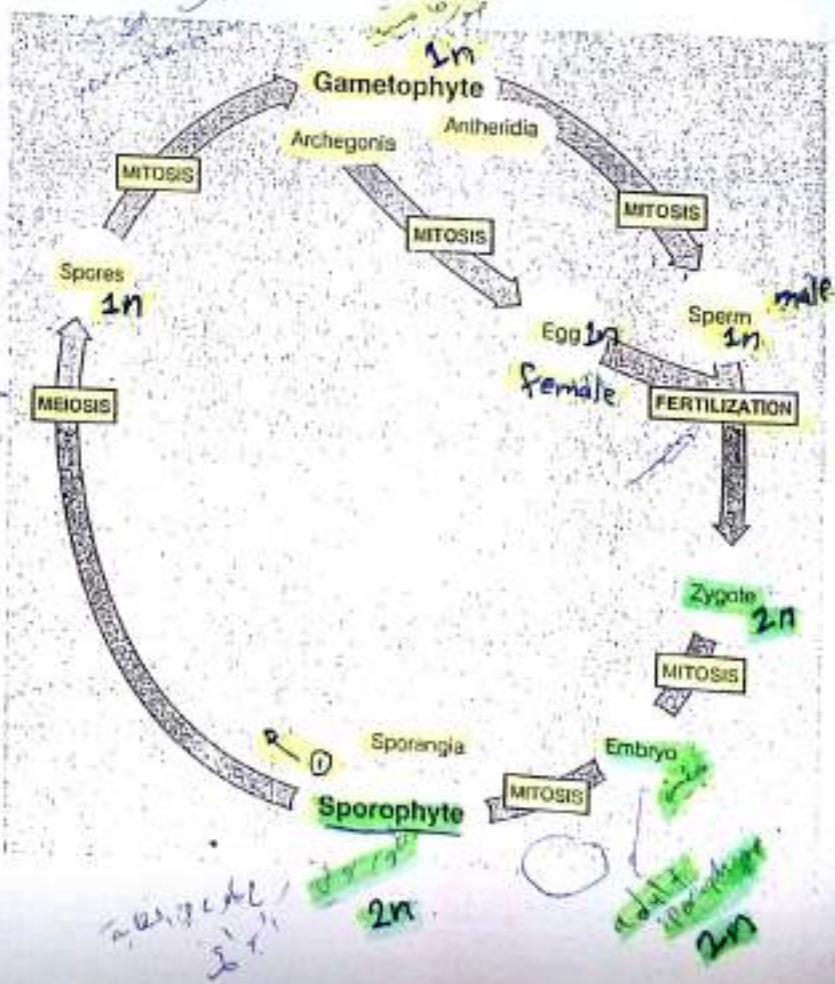


Figure 15.2. Alternation of generations. In this life cycle, a diploid sporophyte plant alternates with a haploid gametophyte plant. Note that haploid spores are produced on the sporophyte by meiosis, and haploid gametes are produced in the gametophyte by mitosis. *Using a colored pencil, indicate the structures that are haploid, and with another color, note the structures that are diploid.*

- The gametophyte produces gametes inside a jacket of nonreproductive cells forming **gametangia** (sing., **gametangium**).
- Eggs are produced by mitosis in **archegonia** (sing., **archegonium**), and sperm are produced in **antheridia** (sing., **antheridium**).
- The gametes fuse (**fertilization**) usually by entrance of the sperm into the archegonium, forming a diploid **zygote**, the first stage of the next diploid sporophyte generation.

Note that both gametes and spores are haploid in this life cycle. Unlike the animal life cycle, *the plant life cycle produces gametes by mitosis; spores are produced by meiosis*. The difference between these two cells is that gametes fuse with other gametes to form the zygote and restore the diploid number, while haploid spores germinate to form a new haploid gametophyte plant.

Review the generalized diagram of this life cycle in Figure 15.2. *Using colored pencils, distinguish the structures that are diploid and those that are haploid.* As you become familiar with variations of this life cycle through specific examples, you will want to continue referring to this general model for review.

Major trends in the evolution of this life cycle include:

the increased importance of the sporophyte as the photosynthetic and persistent plant that dominates the life cycle; the reduction and protection of the gametophyte within the body of the sporophyte; and the evolution of seeds and then flowers.

Nonvascular Plants (Bryophytes) and Seedless Vascular Plants

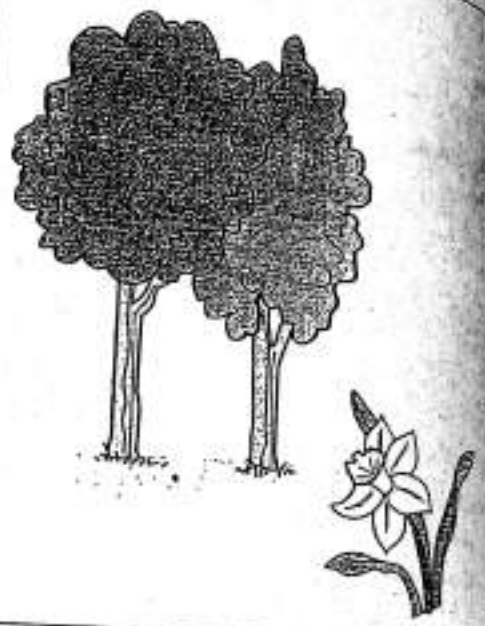
In this lab topic, terrestrial plants will be used to illustrate how life has undergone dramatic changes during the past 500 million years. Not long after the transition to land, plants diverged into at least two separate lineages. One gave rise to the bryophytes, a group of nonvascular plants, including the mosses, and the other to the vascular plants (see Figure 15.1). Nonvascular bryophytes first appear in the fossil record dating over 420 million years ago and remain unchanged, whereas the vascular plants have undergone enormous diversification. As you review the evolution of land plants, refer to the geological time chart for an overview of the history of life on Earth (Figure 15.3).

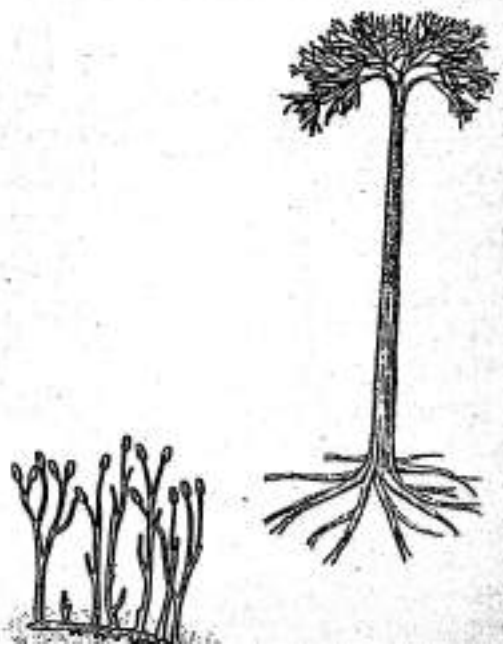
EXERCISE 15.1

Nonvascular Plants (Bryophytes)

The nonvascular plants are composed of three phyla of related plants that share some key characteristics and include mosses (Bryophyta) and liverworts (Hepatophyta). The third phylum, hornworts (Anthocerophyta), will not be seen in lab. (See again Figure 15.1 and Table 15.1.) The term *bryophytes* does not refer to a taxonomic category; rather, bryophytes are an ancient group of nonvascular plants that share a common ancestor, appear to have evolved into several different groups independently, and did not give rise to any other living groups of plants. They are small plants generally lacking vascular tissue (specialized cells for the transport of material),

Period / Epoch	Life on Earth
CENOZOIC Quaternary — Recent, Pleistocene	• Origin of agriculture and artificial selection; *H. sapiens*
Tertiary — Pliocene	• Large carnivores; hominoid apes
Miocene	• Forests dwindle; grassland spreads
Oligocene	• Anthropoid apes
Eocene	• Diversification of mammals and flowering plants
Paleocene	• Specialized flowers; sophisticated pollinators and seed distributors
MESOZOIC Cretaceous	• Flowering plants established and diversified; many modern families present; extinction of many dinosaurs
Jurassic	• Origin of birds; reptiles dominant; cycads and ferns abundant; first modern conifers and immediate ancestors of flowering plants
Triassic	• First dinosaurs and mammals; forests of gymnosperms and ferns; cycads
PALEOZOIC Permian	• Diversification of gymnosperms; origin of reptiles; amphibians dominant
Carboniferous	• First treelike plants; giant woody lycopods and sphenopsids form extensive forests in swampy areas; evolution of early seeds (seed ferns) and first stages of leaves
Devonian	• Diversification of vascular plants; sharks and fishes dominant in the oceans
Silurian	• First vascular plants
Ordovician	• Diversification of algae and plants colonize land
Cambrian	• Diversification of major animal phyla





although water-conducting tubes appear to be present in some mosses. (However, these tubes may be unrelated to the vascular tissue in vascular plants.) The life cycle for the bryophytes differs from all other land plants because the gametophyte is the dominant and conspicuous plant. Because bryophytes are nonvascular, they are restricted to moist habitats for their reproductive cycle and have never attained the size and importance of other groups of plants. The gametophyte plants remain close to the ground, enabling the motile sperm to swim from the antheridium to the archegonium and fertilize the egg. They have a cuticle but lack stomata on the surface of the gametophyte **thallus** (plant body), which is not organized into roots, stems, and leaves. Stomata are present on the sporophyte in some mosses and hornworts.

Bryophytes are not important economically, with the exception of sphagnum moss, which in its harvested and dried form is known as *peat moss*. Peat moss is absorbent, has an antibacterial agent, and was reportedly once used as bandages and diapers. Today peat moss is used in the horticultural industry, and dried peat is burned as fuel in some parts of the world. Peat lands cover more than 1% of the Earth's surface and store 400 billion metric tons of organic carbon. Harvesting and burning peat releases CO_2 to the atmosphere, thus contributing to changes in the global carbon cycle.

Lab Study A. Bryophyta: Mosses

Materials

living examples of mosses
prepared slides of *Mnium* archegonia and antheridia
colored pencils

Introduction

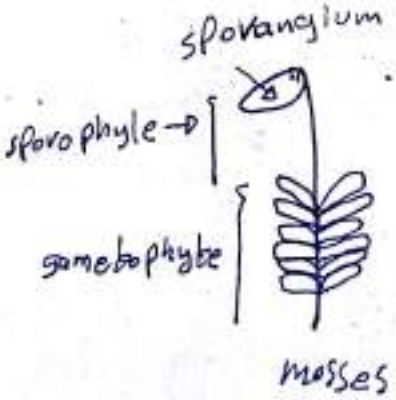
The mosses are the most common group of nonvascular plants, occurring primarily in moist environments but also found in dry habitats that are periodically wet (Color Plate 30). Refer to Figure 15.4 as you investigate the moss life cycle, which is representative of the bryophytes.

Procedure

1. Examine living colonies of mosses on demonstration. Usually you will find the two generations, gametophyte and sporophyte, growing together.
2. Identify the leafy **gametophytes** and the dependent **sporophytes**, which appear as elongated structures growing above them. Tug gently at the sporophyte and notice that it is attached to the gametophyte. Recall that the sporophyte develops and matures while attached to the gametophyte and receives its moisture and nutrients from the gametophyte.

Figure 15.3.
Geological time chart. The history of life can be organized into time periods that reflect changes in the physical and biological environment. Refer to this table as you review the evolution of land plants in Plant Diversity I and II.

* Bryophyta
- gameto phyte is dominant
- small in size
- moist habitat
- need water for fertilization
- Plant body (thallus), have no stomat, have cuticle
- No root, stem or leaf



- mosses life cycle page 396

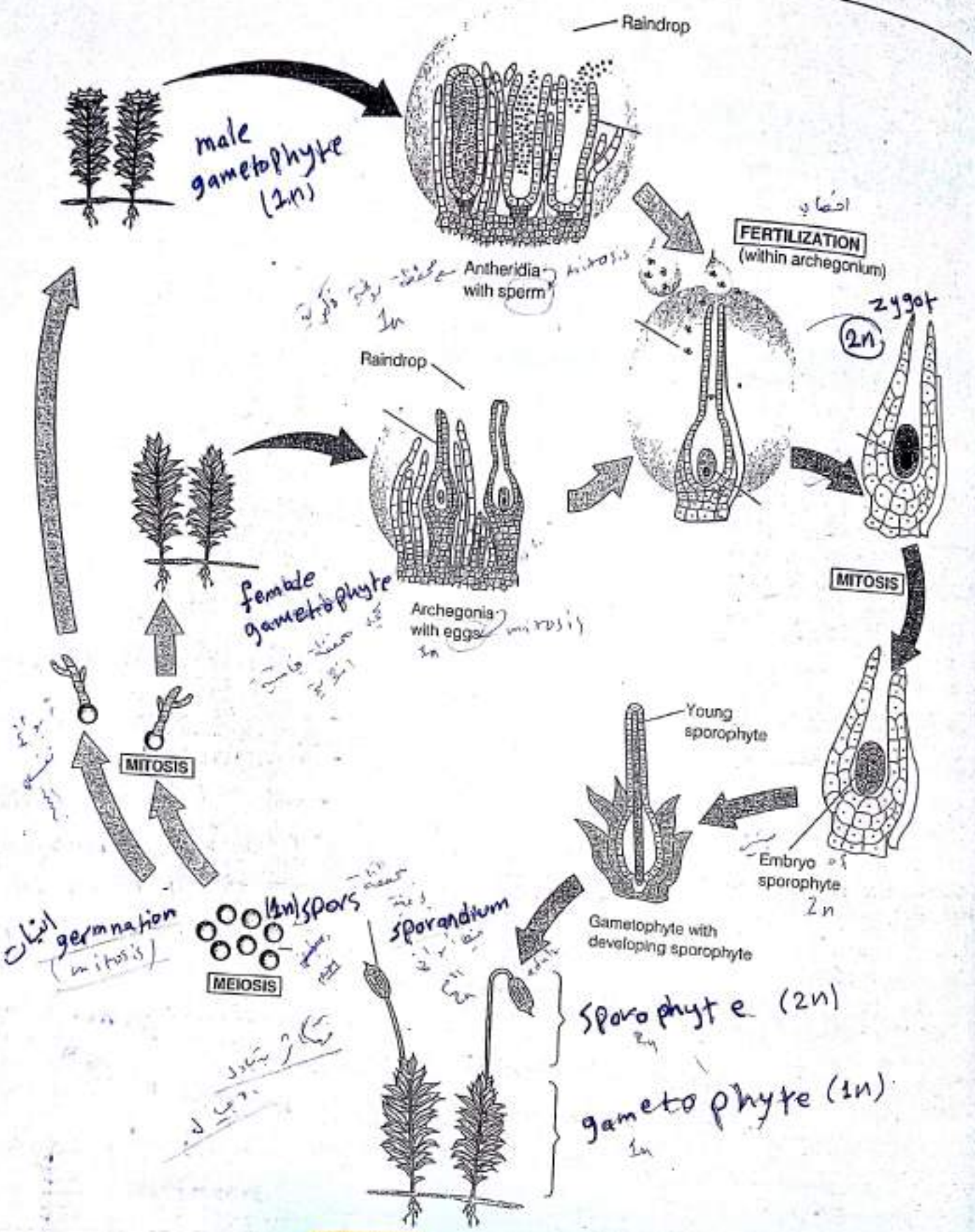


Figure 15.4.
Moss life cycle. The leafy moss plant is the gametophyte, and the sporophyte is dependent on it, deriving its water and nutrients from the body of the gametophyte. Review this variation of alternation of generations and label the structures described in Lab Study A. *Using colored pencils, highlight the haploid and diploid structures in different colors. Circle the processes of mitosis and meiosis.*

3. The gametes are produced by the gametophyte in **gametangia** by *mitosis*. Gametangia protect the gametes but are not readily visible without a microscope. Observe under the microscope's low-power lens prepared slides containing long sections of heads of the unisex moss *Mnium*, which contain the gametangia. One slide has been selected to show the **antheridia** (male); the other is a rosette of **archegonia** (female). Sperm-forming tissue will be visible inside the antheridia. On the archegonial slide, look for an archegonium. The moss archegonium has a very long neck and rounded base. It will be difficult to find an entire archegonium in any one section. Search for a single-celled egg in the base of the archegonium.

