Introduction to Laboratory Work

The laboratory is a scientist’s workshop—the place where ideas are tested. In the laboratory portion of this course, you will see evidence that supports major biological concepts. To pursue your investigations effectively, you need to learn certain basic techniques, including safe laboratory practices, record keeping, report writing, and measurement. The information on the following pages will help you learn these skills and techniques.

Laboratory Safety

The laboratory can be either safe or dangerous. The difference depends on your knowledge of and adherence to safe laboratory practices. It is important that you read the information here and learn how to recognize and avoid potentially hazardous situations. Basic rules for working safely in the laboratory include the following:

1. Be prepared. Study the assigned investigation before you come to class. Be prepared to ask questions about the procedures you do not understand before you begin to work.
3. Maintain a clean, open work area, free of everything except those materials necessary for the assigned investigation. Store books, backpacks, and purses out of the way. Keep laboratory materials away from the edge of the work surface.
4. Tie back long hair, and remove dangling jewelry. Roll up long sleeves, and tuck long neckties into your shirt. Do not wear loose-fitting sleeves or open-toed shoes in the laboratory.
5. Wear a lab apron and safety goggles whenever working with chemicals, hot liquids, lab burners, hot plates, or apparatus that could break or shatter. Wear protective gloves when working with preserved specimens or toxic or corrosive chemicals or when otherwise directed.
6. Never wear contact lenses while conducting any experiment that uses chemicals. If you must wear them (by a physician’s order), inform your teacher prior to conducting any experiment involving chemicals.
7. Never use direct or reflected sunlight to illuminate your microscope or any other optical device. Direct or reflected sunlight can cause serious damage to your retina.
8. Keep your hands away from the sharp or pointed ends of equipment such as scalpels, dissecting needles, and scissors.
9. Observe all cautions in the procedural steps of the investigation. CAUTION, WARNING, and DANGER are signal words used in the text and on labeled chemicals or reagents that tell you about the potential for injury. They remind you to observe specific practices. Always read and follow these statements.
10. Become familiar with caution symbols, identified in Figure A.
FIGURE A  Caution symbols.

**safety goggles**
Safety goggles are for eye protection. Wear goggles whenever you see this symbol. If you wear glasses, be sure the goggles fit comfortably over them. In case of splashes into the eye, flush the eye (including under the lid) at an eyewash station for 15 to 20 minutes. If you wear contact lenses, remove them immediately and flush the eye as directed. Call your teacher.

**lab apron**
A lab apron is intended to protect your clothing. Whenever you see this symbol, put on your lab apron and tie it securely behind you. If you spill any substance on your clothing, call your teacher.

**gloves**
Wear gloves when you see this symbol or whenever your teacher directs you to do so. Wear them when using any chemical or reagent solution. Do not wear your gloves for an extended period of time.

**sharp object**
Sharp objects can cause injury, either as a cut or a puncture. Handle all sharp objects with caution, and use them only as your teacher instructs you. Do not use them for any purpose other than the intended one. If you do get a cut or puncture call your teacher and get first aid.

**irritant**
An irritant is any substance that, on contact, can cause reddening of living tissue. Wear safety goggles, lab apron, and protective gloves when handling any irritating chemical. In case of contact, flush the affected area with soap and water for a least 15 minutes and call your teacher. Remove contaminated clothing.

**reactive**
These chemicals are capable of reacting with any other substance, including water, and can cause a violent reaction. Do not mix a reactive chemical with any other substance, including water, unless directed to do so by your teacher. Wear your safety goggles, lab apron, and protective gloves.

**corrosive**
A corrosive substance injures or destroys body tissue on contact by direct chemical action. When handling any corrosive substance, wear safety goggles, lab apron, and protective gloves. In case of contact with a corrosive material, immediately flush the affected area with water and call your teacher.

**flammable**
A flammable substance is any material capable of igniting under certain conditions. Do not bring flammable materials into contact with open flames or near heat sources unless instructed to do so by your teacher. Remember that flammable liquids give off vapors that can be ignited by a nearby heat source. Should a fire occur, do not attempt to extinguish it yourself. Call your teacher. Wear safety goggles, lab apron, and protective gloves whenever handling a flammable substance.

**poison**
Poisons can cause injury by direct action within a body system through direct contact (skin), inhalation, ingestion, or penetration. Always wear safety goggles, lab apron, and protective gloves when handling any material with this label. Before handling any poison, inform your teacher if you have preexisting injuries to your skin. In case of contact, call your teacher immediately.

**biohazard**
Any biological substance that can cause infection through exposure is a biohazard. Before handling any material so labeled, review your teacher’s specific instructions. Do not handle in any manner other than as instructed. Wear safety goggles, lab apron, and protective gloves. Any contact with a biohazard should be reported to your teacher immediately.

**NO FOOD OR DRINKS SHOULD BE PRESENT IN THE LAB AT ANY TIME.**
11. Never put anything into your mouth, and never touch or taste substances in the laboratory unless specifically instructed to by your teacher.
12. Never smell substances in the laboratory without specific instructions. Even then, do not inhale fumes directly; wave the air above the substance toward your nose and sniff carefully.
13. Never eat, drink, chew gum, or apply cosmetics in the laboratory. Do not store food or beverages in the lab area.
14. Know the location of all safety equipment, and learn how to use each piece of equipment.
15. If you witness an unsafe incident, an accident, or a chemical spill, report it to your teacher immediately.
16. Use materials only from containers labeled with the name of the chemical and the precautions to be used. Become familiar with the safety precautions for each chemical by reading the label before use.
17. When diluting acid with water, always add acid to water.
18. Never return unused chemicals to the stock bottles. Do not put any object into a chemical bottle except the dropper with which it may be equipped.
19. Clean up thoroughly. Dispose of chemicals, and wash used glassware and instruments according to your teacher’s instructions. Clean tables and sinks. Put away all equipment and supplies. Make sure all water, gas jets, burners, and electrical appliances are turned off. Return all laboratory equipment and supplies to their proper places.
20. Wash your hands thoroughly after handling any living organisms or hazardous materials and before leaving the laboratory.
21. Never perform unauthorized experiments. Do only those experiments assigned by your teacher.
22. Never work alone in the laboratory, and never work without a teacher’s supervision.
23. Approach laboratory work with maturity. Never run, push, or engage in horseplay or practical jokes of any type in the laboratory. Use laboratory materials and equipment only as directed.

In addition to observing these general safety precautions, you need to know about some specific categories of safety. Before you do any laboratory work, familiarize yourself with the following precautions:

Heat
1. Use only the source of heat specified in the investigation.
2. Never allow flammable materials, such as alcohol, near a flame or any other source of ignition.
3. When heating a substance in a test tube, point the mouth of the tube away from other students and yourself.
4. Never leave a lighted lab burner, hot plate, or any other hot object unattended.
5. Never reach over an exposed flame or other heat source.
6. Use tongs, test-tube clamps, insulated gloves, or pot holders to handle hot equipment.
Glassware
1. Never use cracked or chipped glassware.
2. Use caution and proper equipment when handling hot glassware; remember that hot glass looks the same as cool glass.
3. Make sure glassware is clean before you use it and clean when you store it.
4. When putting glass tubing into a rubber stopper, use a lubricant such as glycerine or petroleum jelly on both the stopper and the glass tubing. When putting glass tubing into or removing it from a rubber stopper, protect your hands with heavy cloth. Never force or twist the tubing.
5. Sweep up broken glassware immediately (never pick it up with your fingers), and discard it in a special labeled container for broken glass.

Electrical Equipment and Other Apparatus
1. Before you begin any work, always be sure you learn how to use each piece of apparatus safely and correctly to obtain accurate scientific information.
2. Never use equipment with frayed insulation or with loose or broken wires.
3. Make sure the area under and around electrical equipment is dry and free of flammable materials. Never touch electrical equipment with wet hands.
4. Turn off all power switches before plugging an appliance into an outlet. Never jerk wires from outlets or pull appliance plugs out by the wire.

Living and Preserved Specimens
1. Properly mount and support specimens for dissection. Do not cut a specimen while holding it in your hand.
2. Wash down your work surface with a disinfectant solution both before and after using living microorganisms.
3. Always wash your hands with soap and water after working with live or preserved specimens.
4. Care for animals humanely. General rules are
   a. Always carefully follow your teacher’s instructions concerning the care of laboratory animals.
   b. Provide a suitable escape-proof container in a location where the animal will not be constantly disturbed.
   c. Keep the container clean. Cages of small birds and mammals should be cleaned daily. Provide proper ventilation, light, and temperature.
   d. Provide water at all times.
   e. Feed the animal regularly, depending on its needs.
   f. Treat laboratory animals gently and with kindness in all situations.
   g. If you are responsible for the regular care of any animals, be sure to make arrangements for weekends, holidays, and vacations.
   h. When animals must be disposed of or released, your teacher will provide a suitable method.
5. Many plants or plant parts are poisonous. Work only with the plants specified by your teacher. Never put any plant or plant parts in your mouth.
6. Handle plants carefully and gently. Most plants must have light, soil, and water, although requirements differ.

**Accident Procedures**
1. Report *all* incidents, accidents, injuries, breakages, and spills, no matter how minor, to your teacher.
2. If a chemical spills on your skin or clothing, wash it off immediately with plenty of water and notify your teacher.
3. If a chemical gets into your eyes or on your face, wash immediately at the eyewash station with plenty of water. Wash for at least 15 minutes, flushing the eyes—including under each eyelid. Have a classmate notify your teacher.
4. If a chemical spills on the floor or work surface, do not clean it up yourself. Notify your teacher immediately.
5. If a thermometer breaks, do not touch the broken pieces with your bare hands. Notify your teacher immediately.
6. Smother small fires with a wet towel. Use a blanket or the safety shower to extinguish clothing fires. Always notify your teacher.
7. Report all cuts and abrasions (no matter how small) received in the laboratory to your teacher.

**Chemical Safety**
All chemicals are hazardous in some way. A hazardous chemical is defined as a substance that is likely to cause injury. Chemicals can be placed in four hazard categories: flammable, corrosive, toxic, and reactive.

In the laboratory investigations for this course, every effort is made to minimize the use of dangerous materials. However, many “less hazardous” chemicals can cause injury if not handled properly. The following information will help you become aware of the types of chemical hazards that exist and of how you can reduce the risk of injury when using chemicals. Be sure also to review the basic safety rules described previously before you work with any chemical.

**Flammable/Combustible Substances.** Flammable/combustible substances are solids, liquids, or gases that will sustain burning. The process of burning involves three interrelated components—fuel (any substance capable of burning), oxidizer (often air or a specific chemical), and ignition source (a spark, flame, or heat). The three components are represented in Figure B. For burning to occur, all three components (sides) of the fire triangle must be present. To control a fire hazard, you must remove, or otherwise make inaccessible, at least one side of the fire triangle. Flammable chemicals should not be used in the presence of ignition sources such as lab burners, hot plates, and sparks from electrical equipment or static electricity. Containers of flammables should be closed when not in use. Sufficient ventilation in the laboratory will help keep the concentration of flammable vapors to a minimum. Wearing safety goggles, lab aprons, and protective gloves are important precautionary measures when using flammable/combustible materials.
Toxic Substances. Most of the chemicals in a laboratory are toxic, or poisonous to life. The degree of toxicity depends on the properties of the specific substance, its concentration, the type of exposure, and other variables. The effects of a toxic substance can range from minor discomfort to serious illness or death. Exposure to toxic substances can occur through ingestion, skin contact, and inhaling vapors. Wearing a lab apron, safety goggles, and protective gloves are important precautions when using toxic chemicals. A clean work area, prompt spill cleanup, and good ventilation also are important.

Corrosive Substances. Corrosive chemicals are solids, liquids, or gases that by direct chemical action either destroy living tissue or cause permanent change in the tissue. Corrosive substances can destroy eye and respiratory-tract tissues, causing impaired sight or permanent blindness, severe disfigurement, permanent severe breathing difficulties, and even death. Lab aprons, safety goggles, and protective gloves should be worn when handling corrosive chemicals to prevent contact with the skin or eyes. Splashes on the skin or in the eye should be washed off immediately while a classmate notifies your teacher.

Reactive Substances. Reactive chemicals promote violent reactions under certain conditions. A chemical may explode spontaneously or when mechanically disturbed. Reactive chemicals also include those that react rapidly when mixed with another chemical, releasing a large amount of energy. Keep chemicals separate from each other unless they are being combined according to specific instructions in an investigation. Heed any other cautions your teacher may give you. Always wear your lab apron, safety goggles, and protective gloves when handling reactive chemicals.