4. Refer to Figure 15.4 as you follow the steps of fertilization through formation of the gametophyte in the next generation. The sperm swim through a film of water to the archegonium and swim down the neck to the egg, where fertilization takes place. The diploid zygote divides by mitosis and develops into an embryonic sporophyte within the archegonium. As the sporophyte matures, it grows out of the gametophyte but remains attached, deriving water and nutrients from the gametophyte body. **Spores** develop *by meiosis* in the **sporangium** at the end of the sporophyte. The haploid spores are discharged from the sporangium and in a favorable environment develop into new haploid gametophytes.

Results

1. Review the structures and processes observed and then label the moss life cycle diagram in Figure 15.4.
2. Using colored pencils, indicate if structures are haploid or diploid and circle the processes of mitosis and meiosis.

Discussion

Refer to Plant Life Cycles in the Introduction and Figure 15.2, the generalized diagram of the plant life cycle.

1. Are the spores produced by the moss sporophyte formed by meiosis or mitosis? Are they haploid or diploid?

2. Do the spores belong to the gametophyte or sporophyte generation?

3. Are the gametes haploid or diploid? Are they produced by meiosis or mitosis?

4. Is the dominant generation for the mosses the gametophyte or the sporophyte?

6. What feature of the life cycle differs for bryophytes compared with other land plants?

Lab Study B. Hepatophyta: Liverworts

Materials

living liverworts

Introduction

Liverworts are so named because their bodies are flattened and lobed (Color Plate 31). Early herbalists thought that these plants were beneficial in the treatment of liver disorders. Although less common than mosses, liverworts can be found along streams on moist rocks, but because of their small size you must look closely to locate them.

Procedure

Examine examples of liverworts on demonstration. Liverworts have a flat **thallus** (plant body). Note the **rhizoids**, rootlike extensions on the lower surface, that primarily anchor plants. Observe the **pores** on the surface of the leaflike thallus. These openings function in gas exchange; however, they are always open since they lack guard cells. On the upper surface of the thallus you should see circular cups called **gemmae cups**, which contain flat disks of green tissue called **gemmae**. The gemmae are washed out of the cups when it rains, and they grow into new, genetically identical liverworts.

Results

Sketch the overall structure of the liverwort in the margin of your laboratory manual. Label structures where appropriate.

Discussion

1. Is the plant you observed the gametophyte or sporophyte?

2. Are the gemmae responsible for asexual or sexual reproduction? Explain.

3. Why are the

4. In this lab topic, as in Plant Diversity II (Lab Topic 16) and Plant Anatomy (Lab Topic 20), you are asked to complete tables that summarize features advantageous to the adaptation of plant groups to the land environment. You may be asked to compare these **derived** features with others that have changed little (**ancestral**) in the evolution of land plants. For example, for nonvascular plants, motile sperm might be considered an ancestral feature, while the cuticle would be considered derived.

Complete Table 15.2, relating the features of nonvascular plants to their success in the land environment. Refer to the lab topic introduction for assistance.

Table 15.2
Ancestral and Derived Features of Nonvascular Plants as They Relate to Adaptation to Land

Ancestral Features	Derived Features

EXERCISE 15.2
Seedless Vascular Plants

Seedless, terrestrial plants are analogous to the first terrestrial vertebrate animals, the amphibians, in their dependence on water for external fertilization and development of the unprotected, free-living embryo. Both groups were important in the Paleozoic era but have undergone a steady decline in importance since that time. Seedless plants were well suited for life in the vast swampy areas that covered large areas of the Earth in the Carboniferous period but were not suited for the drier areas of the Earth at that time or for later climatic changes that caused the vast swamps to decline and disappear. The fossilized remains of these swamp forests are the coal deposits of today (Figures 15.3 and 15.7).

Although living representatives of the seedless vascular plants have survived for millions of years, their limited adaptations to the land environment have restricted their range. All seedless vascular plants have vascular tissue, which is specialized for conducting water, nutrients, and photosynthetic products. Their life cycle is a variation of alternation of generations, in which the sporophyte is the dominant plant; the gametophyte is usually independent of the sporophyte. These plants generally have well-developed leaves and roots, stomata, and structural support tissue. However, since they still retain the ancestral feature of motile sperm that require water for fertilization, the gametophyte is small and restricted to moist habitats.

- Seedless vascular plant
- sporophyte is dominants
- gametophyte restricted to moist habitat
- need water for fertilization

Economically, the only important members of this group are the ferns, a significant horticultural resource.

The phyla included in the seedless vascular plants are Lycophyta and Pterophyta (see again Table 15.1 and Figure 15.1).

The living examples of lycophytes are small club mosses, spike mosses, and quillworts. (Though named "mosses," these plants have vascular tissue and therefore are not true mosses.) The pterophytes include ferns, horsetails, and whisk ferns, which are remarkably different in overall appearance. Current evidence from molecular biology indicates that these diverse plants share a common ancestor and should all be included in the phylum Pterophyta. This evidence also suggests that pterophytes are more closely related to seed plants than they are to lycophytes.

Lab Study A. Lycophyta: Club Mosses

Materials

living *Selaginella* and *Lycopodium*
preserved *Selaginella* with microsporangia and megasporangia
prepared slide of *Selaginella* strobilus, l.s.

Introduction

Living members of Lycophyta are usually found in moist habitats, including bogs and streamsides (Color Plates 32 and 33). However, one species of *Selaginella*, the resurrection plant, inhabits deserts. It remains dormant throughout periods of low rainfall, but then comes to life—resurrects—when it rains. During the Carboniferous period, lycophytes were not inconspicuous parts of the flora but rather formed the forest canopy; they were the ecological equivalent of today's oaks, hickories, and pines (Figure 15.7).

Nonvascular plants and most seedless vascular plants produce one type of spore (**homospory**), which gives rise to the gametophyte by mitosis. One advanced feature occasionally seen in seedless vascular plants is the production of two kinds of spores (**heterospory**). Large spores called **megaspores** divide by mitosis to produce the female gametophyte. The numerous small spores, **microspores**, produce the male gametophytes by mitosis. Heterospory and separate male and female gametophytes, as seen in *Selaginella*, are unusual in seedless vascular plants, but characteristic of seed-producing vascular plants.

Procedure

1. Examine living club mosses, *Selaginella* and *Lycopodium*. Are they dichotomously branched? (The branches would split in two, appearing to form a Y.) Locate sporangia, which may be present either clustered at the end of the leafy stem tips, forming **strobili**, or **cones**, or dispersed along the leafy stems. Note that these plants have small leaves, or bracts, along the stem.
2. Examine preserved strobili of *Selaginella*. Observe the round sporangia clustered in sporophylls (leaflike structures) at the tip of the stem (Figure 15.5a). These sporangia contain either four megaspores or numerous microspores. Can you observe any differences in the sporangia or spores?

3. Are microspores and megaspores produced by mitosis or meiosis? (Review the life cycle in Figure 15.2.)

4. Will megaspores divide to form the female gametophyte or the sporophyte?

Having trouble with life cycles? Return to the Introduction and review the generalized life cycle in Figure 15.2. Reread the introduction to the study of seedless vascular plants. The key to success is to determine where meiosis occurs and to remember the ploidal level for the gametophyte and the sporophyte.

Lab Study B. Pterophyta: Ferns, Horsetails, and Whisk Ferns

Materials

living and/or preserved horsetails (*Equisetum*)
living and/or preserved whisk ferns (*Psilotum*)
living ferns

Introduction

If a time machine could take us back 400 million years to the Silurian period, we would find that vertebrate animals were confined to the seas, and early vascular plants had begun to diversify on land (Figure 15.3). By the Carboniferous period, ferns, horsetails, and whisk ferns grew alongside the lycophytes. Until recently, these three groups of seedless vascular plants were placed in separate phyla: Pterophyta (ferns), Sphenophyta (horsetails), and Psilophyta (whisk ferns). Strong evidence from molecular biology now reveals a close relationship among these three groups, supporting a common ancestor for the group and their placement in one phylum, Pterophyta.

Psilophytes (**whisk ferns**) are diminutive, dichotomously branched (repeated Y branches), photosynthetic stems that reproduce sexually by aerial spores. Today, whisk ferns can be found in some areas of Florida and in the tropics (Color Plate 34). Sphenophytes (**horsetails**) have green jointed stems with occasional clusters of leaves or branches. Their cell walls contain silica that give the stem a rough texture. These plants were used by pioneers to scrub dishes—thus their name, scouring rushes. In cooler regions of North America, horsetails grow as weeds along roadsides (Color Plate 35).

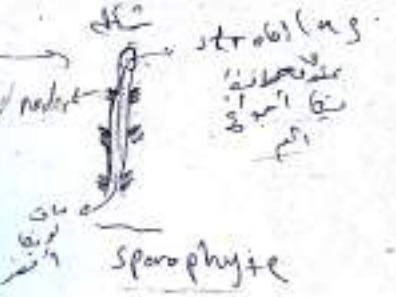
Ferns are the most successful group of seedless vascular plants, occupying habitats from the desert to tropical rain forests. Most ferns are small plants that lack woody tissue (Color Plate 36). An exception is the tree ferns found in tropical regions. Many cultivated ferns are available for home gardeners.

In this lab study you will investigate the diversity of pterophytes, including whisk ferns, horsetails, and a variety of ferns. The plants on demonstration

are sporophytes, the dominant generation in seedless vascular plants. You will investigate the life cycle of a fern in Lab Study C, Fern Life Cycle.

Procedure

1. Examine a living **whisk fern** (*Psilotum nudum*) on demonstration. This is one of only two extant genera of psilophytes.
2. Observe the spherical structures on the stem. If possible, cut one open and determine the function of these structures. Note the dichotomous branching, typical of the earliest land plants.
3. Examine the **horsetails** (*Equisetum* sp.) on demonstration. Note the ribs and ridges in the stem. Also examine the nodes or joints along the stem where branches and leaves may occur in some species. Locate the **strobili** in the living or preserved specimens on demonstration. These are clusters of **sporangia**, which produce **spores**.
4. Examine the living **ferns** on demonstration. Note the deeply dissected leaves, which arise from an underground stem called a **rhizome**, which functions like a root to anchor the plant. Roots arise from the rhizome. Observe the dark spots, or **sori** (sing, **sorus**), which are clusters of sporangia, on the underside of some leaves, called **sporophylls**. (See Color Plate 37.)



Results

1. Sketch the overall structure of the whisk fern, horsetail, and fern in the margin of your lab manual. Label structures where appropriate.
2. Are there any leaves on the whisk fern? On the horsetails?
3. Are sporangia present on the whisk fern? On the horsetails? On the ferns?

Discussion

1. Are the spores in the sporangia produced by mitosis or meiosis?
2. Are the sporangia haploid or diploid? Think about which generation produces them.
3. Once dispersed, will these spores produce the gametophyte or sporophyte generation?

Lab Study C. Fern Life Cycle

Materials

living ferns
living fern gametophytes with archegonia and antheridia
living fern gametophytes with young sporophytes attached
stereoscopic microscope
compound microscope
prepared slide of fern gametophytes with archegonia, c.s.
colored pencils
Protoslo®
glycerol in dropping bottle

Introduction

In the previous Lab Study you examined the features of the fern sporophyte. In this lab study you will examine the fern life cycle in more detail, beginning with the diploid sporophyte.

Procedure

1. Examine the sporophyte leaf with sori (sporophyll) at your lab bench (Color Plate 37). Make a wet mount of a sorus, using a drop of glycerol, and do not add a cover slip. Examine the sporangia using a dissecting microscope. You will find the stalked **sporangia** in various stages of development. Find a sporangium still filled with **spores** and observe carefully for a few minutes, watching for movement. The sporangia will open and fling the spores into the glycerol.
2. Refer to Figure 15.6 as you observe the events and important structures in the life cycle of the fern. The haploid spores of ferns fall to the ground and grow into heart-shaped **gametophyte** plants. All seedless terrestrial plants depend on an external source of water for a sperm to swim to an egg to effect fertilization and for growth of the resulting sporophyte plant. The gametangia, which bear male and female gametes, are borne on the underside of the gametophyte. Egg cells are produced by mitosis in urnlike structures called **archegonia**, and sperm cells are produced by mitosis in globular structures called **antheridia**. Archegonia are usually found around the notch of the heart-shaped gametophyte, while antheridia occur over most of the undersurface.
3. To study whole gametophytes, make a slide of living gametophytes. View them using the stereoscopic microscope or the scanning lens on the compound microscope. Note their shape and color and the presence of **rhizoids**, rootlike multicellular structures. Locate archegonia and antheridia. Which surface will you need to examine? Sketch in the margin of your lab manual any details not included in Figure 15.6.
4. If you have seen antheridia on a gametophyte, remove the slide from the microscope. Gently but firmly press on the coverslip with a pencil eraser. View using the compound microscope first on intermediate and then on high power. Look for motile **sperm** swimming with a spiral motion. Each sperm has two flagella. Add a drop of Protoslo to slow down movement of sperm.
5. Observe the cross section of a fern gametophyte with archegonia. Each archegonium encloses an **egg**, which may be visible on your slide.
6. Make a wet mount of a fern gametophyte with a **young sporophyte** attached. Look for a young **leaf** and **root** on each sporophyte.
7. Share slides of living gametophytes with archegonia, antheridia and sperm, and sporophytes until everyone has observed each structure.

Results

1. Review the structures and processes observed, and then label the stages of fern sexual reproduction outlined in Figure 15.6.
2. Using colored pencils, circle those parts of the life cycle that are sporophytic (diploid). Use another color to encircle the gametophytic (haploid) stages of the life cycle. Highlight the processes of meiosis and mitosis.

Discussion

Refer to Figure 15.2, the generalized diagram of the plant life cycle, and Figure 15.6, a representation of the fern life cycle.