Record Keeping
Science deals with verifiable observations. No one—not even the original observer—can trust the accuracy of a confusing, indefinite, or incomplete observation. Scientific record keeping requires clear and accurate records made at the time of observation.
The best method of keeping records is to jot them down in a logbook. It should be a hardcover book, permanently bound (not loose-leaf).

Keep records in diary form, recording the date first. Keep observations of two or more investigations separate. Data recorded in words should be notes that are brief but to the point. Complete sentences are not necessary, but single words are seldom descriptive enough to represent accurately what you have observed.

You may choose to sketch your observations. A drawing often records an observation more easily, completely, and accurately than words can. Your sketches need not be works of art. Their success depends on your ability to observe, not on your artistic talent. Keep the drawings simple, use a hard pencil, and include clearly written labels.

Data recorded numerically as counts of measurements should include the units in which the measurements are made. Often numerical data are most easily recorded in a table.

Do not record your data on other papers to copy into the logbook later. Doing so might increase neatness, but it will decrease accuracy. Your logbook is your record, regardless of the blots and stains that are a normal circumstance of field and laboratory work.

You will do much of your laboratory work as a member of a team. Your logbook, therefore, will sometimes contain data contributed by other members of your team. Keep track of the source of observations by circling (or recording in a different color) the data reported by others.

**Writing Laboratory Reports**

Discoveries become a part of science only when they are made known to others. Communication, therefore, is an important part of science. In writing, scientists must express themselves so clearly that another person can repeat their procedures exactly. The reader must know what material was used (in biology this includes the species of organism) and must understand every detail of the work. Scientific reports frequently are written in a standard form as follows:

1. Title
2. Introduction: a statement of how the problem arose, often including a summary of past work
3. Materials and equipment: usually a list of all equipment, chemicals, specimens, and other materials
4. Procedure: a complete and exact account of the methods used in gathering data
5. Results: data obtained from the procedure, often in the form of tables and graphs
6. Discussion: a section that demonstrates the relationship between the data and the purpose of the work
7. Conclusion: a summary of the meaning of the results, often suggesting further work that might be done
8. References: published scientific reports and papers that you have mentioned specifically

Your teacher will tell you what form your laboratory reports should take for this course. Part of your work may include written answers to the Analysis questions at the end of each investigation. In any event, the material in your logbook is the basis for your reports.

**Measurement**

Measurement in science is made using the System International d’Unités (international system of units), more commonly referred to as SI. A modification of the older metric system, it was used first in France and now is the common system of measurement throughout the world.

Among the basic units of SI measurement are the meter (length), the kilogram (mass), the kelvin (temperature), and the second (time). All other SI units are derived from these four. Units of temperature that you will use in this course are degrees Celsius, which are equal to kelvins.

SI units for volume are based on meters cubed. In addition, you will use units based on the liter for measuring volumes of liquids. Although not officially part of SI, liter measure is used commonly. The liter is a metric measurement (1 L = 0.001 m$^3$). It is equivalent to 1,000 cubic centimeters (cc).

Below are some of the SI units derived from the basic units for length, mass, volume, and temperature. Note, especially, those units described as most common to your laboratory work.

**Length**

1 kilometer (km) = 1,000 meters
1 hectometer (hm) = 100 meters
1 dekameter (dkm) = 10 meters
1 meter (m)
1 decimeter (dm) = 0.1 meter
1 centimeter (cm) = 0.01 meter
1 millimeter (mm) = 0.001 meter
1 micrometer ($\mu$m) = 0.000001 meter
1 nanometer (nm) = 0.000000001 meter

Measurements under microscopes often are made in micrometers (also called microns). Still smaller measurements, as for wavelengths of light used by plants in photosynthesis, are made in nanometers. The unit of length you will use most frequently in the laboratory is centimeter (cm).

Units of area are derived from units of length by multiplication. One hectometer squared is a measure often used for ecological studies; it is commonly called a hectare and equals 10,000 m$^2$. Measurements of area made in the laboratory most frequently will be in centimeters squared (cm$^2$).

Units of volume are also derived from units of length by multiplication. One meter cubed (m$^3$) is the standard, but it is too large for practical use in the laboratory. Centimeters cubed (cm$^3$) are the more common units you will
see. For the conditions you will encounter in these laboratory investigations, 1 cm³ measures a volume equal to 1 mL.

**Mass**
1 kilogram (kg) = 1,000 grams
1 hectogram (hg) = 100 grams
1 dekagram (dkg) = 10 grams
1 gram (g)
1 decigram (dg) = 0.1 gram
1 centigram (cg) = 0.01 gram
1 milligram (mg) = 0.001 gram
1 microgram (μg) = 0.000001 gram
1 nanogram (ng) = 0.000000001 gram

Measurements of mass in your biology laboratory usually will be made in kilograms, grams, centigrams, and milligrams.

**Volume**
1 kiloliter (kL) = 1,000 liters
1 hectoliter (hL) = 100 liters
1 dekaliter (dkL) = 10 liters
1 liter (L)
1 centiliter (cL) = 0.01 liter
1 milliliter (mL) = 0.001 liter

Your measurements in the laboratory will usually be made in milliliters and liters.

**Temperature**
On the Celsius scale, 0ºC is commonly known as the freezing point of water and 100ºC is the boiling point of water. (Atmospheric pressure affects both of these temperatures.) Figure C illustrates the Celsius scale alongside the Fahrenheit scale, which is still used in the United States. On the Fahrenheit scale, 32ºF is the freezing point of water and 212ºF is the boiling point of water. The figure is useful in converting from one scale to the other.

**Concentration**
Another type of measurement you will encounter in this course is molarity (labeled with the letter M). Molarity measures the concentration of a dissolved substance in a solution. A high molarity indicates a high concentration. The solutions you will use in the investigations frequently will be identified by their molarity.

If you wish to learn more about SI measure, write to the U.S. Department of Commerce, National Institute of Standards and Technology (NIST), Washington, DC 20234.
Investigations for the Prologue
Biology and the Molecular Perspective

Investigation PA ◆ Analyzing Ethical Issues

Biological Challenges in the prologue describes bioethics as the “application of ethics to biological issues.” Ethical analysis is a process of critical inquiry. There are various models for conducting such inquiries; this investigation introduces you to one such model, which focuses on goals, rights, and duties. You will apply this model to a case study that involves the use of biosynthetic human growth hormone (GH).

Materials (per person)
paper and pen

Procedure
1. Read the following case study:
   Sharon has just completed ninth grade at Deerfield Junior High School in Iowa. She attended summer basketball camp for the past 3 years and already is one of the best players in the state. Deerfield’s girl’s basketball team has won two district championships, and Sharon—a forward—has been the leading scorer. It is clear she has great potential both as a high school and college player.

   Sharon and her coaches, however, are concerned about her height. She is only 5 feet 7 inches (170 cm) tall. Sharon’s coaches want to develop her skills through high school and would like her to attain as much height as possible. She is not likely to gain much more height naturally. Sharon and her parents have discussed her problem with an endocrinologist and asked that he provide her with biosynthetic GH. Thus far he has refused, saying that GH is used only to treat children who are abnormally short. The biotech company that sells the hormone recommends that it be prescribed only to children who are among the shortest 3% in the population. Furthermore, a deficiency of GH is not the only reason for being short of stature. The doctor maintains that Sharon is not handicapped and should not risk the possible side effects of GH when she does not need the hormone for a legitimate medical reason. Although the consequences of receiving extra GH are not fully understood, there is reason for concern. Mice that received extra GH attained twice their normal size and appeared to be otherwise healthy. Pigs, on the other hand, did not fare as well. Most pig embryos that received genes for GH did not survive, and of those that did, many were arthritic, had vision problems, and displayed deficient immune systems. The potential side effects for humans, which are irreversible, include the development of diabetes and heart problems and elongation of the facial bones, hands, and feet.

   Sharon has told the doctor that she can get GH on the black market and will do so if he does not prescribe it for her. She prefers to have the hormone administered under the doctor’s supervision because the hormone will be pure and he can monitor her progress. What should the doctor do?

2. Read the following information about goals, rights, and duties.

   Goals: One way to judge the morality of an action is by looking at what it intends to accomplish. If this view is the basis for determining whether an action is morally correct, then a “good” outcome may be judged morally correct no matter how the outcome is achieved. Assume, for example, that a given physician’s primary goal is the preservation of life. She might then refuse to disconnect a respirator that is keeping a terminally ill patient alive, even if the patient or the patient’s family wishes her to do so. In her view, going against the rights of the patient is justified by her goal of the preservation of life.

   Rights: Moral arguments based on rights are familiar to all of us. Our Constitution, for example, guarantees the right to free speech, the right to religious freedom, and the right to trial by a jury of one’s peers. Most physicians agree that a patient has the right to know all of the relevant information about a given treatment. Consider a situation in which the physician knows that a patient will refuse lifesaving
treatment if the patient knows all of the potential side effects. Should the physician violate the patient’s right to the information to further the physician’s goal of the preservation of life?

Duties: Some moral arguments are based on the obligation, or duty, to act in a certain way. For example, we generally have a duty to tell the truth, keep a promise, or help a friend. The justification for a duty often is based on the achievement of a worthy goal or on the basis of someone’s right. Duties, therefore, can be derived from goals or rights, but they can also be in conflict with goals or rights. Suppose, for example, a dying man asks a physician not to prolong his life. Does the physician have a duty to respect the man’s right to die? Or does the physician have a duty to pursue his own goal of the preservation of life?

3. Work in teams of three or four to discuss the case study of Sharon and her desire to use GH to increase her height. List the goals, rights, and duties for Sharon, the doctor, Sharon’s parents, and the basketball coaches in this situation. When you have completed your discussion, your teacher will compile a list of the responses from each group so you can discuss the Analysis questions as a class.

Analysis
1. Where are the major conflicts in the goals, rights, and duties for Sharon, the doctor, Sharon’s parents, and the basketball coaches?
2. Should the doctor prescribe GH for Sharon?
3. Is there any additional scientific information that would affect your ethical analysis?
4. Can you think of reasons for short stature besides a lack of GH?
5. What are the most important justifications for the position you have chosen?
6. Two U.S. companies now produce GH for distribution through prescription and under a physician’s supervision. What are the goals, rights, and duties of these companies with respect to production and distribution of GH?
7. Is the recommendation that GH be administered to the shortest 3% of the population appropriate? Why or why not?
8. Who should determine the population eligible for receiving GH?
9. Is Sharon handicapped?
10. Assuming that GH is completely safe, would you take it?

Investigation PB ◆ Scientific Observation

Careful observation is important in any science. The results you obtain from an experiment must be replicable. Detailed observations and notes not only help identify possible areas of error, but also allow others to duplicate, and thus verify, your work. This investigation is an exercise in observation.

Sometimes careful observation is needed to distinguish one species from another, or even one sex from another within the same species. In this investigation, you will examine a mixed population of male and female fruit flies and attempt to separate them into two groups based on their sex (Figure PB.1). It is not expected that you will know which group corresponds to males and which group corresponds to females. The challenge is to develop observational criteria that will allow not only you but others to accurately distinguish between the sexes of fruit flies.

Materials (per team of 3)
- hand lens
- metric ruler
- small paintbrush
- mixture of male and female fruit flies (containing an equal number of each sex)

Procedure
1. Your team will be given a petri dish containing an equal number of male and female fruit flies. As you observe individual flies, use the paintbrush to gently move them about the dish.

FIGURE PB.1
What observations best describe the sex differences in fruit flies?
2. Use the hand lens and ruler to measure the length of each fly to the nearest 0.5 mm, and record it in your logbook. Observe each fly carefully, and record all of your observations in your logbook. Describe the shapes and colors of the major body parts (or sketch them).

3. When you have recorded as many observations as you can, discuss with your lab partners which observations allow you to separate the flies into two equal groups.

4. Recombine the two groups of flies, and use your agreed-upon criteria to re-sort the flies. If your criteria do not sort the flies into two equal groups, refine your observations so that you can clearly sort each fly into one of the two groups.

5. When you and your lab partners are satisfied with your sexing criteria, your teacher will give you instructions for another part to this exercise. It will be a realistic test of how careful you were in your observations and note taking.

Analysis

1. What were the different ways you found to distinguish male from female fruit flies?
2. What proved to be the most helpful information in distinguishing the sexes of fruit flies?
3. What percentage of your class could reproducibly sort their flies into two groups?
4. What percentage of your class could use another team’s criteria to accurately sort flies by sex?
5. What steps could be taken to improve both percentages?
6. People often confuse observations with inferences. Observations are collected on the scene, using your senses. Inferences are ideas or conclusions based on what you observe or already know. Based on this distinction, which of the following statements are observations and which are inferences?
   - The male flies have bristles (sex combs) on their front legs.
   - Both groups of flies have red eyes.
   - One group has a striped abdomen.
   - The female’s abdomen becomes expanded prior to egg laying.

7. Look at your notes and label any inferences that you included.

Investigation PC ◆ The Compound Microscope

The human eye cannot distinguish objects much smaller than 0.1 mm. The microscope is a tool that extends vision and allows observation of much smaller objects. The most commonly used compound microscope (Figure PC.1) is monocular (one eyepiece). Light reaches the eye after passing through the objects to be examined.

In this investigation, you will learn how to use and care for a microscope.

Materials (per team of 2)
3 coverslips
dropping pipette
3 microscope slides
compound microscope
newspaper
scissors
transparent metric ruler
prepared slide of colored threads

Procedure

PART A  Care of the Microscope
1. The microscope is a precision instrument that requires proper care. Always carry the microscope with both hands—one hand under its base, the other on its arm.
2. When setting the microscope on a table, keep it away from the edge. If a lamp is attached to the microscope, keep its wire out of the way. Keep everything not needed for microscope studies off your lab table.
3. Avoid tilting the microscope when using temporary slides made with water.
4. The lenses of the microscope cost almost as much as all the other parts put together. Never
clean lenses with anything other than the lens paper designed for this task.

5. Before putting away the microscope, *always* return it to the low-power setting. The high-power objective reaches too near the stage to be left in place safely.

PART B  Setting Up the Microscope

6. Rotate the low-power objective into place if it is not already there. When you change from one objective to another, you will hear a click as the objective sets into position.

7. Move the mirror so that even illumination is obtained through the opening in the stage, or turn on the substage lamp. Most microscopes are equipped with a diaphragm for regulating light. Some materials are best viewed in dim light; others, in bright light. Remember that direct sunlight can damage eyes. If you use natural light as your light source, do not reflect direct sunlight through the diaphragm.

8. Make sure that the lenses are dry and free of fingerprints and debris. Wipe lenses with lens paper only.

PART C  Using the Microscope

9. In your logbook, prepare a table like Table PC.1.

<table>
<thead>
<tr>
<th>Object being viewed</th>
<th>Observations and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter o</td>
<td></td>
</tr>
<tr>
<td>Letter c</td>
<td></td>
</tr>
<tr>
<td>Etc.</td>
<td></td>
</tr>
<tr>
<td>Millimeter ruler</td>
<td></td>
</tr>
</tbody>
</table>

10. Cut a lowercase o from a piece of newspaper. Place it right side up on a clean slide. With a dropping pipette, place one drop of water on the letter. This type of slide is called a wet mount.

⚠️ CAUTION: Scissors are sharp. Handle with care.

11. Wait until the paper is soaked before adding a coverslip. Hold the coverslip at about a 45% angle to the slide, and slowly lower it. Figure PC.2 shows these first steps.
12. Place the slide on the microscope stage, and clamp it down. Move the slide so the letter is in the middle of the hole in the stage. Use the coarse-adjustment knob to lower the low-power objective to the lowest position.

13. Look through the eyepiece, and use the coarse-adjustment knob to raise the objective slowly until the letter o is in view. Use the fine-adjustment knob to sharpen the focus. Position the diaphragm for the best light. Compare the way the letter looks through the microscope with the way it looks to the naked eye.

14. To determine how greatly magnified the view is, multiply the number inscribed on the eyepiece by the number on the objective being used. For example, eyepiece (10×) × objective (10×) = total magnification (100×).

15. Follow the same procedure with a lowercase c. In your logbook, describe how the letter looks when viewed through a microscope.

16. Make a wet mount of the letter e or the letter r. Describe how the letter looks when viewed through the microscope. What new information (not revealed by the letter c) is revealed by the e or r?

17. Look through the eyepiece at the letter as you use your thumbs and forefingers to move the slide away from you. Which way does your view of the letter move? Move the slide to the right. In which direction does the image move?

18. Make a pencil sketch of the letter as you see it under the microscope. Label the changes in image and movement that occur under the microscope.

19. Make a wet mount of two different-colored hairs, one light and one dark. Cross one hair over the other. Position the slide so that the hairs cross in the center of the field. Sketch the hairs under low power; then go to Part D.

PART D  Using High Power

20. With the crossed hairs centered under low power, adjust the diaphragm for the best light.

21. Turn the high-power objective into viewing position. Do not change the focus.

22. Sharpen the focus with the fine-adjustment knob only. Do not focus under high power with the coarse-adjustment knob.

23. Readjust the diaphragm to get the best light. If you are not successful in finding the object under high power the first time, return to step 20 and repeat the whole procedure carefully.

24. Using the fine-adjustment knob, focus on the hairs at the point where they cross. Can you see both hairs sharply at the same focus level? How can you use the fine-adjustment knob to determine which hair crosses over the other? Sketch the hairs under high power.

25. Remove the wet mount of the hairs, and replace it with the prepared slide of the colored threads. The prepared slide contains three colored threads that overlap in a specific order.

26. Focus the threads under low power, and adjust the diaphragm for best light.

27. Turn the high-power objective into viewing position. Do not change the focus.

28. Sharpen the focus with the fine-adjustment knob only.

29. Readjust the diaphragm to get the best light. If you are not successful in finding the threads under high power, return to step 26 and repeat the procedure.

30. Using the fine-adjustment knob, focus on an area where the threads overlap. Use the fine-adjustment knob to determine the order in which the colored threads lie on the slide.

PART E  Measuring with a Microscope

31. Because objects examined with a microscope usually are small, biologists use units of length smaller than centimeters or millimeters for microscopic measurement. One such unit is the micrometer, which is 1/1,000 of a millimeter. The symbol for micrometer is μm, the Greek letter μ (called mu) followed by m.

32. You can estimate the size of a microscopic object by comparing it with the size of the circular field of view. To determine the size of the field, place a plastic metric ruler on the stage. Use the low-power objective to obtain a clear image of the divisions on the ruler. Carefully move the ruler until its marked edge passes through the exact center of the field of view. Count the number of divisions that you can see in the field of view. The marks on the
ruler will appear quite wide; 1 mm is the distance from the center of one mark to the center of the next. Record the diameter, in millimeters, of the low-power field of your microscope.

33. Remove the plastic ruler, and replace it with the wet mount of the letter e. (If the wet mount has dried, lift the coverslip and add water.) Using low power, compare the height of the letter with the diameter of the field of view. Estimate as accurately as possible the actual height of the letter in millimeters.

Analysis
1. Summarize the differences between an image viewed through a microscope and the same image viewed with the naked eye.
2. When viewing an object through the high-power objective, not all of the object may be in focus. Explain your answer.
3. What was the order of the overlapping colored threads in step 30?
4. What is the relationship between magnification and the diameter of the field of view?
5. Calculate the diameter in micrometers of your high-power field. Use the following equations:

\[
\frac{\text{magnification number of high-power objective}}{\text{magnification number of low-power objective}} = A
\]

\[
\frac{\text{diameter of low-power field of view}}{A} = \text{diameter of high-power field of view}
\]

For example, if the magnification of your low-power objective is 12× and that of your high-power objective is 48×, \( A = 4 \). If the diameter of the low-power field of view is 1,600 μm, the diameter of the high-power field of view is 1,600 μm + 4, or 400 μm.

7. Use your sketch of the hairs under high power and the diameter of your high-power field calculated above to estimate the diameter of your human hair.

Investigation PD ◆ Developing Concept Maps

As you study biology, you will be exposed to many new ideas and processes. At times the amount of information and the number of new words can seem overwhelming. There is, however, a method you can use to manage this new information. Concept maps are tools that can help you organize and review ideas in a way that emphasizes the relationships among ideas. Ideas are much easier to learn and remember once you understand how they are related to one another.

Concept maps are constructed using ideas, objects, processes, and actions as the concept words. Other words that explain the relationship between two concepts are the linking words. For example, look at the simple concept map in Figure PD.1 of the information about AIDS presented in Section P2.

The words that appear in the boxes are the concepts. Notice that the concepts become increasingly specific as you travel down the map. The relationships between the concepts are shown by the connecting lines. The words that appear with the lines are the linking words that describe the relationships among the concepts.

Examine a second concept map of the AIDS discussion in Figure PD.2. Notice that this map makes different connections between concepts.

There is no single, correct concept map for a body of information. Each map may be different and may emphasize different concepts. In the second map, notice that some of the connecting lines do not follow the downward trend. These lines are cross-
linkages. They describe additional, and perhaps more complex, relationships. You can follow a few simple steps that will help you build your own concept maps.

1. Identify and list the major concepts you want to map.
2. Decide which idea is the main concept. Group similar remaining concepts together, and rank them with each group from the more general to the most specific.
3. Choose linking words that identify the relationships between the concepts. Be sure that the linking words are not concepts themselves.
4. Begin constructing your map by branching one or two concepts from your major concept. Add the remaining more specific concepts as you progress. Be sure to look for opportunities to establish cross-linkages.

Materials (per team of 3)
pictures of laboratory equipment
3 pairs of scissors

PART A Laboratory Equipment

Procedure
You will work in cooperative teams during this investigation, which means you must share your information to build a complete concept map. Each of you has received only part of a set of pictures. You will need to build a map of your set of pictures and then work with your teammates to construct a team map for all the pictures.

1. Cut out each individual picture of equipment from the sheet you receive.
2. Group the pieces of equipment that are somehow similar.
3. Subdivide the groups according to more specific characteristics. For example, the microscope is larger than the slides, and the slides are larger than the coverslips.
4. Using laboratory equipment as your main concept, arrange the pictures on a sheet of paper in the groups you have determined.
5. Link the pictures with linking words. For example, laboratory equipment may be glass or may be metal.
6. Try to establish some cross-linkages in your map. For example, glass items can be used with metal items.
7. Once you have completed your own map, work with your teammates to construct a team map of all the items, still using laboratory equipment as your main concept. Your teacher will assign you a job to do during the construction of the team map: The checker will make sure all concepts have been included and determine whether all team members agree on the structure of the map. The recorder will draw the map and make changes and corrections suggested by the team. The arranger will make sure linking words are accurate and that cross-linkages have been identified.
8. Once you have completed your map, your teacher will randomly select one member of your team to explain your map to the class. Any teammate may be called on, so all members must be able to explain the team’s work.

Analysis
1. Compare your map with your classmates’ maps. How is your concept map different?
2. Which concept map is easiest for you to understand?
3. What did members of your team do that worked well? What did not work well? What problems did you encounter? How did you solve those problems?

4. What would you do differently next time you work in a group?

PART B Evolution

9. Working with your teammates, use the concepts and linking words listed here to complete the concept map for evolution in Figure PD.3, based on Sections P5 and P6. (Linking words can be used more than once.) Add other concepts to the map if you wish.

**Concept Words**
- evolution
- natural selection
- observations
- predictions
- acquired characteristics
- Darwin
- Lamarck
- theory
- diversity
- variation

**Linking Words**
- is a
- developed by
- based on
- lead to
- accounts for
- does not account for
- that can make
- based on
- from
- affects
- results in
- may be
- may include
- as
- such as

**Analysis**

1. Compare the concept maps you drew in Parts A and B. Do their characteristics differ? If so, how?

2. Compare your own concept maps for Part B with the maps of other teams. How are the maps different? How are they the same?

PART C Science and Pseudoscience

10. Construct a concept map dealing with science and pseudoscience based on Section P8 in the text.

11. Use the following concepts and linking words. You may add additional concepts and linking words as you need them.

**Concept Words**
- theory
- hypothesis
- data
- observation

**Linking Words**
- may be
- based on
- may include
- such as

**Analysis**

1. Compare your concept map for Part C with the maps of other teams. How are they similar? How are they different?

Investigations for Chapter 1
*The Chemistry of Life*

**Investigation 1A ◆ Organisms and pH**

Individual organisms and cells must maintain a relatively stable internal environment, but many
factors can affect the stability—for example, the relative concentrations of hydrogen ions (H+) and hydroxide ions (OH–). The biochemical activities of living tissues frequently affect pH, yet life depends on maintaining a pH range that is normal for each tissue or system. Using a pH meter or wide-range pH paper, you can compare the responses of several materials to the addition of an acid and a base.

**Hypothesis:** Before you begin, study the investigation and develop a hypothesis that addresses the question “How do organisms survive and function despite metabolic activities that tend to shift pH toward either acidic or basic ends of the scale?”