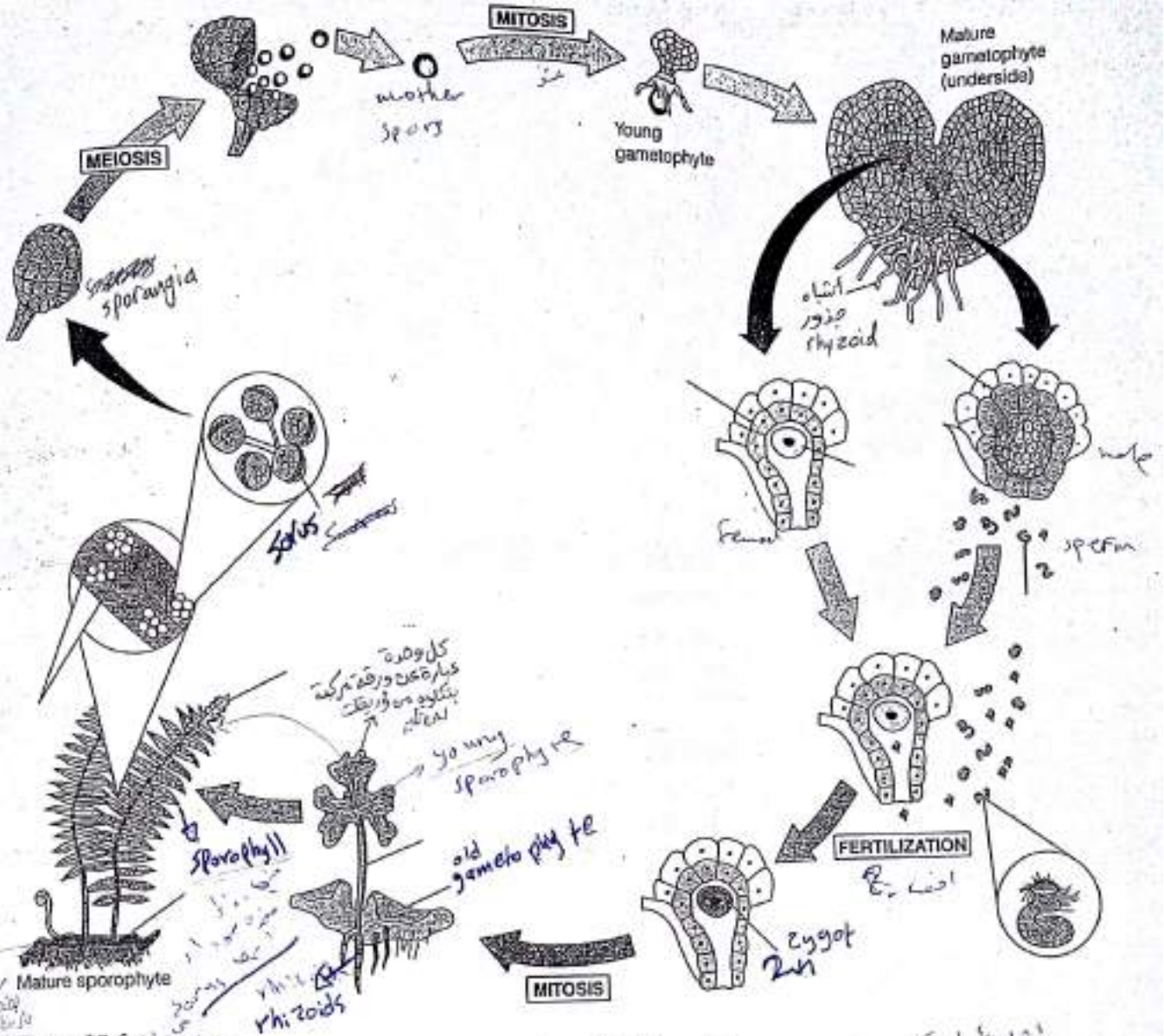


Figure 15.6.
Fern life cycle. The familiar leafy fern plant is the sporophyte, which alternates with a small, heart-shaped gametophyte. Review this life cycle, a variation of alternation of generations, and label the structures and processes described in Lab Study C. *Using colored pencils, highlight the haploid and diploid structures in different colors.*

1. Are the spores produced by the fern sporophyte formed by meiosis or mitosis?

2. Do the spores belong to the gametophyte or the sporophyte generation?

3. Are the gametes produced by mitosis or meiosis?

* sours : (sori) cluster of sporangium
* strobilus : cluster of sporangium

Lab Topic 16

Plant Diversity II: Seed Plants

Before lab, read the following material on gymnosperms and angiosperms and complete Table 16.1 by listing (and comparing) the traits of each.

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify examples of the phyla of seed plants.
2. Describe the life cycle of a gymnosperm (pine tree) and an angiosperm.
3. Describe features of flowers that ensure pollination by insects, birds, bats, and wind.
4. Describe factors influencing pollen germination.
5. Identify types of fruits, recognize examples, and describe dispersal mechanisms.
6. Relate the structures of seed plants to their functions in the land environment.
7. Compare the significant features of life cycles for various land plants and state their evolutionary importance.
8. Summarize major trends in the evolution of land plants and provide evidence from your laboratory investigations.

Gymnosperms

For over 500 million years, plants have been adapting to the rigors of the land environment. The nonvascular bryophytes with their small and simple bodies survived in moist habitats, habitats moist at least for part of their life cycle. During the cool Carboniferous period, vascular seedless plants dominated the landscape of the swamp forests that covered much of the earth. Although these plants were more complex and better adapted to the challenges of the land environment, they still were dependent on water for sperm to swim to the egg. During the Mesozoic era, 150 million years ago, Earth became warmer and drier and the swamp forests declined, presenting another challenge to terrestrial plants and animals. Earth at that time was a world dominated by reptilian vertebrates, including the flying,

* Gymnosperms
- Naked - seed
- have very reduced gamyna plyta
- ♂ ≡ Pollen grain
- ♀ ≡ in ovule sporangium
- wind Fertilization = Pollination
- heterosporous { microspore = ♂ gamytoplyte; megaspore = ♀ gamytoplyte
- trees or shrubs
- life cycle is long From seed to seed

- Phylum Coniferophyta e.g Pine (Pinus)

Cones < ♀ ♂

- Seeds: dorman embryo + Nutrient + Protective cover

" Kingdom of Plant "

- Vascular Plant (النباتات الوعائية)
  - seedless
  - seed
    - Gymnosperms (عراة البذور)
      - ↓
      - Coniferophyta (مخروطيات)
      - eg: Pinus (الصنوبر) (Pine)
    - Angiosperm (مغلفة البذور)
      - ↓
      - anthophyta
      - eg: Flowering Plant
- non vascular plant

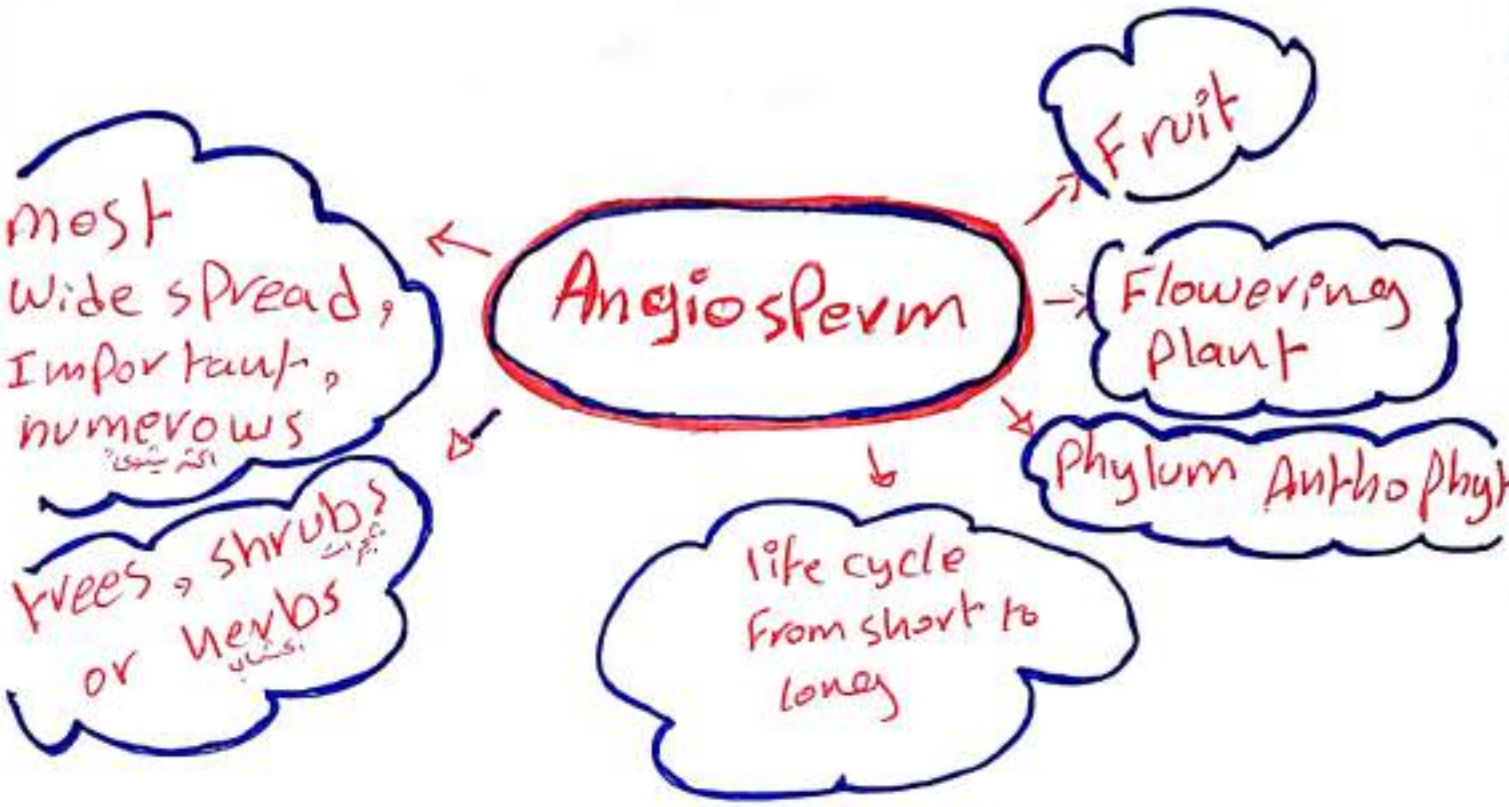
Gymnosperms

♂ = Pollen grain (حبوب لقاح)

♀ = in ovule sporangium

- ← Gymnosperms → Naked seed (بذور بدون ثمرة)
- trees and shrubs (أشجار)
- long life cycle (from seed to seed)
- * Cones : مخاريط
- wind Fertilization = Pollination
- have very reduced gamyto phyte (smaller size)

- * heterosporous
  - microspores gamyto phyte (♂)
  - megaspores gamyto phyte (♀)
- * seeds:
  - dorman embryo
  - + Nutrient
  - + protictive cover



* Pollination :
water, wind, animal, birds, bats, insects

* Flower :
color, nectar odor, Pollen

"showy Flower"

* ovary → Fruit
* ovule → mature to give seed

running, and climbing dinosaurs. The landscape was dominated by a great variety of seed-bearing plants called **gymnosperms** (literally, "naked seeds"), which in the Carboniferous period had been restricted to dry sites. During the Mesozoic, a number of distinct gymnosperm groups diversified, and a few of the spore-bearing plants survived (see Figure 15.1 and Table 15.1 in Lab Topic 15). As you review the evolution of land plants, refer to the geological time chart in Figure 15.3 for an overview of the history of life on Earth.

Vertebrate animals became fully terrestrial during the Mesozoic with the emergence of reptiles, which were free from a dependence on water for sexual reproduction and development. The development of the amniotic egg along with an internal method of fertilization made this major transition possible. The amniotic egg carries its own water supply and nutrients, permitting early embryonic development to occur on dry land, a great distance from external water. In an analogous manner, the gymnosperms became free from dependence on water through the development of a process of internal fertilization via the **pollen grain** and development of a **seed**, which contains a dormant embryo with a protective cover and special nutrient tissue.

Several features of the gymnosperms have been responsible for their success. They have reduced (smaller-sized) gametophytes; the male gametophyte is a multinucleated pollen grain, and the female gametophyte is small and retained within the sporangium in the ovule of the sporophyte generation. The pollen grain is desiccation resistant and adapted for wind pollination, removing the necessity for fertilization in a watery medium. The pollen tube conveys the sperm nucleus to an egg cell, and the embryonic sporophyte develops within the gametophyte tissues, which are protected by the previous sporophyte generation. The resulting seed is not only protected from environmental extremes, but also is packed with nutritive materials and can be dispersed away from the parent plant. In addition, gymnosperms have advanced vascular tissues: xylem for transporting water and nutrients and phloem for transporting photosynthetic products. The xylem cells are called *tracheids* and are more efficient for transport than those of the seedless vascular plants.

Angiosperms

A visit to Earth 60 million years ago, during the late Cretaceous period, would reveal a great diversity of mammals and birds and a landscape dominated by **flowering plants**, or **angiosperms** (phylum **Anthophyta**). Ultimately, these plants would diversify and become the most numerous, widespread, and important plants on Earth. Angiosperms now occupy well over 90% of the vegetated surface of Earth and contribute virtually 100% of our agricultural food plants.

The evolution of the **flower** resulted in enormous advances in the efficient transfer and reception of pollen. Whereas gymnosperms are all wind-pollinated, producing enormous amounts of pollen that reach the appropriate species by chance, the process of flower pollination is mediated by specific agents—insects, birds, and bats—in addition to water and wind.

pollination agents such as the insect are attracted to the flower with its rewards of nectar and pollen. Animal movements provide precise placement of pollen on the receptive portion of the female structures, increasing the probability of fertilization. The process also enhances the opportunity for cross-fertilization among distant plants and therefore the possibility of increased genetic variation.

Angiosperm reproduction follows the trend for reduction in the size of the gametophyte. The pollen grain is the male gametophyte, and the eight-nucleated embryo sac is all that remains of the female gametophyte. This generation continues to be protected and dependent on the adult sporophyte plant. The female gametophyte provides nutrients for the developing sporophyte embryo through a unique triploid **endosperm** tissue. Another unique feature of angiosperms is the **fruit**. The seeds of the angiosperm develop within the flower ovary, which matures into the fruit. This structure provides protection and enhances dispersal of the young sporophyte into new habitats.

In addition to advances in reproductive biology, the angiosperms evolved other advantageous traits. All gymnosperms are trees or shrubs, with a large investment in woody, persistent tissue; and their life cycles are long (5 or more years before they begin to reproduce and 2 to 3 years to produce a seed). Flowering plants, on the other hand, can be woody, but many are herbaceous, with soft tissues that survive up to a few years. It is possible for angiosperms to go from seed to seed in less than 1 year. As you perform the exercises in this lab, think about the significance of this fact in terms of the evolution of this group. How might generation length affect the rate of evolution? Angiosperms also have superior conducting tissues. Xylem tissue is composed of *tracheids* (as in gymnosperms), but also contains large-diameter, open-ended *vessels*. The phloem cells, called *sieve-tube members*, provide more efficient transport of the products of photosynthesis. The cell structure and organization of plants will be investigated in Lab Topic 20 Plant Anatomy.

Review the characteristics of gymnosperms and angiosperms described in this introduction, and *summarize in Table 16.1 the advantages of these groups relative to their success on land*. You should be able to list several characteristics for each. At the end of the lab, you will be asked to modify and complete the table, based on your investigations.

You will want to return to this table after the laboratory to be sure that the table is complete and that you are familiar with all these important features.