**Materials** (per team of 4)
- 4 pairs of safety goggles
- 4 lab aprons
- 50-mL beaker or small jar
- 50-mL graduated cylinder
- 3 colored pencils
- pH meter or wide-range pH paper
- forceps
- tap water
- HCl (0.1 M) in dropping bottle
- NaOH (0.1 M) in dropping bottle
- sodium phosphate pH 7 buffer solution
- liver homogenate
- potato homogenate
- egg white (diluted 1:5 with water)
- warm gelatin suspension (2%)

**Procedure**
1. In your logbook, prepare a table similar to Table 1A.1, or tape in the table provided by your teacher.
2. Pour 25 mL of tap water into a 50-mL beaker.
3. Record the initial pH by using a pH meter, or use forceps to dip small strips of pH paper into the water and compare the color change to a standard color chart.
4. Add 0.1 M HCl a drop at a time. Gently swirl the mixture after each drop. Determine the pH after 5 drops have been added. Repeat this procedure until 30 drops have been used. Record the pH measurements in your table.

   **CAUTION:** 0.1 M HCl is a mild irritant. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher.
5. Rinse the beaker thoroughly, and pour into it another 25 mL of tap water. Record the initial pH of the water, and add 0.1 M NaOH drop by drop, recording the pH changes in exactly the same way as for the 0.1 M HCl.

| TABLE 1A.1
Testing pH |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests with 0.1 M HCl</strong></td>
<td><strong>Tests with 0.1 M NaOH</strong></td>
<td></td>
</tr>
<tr>
<td>Solution Tested</td>
<td>pH after addition of 0</td>
<td>5</td>
</tr>
<tr>
<td>Tap water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg white</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CAUTION: 0.1M NaOH is a mild irritant. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher.

6. Using the biological material assigned by your teacher, repeat steps 2–5. Record the data in your table.

7. Test the buffer solution (a nonliving chemical solution) using the same method outlined in steps 2–5. Record the data in your table.

8. Wash your hands thoroughly before leaving the laboratory.

Analysis

1. Summarize the effects of HCl and NaOH on tap water.

2. What was the total pH change for the 30 drops of HCl added to the biological material? for the 30 drops of NaOH added? How do these data compare with the changes in tap water?

3. In your logbook, prepare a simple graph of pH versus the number of drops of acid and base solutions added to tap water. Plot two lines—a solid line for changes with acid and a dashed line for changes with base. Using different colored solid and dashed lines, add the results for your biological material. Compare your graph to the graphs of teams who used a different biological material. What patterns do the graphs indicate for biological materials?

4. How do biological materials respond to changes in pH?

5. Use different colored solid and dashed lines to plot the reaction of the buffer solution on the same graph. How does the buffer system respond to the HCl and NaOH?

6. Is the pH response of the buffer system more like that of water or of the biological material?

7. How does the reaction of the buffer solution serve as a model for the response of biological materials to pH changes?

8. Would buffers aid or hinder the maintenance of a relatively stable environment within a living cell in a changing external environment?

9. What does the model suggest about a mechanism for regulating pH in an organism?

SAFETY Put on your safety goggles, lab apron, and gloves. Tie back long hair.

Investigation 1B  Compounds of Living Things

The compounds your body needs for energy and growth are carbohydrates, proteins, fats, vitamins, and other nutrients. These compounds are present in the plants and animals you use as food. In this investigation, you will observe tests for specific compounds and then use those tests to determine which compounds are found in ordinary foods.

Materials (per team of 4)
- 4 pairs of safety goggles
- 4 lab aprons
- 4 pairs of plastic gloves
- 250-mL beaker
- 10-mL graduated cylinder
- 6 18-mm × 150-mm test tubes
- test-tube clamp
- test-tube rack
- hot plate
- Benedict’s solution in dropping bottle
- Biuret solution in dropping bottle
- indophenol solution in dropping bottle
- Lugol’s iodine solution in dropping bottle
- silver nitrate solution (1%) in dropping bottle
- isopropyl alcohol (99%) in screw-top jar
- brown wrapping paper
- 3 foods: apple, egg white, liver, onion, orange, or potato

Procedure

PART A  Test Demonstration

1. In your logbook, prepare a table similar to Table 1B.1.

2. Reagents are chemical solutions that scientists use to detect the presence of certain compounds. Observe the six reagent tests your teacher performs. In your table, describe the results of each test.
PART B  Compounds in Food

3. In your logbook, prepare a table similar to Table 1B.2. Record the presence (+) or absence (–) of each chemical substance in the foods you test.

WARNING: The reagents you will use in this procedure may be corrosive, poisonous, and/or irritants, and they may damage clothing. Avoid skin and eye contact; do not ingest. If contact occurs, flush the area with water for 15 minutes; rinse mouth with water; call your teacher immediately.

4. Predict the substances you will find in each sample your teacher assigns to you. Then test the samples as your teacher demonstrated or as described in steps 5–10. Record the result of each test in your logbook, using a + or –.

5. Protein test: Place 5 mL of the assigned food in a test tube. Add 10 drops of Biuret solution.

6. Glucose test: Add 3 mL of Benedict’s solution to 5 mL of the assigned food. Place the test tube in a beaker of boiling water, and heat for 5 minutes.

7. Starch test: Add 5 drops of Lugol’s iodine solution to 5 mL of the assigned food.

8. Vitamin C test: Add 8 drops of indophenol to 5 mL of the assigned food.

9. Chloride test: Add 5 drops of silver nitrate solution to 5 mL of the assigned food.

10. Fat test: Rub the assigned food on a piece of brown wrapping paper. Hold the paper up to the light. When food contains only a small amount of fat, the fat may not be detected by this method. If no fat has been detected, place the assigned food in 10 mL of a fat solvent such as isopropyl alcohol. Allow the food to dissolve in the solvent for about 5 minutes. Pour the solvent on brown paper. The spot should dry in about 10 minutes. Check the paper.

WARNING: Isopropyl alcohol is flammable and is a poison. Do not expose the liquid or its vapors to heat, sparks, open flame, or other ignition sources. Do not ingest; avoid skin/eye contact. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water. If a spill occurs, flood spill area with water; then call your teacher.

11. Wash your hands thoroughly before leaving the laboratory.

TABLE 1B.1
Reagent Tests of Known Food Substances

<table>
<thead>
<tr>
<th>Food substance</th>
<th>Reagent test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin</td>
<td>Biuret solution</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Benedict’s solution</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>Lugol’s iodine solution</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>indophenol solution (0.1%)</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>silver nitrate solution (1%)</td>
<td></td>
</tr>
<tr>
<td>Butter or vegetable oil</td>
<td>brown paper</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1B.2
Analysis of Compounds in Common Food

<table>
<thead>
<tr>
<th>Substance</th>
<th>Protein</th>
<th>Glucose</th>
<th>Starch</th>
<th>Vitamin C</th>
<th>Chloride</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>Prediction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Prediction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etc.</td>
<td>Prediction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Analysis
1. How did your predictions compare with the test results?
2. Which of your predictions was totally correct?
3. Which foods contained all the compounds for which you tested?
4. On the basis of your tests, which food could be used as source of protein? glucose? starch? vitamin C? fat?
5. How might the original colors of the test materials affect the results?

Investigations for Chapter 2
Energy, Life, and the Biosphere

Investigation 2A ◆ Are Corn Seeds Alive?

Observe a corn seed carefully. Can you tell whether the seed is alive? How could you determine if it is? If a seed is alive, it is said to be viable—capable of growing and developing. There are two ways to investigate whether or not a seed is viable. One way is to perform a tetrazolium test. Tetrazolium is a colorless chemical that turns pink or red in the presence of hydrogen, which is released by all living organisms as they carry on their daily chemical activities. The second way is to do a germination test. You will do both these tests using corn seeds from two different batches, labeled I and II. Keep the two groups separate.

Materials (per team of 4)
4 pairs of safety goggles
4 lab aprons
4 pairs of plastic gloves
10-mL graduated cylinder
2 jars
2 petri-dish halves
forceps
glass-marking pencil
2 paper towels
2 rubber bands
2 scalpels
wax paper
20 mL tetrazolium reagent (1%)
soaked corn seeds (50 type I and 50 type II)

Procedure

PART A Tetrazolium Test
1. Mark the outside bottom of one petri-dish half type I and the outside bottom of the other petri-dish half type II.
2. Obtain 25 corn seeds of type I. Using the scalpel, cut each kernel lengthwise down the middle, as shown in Figure 2A.1a. The seeds may have been treated with a pesticide. Do not handle them without gloves. You should be able to see the miniature plant (the embryo) inside the seed after it is cut.

◆ CAUTION: Scalpels are sharp; handle with care.

3. Discard one half of each seed, and place the other half in the team’s petri dish with the cut surface down.
4. Cover the seed halves with 10 mL of tetrazolium reagent.

◆ WARNING: Tetrazolium is a contact irritant and poison. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher immediately.

5. Repeat steps 2–4 with 25 seeds of type II.
6. After 25 minutes, use gloves and forceps to remove the seeds. Examine the cut surface of each seed for a color change. A red or pink color indicates a living substance.

7. Copy Table 2A.1 in your logbook, and record your results there.

PART B Germination Test
8. Place a wet paper towel on a sheet of wax paper (see Figure 2A.1b).
9. Place 25 type I corn seeds in five rows of five across the wet paper towel. Place the seeds so the pointed end is toward the top of the towel.
10. Cover the seeds with another wet paper towel.
11. Fold the left side of the wax paper over the edge of the wet paper towels. Roll the wax paper, wet paper towels, and corn seeds from the left side toward the right side of the paper. Make the roll tight.
12. Secure the roll with a rubber band so it will stay rolled up, and place it upright in a jar of water (see Figure 2A.1b).
13. Repeat steps 8–12 with 25 type II corn seeds.
14. Label each jar either type I or type II. Place the jars in a dark area designated by your teacher.
15. Wash your hands thoroughly before leaving the laboratory.
16. After 3 days, determine the total number of seeds in each jar that have begun to germinate (grow). Record the data in the table.

Analysis
1. For each treatment done per team and per class, calculate the percentage viability as follows and enter the percentages in the table.

\[
\frac{\text{number of seeds showing viability}}{\text{total number of seeds per treatment}} \times 100 = \% \text{ viability}
\]

2. In Part A, if a color change indicates activity in living things, what can you conclude about the type I and type II seeds?
3. What evidence have you found that the type I seeds are alive? not alive?
4. What evidence have you found that the type II seeds are alive? not alive?

<table>
<thead>
<tr>
<th>TABLE 2A.1</th>
<th>Results of Tetrazolium Test and Germination Test on Corn Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tetrazolium test</td>
</tr>
<tr>
<td></td>
<td>Type I seeds</td>
</tr>
<tr>
<td>Team</td>
<td>Class</td>
</tr>
<tr>
<td>Number of seeds used</td>
<td></td>
</tr>
<tr>
<td>Number of seeds viable or germinated</td>
<td></td>
</tr>
<tr>
<td>Percentage of seeds viable or germinated</td>
<td></td>
</tr>
</tbody>
</table>
5. Discuss in class the results of both experiments. Does the information provided by your teacher change any of your conclusions? Explain your answer.

6. How does the percentage of viability as determined from the tetrazolium test compare with the percentage from the germination test?

7. Are the two percentages the same? If not, can you suggest a reason for the difference?

8. Is it possible to tell if seeds are alive by just looking at them? Why or why not?

9. What is the advantage of combining data from several teams in the class?

Investigation 2B ◆ Food Energy

All foods contain energy, but the amount varies greatly from one food to another. You can use a calorimeter (Figure 2B.1) to measure the amount of energy, in calories, in some foods. A calorie is the amount of heat required to raise the temperature of 1 g (1 mL) of water 1°C. Calorie values of food on diet charts are given in kilocalories (1,000 calories), or kcals. (The kilocalorie is also referred to as the Calorie.) Your teacher will provide tables listing caloric values for common foods.

Using a thermometer, you can measure the change in temperature (ΔT) of a known volume of water. The water changes temperature by absorbing the heat given off by the burning of a known mass of food. Based on ΔT, you can calculate the amount of energy in the food.

**Materials** (per team of 3)
- 3 pairs of safety goggles
- 3 lab aprons
- 100-mL graduated cylinder
- 250-mL flask
- Nonmercury thermometer
- Balance

![The calorimeter setup.](image-url)
16-oz. can with cutout air and viewing holes
cork with sample holder
kitchen matches
20-cm × 30-cm piece of heavy-duty aluminum foil
2 pot holders
small container of water
3 whole peanuts
3 walnut halves

**SAFETY** Put on your safety goggles and lab apron. Tie back long hair, and roll up long, loose sleeves.

**Procedure**

1. Decide who in your team will be the experimenter, who will be the recorder, and who will be the safety monitor to assure correct safety procedures are followed.

2. Copy Table 2B.1 in your logbook, or tape in the table your teacher provides.

3. Using the balance, determine the mass to the nearest 0.1 g of each peanut and each walnut half. Record the masses in the table.

4. Obtain a 250-mL flask, a can, a cork with sample holder, and a piece of heavy-duty aluminum foil. Use equipment to make a calorimeter like the one shown in Figure 2B.1. Practice assembling and disassembling the equipment.

5. With the calorimeter disassembled, measure 100 mL of tap water and pour it into the flask.

6. Set the thermometer in the flask.

7. Measure the temperature of the water, and record it in the table.

8. Place a peanut in the wire holder anchored in the cork. Then place the cork on the piece of aluminum foil.

9. Carefully set fire to the peanut. This may require several matches. Discard burned matches in the container of water.

**WARNING:** Matches are flammable solids. In case of burns, **immediately** place burned area under cold running water; *then* call your teacher.

10. Place the can over the burning sample with the viewing hole facing you. Place the flask of water on top of the can.

11. Take temperature readings as soon as the sample has burned out and then at 30-second intervals until the water temperature begins to decrease. (The temperature will continue to rise after the sample has burned out as the water absorbs heat from the can.)

12. Allow the calorimeter to cool about 2 minutes before disassembling.

**TABLE 2B.1**

**Energy Content of Nut Samples**

<table>
<thead>
<tr>
<th></th>
<th>Temperature of water, °C</th>
<th>Food energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before burning</td>
<td>After burning</td>
</tr>
<tr>
<td>Walnut sample 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walnut sample 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walnut sample 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut sample 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut sample 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut sample 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
WARNING: Use pot holders to handle hot flasks. Boiling water will scald, causing second-degree burns. Do not touch the flask or allow boiling water to contact your skin. Avoid vigorous boiling. If a burn occurs, immediately place burned area under cold running water; then call your teacher.

13. Repeat steps 5–12 until you have data for three samples each of a peanut and walnut half. Change the water in the flask each time.
14. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. Prepare graphs of the data. Determine the average change in temperature for each sample. Calculate the number of kilocalories produced per gram. To do this, multiply the increase in water temperature (average change) by 100 (the number of milliliters of water used). This step will give you the number of calories. To convert to kilocalories, divide by 1,000 calories/kilocalorie. To calculate kilocalories produced per gram of food, divide this number by the number of grams of food burned. Enter all data in the table.
2. How do your data (adjusted for 100 g) compare with the values for 100 g of the same or similar food listed by your teacher? (The kilocalories listed in most diet charts are per 100 g, per ounce, per cup, or per serving. To compare your results, you may need to convert to common units.)
3. How do you account for any differences?
4. If the same amount of food you tested were completely burned in the cells of the human body, would you expect the energy release to be greater or less than your results? greater or less than published charts of the same caloric content of foods?
5. Which of the two foods tested seems to be the better energy source?
6. Why might some foods with fewer kilocalories be better energy sources than other foods with more kilocalories?
7. What was the original source of energy in all the foods tested?

Investigation 2C ◆ Enzyme Activity

Enzymes are biological catalysts (usually proteins) that speed up the rates of chemical reactions that take place within cells. In this investigation, you will study several factors that affect the activity of enzymes. The specific enzyme you will use is catalase, which is present in most cells and found in high concentrations in liver and blood cells. You will use liver homogenate as the source of catalase. Catalase promotes the decomposition of hydrogen peroxide (H₂O₂) in the following reaction:

\[
\text{catalase} \quad 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

Hydrogen peroxide is formed as a by-product of chemical reactions in cells. It is toxic and soon would kill cells if not immediately removed or broken down. (Hydrogen peroxide is also used as an antiseptic. It is not a good antiseptic for open wounds, however, as it quickly is broken down by the enzyme catalase, which is present in human cells.)

Materials (per team of 3)
- 3 pairs of safety goggles
- 3 lab aprons
- 50-mL beaker
- 2 250-mL beakers
- 10-mL graduated cylinder
- 50-mL graduated cylinder
- reaction chamber
- 6 18-mm × 150-mm test tubes
- forceps
- square or rectangular pan
- test-tube rack
- nonmercury thermometer
- filter-paper disks
- ice
- water bath at 37°C
- buffer solutions: pH 5, pH 6, pH 7, pH 8
- catalase solution
- fresh H₂O₂ (3%)
Procedure
In all experiments, make certain that your reaction chamber is scrupulously clean. Catalase is a potent enzyme. If the chamber is not washed thoroughly, the enzyme will adhere to the sides and make subsequent tests inaccurate. Measure all substances carefully. Results depend on comparisons between experiments, so the amounts measured must be equal or your comparisons will be valueless. Before you do the experiments, read through the instructions completely. Make sure that you have all required materials on hand, that you understand the sequence of steps, and that each member of your team knows his or her assigned function.

PART A  The Time Course of Enzyme Activity
1. Prepare two tables in your logbook similar to Table 2C.1. One will record your team’s data while the other will record data averaged for the entire class.
2. Obtain a small amount of catalase solution in a 50-mL beaker.
3. Obtain a reaction chamber and a number of filter-paper disks.
4. Place four catalase-soaked filter-paper disks on one interior sidewall of the reaction chamber. (They will stick to the sidewall.) Prepare a disk for use in the reaction chamber by holding it by its edge with a pair of forceps and dipping it into the catalase solution for a few seconds. Drain excess solution from the disk by holding it against the side of the beaker before you transfer it to the reaction chamber.
5. Stand the reaction chamber upright, and carefully add 10 mL of 3% hydrogen peroxide (H₂O₂) solution. Do not allow the peroxide to touch the filter-paper disks.

6. Tightly stopper the chamber.
7. Fill a pan almost full with water.
8. Lay the 50-mL graduated cylinder on its side in the pan so that it fills with water completely. If any air bubbles are present, carefully work these out by tilting the cylinder slightly. Turn the cylinder upside down into an upright position, keeping its mouth underwater at all times.
9. Making certain the side with the disks is at the top, carefully place the reaction chamber and its contents on its side in the pan of water.
10. Move the graduated cylinder into a position (Figure 2C.1) where its mouth lies directly over

<table>
<thead>
<tr>
<th>TABLE 2C.1</th>
<th>Catalase Activity under Various Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>mL O₂ evolved/30 sec</td>
</tr>
<tr>
<td>Full concentration</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20</td>
</tr>
<tr>
<td>3/4 concentration</td>
<td></td>
</tr>
<tr>
<td>Etc.</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 2C.1
Apparatus for measuring oxygen production in a reaction between catalase and hydrogen peroxide.

CAUTION: H₂O₂ is reactive. Avoid contact between H₂O₂ and other chemicals unless instructed otherwise. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher immediately.
the tip of the dropping pipet that extends from the reaction chamber. One member of the team should hold it in this position for the duration of the experiment.

11. Rotate the reaction chamber 180 degrees on its side so that the hydrogen peroxide solution comes into contact with the soaked disks.

12. Measure the gas levels in the graduated cylinder at 30-second intervals for 10 minutes. Record the levels in your data table.

13. Pool your team’s data with the other teams in the class. Record class average values of gas levels for each 30-second interval in your second data table.

PART B  The Effect of Enzyme Concentration on Enzyme Activity

14. Test 3/4, 1/2, and 1/4 concentrations of enzyme solution using the procedures for Part A with the following changes:
   • 3/4 concentration: Use three catalase-soaked disks instead of 4.
   • 1/2 concentration: Use two catalase-soaked disks and a 10-mL graduated cylinder.
   • 1/4 concentration: Use one catalase-soaked disk and a 10-mL graduated cylinder.

15. Record all data in your data table.

16. Pool data over the entire class, and record average values in your second data table.

PART C  The Effect of Temperature on Enzyme Activity

17. Add 10 mL of 3% H₂O₂ to each of two test tubes. Place one tube in a beaker of ice water and the other in a beaker with water maintained at 37°C.

18. When the temperature of the contents of the chilled H₂O₂ reaches approximately 10°C, repeat Part A with the following changes:
   • In step 5, use 10 mL of chilled 3% H₂O₂.
   • In step 7, add ice to the pan to chill the water to approximately 10°C.

19. When the temperature of the warmed H₂O₂ reaches approximately 37°C, repeat Part A with the following changes:
   • In step 5, use 10 mL of warmed 3% H₂O₂.
   • In step 7, fill the pan with water warmed to approximately 37°C.

20. Record the data in your data table.

21. Pool data over the entire class, and record average values in your second data table.

PART D  The Effect of pH on Enzyme Activity

22. Label four test tubes as follows: pH 5, pH 6, pH 7, and pH 8. Add to each of these 8 mL of 3% H₂O₂.

23. Add 4 mL of pH 5 buffer solution to the pH 5 test tube, shaking well to ensure mixing. To each of the other three test tubes, add 4 mL of pH 6, pH 7, and pH 8 buffer solutions, shaking each test tube well.

24. Repeat the procedure of Part A for each pH value, substituting the buffered 3% H₂O₂ solutions in step 5.

25. Record the results in your data table.

26. Pool data over the entire class, and record average values in your second data table.

27. Wash your hands thoroughly before leaving the laboratory.

Analysis

1. Why is it a good idea to pool data from the entire class? Use the class averaged data to answer the following questions.

2. In your logbook, plot the data from Part A on the graph. Label the horizontal axis Time (sec) and the vertical axis mL O₂ evolved. Does the action of the catalase change through time? Explain your answer.

3. Plot the data from Part B on the grid used for Part A, and label the enzyme concentrations on the graph. Based on these data, how does enzyme activity vary with concentration?

4. Copy the graph for Part A, and plot the data from Part C on it. Based on these data, how does temperature affect enzyme action?

5. Plot the results for all four runs of Part D on a third graph. How does pH affect the activity of enzymes?

6. What is a buffer? Would the results of Parts A, B, and C have been different if buffers also had been used in those experiments? If so, how?

7. Summarize the general conditions necessary for effective enzyme action. Are these conditions the same for each enzyme? Why or why not?
8. How would you design an experiment to show how much faster \( \text{H}_2\text{O}_2 \) decomposes in the presence of catalase than it does without the enzyme?

9. Explain why the enzyme catalase was still active even though the liver cells from which you obtained the enzyme were no longer living.

Investigation 2D ◆ Starch Digestion

How do you obtain the energy you need for your activities? Starch makes up a large part of the food of many organisms and is a major source of energy for them. Human saliva contains an enzyme, amylase, which begins the breakdown of starch into sugar molecules that can be absorbed into the bloodstream and taken up by cells. In this investigation, you will explore the action of amylase on starch and identify some sources of this enzyme.

To determine if the results you observe are caused by amylase or by some other factor, such as elapsed time, you will set up controls. In an experiment, all the factors that might cause the observed results are referred to as variables. When scientists test a particular variable, they must control all the other variables so they know what causes the results. One way to do this is to set up additional tests, called controls, to rule out the other variables. For example, when starch and amylase are dissolved in water, a change in the starch is observed. To be sure this change is caused by the amylase rather than by the water, you could set up one test with starch and amylase and another with starch and water. The starch and water become the control for the test of the effect of amylase on starch.

Scientists used special chemical solutions called indicators to detect the presence of certain substances. For example, Lugol's iodine solution is used to detect the presence of starch.

Materials (per team of 4)
- 4 pairs of safety goggles
- 4 lab aprons
- 7 dropping pipettes
- 6 18-mm × 150-mm test tubes
- 10-mL graduated cylinder
- spot plate
- test-tube rack
- 6 rubber stoppers for test tubes
- glass-marking pencil
- funnel
- scissors
- mortar and pestle
- cheesecloth (several layers)
- pinch of sand
- 10 mL 5% starch suspension
- 10 mL 0.1% starch suspension
- 10 mL 1% amylase solution
- 25 mL distilled water
- Lugol's iodine solution in dropping bottle
- 7 glucose test strips
- 5 g fresh spinach

**SAFETY** Put on your safety goggles and lab apron. Tie back long hair.

PART A Designing a Controlled Experiment
Participate in the discussion/demonstration that your teacher leads, keeping notes in your logbook.

PART B Starch Digestion in Animals
Half of the teams will conduct this part of the activity.

Day 1
1. Read through the procedure for day 1, and prepare a logbook in which to record predictions, observations, and results of any tests.
2. Place 5 mL of starch suspension and 5 mL of amylase solution into a test tube labeled \( \text{starch/amylace} \).
3. What factors (variables) might affect your results in step 2? Set up controls for those variables in additional test tubes. Label the controls appropriately, and record the contents of each control in your data table.
4. Stopper the tubes, and store them in a dark place for 24 hours.
5. Based on the introduction to this activity, predict what substances will be present in each tube after 24 hours, recording your predictions in your data table.
6. Wash your hands before leaving the laboratory.
Day 2
7. Observe the tubes that you set up yesterday, and record your observations in the table.
8. What indicator tests should you use to determine what has occurred in each tube? Perform those tests, recording the results in your data table and in the class data table that your teacher has set up.

**WARNING:** Lugol's iodine solution is a poison if ingested, is a strong irritant, and can stain clothing. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher immediately.