EXERCISE 16.1
Gymnosperms

The term *gymnosperms* refers to a diverse group of seed plants that do not produce flowers. Although they share many characteristics, including the production of pollen, they represent four distinct groups, or phyla. In this exercise, you will observe members of these phyla and investigate the life cycle of a pine, one of the most common gymnosperms.

Table 16.1
Traits for Gymnosperms and Angiosperms Relative to Their Success on Land

	Adaptations to the Land Environment
Gymnosperms 	
Angiosperms 	

Lab Study A. Phyla of Gymnosperms

Materials

living or pressed examples of conifers, ginkgos, cycads, and Mormon tea

Introduction

Gymnosperms are composed of several phyla (see Figure 15.1 and Table 15.1 in Lab Topic 15). The largest and best known phylum is **Coniferophyta**, which includes pines and other cone-bearing trees and shrubs (Color Plate 38). Cycads (**Cycadophyta**), which have a palmlike appearance, are found primarily in tropical regions scattered around the world (Color Plate 39). Ginkgos (**Ginkgophyta**), with their flat fan-shaped leaves, are native to Asia and are prized as urban trees. An extract of Ginkgo is used as an herbal medicine purported to improve memory (Color Plates 40 and 41). **Gnetophyta** is composed of three distinct and unusual groups of plants: gnetums, which are primarily vines of Asia, Africa, and South America; *Welwitschia*, a rare desert plant with two leathery leaves; and Mormon tea (*Ephedra*), desert shrubs of North and Central America (Color Plate 42). Compounds from *Ephedra*, ephedrines, used in diet aids and decongestants, have raised serious concerns due to side effects including

3. What economically important products are provided by conifers?

4. What economically important products are provided by other gymnosperms?

Lab Study B. Pine Life Cycle

Materials

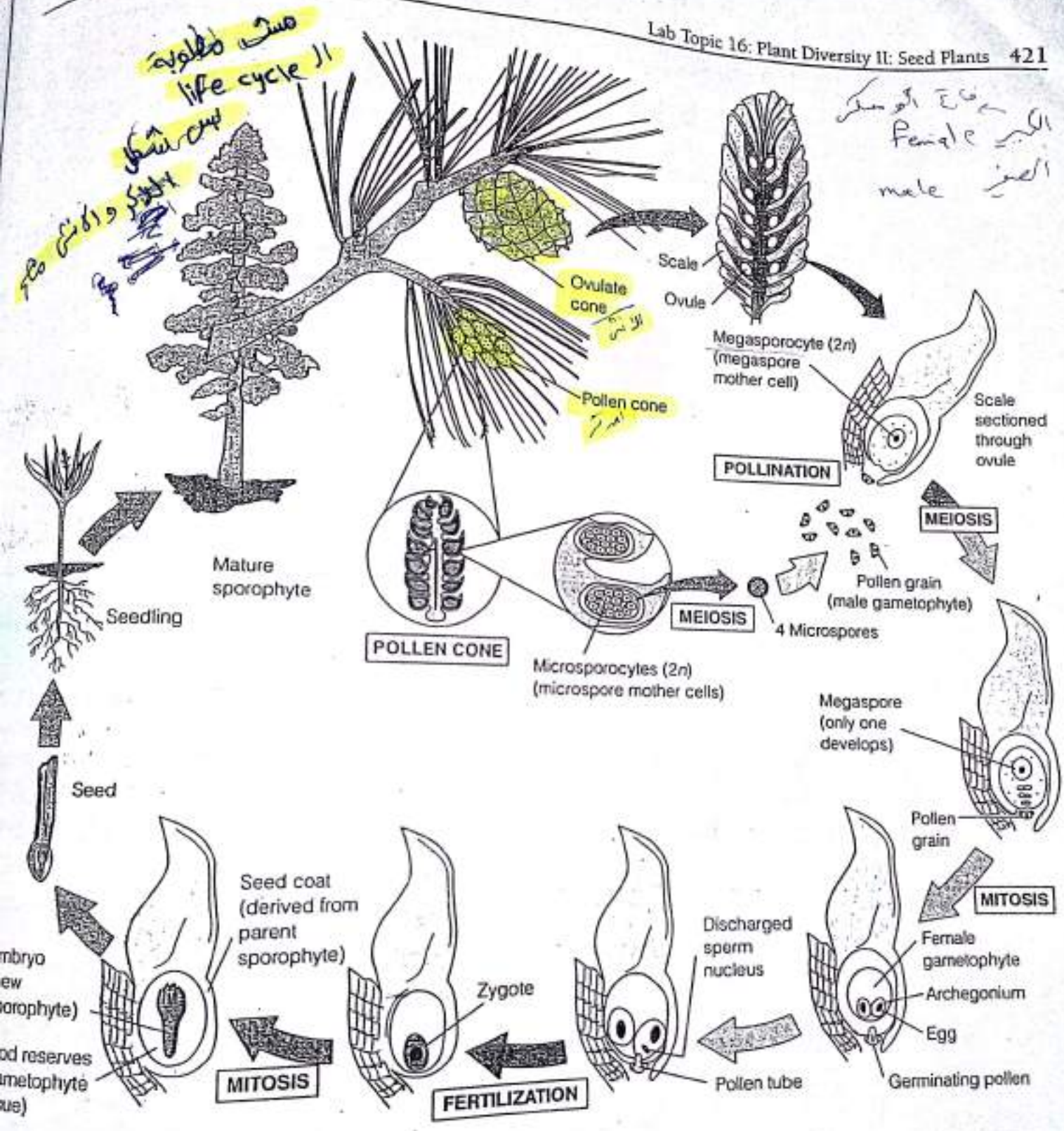
living or preserved pine branch, male and female cones (1, 2, and 3 years old)
fresh or dried pine pollen or prepared slide of pine pollen
coverslips
prepared slides of male and female pine cones
colored pencils
slides

Review the pine life cycle (Figure 16.1) before you begin. Follow along as you complete the exercise.

Introduction

All gymnosperms are **wind-pollinated** trees or shrubs, most bearing unisexual, male, and female reproductive structures on different parts of the same plant. Gymnosperms are **heterosporous**, producing two kinds of spores: male **microspores**, which develop into **pollen**, and female **megaspores**. The megaspore develops into the female gametophyte, which is not free-living as with ferns but retained within the **megasporangium** and nourished by the sporophyte parent plant. The female gametophyte develops archegonia, each containing an egg. Numerous pollen grains (the male gametophytes) are produced in each **microsporangium**, and when they are mature they are released into the air and conveyed by wind currents to the female cone. **Pollen tubes** grow through the tissue of the megasporangium, and the **sperm nucleus** is released to fertilize the egg. After fertilization, development results in the formation of an **embryo**. A **seed** is a dormant embryo embedded in nutrient tissue of the female gametophyte and surrounded by the hardened sporangium wall, or **seed coat**.

Having trouble with life cycles? Return to Lab Topic 15 (Plant Diversity I) and review the generalized life cycle (Figure 15.2). The key to success is to determine where meiosis occurs and to remember the ploidal level for the gametophyte and sporophyte.



re 16.1.

life cycle. Observe the structures and processes as described in Exercise 16.1. colored pencils, indicate the structures that are haploid or diploid. Circle the terms is, meiosis, and fertilization.

...view the structures and processes observed.
...Using colored pencils, indicate the structures of the pine life cycle in Figure 16.1 that are haploid or diploid, and circle the processes of mitosis, meiosis, and fertilization.

...cussion

1. What is the function of the wings on the pollen grain?

2. Why is wind-dispersed pollen an important phenomenon in the evolution of plants?

3. Are microspores and megaspores produced by mitosis or meiosis?

4. Describe the structure and function of a seed.

5. Can you think of at least two ways in which pine seeds are dispersed?

6. One of the major trends in plant evolution is the reduction in size of the gametophytes. Describe the male and female gametophyte in terms of size and location.

EXERCISE 16.2

Angiosperms

All flowering plants (angiosperms) are classified in the phylum **Anthophyta** (Gk. anthos, "flower"). A unique characteristic of angiosperms is the **carpel**, a structure in which **ovules** are enclosed. After fertilization, the ovule develops into a **seed** (as in the gymnosperms), while the carpel matures into a **fruit** (unique to angiosperms). Other important aspects of angiosperm reproduction include additional reduction of the gametophyte, double fertilization, and an increase in the rapidity of the reproduction process.

* Angiosperm
- Fruit
- Flowering plant
- phylum Anthophyta
- Most widespread, important, numerous
- trees, shrubs or herb
- life cycle from short to long
* Pollination by
= water, wind, animal, birds, bats, insects,...
- flower ≡ color, nectar (sugar), odor,
* showy flower
* ovary → fruit
* ovule → mature to give seed
* endosperm
* numerous

The flowers of angiosperms are composed of male and female reproductive structures, which are frequently surrounded by attractive or protective leaflike structures collectively known as the **perianth** (Color Plate 43). The flower functions both to protect the developing gametes and to ensure **pollination and fertilization.** Although many angiosperm plants are self-fertile, cross-fertilization is important in maintaining genetic diversity. Plants, rooted and stationary, often require transfer agents to complete fertilization. A variety of insects, birds, and mammals transfer pollen from flower to flower. The pollen then germinates into a pollen tube and grows through the female carpel to deliver the sperm to the egg.

Plants must attract pollinators to the flower. What are some features of flowers that attract pollinators? Color and scent are important, as is the shape of the flower. Nectar and pollen provide nutritive rewards for the pollinators as well. The shape and form of some of the flowers are structured to accommodate pollinators of specific size and structure, providing landing platforms, guidelines, and even special mechanisms for the placement of pollen on body parts. While the flower is encouraging the visitation by one type of pollinator, it also may be excluding visitation by others. The more specific the relationship between flower and pollinator, the more probable that the pollen of that species will be successfully transferred. But many successful flowers have no specific adaptations for particular pollinators and are visited by a wide variety of pollinators.

Some plants do not have colorful, showy flowers and are rather inconspicuous, often dull in color, and lacking a perianth. These plants are usually wind-pollinated, producing enormous quantities of pollen and adapted to catch pollen in the wind (Color Plate 44).

The origin and diversification of angiosperms cannot be understood apart from the coevolutionary role of animals in the reproductive process. Colorful petals, strong scents, nectars, food bodies, and unusual perianth shapes all relate to pollinator visitation. Major trends in the evolution of angiosperms involve the development of mechanisms to exploit a wide variety of pollinators (Color Plates 45, 46, and 47).

In Lab Study A, you will investigate a variety of flowers, observing their shape, structure, and traits that might attract pollinators of various kinds. Following this, in Lab Study B, you will use a key to identify the probable pollinators for some of these flowers. You will follow the life cycle of the lily in Lab Study C and complete the lab by using another key to identify types of fruits and their dispersal mechanisms. *Note: Before beginning Lab Study A, turn to Lab Study C, Procedure step 3, to set up pollination experiments, since the pollen must incubate for 30–60 minutes. Then return to Lab Study A below.*

Lab Study A. Flower Morphology

Materials

living flowers provided by the instructor and/or students
stereoscopic microscope

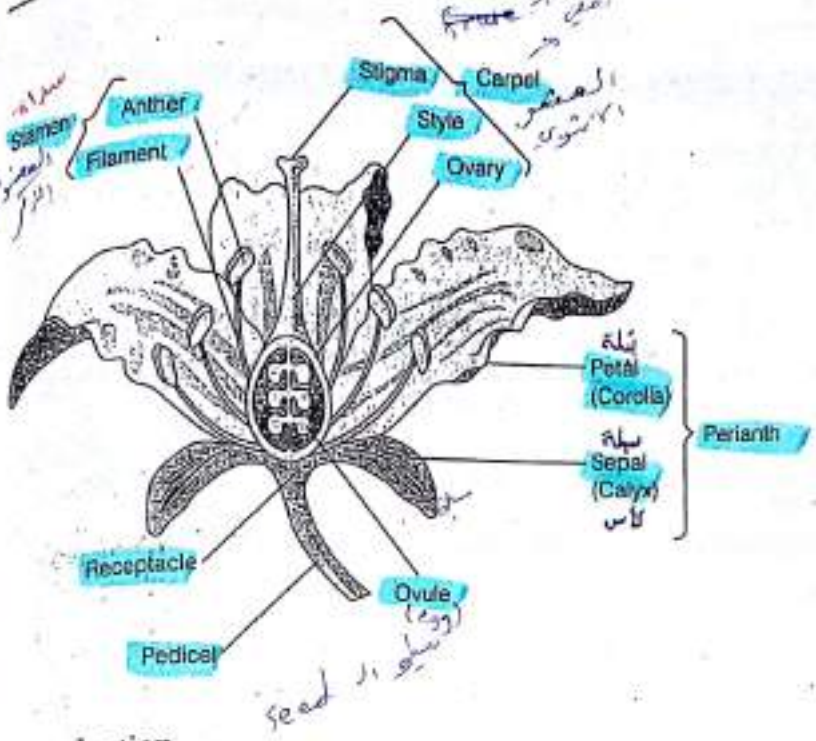


Figure 16.2.
Flower structures. Determine the structures of flowers in the laboratory by reviewing this general diagram.

Introduction

Working in teams of two students, you will investigate the structure of the flower (Figure 16.2, Color Plate 43). The instructor will provide a variety of flowers, and you may have brought some with you to lab. You will need to take apart each flower carefully to determine its structure, since it is unlikely that all your flowers will follow the simple diagram used to illustrate the structures. Your observations will be the basis for predicting probable pollinators in Lab Study B.

Procedure

1. Examine fresh flowers of four different species, preferably with different floral characteristics.
2. Identify the parts of each flower using Figure 16.2 and the list provided following the heading Floral Parts. You may be able to determine the floral traits for large, open flowers by simply observing. However, to really understand the flower structure, you should remove the flower parts stepwise, beginning with the outer sepals and continuing toward the center of the flower. To observe the details of smaller flowers and structures, use the stereoscopic microscope. For example, the ovary is positively identified by the presence of tiny crystal-like ovules, and these are best seen with the stereoscopic scope.
3. In the margin of your lab manual, sketch any flower shapes or structures that you might need to refer to in the future.
4. Record the results of your observations in Table 16.3. You will determine pollinators in Lab Study B.

Floral Parts:

Pedicel: stalk that supports the flower.
Receptacle: tip of the pedicel where the flower parts attach.
Sepal: outer whorl of bracts, which may be green, brown, or colored like the petals; may appear as small scales or be petal-like.
Calyx: all the sepals, collectively.

Petal: colored, white, or even greenish whorl of bracts located just inside the sepals.
Corolla: all the petals, collectively
Stamen: pollen-bearing structure, composed of filament and anther.
Filament: thin stalk that supports the anther.
Anther: pollen-producing structure.
Carpel: female reproductive structure, composed of the stigma, style, and ovary, often pear-shaped and located in the center of the flower.
Stigma: receptive tip of the carpel, often sticky or hairy, where pollen is placed; important to pollen germination.
Style: tissue connecting stigma to ovary, often long and narrow, but may be short or absent; pollen must grow through this tissue to fertilize the egg.
Ovary: base of carpel; protects ovules inside, matures to form the fruit.