9. Wash your hands thoroughly before leaving the laboratory.

### PART C  Starch Digestion in Plants
Half of the teams will conduct this part of the activity.

Day 1
1. Using scissors, cut up about 5 g of fresh spinach leaves into small pieces.
2. Put the pieces in a mortar with 10 mL of distilled water and a pinch of sand. Grind them thoroughly with a pestle.
3. Line the funnel with several layers of cheesecloth, and set it in a test tube labeled filtrate. Filter the crushed plant material through the funnel. Save the resulting filtrate (plant juice).
4. Prepare a data table in which to record predictions, observations, and results of any tests.
5. Using the indicators you observed in Part A, can you tell what substances probably are in the filtrate? Perform appropriate tests to find out. Record your results in your data table.

**WARNING:** Use dropping pipettes to remove samples, a spot plate for the tests, and the test indicators you observed in Part A.

6. Into a test tube labeled filtrate/starch, place 5 mL of filtrate and 5 mL of starch suspension, swirling to mix the solutions.
7. What factors (variables) might affect your results in step 6? Set up controls for those variables in additional test tubes. Label the controls appropriately, and record the contents of each control in your data table.
8. Stopper the tubes, and store them in a dark place for 24 hours.
9. Based on the results from step 5, predict what substances will be present in each tube after 24 hours. Record your predictions in your data table.
10. Wash your hands thoroughly before leaving the laboratory.

Day 2
11. Using the procedures of step 5, test the contents of the tubes that you set up in steps 6 and 7. Record the results in your data table and in the class data table that your teacher has set up.
12. Wash your hands before leaving the laboratory.

### Analysis
1. From the results of Part B, what can you conclude about the effect of amylase on starch?
2. From the results of Part C, what can you conclude about a plant's ability to digest starch?
3. If you had not controlled the variables in this experiment, what conclusions could you have drawn?
4. Of what value to an organism is the ability to digest starch?
5. Animals have special glands for the production of enzymes that break large food molecules into small ones. What, if any, evidence from the results of this experiment indicates that plant cells possess similar enzymes?
6. Describe the processes responsible for food breakdown in the major compartments of the digestive system and the role of each.

7. Why do doctors give glucose rather than starch as intravenous injections to patients whose digestive systems are not functioning properly?

Investigations for Chapter 3
Exchanging Materials with the Environment

Investigation 3A ◆ Cells and Movement of Materials

To survive, all organisms need to balance their internal environment despite constantly changing conditions. Multicellular organisms have complex systems of organs that maintain this balance, but a single cell must rely on a solitary structure. The contents of a cell are surrounded by a thin membrane—the plasma membrane. Anything entering or leaving the cell must pass through this membrane. Certain materials can pass into the cell, whereas others cannot. Similarly, some materials pass out of the cell, while others that are vital to the cell’s existence remain inside.

In this investigation, you will explore the movement of substances through membranes—both living plasma membranes and nonliving material that models plasma membranes. A nonliving material—called dialysis tubing—serves to separate larger molecules from smaller molecules. The size of the pores in the tubing determines which molecules may pass through it. A balloon also serves as a model of a plasma membrane, allowing some molecules to pass through easily while retaining others.

Materials (per team of 2)
2 pairs of safety goggles
2 lab aprons
2 250-mL beakers
5 coverslips
4 dropping pipettes
5 microscope slides
2 test tubes
glass-marking pencil
compound microscope
2 15-cm pieces of dialysis tubing
3 balloons
hot plate
paper towel
4 10-cm pieces of string
test-tube clamp
test-tube rack
glucose test strips
brilliant cresyl blue solution in dropping bottle (5%)
distilled water
mineral oil
white vinegar
vanilla extract
15 mL glucose solution
salt solution (5%)
15 mL soluble-starch solution
Lugol’s iodine solution in dropping bottle
yeast suspension
elodea leaf (Anacharis)

SAFETY Put on your safety goggles and lab apron. Tie back long hair.

PART A Diffusion through Dialysis Tubing

Procedure
1. Twist one end of a piece of dialysis tubing. Fold the twisted end over, and tie it tightly with a piece of string. Prepare the other piece the same way.
2. Pour soluble-starch solution to within 4 cm of the top of one piece of tubing. Twist and tie the end as in step 1. Rinse the tubing under running water to remove any starch from the outside.
3. Place the tubing in a beaker of water labeled A (Figure 3A.1). Add enough Lugol’s iodine solution to give the water a distinct yellowish color.

WARNING: Lugol’s iodine solution is a poison if ingested, is a strong irritant, and can stain clothing. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher immediately.
4. Repeat step 2 with the second piece of dialysis tubing, using glucose solution instead of the soluble starch. Place this tubing in a beaker of water labeled B.

5. Allow the pieces of tubing to stand for about 20 minutes. Dip a glucose test strip into the water in beaker B. Record the color on the strip.

6. Observe the tubing in beaker A. Record any changes, including color, that you see in either the tubing or the water in the beaker.

7. Let beakers A and B stand overnight. Record any changes observed the next day.

8. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. On the basis of the results from steps 3 and 6, what must have happened to the iodine molecules in beaker A?

2. On the basis of the chemical test for glucose (step 5), what must have happened to the glucose molecules in beaker B?

3. From the evidence obtained after the beakers stood overnight, what other substances passed through the membrane in beaker B?

4. Which substance did not pass through a membrane? Explain your answer.

5. Physicists can show that all the molecules of a given substance are about the same size but that molecules of different substances are different in size. Measurements show that iodine molecules and water molecules are very small, glucose molecules are considerably larger, and starch molecules are very large. On the basis of this information, suggest a hypothesis to account for your observations.

6. What assumption did you make about the structure of the dialysis tubing?

PART B Diffusion through a Balloon

Procedure
9. Blow up a balloon, and tie off the end.

10. Use a dropping pipette to place one drop of water on the surface of the balloon. Note how the water drop behaves as it interacts with the balloon surface. Use a paper towel to wipe the water drop off the surface of the balloon.

11. Use a dropping pipette to place one drop of mineral oil on the surface of the balloon. Note how the oil drop behaves as it interacts with the balloon surface.

12. Use a dropping pipette to add about 2 mL of white vinegar to the inside of an uninflated balloon. (White vinegar contains water and acetic acid.) Blow up the balloon, and tie off the end.

13. Consider your observations from step 10, and predict whether you will be able to smell the vinegar inside the balloon. Take the balloon to an area of the room away from open containers or pipettes that contain vinegar, and conduct the smell test. Record your observations in your logbook.

14. Use a dropping pipette to place about 2 mL of vanilla extract inside a second uninflated balloon. Blow up the balloon, and tie off the end.

15. Consider your observations from step 11, and predict whether you will be able to smell the vanilla extract inside the balloon. Take the balloon to an area of the room away from open containers or pipettes that contain vanilla extract, and conduct the smell test. Record your observations in your logbook.

Analysis
1. On the basis of your smell test, did the vinegar molecules pass through the balloon?
2. On the basis of your smell test, did the vanilla extract molecules pass through the balloon?

3. Although a balloon membrane is much thicker than a plasma membrane of a cell, both can be described as nonpolar membranes. Nonpolar membranes repel charged particles and polar molecules but let nonpolar (fat soluble) molecules pass through. Use this information to suggest a hypothesis that explains your observations.

4. Why can polar water molecules pass through a cell’s plasma membrane?

PART C Osmosis and the Living Cell

Procedure

16. Place a leaf from the growing tip of an elodea plant in a drop of water on a clean slide. Add a coverslip and examine under low power. Position the slide so that the cells along one edge of the leaf are near the center.

17. Switch to high power, and focus sharply on a few cells near the edge of a leaf. Place a small piece of absorbent paper at the edge of the coverslip opposite the side of the leaf you are observing. (Remember, directions are reversed when you look through a microscope.) Have your lab partner place several drops of glucose solution at the coverslip edge nearest the part of the leaf being observed. Use the fine adjustment to adjust the focus while the water is being replaced. Continue observing the cells until you see changes in them.

18. Make simple sketches showing cells both before and after the glucose solution was added.

19. Remove the glucose solution, and replace it with distilled water. Use a new piece of absorbent paper, and allow 2 or 3 drops of distilled water to flow across the slide into the paper to make sure that most of the glucose solution is washed away. Make observations while this is being done.

20. Exchange places with your lab partner. Repeat steps 17–19.

21. Repeat steps 17–19 with salt solution in place of the glucose solution.

Analysis

1. Did water move into or out of the cells while the leaf was surrounded by the glucose solution? by the salt solution? What evidence do you have to support your answer.

2. In which direction did water move through the plasma membrane when the cell was surrounded by distilled water?

3. What do you think would happen to elodea cells if they were left in the glucose solution for several hours? Could elodea from a freshwater lake be expected to survive if transplanted into the ocean? (Assume that the salt concentration of the ocean is about the same as the salt solution used in this experiment.)

4. An effective way to kill plants is to pour salt on the ground around them. Using principles discovered in this investigation, explain why the plants die.

5. Bacteria cause food to spoil and meat to rot. Explain why salted pork, strawberry preserves, and sweet pickles do not spoil even though they are exposed to bacteria. Name other foods preserved in the same manner.

PART D Membranes in Living and Dead Cells

Procedure

22. Place one drop of yeast suspension on a slide, add a coverslip, and observe the yeast cells under low power and then high power. Describe what you see, and sketch two or three cells to show their general appearance.

23. Place about 1 mL of yeast suspension in each of two small test tubes. Label one tube boiled and the other unboiled. Heat one of the test tubes in a beaker of boiling water until the contents have boiled for at least 2 minutes. This action will kill the yeast cells. Allow the test tube and its contents to cool for a few minutes.

WARNING: Use test-tube clamps to hold hot test tubes. Boiling water will scald, causing second-degree burns. Do not touch the beaker or allow boiling water to contact your skin. Avoid vigorous boiling. If a burn occurs, immediately place the burned area under cold running water; then call your teacher.
24. Add 5 drops of brilliant cresyl blue solution to the boiled yeast suspension and 5 drops to the unheated yeast suspension. 

**CAUTION:** Brilliant cresyl blue solution is a mild irritant. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher.

25. Label one microscope slide boiled and another unboiled. Prepare a slide from each test tube and examine under high power. Record any differences between the yeast cells in the two suspensions.

26. Wash your hands thoroughly before leaving the laboratory.

**Analysis**
1. What effect does heat seem to have on the yeast plasma membrane?
2. In a preparation of unheated yeast solution and brilliant cresyl blue, a few blue yeast cells are usually visible. What assumption can you make concerning these cells?
3. Which passes more easily through membranes of living cells, brilliant cresyl blue molecules or water molecules? Develop a hypothesis to account for your observation and answer.

**Investigation 3B ◆ Diffusion and Cell Size**

Does diffusion proceed rapidly enough to supply a cell efficiently with some of its materials? The same question can be asked about removing cell wastes. In this investigation, you will discover how the rate of diffusion and the size of a cell are related.

**Materials** (per team of 2)
- 2 pairs of safety goggles
- 2 lab aprons
- 2 pairs of plastic gloves
- 250-mL beaker
- metric ruler
- paper towel
- plastic knife
- plastic spoon
- 3 cm × 3 cm × 6 cm cube of phenolphthalein agar
- 150 mL HCl (0.1%)

**Procedure**
1. Using a plastic knife, trim the agar block to make three cubes—3 cm, 2 cm, and 1 cm on a side.
2. Place the cubes in the beaker, and add 0.1% HCl until the cubes are submerged. Record the time. Use the plastic spoon to turn the cubes frequently for the next 10 minutes.

**CAUTION:** 0.1% HCl is a mild irritant. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher.

3. Prepare a table similar to Table 3B.1, and do the calculations necessary to complete it. The ratio of surface area to volume is calculated as follows:

\[
\text{ratio of surface area to volume} = \frac{\text{surface area}}{\text{volume}}
\]

This ratio also may be written as “surface area:volume.” The ratio should be expressed in its simplest form (for example, 3:1 rather than 24:8).

4. Wear gloves and use the plastic spoon to remove the agar cubes from the HCl after 10 minutes. Blot them dry. Avoid handling the cubes until they are blotted dry. Use the plastic knife to slice each cube in half. Rinse and dry the knife between cuts. Record your observations of the sliced surface. Measure the depth of diffusion of the HCl in each of the three cubes.

**Analysis**
1. List the agar cubes in order of size, from largest to smallest. List them in order of the ratios of surface area to volume, from the largest to the smallest ratio. How do the lists compare?
2. Calculate the ratio of surface area to volume for a cube 0.01 cm on a side.
3. Which has the greater surface area, a cube 3 cm on a side or a microscopic cube the size of an onion-skin cell? (Assume the cell to be 0.01 cm
on a side.) Which has the greater surface area in proportion to its volume?

4. What evidence is there that HCl diffuses into an agar cube? What evidence is there that the rate of diffusion is about the same for each cube?  
   Explain your answer.

5. What happens to the ratio of surface area to volume of cubes as they increase in size?

6. Most cells and microorganisms measure less than 0.01 cm on a side. What is the relationship between rate of diffusion and cell size?

7. Propose a hypothesis to explain one reason why large organisms have developed from more cells rather than larger cells.

Investigation 3C • The Kidney and Homeostasis

The cells of the human body are surrounded by liquid that is remarkably constant in its properties. The continuous regulation of the many dissolved compounds and ions in this internal environment is referred to as homeostasis.

The kidneys play an important role in homeostasis by regulating blood composition and by regulating the levels of many important chemicals and ions. The production of urine and its elimination from the body are critical functions of the kidneys and the urinary system (Figure 3C.1).

**Materials** (per team of 3)  
pencil and paper

**PART A Blood versus Urine**

**Procedure**
The relationship of structure and function in the kidney is illustrated in Figure 3.21 in Chapter 3. Use this illustration and the data in Table 3C.1 to answer the Analysis questions.

**Analysis**
1. What do the data for water indicate?

2. Protein molecules are not normally found in the urine. Explain why.

3. The information for glucose is similar to that for protein. Explain these data.

4. Based on what the sodium data indicate, what do you think may happen to the sodium content in the urine of a person who increases his or her intake of sodium chloride?

---

**TABLE 3B.1**
Comparison of Agar Cubes

<table>
<thead>
<tr>
<th>Cube dimension</th>
<th>Surface area (cm²)</th>
<th>Volume (cm³)</th>
<th>Simplest ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3C.1**
Comparison of Substances in Blood and Urine

<table>
<thead>
<tr>
<th></th>
<th>% in blood as it enters kidney</th>
<th>% in urine as it leaves kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>91.5</td>
<td>96.0</td>
</tr>
<tr>
<td>Protein</td>
<td>7.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.33</td>
<td>0.29</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.02</td>
<td>0.24</td>
</tr>
<tr>
<td>Urea</td>
<td>0.03</td>
<td>2.70</td>
</tr>
</tbody>
</table>

**FIGURE 3C.1**
Micrograph of the glomerulus of a nephron (×260). The glomerulus is a capillary network through which fluid and other materials pass from the blood into the tubules of the nephron.
5. How do the data for potassium differ from those for sodium?
6. How would you interpret the data for urea?
7. Summarize the processes that take place between blood and urine, and identify the structures where they occur.

PART B Filtration, Reabsorption, and Secretion

Procedure
The micropuncture method was used in a second study of the six materials listed in Table 3C.1. Under a microscope, a very fine pipet was used to withdraw samples of fluid at four points along the nephron (see Figures 3.21, 3.22, and 3.23). Study Table 3C.2, which shows the data that were collected using this technique. Use the data to answer the Analysis questions.

Analysis
1. Which function, secretion or reabsorption, involves the movement of a greater amount of water in the kidney? Explain your answer.
2. Proteins are involved in which of the three kidney functions?
3. Compare the protein data with the glucose data. What is the difference? Explain the difference.
4. In some samples, glucose is found in the urine. What might cause this condition?
5. Why are excess glucose molecules in the blood excreted?
6. The data tell us that the concentration of sodium in the blood is greater than in the urine, yet most of the sodium ions in the urine move back into the blood. What process makes this movement possible?
7. Urea is a by-product of amino-acid metabolism. Next to water, urea is the most abundant material found in urine. If urea were allowed to accumulate in the blood, what might happen?
8. Homeostasis is the maintenance of a relatively stable internal environment in an organism. Summarize how the kidney functions as a homeostatic organ.

Investigations for Chapter 4
Autotrophy: Collecting Energy from the Nonliving Environment

Investigation 4A ♦ Photosynthesis

Section 4.2 of your text developed the following equation to represent the materials and products of photosynthesis:

\[ 3\text{CO}_2 + 3\text{H}_2\text{O} \xrightarrow{\text{light energy}} \text{C}_6\text{H}_{12}\text{O}_6 + 3\text{O}_2 \]

carbon dioxide water chlorophyll 3-carbon sugar oxygen gas
This equation raises several questions, which can serve as a basis for experiments that will help you understand the process of photosynthesis.

A. Does a green plant use carbon dioxide in the light?
B. Is light necessary in order for this reaction to take place?
C. Are the materials in the equation involved in any plant process other than photosynthesis?
D. Do plants release the oxygen produced in photosynthesis?

In this investigation, you are asked to design experiments to answer the questions above. You will need to consider several factors before starting work.

- What type of plant could best serve your purpose, a water plant or a land plant?
- What factor affecting photosynthesis could best be used to start and stop the process?
- What type of detector can be used to show that photosynthesis has or has not occurred?
- How can you identify the substances that are produced or given off during photosynthesis?
- What type of controls are necessary?

**Materials** (per team of 2)
- 2 lab aprons
- 2 pairs of safety goggles
- 18-mm x 150-mm test tubes (number used will vary)
- wrapped drinking straws
- rubber stoppers for test tubes
- carbonated water
- bromthymol blue solution
- elodea (*Anacharis*)

**Procedure**

**PART A Use of Carbon Dioxide in Light**

1. Add enough bromthymol blue solution to a test tube to give a light blue color, and using a drinking straw, gently bubble your breath through it until you see a color change. Discard the straw after use.

2. Add a few drops of carbonated water to a small amount of bromthymol blue in a test tube, and observe any color change. What do carbonated water and your breath have in common that might be responsible for the similar result? What action would be necessary to restore the original color of the bromthymol blue?

3. Using elodea, bromthymol blue solution, and test tubes, set up an experiment to answer Question A. (Hint: Bromthymol blue solution is not poisonous to elodea.)

4. Using Table 4A.1 as a guide, prepare a table listing the test tubes in your experiment. Show what you added to each tube, what change you expected in the bromthymol blue solution, and what change actually occurred. Explain the change. Fill in the first three columns of the table on the day the experiments are set up, and fill in the last two columns the next day.

5. Wash your hands before leaving the laboratory.

**PART B Light and Photosynthesis**

6. Using the same types of material as in Part A, set up an experiment to answer question B. Use as many plants and test tubes as necessary to be sure of your answer.

7. Prepare and complete a data table as in Part A.

<table>
<thead>
<tr>
<th>Test tube</th>
<th>Material added (procedure)</th>
<th>Expected indicator change (hypothesis)</th>
<th>Actual indicator change (data)</th>
<th>What the change shows (interpretation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bromthymol blue solution, elodea, CO₂, and light</td>
<td>Yellow bromthymol blue solution will turn blue.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PART C  Carbon Dioxide in Other Plant Processes

8. Using the same types of materials as in Part A, set up an experiment to answer question C for carbon dioxide.

9. Prepare and complete a data table as in Part A.

PART D  Oxygen and Photosynthesis

10. Your teacher or a selected group of students will set up a demonstration experiment to answer question D. What observations in Parts A, B, or C indicate that the elodea in light was giving off a gas? How might some of this gas be collected and tested to determine its identity?

Analysis

1. Do you have evidence from Part A that light alone does not change the color of the bromthymol blue solution? Explain your answer.

2. What test tubes show that light is necessary for a plant to carry on photosynthesis?

3. How is carbon dioxide used by a plant that is not carrying on photosynthesis? What test tubes show carbon dioxide’s role? What biological process accounts for your findings?

4. Determine where your expected changes disagree with the actual changes. Are the differences, if any, due to experimental error or a wrong hypothesis? Explain your answer.

Investigation 4B  ◆ Rate of Photosynthesis

There are several ways to measure the rate of photosynthesis. In this investigation, you will use elodea and pH paper. The rate of photosynthesis is determined indirectly by measuring the amount of carbon dioxide removed from water by elodea. Carbon dioxide is added by bubbling breath into the water, which absorbs the carbon dioxide. The carbon dioxide combines with water to produce carbonic acid ($\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3$), a reversible reaction. As elodea uses carbon dioxide in photosynthesis, less carbonic acid is present, and the pH of the water increases.

**Hypothesis:** After reading the procedure, develop hypotheses that predict the effects of light intensity and color on the rate of photosynthesis.

**Materials** (per team of 4)

- 4 pairs of safety goggles
- 2-L beaker
- 100-mL beaker
- 250-mL flask
- 2 1-mL pipettes
- 2 25-mm x 200-mm test tubes
- forceps
- 2 wrapped drinking straws
- lamp with 100-watt flood-lamp bulb
- narrow-range pH paper
- nonmercury thermometer

**TABLE 4B.1**

<table>
<thead>
<tr>
<th>Reading</th>
<th>30 cm</th>
<th>10 cm</th>
<th>50 cm</th>
<th>Red</th>
<th>Blue</th>
<th>Green</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH1</td>
<td>pH2</td>
<td>°C</td>
<td>pH1</td>
<td>pH2</td>
<td>°C</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Experimental condition**

- 30 cm
- 10 cm
- 50 cm
- Red
- Blue
- Green
red, blue, and green cellophane
distilled water
ice water
tap water at 25ºC
2 15-cm sprigs of young elodea

SAFETY Put on your safety goggles.

FIGURE 4B.1
Experimental setup.

Part A Equipment Assembly
1. In your logbook, prepare a table similar to Table 4B.1, or tape in the table your teacher provides.
2. Put 125 mL of distilled water in the flask. Blow through a small straw into the water for 2 minutes. Discard the straw after use as your teacher directs. What is the purpose of blowing into the water in the flask?
CAUTION: Be careful not to suck any liquid through the straw.

3. Place two sprigs of elodea, cut end up, into one of the test tubes (experimental tube).
4. Fill both test tubes three-fourths full with the water you blew into. What is the purpose of the second test tube?
5. Stand the test tubes in a 2-L beaker; add 25ºC tap water to the beaker until it is about two-thirds full (Figure 4B.1).
6. Insert a thermometer into the water in the 2-L beaker. Determine the temperature throughout the experiment. (Use the small beaker to add ice water and/or to remove water from the 2-L beaker.)
7. Let the entire assembly stand for about 5 minutes to permit the temperature to become uniform throughout the system. Note the initial pH in the test tubes, and record both readings in the data table. Use the 1-mL pipettes to transfer a drop of water from halfway down each test tube to a piece of narrow-range pH paper, and read the pH from the comparison chart on the strip.
PART B Basic Photosynthetic Rate
8. Place the lamp with the 100-watt bulb 30 cm from the beaker, and illuminate the two test tubes.
9. Take pH and temperature readings every 5 minutes for a total of 30 minutes. Record the readings in the data table.
10. Wash your hands thoroughly before leaving the laboratory.

PART C Effects of Light Intensity on Photosynthetic Rate
11. Repeat steps 2–9 with the light source first 10 cm and then 50 cm away from the test-tube assembly.

PART D Effects of Light Color on Photosynthetic Rate
12. Repeat Steps 2–9 using first red, then blue, and then green cellophane over the beaker.
13. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. What chemical change occurred in the water you blew into?
2. What happens during photosynthesis that causes the pH to increase in the test tubes?
3. What two environmental factors are being controlled by the test tube without the elodea?
4. How much did the pH change during the 30-minute period for each condition tested?
5. How would you use any change in pH in the test tube without elodea to correct the data for the experimental test tube?
6. Prepare graphs showing pH every 5 minutes under the various conditions being tested. What variables are on the horizontal and vertical axes of the graphs?
7. Use the change in pH that occurs at the three light intensities you tested to determine the effect of light intensity on the rate of photosynthesis. Do your data support your hypothesis?
8. Use the change in pH with the three colors (red, blue, and green) to determine the effect of light color on the rate of photosynthesis. Do your data support the hypothesis you constructed?

Investigation 4C Chemoautotrophs
All life on Earth can be placed into categories according to the organism’s sources of energy and carbon. Phototrophs obtain their energy from sunlight, while chemotrophs obtain energy by oxidizing various organic or inorganic molecules. Autotrophs use carbon dioxide as their source of carbon, while heterotrophs get carbon from a variety of organic compounds.

A Winogradsky column is a self-contained microbial ecosystem. Mud and water from a freshwater or marine source supply the microorganisms. Energy is derived from sunlight and organic molecules. Careful observation of a Winogradsky column over a period of weeks or months reveals a great deal of microbial diversity, including examples of all four basic life strategies described above.