Results

Summarize your observations of flower structure in Table 16.3.

ble 16.3
ower Morphology and Pollinators

eatures	Plant Names			
	1	2	3	4
umber of petals				
umber of sepals				
rts absent (petals, amens, etc.)				
lor				
nt (+/–)				
air (+/–)				
e (including la-shape: ar, star, etc.)				
al features ng-platform, ines, nectar				

Discussion

What structures or characteristics did you observe in your (or other teams') investigations that you predict are important to pollination?

Student Media Videos—Ch. 30: Flower Blooming (Time Lapse); Flowering Plant Life Cycle

Lab Study B. Pollinators

Materials

living flowers provided by the instructor and/or students
stereoscopic microscope

Introduction

Flowers with inconspicuous sepals and petals are usually pollinated by wind (Color Plate 44). Most showy flowers are pollinated by animals. Some pollinators tend to be attracted to particular floral traits, and, in turn, some groups of plants have coevolved with a particular pollination agent that ensures successful reproduction. Other flowers are generalists, pollinated by a variety of organisms, and still others may be visited by only one specific pollinator (Color Plates 45, 46, and 47). Based on the floral traits that attract common pollinators (bees, flies, butterflies, and hummingbirds), you will predict the probable pollinator for some of your flowers using a **dichotomous key**. (Remember, *dichotomous* refers to the branching pattern and means "divided into two parts.")

In biology, we use a key to systematically separate groups of organisms based on sets of characteristics. Most keys are based on couplets, or pairs of characteristics, from which you must choose one or the other, thus, the term *dichotomous*. For example, the first choice of characteristics in a couplet might be *plants with showy flowers and a scent*, and the other choice in the pair might be *plants with tiny, inconspicuous flowers with no scent*. You must choose one or the other statement. In the next step, you would choose from a second pair of statements listed directly below your first choice. With each choice, you would narrow the group more and more until, as in this case, the pollinator is identified. *Each couplet or pair of statements from which you must choose will be identified by the same letter or number.*

519 – 522

Plant tissue :
- dermal tissue
- ground tissue
- Vascular =
- meistematic =

Lab Topic 20

Plant Anatomy

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify and describe the structure and function of each plant cell type and tissue type.
2. Describe the organization of tissues and cells in each plant organ.
3. Relate the function of an organ (root, stem, and leaf) to its structure.
4. Describe primary and secondary growth and identify the location of each in the plant.
5. Relate primary and secondary growth to the growth habit (woody or herbaceous).
6. Discuss adaptation of land plants to the terrestrial environment as illustrated by the structure and function of plant anatomy.
7. Apply your knowledge of plants to the kinds of produce you find in the grocery store.

Introduction

Vascular plants have been successful on land for over 420 million years, and their success is related to their adaptations to the land environment. An aquatic alga lives most often in a continuously homogeneous environment: The requirements for life are everywhere around it, so relatively minor structural adaptations have evolved for functions such as reproduction and attachment. In contrast, the terrestrial habitat, with its extreme environmental conditions, presents numerous challenges for the survival of plants. Consequently, land plants have evolved structural adaptations for functions such as the absorption of underground water and nutrients, the anchoring of the plant in the substrate, the support of aerial parts of the plant, and the transportation of materials throughout the relatively large plant body. In angiosperms, the structural adaptations required for these and other functions are divided among three vegetative plant organs: stems, roots, and leaves. Unlike animal organs, which are often composed of unique cell types (for example, cardiac muscle fibers are found only in the heart, osteocytes only in bone), plant organs have many tissues and cell types in common, but they are organized in different ways. The structural organization of basic tissues and cell types in different plant organs is directly related to their different functions. For example, leaves function as the primary photosynthetic organ and generally have thin, flat blades that maximize light absorption and gas

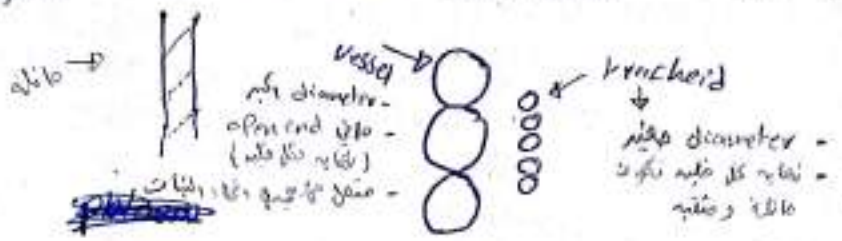
* Vascular tissue

* النسيج الي بينقل المواد في النبات
(complex tissue)

1 Xylem

- transport / carry / conduct of
  - water and minerals
  - support ← بما انو خلاياه قويه وصلبه في كل النبات بعطي وظيفة ثالثة

tracheid and vessel element ← main cells for Xylem

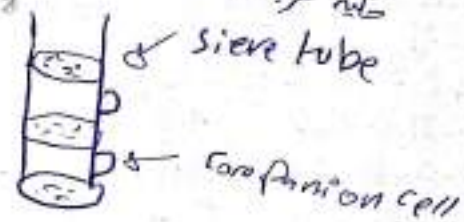


- Parenchyma → وظيفتها مخزن : storage , lateral transport
- Fiber → وظيفتها مخزن : extra support for xylem

خلايا اضافية في Xylem

2 Phloem

- transport Photosynthetic Product
- Sieve-tubes , Companion cell → F: contain sieve tubes
- lack nucleus
- when mature
- Fibre
- Parenchyma



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* meristematic tissue → undifferentiated cell

1 1° mtri
responsable for 1° growth , root tip , shoot buds

2 Pericycle root → lateral root

3 vascular cambium
2° growth

4 cork cambium
2° growth

exchange. Specialized cells of the root epidermis are long extensions that promote one of the root functions, absorption. The interrelationship of structure and function is a major theme in biology, and you will continue to explore it in this lab topic.

Use the figures in this lab topic for orientation and as a study aid. Be certain that you can identify all items by examining the living specimens and microscope slides. These, and not the diagrams, will be used in the laboratory evaluations.

Summary of Basic Plant Tissue Systems and Cell Types

The plant body is constructed into tissue systems based on their shared structural and functional features. There are three tissue systems—dermal, ground, and vascular—that are continuous throughout the organs of roots, stems, and leaves. The plant tissues that actively divide by mitosis are called **meristematic tissues**. These are located in specific regions—for example the root tip. Following is a review of plant tissue systems and the most common types of cells seen in plant organs, as well as their functions. Other specialized cells will be described as they are discussed in lab. Refer back to this summary as you work through the exercises.

Dermal Tissue System: Epidermis

The epidermis forms the outermost layer of cells, usually one cell thick, covering the entire plant body. The epidermal cells are often flattened and rectangular in shape (Figure 20.1a and 1b). Specialized epidermal cells include the **guard cells** of the stomata, hairs called **trichomes**, and unicellular **root hairs**. Most epidermal cells on aboveground structures are covered by a waxy **cuticle**, which prevents water loss. The epidermis provides protection and regulates movement of materials.

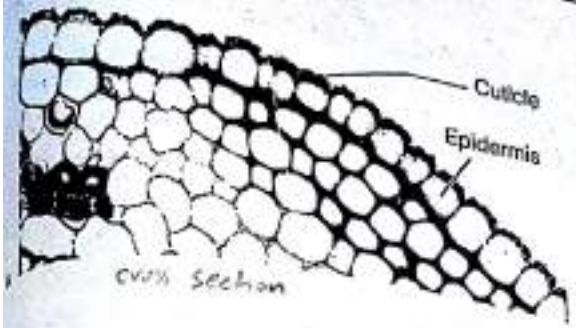
Ground Tissue System: Parenchyma, Collenchyma, and Sclerenchyma

The ground tissue system is distributed throughout the plant beneath the epidermis and surrounding the vascular tissues. Parenchyma, collenchyma, and/or sclerenchyma cells are typically found in ground tissue as seen in the cross section of a pumpkin stem (Figure 20.1c and Color Plate 61).

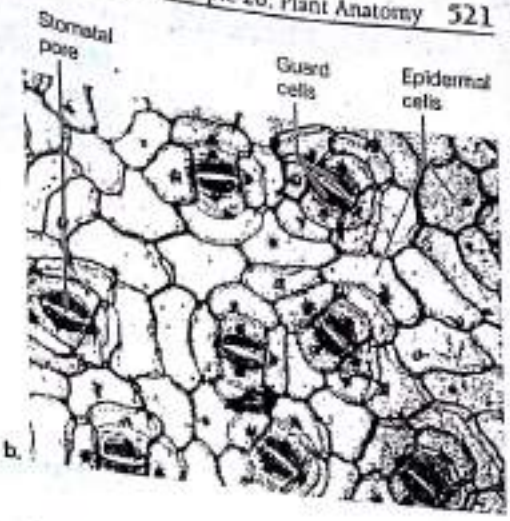
Parenchyma cells are the most common cell in plants and are characteristically thin-walled with large vacuoles. These cells may function in photosynthesis, support, storage of materials, and lateral transport.

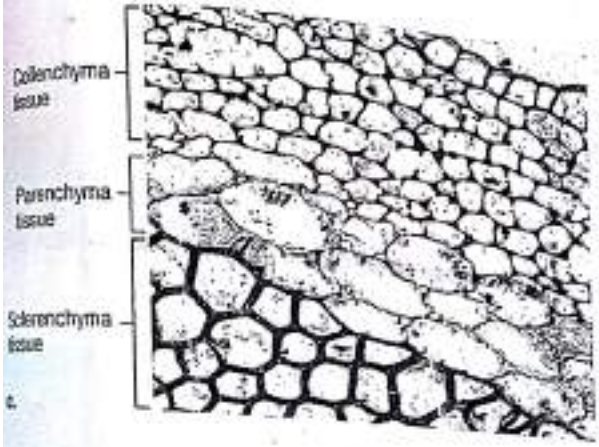
Collenchyma cells are usually found near the surface of the stem, leaf petioles, and veins. These living cells are similar to parenchyma cells but are characterized by an uneven thickening of cell walls. They provide flexible support to young plant organs.

Sclerenchyma cells have thickened cell walls that may contain lignin. They provide strength and support to mature plant structures and may be dead at functional maturity. The most common type of sclerenchyma cells are long, thin **fibers**.



Cross Section





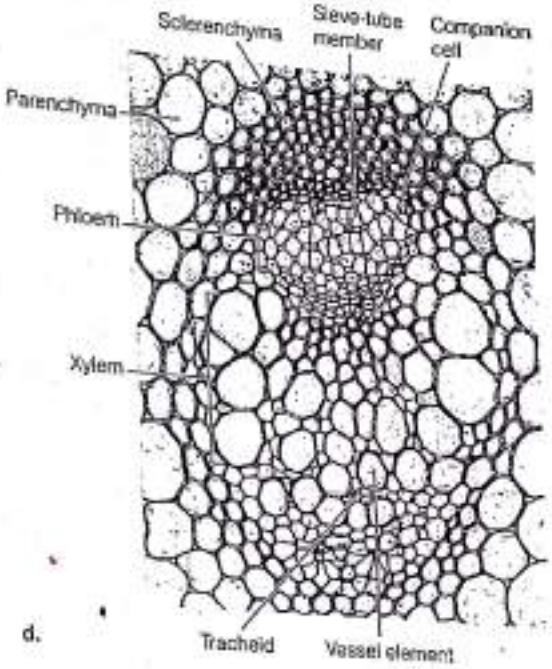


Figure 20.1.
Plant tissue systems and cell types.
(a) Dermal tissue—a single layer of epidermis covered by waxy cuticle; (b) Leaf surface showing epidermis with stomata and guard cells; (c) Ground tissue—cross section of pumpkin stem (Color Plate 61); (d) Vascular tissue—cross section of a vascular bundle in a buttercup stem; (e) Long section through xylem and phloem of pumpkin stem.

Vascular Tissue System: Xylem and Phloem

The vascular tissue system functions in the transport of materials throughout the plant body. Xylem tissue and phloem tissue are complex tissues (composed of several cell types) seen in the cross section of a buttercup stem (Figure 20.1d) and a long section of a pumpkin stem (Figure 20.1e).

Xylem cells form a complex vascular tissue that functions in the transport of water and minerals throughout the plant and provides support. **Tracheids** and **vessel elements** are the primary water-conducting cells. Tracheids are long, thin cells with perforated tapered ends. Vessel elements are larger in diameter, open-ended, and joined end to end, forming continuous transport systems referred to as **vessels**. Parenchyma cells are present in the xylem and function in storage and lateral transport. Fibers in the xylem provide additional support.

Phloem tissue transports the products of photosynthesis throughout the plant as part of the vascular tissue system. This complex tissue is composed of living, conducting cells called **sieve-tube members**, which lack a nucleus and have sieve plates for end walls. The cells are joined end to end throughout the plant. Each sieve-tube member is associated with one or more adjacent **companion cells**, which are thought to regulate sieve-tube member function. Phloem parenchyma cells function in storage and lateral transport, and phloem fibers provide additional support.

Meristematic Tissue: Primary Meristem, Cambium, and Pericycle

Primary meristems consist of small, actively dividing, undifferentiated cells located in buds of the shoot and in root tips of plants. These cells produce the primary tissues along the plant axis throughout the life of the plant. You will study primary meristems in the apical bud in Exercise 20.2 (Figure 20.3).

Pericycle is a layer of meristematic cells just outside the vascular cylinder in the root. These cells divide to produce lateral branch roots (Exercise 20.3 Lab Study B, Figure 20.6c).

Vascular cambium is a lateral meristem also composed of small, actively dividing cells that are located between the xylem and phloem vascular tissue. These cells divide to produce secondary growth, which results in an increase in circumference (Exercise 20.4, Figure 20.10).

Cork cambium is a lateral meristem located just inside the cork layer of a woody plant. These cells divide to produce secondary growth (Exercise 20.4, Figure 20.10).

This lab topic begins with a study of the shape and form of the whole plant, then moves to an investigation of the primary plant body derived from apical meristems. You will look at a slide of the apical bud from the tip of the stem. Next you will look at the structure of the three organs of the primary plant body: stems, roots, and leaves. Some plants continue to grow from lateral meristems producing secondary tissues, which you will investigate in stems of a woody plant. The lab topic concludes with an application of your knowledge to plants commonly found in the grocery store.

EXERCISE 20.1
Plant Morphology

Materials

living bean or geranium plant
squirt bottle of water
paper towel

Introduction

As you begin your investigation of the structure and function of plants, you need an understanding of the general shape and form of the whole plant. In this exercise, you will study a bean or geranium plant, identifying basic features of the three vegetative organs: *roots*, *stems*, and *leaves*. In the following exercises, you will investigate the cellular structure of these organs as viewed in cross sections. Refer to the living plant for orientation before you view your slides.

Procedure

1. Working with another student, examine a living herbaceous (nonwoody) plant and identify the following structures in the *shoot (stems and leaves)*:
   a. **Nodes** are regions of the stem from which leaves, buds, and branches arise and which contain meristematic tissue (areas of cell division).
   b. **Internodes** are the regions of the stem located between the nodes.
   c. **Terminal buds** are located at the tips of stems and branches. They enclose the shoot apical meristem, which gives rise to leaves, buds, and all primary tissue of the stem. Only stems produce buds.
   d. **Axillary**, or **lateral, buds** are located in the leaf axes at nodes; they may give rise to lateral branches.
   e. Leaves consist of flattened **blades** attached at the node of a stem by a stalk, or **petiole**.
2. Observe the *root* structures by gently removing the plant from the pot and loosening the soil from the root structure. You may need to rinse a few roots with water to observe the tiny, active roots. Identify the following structures.
   a. **Primary and secondary roots.** The primary root is the first root produced by a plant embryo and may become a long taproot. Secondary roots arise from meristematic tissue deep within the primary root.
   b. Root tips consist of a **root apical meristem** that gives rise to a **root cap** (protective layer of cells covering the root tip) and to all the primary tissues of the root. A short distance from the root tip is a zone of **root hairs** (specialized epidermal cells), the principal site of water and mineral absorption.

Results

1. Label Figure 20.2.
2. Sketch in the margin of your lab manual any features not included in this diagram that might be needed for future reference. For example, your plant may have small green bracts (leaflike structures) at the base of the petiole. These are called stipules.

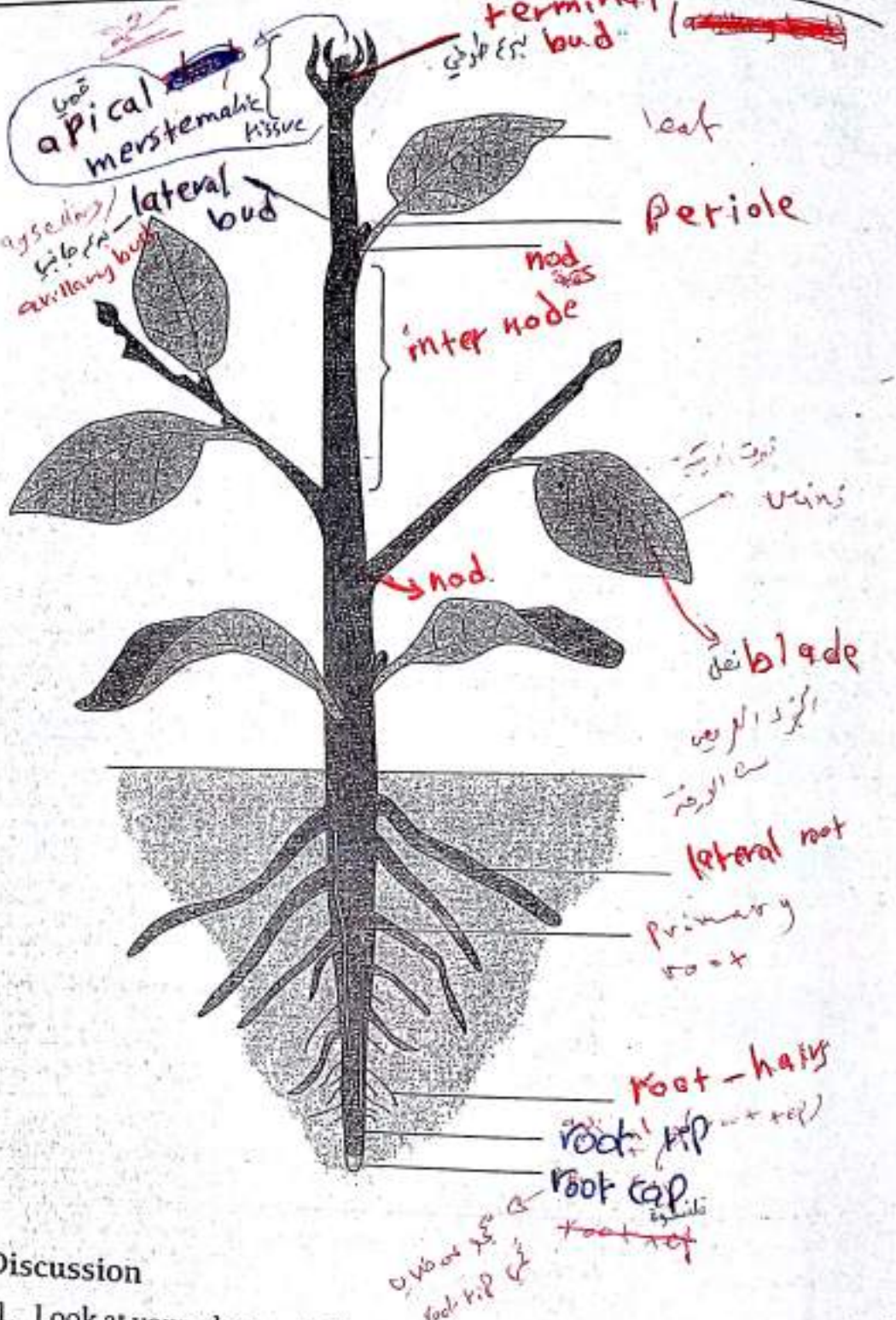


Figure 20.2.
A herbaceous plant. The vegetative plant body consists of roots, stems, and leaves. The buds are located in the axils of the leaves and at the shoot tip. The roots also grow from meristem tissues in the root tip. Label the diagram based on your observations of a living plant and the structures named in Exercise 20.1.

Discussion

1. Look at your plant and discuss with your partner the possible functions of each plant organ. Your discussion might include evidence observed in the lab today or prior knowledge. Describe proposed functions (more than one) for each organ.

   Stems:

2. Imagine that you have cut each organ—roots, stems, and leaves—in cross section. Sketch the overall shape of that cross section in the margin of your lab manual. Remember, you are not predicting the internal structure, just the overall shape.

EXERCISE 20.2
Plant Primary Growth and Development

Materials

prepared slides of *Coleus* stem (long section)
compound microscope

Introduction

Plants produce new cells throughout their lifetime as a result of cell divisions in meristems. Tissues produced from apical meristems are called **primary tissues**, and this growth is called **primary growth**. Primary growth occurs along the plant axis at the shoot and the root tip. Certain meristem cells divide in such a way that one cell product becomes a new body cell and the other remains in the meristem. Beyond the zone of active cell division, new cells become enlarged and specialized (differentiated) for specific functions (resulting, for example, in vessels, parenchyma, and epidermis). Using the model plant, *Arabadopsis*, research into the genetic and molecular basis of cell differentiation has rapidly advanced.

In this exercise, you will examine a longitudinal section through the tip of the stem, observing the youngest tissues and meristems at the apex, then moving down the stem, where you will observe more mature cells and tissues.

Procedure

Examine a prepared slide of a longitudinal section through a terminal bud of *Coleus*. Use low power to get an overview of the slide; then increase magnification. Locate the **apical meristem,** a dome of tissue nestled between the **leaf primordia**, young developing leaves. Locate the axillary **bud primordial** between the leaf and the stem.

Move the specimen under the microscope so that cells may be viewed at varying distances from the apex. The youngest cells are at the apex of the bud, and cells of increasing maturity and differentiation can be seen as you move away from the apex. Follow the early development of vascular tissue, which differentiates in relation to the development of primordial leaves.

a. Locate the narrow, dark tracks of **undifferentiated vascular tissue** in the leaf primordia.
b. Observe changes in cell size and structure of the vascular system as you move away from the apex and end with a distinguishable vessel with its spiral cell wall thickening in the older [illegible] to use the highest power on

Results

1. Label Figure 20.3, indicating the structures visible in the young stem tip.
2. Modify the figure or sketch details in the margin of the lab manual for future reference.

Discussion

1. Describe the changes in cell size and structure in the stem tip. Begin at the youngest cells at the apex and continue to the xylem cells.

2. The meristems of plants continue to grow throughout their lifetime, an example of **indeterminate growth**. Imagine a 200-year-old oak tree, with active meristem producing new buds, leaves, and stems each year. Contrast this with the growth pattern in humans.

EXERCISE 20.3

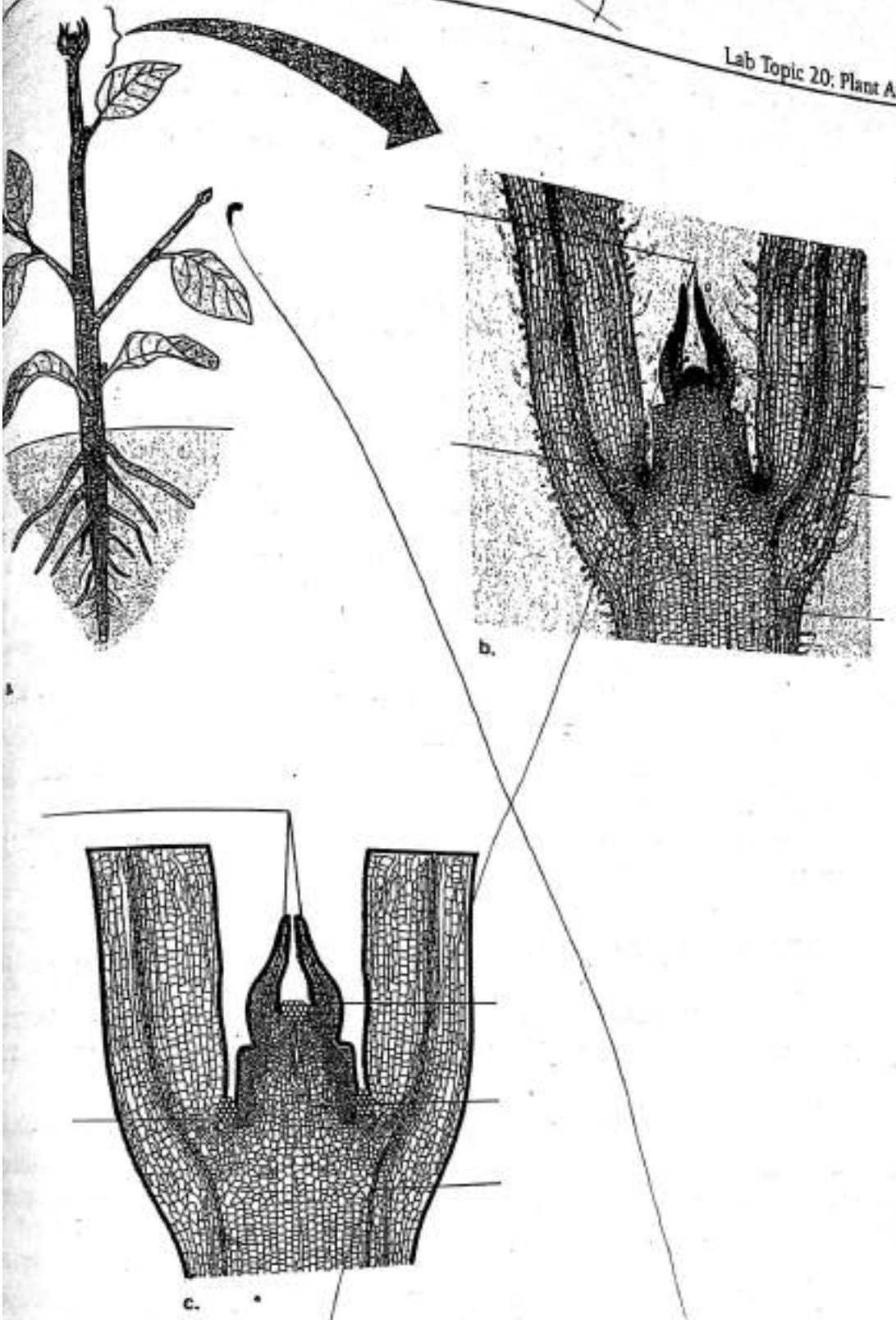
Cell Structure of Primary Tissues

All **herbaceous** (nonwoody) flowering plants produce a complete plant body composed of primary tissue, derived from apical primary meristem. This plant body consists of *organs*—roots, stems, leaves, flowers, fruits, and seeds—and *tissue systems*—**dermal**, **ground**, and **vascular**. In this exercise, you will investigate the cellular structure and organization of plant organs and tissues by examining microscopic slides. You will make your own thin cross sections of stems, and view prepared slides of stems, roots, and leaves. Woody stems will be examined in Exercise 20.4.

Lab Study A. Stems

Materials

prepared slide of herbaceous dicot stem
dropper bottle of distilled water
small petri dish with 50% ethanol
dropper bottle of 50% glycerine
dropper bottle of 0.2% toluidine blue stain
nut-and-bolt microtome
warm paraffin
living plant for sections
new single-edged razor blade
forceps
microscope slides
coverslips
compound microscope
dissecting needles



gure 20.3.
leus stem tip. (a) Diagram of entire plant body. (b) Photomicrograph of a
gitudinal section through the terminal bud. (c) Line diagram of the growing
oot tip with primordial leaves surrounding the actively dividing apical
eristem. The most immature cells are at the tip of the shoot and increase in
ages of development and differentiation farther down the stem. Label the cells
d structures described in Exercise 20.2.

Introduction

A stem is usually the main stalk, or axis, of a plant and is the only organ that produces buds and leaves. Stems support leaves and conduct water and inorganic substances from the root to the leaves and carbohydrate products of photosynthesis from the leaves to the roots. Most herbaceous stems are able to photosynthesize. Stems exhibit several interesting adaptations, including water storage in cacti, carbohydrate storage in some food plants, and thorns that reduce herbivory in a variety of plants.

You will view a prepared slide of a cross section of a stem, and, working with another student, you will use a simple microtome—an instrument used for cutting thin sections for microscopic study—to make your own slides. You will embed the stem tissue in paraffin and cut thin sections. You will stain your sections with toluidine blue, which will help you distinguish different cell types. This simple procedure is analogous to the process used to make prepared slides for subsequent lab studies.

Read through the procedure and set up the materials before beginning.

Procedure

1. Embed the sections of the stem.
   a. Using a new single-edged razor blade, cut a 0.5 cm section of a young bean stem.
   b. Obtain a nut-and-bolt microtome. The nut should be screwed just into the first threads of the bolt. Using forceps, carefully hold the bean stem upright inside the nut.
   c. Pour the warm paraffin into the nut until full. Continue to hold the top of the stem until the paraffin begins to harden. While the paraffin completely hardens, continue the exercise by examining the prepared slide of the stem.
2. Examine a prepared slide of a cross section through the herbaceous dicot stem (Color Plate 62). As you study the stem tissues and cells, refer to "Summary of Basic Plant Tissue Systems and Cell Types," p. 520, Figure 20.1, and Color Plate 61.
3. Identify the **dermal tissue system**, characterized by a protective cell layer covering the plant. It is composed of the **epidermis** and the **cuticle**. Occasionally, you may also observe multicellular **trichomes** on the outer surface of the plants.
4. Locate the **ground tissue system**, background tissue that fills the spaces between epidermis and vascular tissue. Identify the **cortex** region located between the vascular bundles and the epidermis. It is composed mostly of **parenchyma**, but the outer part may contain **collenchyma** as well.

Next find the **pith region**, which occupies the center of the stem, inside the ring of vascular bundles; it is composed of parenchyma. In herbaceous stems, these cells provide support through turgor pressure. This region is also important in storage.

Now identify the **vascular system**, a continuous system of xylem and phloem providing transport and support. In your stems and in many stems, the **vascular bundles** (clusters of xylem and phloem) occur in rings that surround the pith; however, in some groups of flowering plants, the vascular tissue is arranged in a complex network.

Observe that each bundle consists of phloem tissue toward the outside and xylem tissue toward the inside. A narrow layer of vascular cambium, which may become active in herbaceous stems, is situated between the xylem and the phloem. Take note of the following information as you make your observations.

Phloem tissue is composed of three cell types:

a. Dead, fibrous, thick-walled **sclerenchyma cells** that provide support for the phloem tissue and appear in a cluster as a **bundle cap**.

b. **Sieve-tube members**, which are large, living, elongated cells that lack a nucleus at maturity. They become vertically aligned to form sieve tubes, and their cytoplasm is interconnected through sieve plates located at the ends of the cells. Sieve plates are not usually seen in cross sections.

c. **Companion cells**, which are small, nucleated parenchyma cells connected to sieve-tube cells by means of cytoplasmic strands.

Xylem tissue is made up of two cell types:

a. **Tracheids**, which are elongated, thick-walled cells with closed, tapered ends. They are dead at functional maturity, and their lumens are interconnected through pits in the cell walls.

b. **Vessel elements**, which are cylindrical cells that are large in diameter and dead at functional maturity. They become joined end to end, lose their end walls, and form long, vertical vessels.

Vascular cambium is a type of tissue that is located between the xylem and the phloem and which actively divides to give rise to secondary tissues.

8. Complete the Results section on the next page for this slide, then return to step 9 to prepare and observe your own handmade sections of stem preparations.

9. Cut the stem sections in the hardened paraffin.

   a. Support the nut-and-bolt microtome with the bolt head down and, using the razor blade, carefully slice off any excess paraffin extending above the nut. Be careful to slice in a direction away from your body and to keep your fingers away from the edge of the razor blade (Figure 20.4).

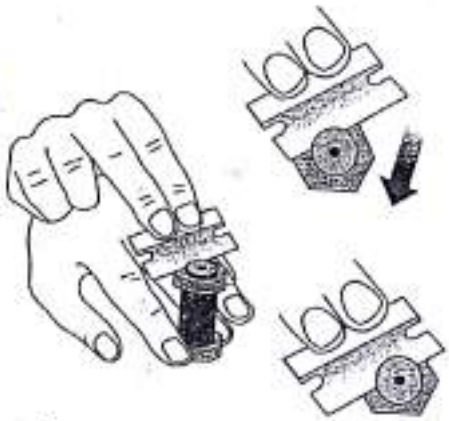
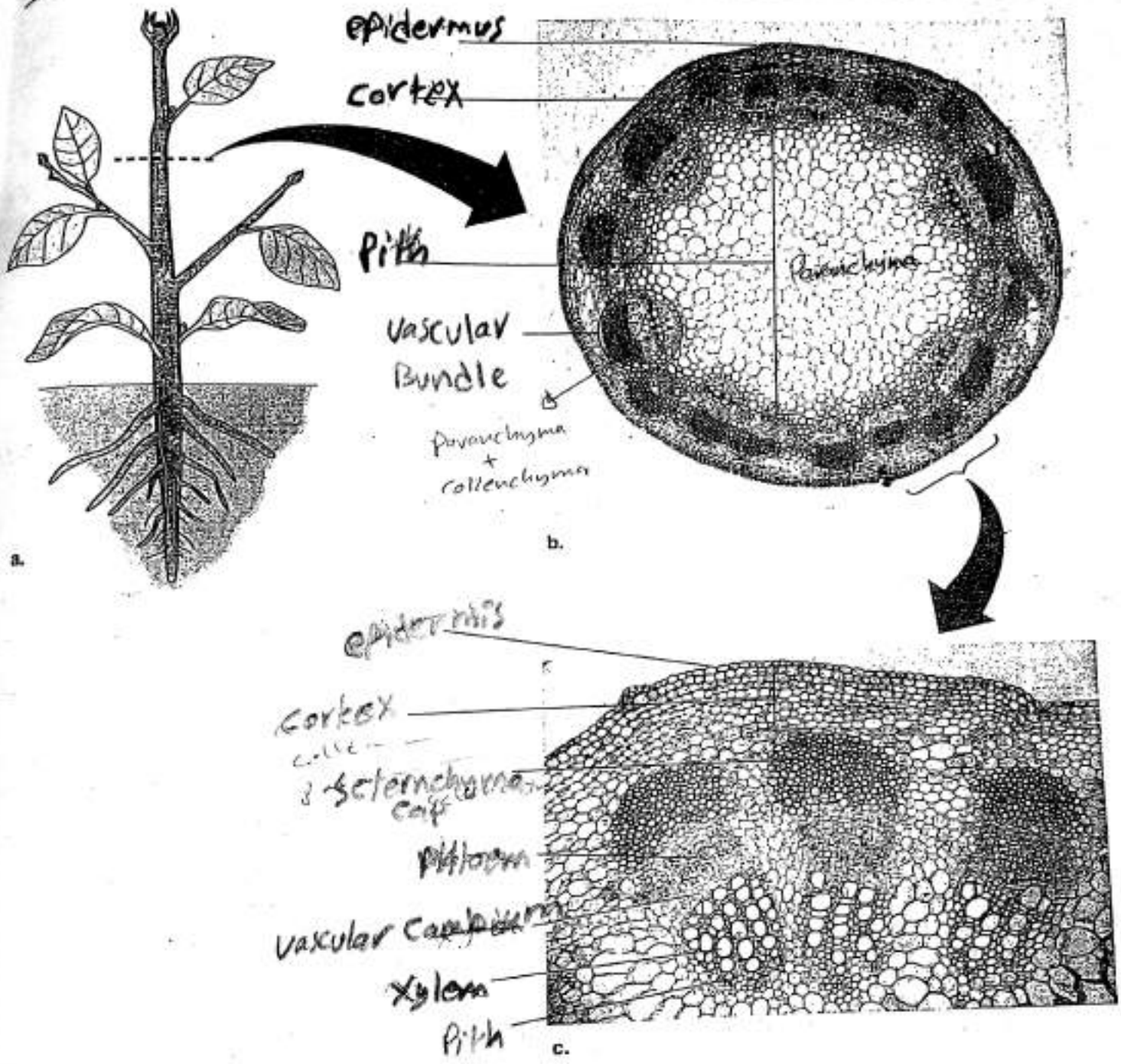


Figure 20.4.
Using the nut-and-bolt microtome. A piece of stem is embedded in paraffin in the bolt. As you twist the bolt up, slice thin sections to be stained and viewed. Slide the entire blade through the paraffin to smoothly slice thin sections. Follow the directions in Exercise 20.3, Lab Study

Be careful to keep fingers and knuckles away from the razor blade. Follow directions carefully.



igure 20.5.
tem anatomy. (a) Diagram of whole plant. (b) Photomicrograph of cross section
rough the stem portion of the plant. (c) Enlargement of one vascular bundle as
en in cross section of the stem (Color Plate 62).

3. For the cells described in your preceding answer, how does their observed structure relate to their function, which is support?

4. What is the function of xylem? Of phloem?

5. The pith and cortex are made up of parenchyma cells. Describe the many functions of these cells. Relate parenchyma cell functions to their observed structure.

6. What differences did you observe in the prepared stem sections and your hand sections? What factors might be responsible for these differences?

* Rizome (stem)
* adventitious root
* fibrous Root

* tap root

Lab Study B. Roots

Materials

prepared slide of buttercup (*Ranunculus*) root (cross section)
demonstration of fibrous roots and taproots
colored pencils
compound microscope

Introduction

Roots and stems often appear to be similar, except that roots grow in the soil and stems above the ground. However, some stems (rhizomes) grow underground, and some roots (adventitious roots) grow aboveground. Roots and stems may superficially appear similar, but they differ significantly in their functions. One of the major themes of biology is that structure and function are closely related at all levels of the hierarchy of life. Therefore, we would expect that the structure of stems and roots might differ in important ways.

What are the primary functions of stems?

Roots have four primary functions:

1. anchorage of the plant in the soil
2. absorption of water and minerals from the soil
3. conduction of water and minerals from the region of absorption to the base of the stem
4. starch storage to varying degrees, depending on the plant

Hypothesis

The working hypothesis for this investigation is that the *structure* of the plant body is related to particular *functions*.

Prediction

Based on the hypothesis, make a prediction about the similarity of root and stem structures that you expect to observe (if/then).

You will now test your hypothesis and predictions by observing the external structure of roots and their internal cellular structure and organization in a prepared cross section. This activity is an example of collecting evidence from observations rather than conducting a controlled experiment.

Procedure

1. Examine the external root structure. When a seed germinates, it sends down a **primary root**, or **radicle**, into the soil. This root sends out side branches called lateral roots, and these in turn branch out until a root system is formed.

   If the primary root continues to be the largest and most important part of the root system, the plant is said to have a **taproot system**. If many main roots are formed, the plant has a **fibrous root system**. Most grasses have a fibrous root system, as do trees with roots occurring within 1 m of the soil surface. Carrots, dandelions, and pine trees are examples of plants having taproots.

   a. Observe examples of fibrous roots and taproots on demonstration in the laboratory.

   b. Sketch the two types of roots in the margin of your lab manual.

2. Examine the internal root structure.

   a. Study a slide of a cross section through a buttercup (*Ranunculus*) root. Note that the root lacks a central pith. The vascular tissue is located in the center of the root and is called the **vascular cylinder** (Figure 20.6b).

      The **cortex** is primarily composed of large

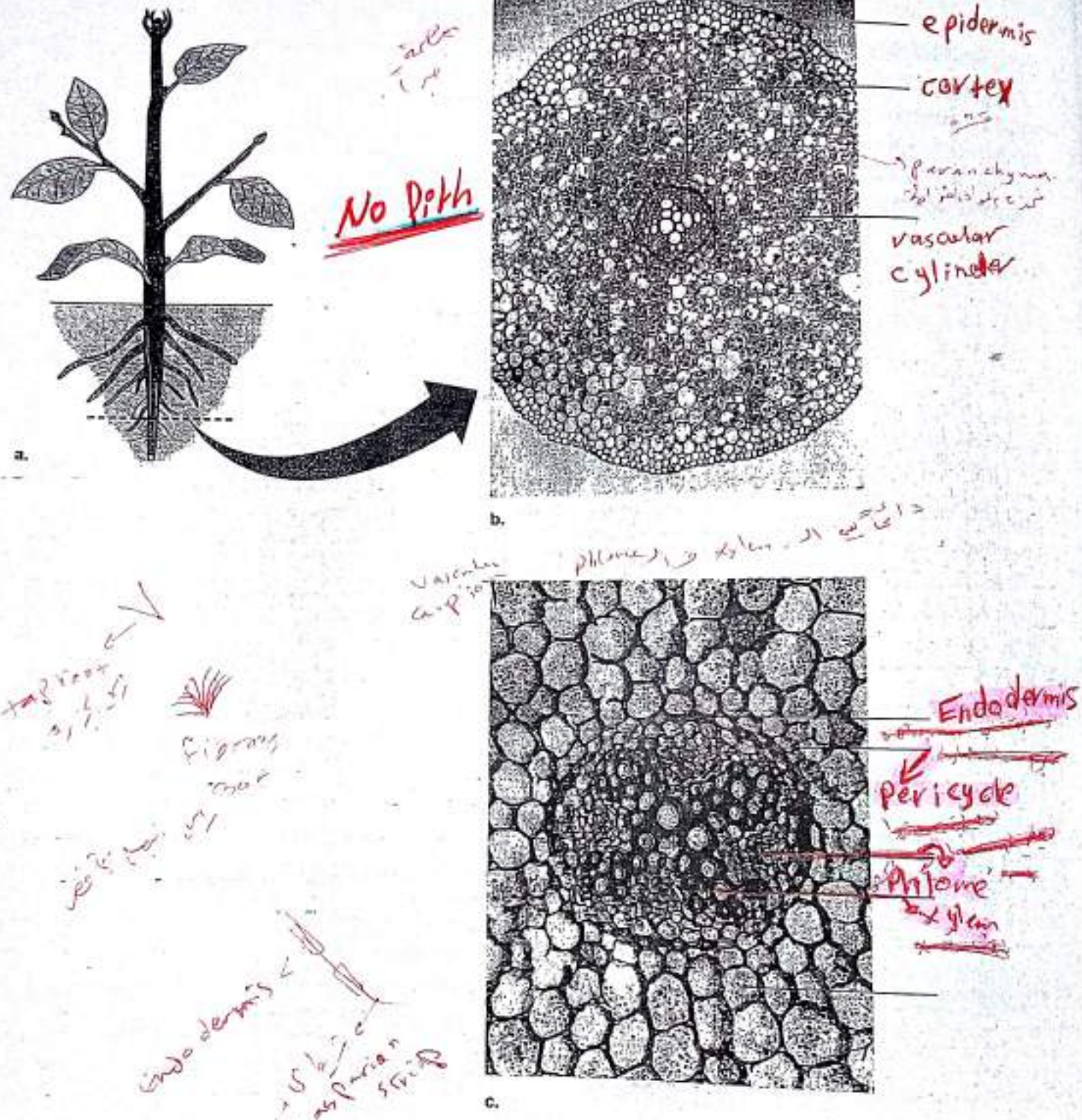


Figure 20.6.
Cross section of the buttercup root. (a) Whole plant. (b) Photomicrograph of a cross section of a root. (c) Enlargement of the vascular cylinder. Label the root based on your observations of a prepared microscope slide.

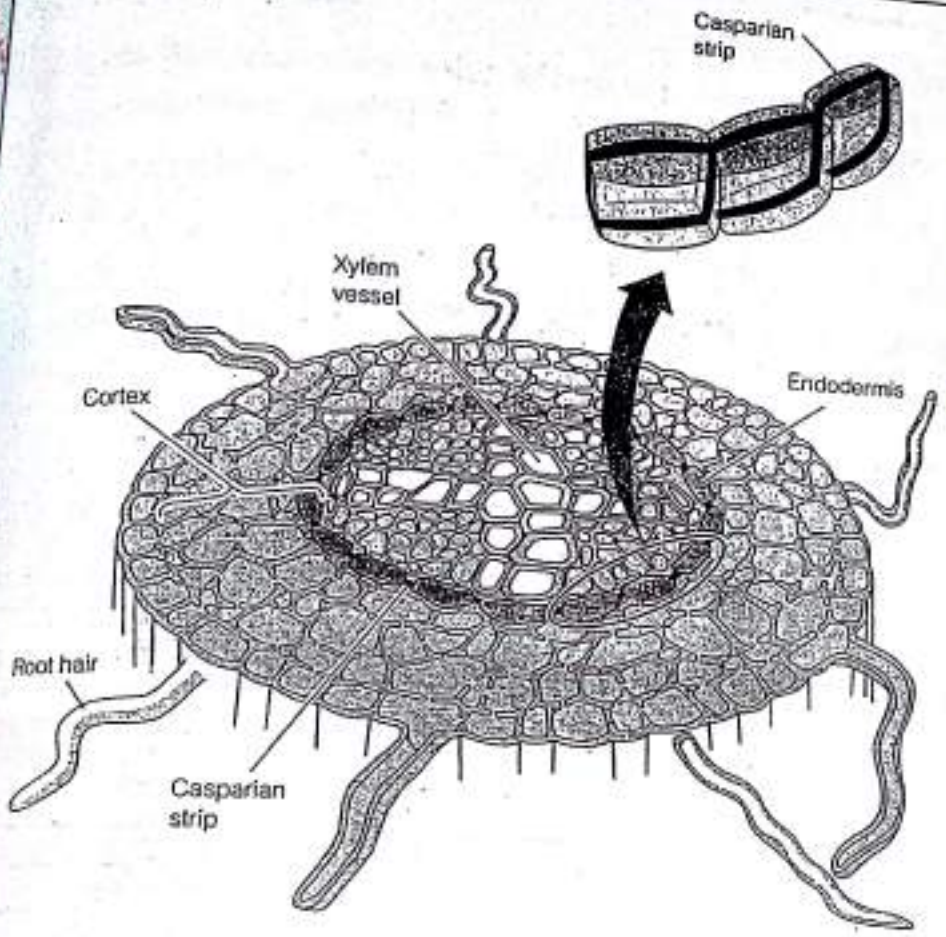


Figure 20.7.
Root endodermis. The endodermis is composed of cells surrounded by a band containing *suberin*, called the *Casparian strip* (seen in enlargement), that prevents the movement of materials along the cells' walls and intercellular spaces into the vascular cylinder. Materials must cross the cell membrane before entering the vascular tissue.

this lab study do you think is related to these cortical cells and their organelles?

c. Identify the following tissues and regions and label Figure 20.6b and 20.6c accordingly: **epidermis**, parenchyma of **cortex**, **vascular cylinder**, **xylem**, **phloem**, **endodermis**, and **pericycle**. The endodermis and the pericycle are unique to roots. The endodermis is the innermost cell layer of the cortex. The walls of endodermal cells have a band called the **Casparian strip**—made of **suberin**, a waxy material—that extends completely around each cell, as shown in Figure 20.7. This strip forms a barrier to the passage of anything moving between adjacent cells of the endodermis. All water and dissolved materials absorbed by the epidermal root hairs and transported inward through the cortex must first pass through the living cytoplasm of endodermal cells before entering the vascular tissues. The pericycle is a layer of dividing cells immediately inside the endodermis; it gives rise to lateral roots. Refer to "Summary of Basic Plant Tissue Systems and Cell Types" and Figure 20.1.

Results

1. Review Figure 20.6 and note comparable structures in Figure 20.7. Highlight the representations of structures or

Lab Study C. Leaves

Materials

prepared slide of lilac (*Syringia*) leaf slides
compound microscope
coverslips
dropper bottles of water
leaves of purple heart (*Setcreasia*) kept in saline and DI water

Introduction

Leaves are organs especially adapted for photosynthesis. The thin blade portion provides a very large surface area for the absorption of light and the uptake of carbon dioxide through stomata. The leaf is basically a layer of parenchyma cells (the **mesophyll**) between two layers of epidermis. The loose arrangement of parenchyma cells within the leaf allows for an extensive surface area for the rapid exchange of gases. Specialized epidermal cells called **guard cells** surround the stomatal opening and allow carbon dioxide uptake and oxygen release, as well as evaporation of water at the leaf surface. Guard cells are photosynthetic (unlike other epidermal cells), and are capable of changing shape in response to complex environmental and physiological factors. Current research indicates that the opening of the stomata is the result of the active uptake of K^+ and subsequent changes in turgor pressure in the guard cells.

In this lab study, you will examine the structure of a leaf in cross section. You will observe stomata on the leaf epidermis and will study the activity of guard cells under different conditions.

Procedure

1. Before beginning your observations of the leaf cross section, compare the shape of the leaf on your slide with Figure 20.8a and 20.8b.
2. Observe the internal leaf structure.
   a. Examine a cross section through a lilac leaf and identify the following cells or structures: **cuticle** (a waxy layer secreted by the epidermis), **epidermis** (upper and lower), parenchyma with chloroplasts (**mesophyll**), **vascular bundle** with **phloem** and xylem, and **stomata** with **guard cells** and **substomatal chamber**. Refer to "Summary of Basic Plant Tissue Systems and Cell Types" and Figure 20.1.
   b. The vascular bundles of the leaf are often called **veins** and can be seen in both cross section and longitudinal sections of the leaf. Observe the structure of cells in the central midvein. Is xylem or phloem on top in the leaf?

4. Observe the distribution of stomata in the upper and lower epidermis. Where are they more abundant?

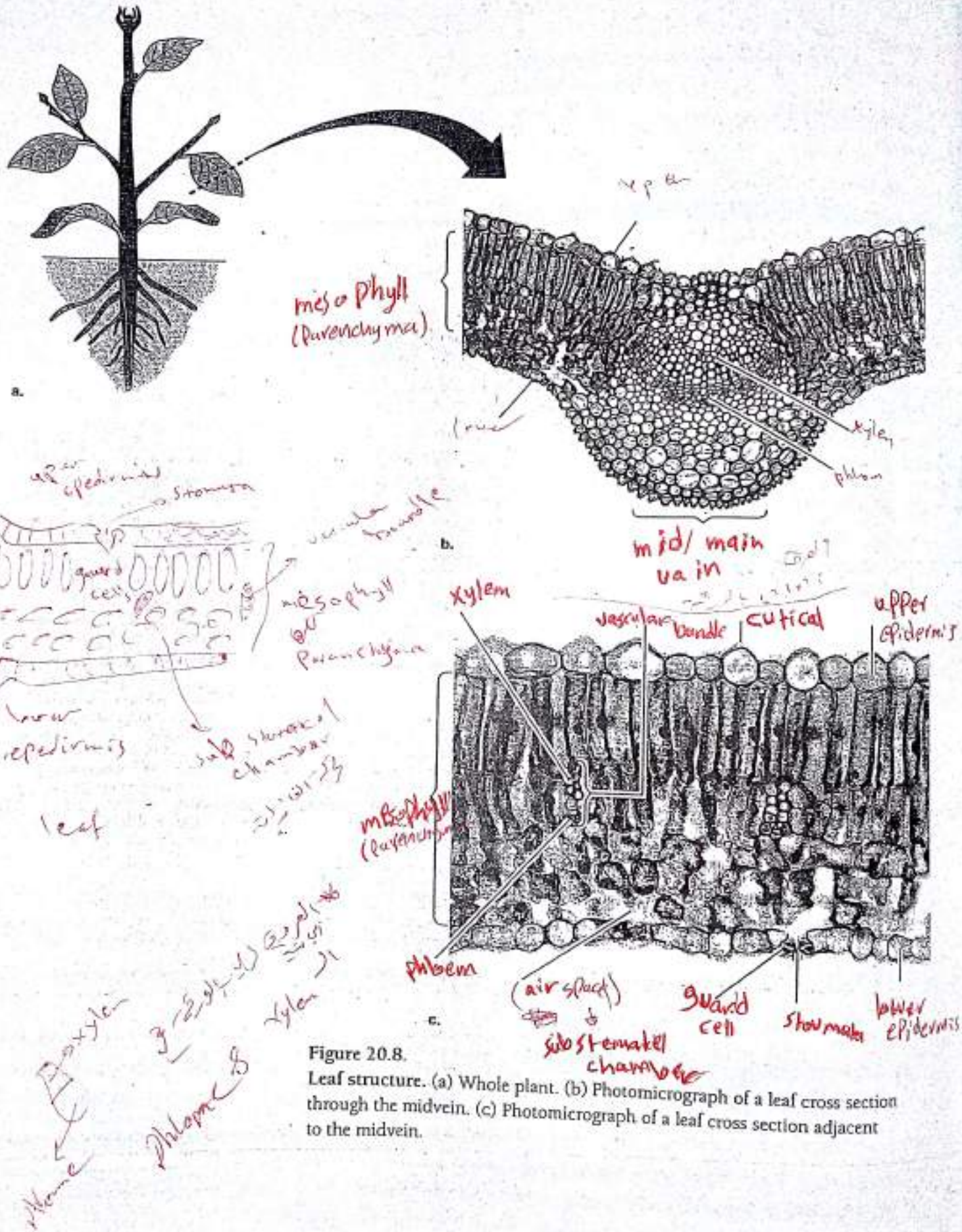


Figure 20.8.
Leaf structure. (a) Whole plant. (b) Photomicrograph of a leaf cross section through the midvein. (c) Photomicrograph of a leaf cross section adjacent to the midvein.

4. Explain the differences observed, if any, between the stomata from leaves kept in DI water and those kept in saline. Utilize your knowledge of osmosis to explain the changes in the guard cells. (In this activity, you stimulated stomatal closure by changes in turgor pressure due to saline rather than K^+ transport.)

EXERCISE 20.4

Cell Structure of Tissues Produced by Secondary Growth

Materials

prepared slides of basswood (*Tilia*) stem
compound microscope

Introduction

Secondary growth arises from meristematic tissue called cambium. Vascular cambium and cork cambium are two types of cambium. The vascular cambium is a single layer of meristematic cells located between the secondary phloem and secondary xylem. Dividing cambium cells produce a new cell at one time toward the xylem, at another time toward the phloem. Thus, each cambial cell produces files of cells, one toward the inside of the stem, another toward the outside, resulting in an increase in stem circumference. The secondary phloem cells become differentiated into sclerenchyma fiber cells, sieve-tube members, and companion cells. Secondary xylem cells become differentiated into tracheids and vessel elements. Certain cambial cells produce parenchyma ray cells that can extend radially through the xylem and phloem of the stem.

The cork cambium is a type of meristematic tissue that divides, producing cork tissue to the outside of the stem and other cells to the inside. The cork cambium and the secondary tissues derived from it are called periderm. The periderm layer replaces the epidermis and cortex in stems and roots with secondary growth. These layers are continually broken and sloughed off as the woody plant grows and expands in diameter.

Procedure

1. Examine a cross section of a woody stem (Color Plate 63).
   a. Observe the cork cambium and periderm in the outer layers of the stem. The outer cork cells of the periderm have thick walls impregnated with a waxy material called **suberin**. These cells are

dead at maturity. The thin layer of nucleated cells that may be visible next to the cork cells is the **cork cambium**. The **periderm** includes the layers of cork and associated cork cambium. The term **bark** is used to describe the periderm and phloem on the outside of woody plants.

b. Observe the cellular nature of the listed tissues or structures, beginning at the periderm and moving inward to the central pith region. **Sclerenchyma fibers** have thick, dark-stained cell walls and are located in bands in the phloem. **Secondary phloem** cells with thin cell walls alternate with the rows of fibers. The **vascular cambium** appears as a thin line of small, actively dividing cells lying between the outer phloem tissue and the extensive secondary xylem. **Secondary xylem** consists of distinctive open cells that extend in layers to the central **pith** region. Lines of parenchyma cells one or two cells thick form **lateral rays** that radiate from the pith through the xylem and expand to a wedge shape in the phloem, forming a **phloem ray**.

2. Note the **annual rings** of xylem, which make up the **wood** of the stem surrounding the pith. Each annual ring of xylem has several rows of **early wood**, thin-walled, large-diameter cells that grew in the spring and, outside of these, a few rows of **late wood**, thick-walled, smaller-diameter cells that grew in the summer, when water is less available.
3. By counting the annual rings of xylem, determine the age of your stem. Note that the phloem region is not involved with determining the age of the tree.

Results

1. Review Figure 20.10 and Color Plate 63.
2. Sketch in the margin of your lab manual any details not represented in the figure that you might need for future reference.
3. Indicate on your diagram the region where primary tissues can still be found.

Discussion

1. What has happened to the several years of phloem tissue production?

Based on your observations of the woody stem, does xylem or phloem ...ctural support for trees?

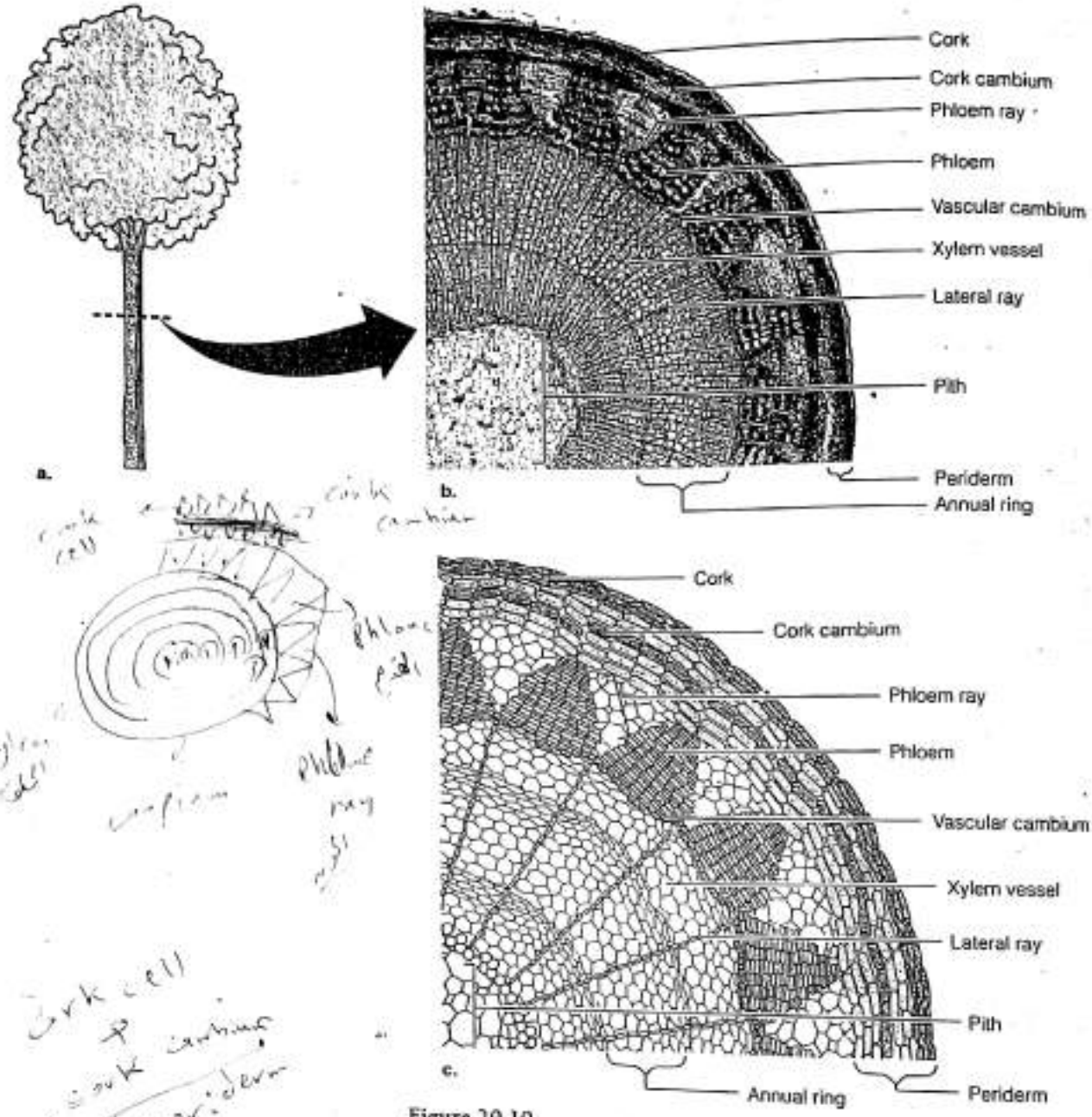


Figure 20.10.
Secondary growth. (a) Whole woody plant. (b) Photomicrograph of a cross section of a woody stem. (c) Compare the corresponding diagram with your observations of a prepared slide. If necessary, modify the diagram to correspond to your specimen (Color Plate 63).

4. How might the structure of early wood and late wood be related to seasonal conditions and the function of the cells? Think about environmental conditions during the growing season.