Materials (per team of 3)
3 pairs of safety goggles
3 lab aprons
3 pairs of plastic gloves
1-L graduated cylinder
mud from freshwater or marine source
water from freshwater or marine source
16 g chalk
8 g newspaper
8 g sodium sulfate
clear plastic wrap
rubber band
balance
light source
scissors
stirring rod
hammer
bucket

SAFETY Put on your safety goggles, lab apron, and gloves. Tie back long hair.
**Procedure**

1. Cut or shred newspaper into small pieces.
2. Weigh out 8 g of shredded newspaper, and add it to the bottom of the 1-L graduated cylinder.
3. Add approximately equal amounts of mud and water to the bucket, and use the stirring rod to mix them together.
4. Pour the mud/water mixture into the graduated cylinder until the column is about half full.
5. Use the stirring rod to mix the mud and water, removing any bubbles.
6. Use a hammer to crush a few pieces of chalk.
7. Weigh out 16 g of crushed chalk.
8. Add the crushed chalk to the column, and mix using the stirring rod.
9. Weigh out 8 g sodium sulfate.
10. Add the sodium sulfate to the column, and mix using the stirring rod.
11. Let the mixture settle in the column for about 5 minutes.
12. Add more of the mud/water mixture as described in steps 4–5 until the volume of packed mud occupies approximately one-third of the column volume.
13. If necessary, add more water to fill the column to the top.
14. Cover the top of the column with clear plastic wrap and secure with a rubber band.
15. Place the column near a window that receives full sunlight.
16. Position a light source to illuminate the side of the column facing away from the window. Do not move or disturb the column once it is in position.
17. During the first week following construction of the column, make daily observations of it in your logbook. Describe the appearance of the column. Include any layers that you see, and note their colors. Also record any odors you can detect.
18. Make weekly observations until your teacher instructs you to stop.

**Analysis**

1. Describe how the appearance of the Winogradsky column changes over time.
2. Which layers in the column are the most aerobic? most anaerobic?
3. Why was newspaper added to the column?
4. Why was sodium sulfate added to the column?
5. Explain how sulfur is recycled in the Winogradsky column.

**Investigations for Chapter 5**

**Cell Respiration: Releasing Chemical Energy**

**Investigation 5A ♦ How Does Oxygen Affect Cells?**

Oxygen is very important in the release of energy from food. Most organisms, including plants and humans, cannot live without a constant supply of oxygen. Some organisms, however, can get energy from food without oxygen. Additionally, there are a few kinds of microorganisms that use oxygen if it is available but still can get energy from food if oxygen is not available. These microorganisms that are both aerobic and anaerobic are called facultative anaerobes. The bacterium *Aerobacter aerogenes* is an example of a facultative anaerobe.

This investigation provides data from an experiment with *Aerobacter aerogenes*. The bacteria were allowed to grow in test tubes containing distilled water to which a few salts and various concentrations of glucose were added. Some of the test tubes were sealed so that no air was available to the cells. Other tubes had a stream of air bubbling through the growth solution. You will work with and interpret the data and develop a hypothesis to explain the findings.

**Materials (per student)**

- graph paper
- pencil
Procedure
1. Graph the data shown in Table 5A.1. Label the vertical axis *millions of cells per mL* and the horizontal axis *glucose (mg/100 mL)*. Plot the data from series A (test tubes without air). Label the line *growth without air*.
2. On the same graph, plot the data from series B (test tubes with air). Label the second line *growth with air*.
3. Use your graphs to help you answer the Analysis questions.

Analysis
1. What are the two obvious differences between the graph curves for series A and B?
2. Look at Table 5A.1, and compare test tubes 4A and 4B. How many times greater was the growth when air was present?
3. Compare the other test tubes in series A and B at the various glucose concentrations. How much greater is the growth in air for each pair of test tubes from 1A and 1B through 5A and 5B?
4. Notice that the number of bacteria is not given for test tubes 6B, 7B, 8B, and 9B. How many bacteria would you predict in test tube 6B? in 7B? These numbers were omitted from the table because the bacteria were too numerous to count.

5. After each test tube reached maximum growth, the solution was tested for the presence of glucose. In all the test tubes from 1A to 6A and from 1B to 9B, there was no glucose. (Test tubes 7A, 8A, and 9A contained some glucose even after maximum growth had been reached.) Compare test tubes 4A and 4B. How many bacteria were produced per milligram of glucose in each case?
6. Develop a hypothesis that explains the numbers you calculated for question 5. Why are there many more bacteria per milligram of glucose in the B test tubes than in the A test tubes?
7. Each milligram of glucose has the same amount of energy available to do work. The series B test tubes produced more bacteria per milligram of glucose than did the series A test tubes. Assuming that each bacterium produced requires a certain amount of energy, which test tube should contain some products of glucose that still contain some “unused” energy?
8. In additional tests, it was determined that alcohol accumulates in the series A test tubes. How does this information relate to your answer concerning “unused” energy in question 7?

Investigation 5B ◆ Rates of Respiration

Precise measurement of the rate of respiration requires elaborate equipment. Reasonably accurate measurements can be obtained by placing living material in a closed system and measuring the amount of oxygen consumed within the system or the amount of carbon dioxide produced over a period of time. In this investigation, you will use a simple volumeter to measure the amount of oxygen taken up by dormant and germinating seeds at different temperatures.

**Hypothesis:** After reading the procedure, develop a hypothesis to explain the movement of the colored drops in the capillary tubes.

**Materials** (per team of 3)
3 pairs of safety goggles
3 lab aprons
volumeter jar with spacers and screw-on lid

---

**Table 5A.1**

<table>
<thead>
<tr>
<th>Concentration of glucose (mg/100 mL of H₂O)</th>
<th>Number of bacteria at maximum growth (millions per mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series A</td>
</tr>
<tr>
<td></td>
<td>Tube</td>
</tr>
<tr>
<td>18</td>
<td>1A</td>
</tr>
<tr>
<td>36</td>
<td>2A</td>
</tr>
<tr>
<td>54</td>
<td>3A</td>
</tr>
<tr>
<td>72</td>
<td>4A</td>
</tr>
<tr>
<td>162</td>
<td>5A</td>
</tr>
<tr>
<td>288</td>
<td>6A</td>
</tr>
<tr>
<td>360</td>
<td>7A</td>
</tr>
<tr>
<td>432</td>
<td>8A</td>
</tr>
<tr>
<td>540</td>
<td>9A</td>
</tr>
</tbody>
</table>
3 volumeters
100-mL graduated cylinder
Pasteur pipette
2 jars
glass or plastic beads or washed gravel
nonmercury thermometer
nonabsorbent cotton
8 1/2" × 11" piece of cardboard
8 1/2" × 11" piece of white paper
forceps
ring stand with support ring
glass-marking pencil
paper clips
paper towels
masking tape
rubber band
wax paper
soda lime packets
colored water
85 Alaska pea seeds
water bath
water at room temperature (about 22ºC)
water at 37ºC
water at 10ºC
ice

Procedure
In this investigation, you will use three volumeters inserted into a jar with spacers and a screw-on lid (Figure 5B.1). Each volumeter consists of a 25-mm × 200-mm test tube, a 30-cm glass capillary tube calibrated at 1-cm intervals, and a stopper assembly. The stopper assembly includes a two-hole rubber stopper, two glass tubes with latex tubing attached, and a 5-mL syringe.

All the test tubes must contain equal volumes of material to ensure that an equal volume of air is present in each tube. A small drop of colored water is inserted into each capillary tube at its outer end. If the volume of gas changes in the tube, the drop of colored water moves, and the direction of water movement depends on whether the volume of gas in the system increases or decreases.

When measuring respiration with the volumeter, consider not only that oxygen enters the living material (and thus leaves the test-tube environment), but also that carbon dioxide leaves the living material (and enters the test-tube environment). To measure the oxygen uptake by the respiring material, a substance is added to absorb the carbon dioxide as it evolves, so it is not added to the volume of gas in the tube.

Day 1
1. Germinate 45 pea seeds according to the procedure used in Investigation 2A, Part B. Label the jars with your team, class, experiment, and date. As the seeds begin to germinate, what biochemical process increases?

   CAUTION: Wash your hands immediately after handling the seeds. They may be treated with a fungicide.

Day 2
2. Select 40 pea seeds from the germination jar. Discard the 5 extra seeds. Determine the volume of the 40 soaked seeds by adding them to a measured volume of water in a graduated cylinder and reading the volume of displaced water. Record the volume of the seeds. Return the 40 seeds to the germination jar.
3. Repeat step 2 using 40 dry ungerminated pea seeds.
4. Wash your hands thoroughly after handling the seeds.
Day 3

5. The volume in the test tubes of the germinated and dry pea seeds must be equal. Measure the volumes of both types of seeds again, and add beads or gravel to the dry seeds until their volume is equal to that of the germinated seeds. What change occurred in the volume of the seeds after 48 hours in the germination jar?

6. Measure an amount of beads or gravel to equal the volume determined for the germinated seeds.

7. Fill the volumeter jar about two-thirds full with room-temperature water. Screw on the lid. Why is water added to the volumeter jar?

8. Clip a piece of white paper to the cardboard, and place it on the ring-stand support ring.

9. Remove the stopper assemblies from each of the three test tubes. Add the germinated peas to one test tube; add the dry peas (and beads or gravel) to another. In the third test tube, place the equal volume of beads or gravel you measured in step 6. This third tube is a thermobarometer, which is used to determine any changes in the system. What two variables will the thermobarometer help you measure?

10. Place a 2-cm plug of dry nonabsorbent cotton about 1 cm above the seeds or beads in each test tube (Figure 5B.2). Use forceps to place a small packet of soda lime wrapped in gauze on top of the plug.

CAUTION: Soda lime is a corrosive solid. Do not touch; do not ingest. If it gets on skin or clothing or in the mouth, rinse thoroughly with water; if in eyes, wash gently but thoroughly for 15 minutes. Call your teacher.

11. Gently but firmly press a stopper assembly into each test tube. Insert the test tubes through the lid into the volumeter jar.

12. Insert the thermometer into the jar through the thermometer hole in the lid. Record the temperature of the water in the volumeter jar, and maintain this temperature throughout the experiment.

13. With a Pasteur pipette, insert a small drop of colored water into the calibrated end of each dry capillary tube. Rotate each tube until the drop is correctly positioned. The drop of colored water in the thermobarometer should be positioned at about the middle of the calibrations, and the other drops should be positioned at the outermost calibration.

14. Carefully attach each capillary tube (by its uncalibrated end) to the longer glass tube in a stopper assembly. Support the tubes in a level horizontal position on the paper prepared in step 8.

15. Use the syringe to reposition the drop in the capillary tube, if necessary. Tape the tubes in place on the paper.

16. Allow the apparatus to stand for about 5 minutes to permit the temperature to become uniform throughout the system.
17. Prepare a table in your logbook similar to Table 5B.1. On the paper beneath the capillary tubes, mark the position of one end of each drop.

18. Record the position of each drop every 5 minutes for 20 minutes. If respiration is rapid, you may need to reposition the colored drop as you did in step 13 or 14. If you do this, be sure to add both measurements of the distance moved by the drop to calculate the total change during the experiment.

19. Disassemble the volumeter, and dry out the capillary tubes.

20. Reassemble the volumeter using 37°C water, and place the jar containing the volumeter tubes in a water bath that also contains 37°C water. Ideally, when the volumeter is placed in the water bath, the water level should reach the same level as the water in the jar.

21. Position a drop of colored water in each capillary tube, attach each capillary tube to its longer glass tube, and record drop positions as described in steps 13–18.

22. Repeat steps 19–21 using water at 10°C. Add ice as necessary to lower the water temperature.

23. Wash your hands thoroughly before leaving the laboratory.

**Analysis**

1. What is the effect of moisture on the respiration rate of germinating pea seeds?
2. Would adding more water to the soaked seeds result in an increased rate of respiration? Explain your answer.
3. What if the carbon dioxide absorbent (soda lime) were not used? Use the equation \( C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 \) to calculate how much, if any, the volume within the volumeter would change if the carbon dioxide were not removed. Do you think the six water molecules that are released per molecule of sugar should be considered? Why or why not?

4. Use any changes in the thermobarometer to determine the corrected distance moved by the drops in the volumeters containing the pea seeds.

5. Calculate the total volume of oxygen used by the pea seeds. Each 1-cm mark on the capillary tube equals 0.063 mL of oxygen by volume. For each temperature, determine the rate of respiration for the pea seeds by calculating oxygen consumed per minute.

6. Does the rate of respiration for pea seeds change with temperature?

7. How does the rate of respiration of the ungerminated seeds compare to the germinated seeds? What is the significance of this difference as far as the seeds’ ability to survive in nature is concerned?

8. Make a linear graph comparing the rates of respiration of pea seeds at the three different temperatures.

**Investigations for Chapter 6**

**Cell Structures and Their Functions**

**Investigation 6A • Cell Structure**

In this investigation, you will examine some cells from unicellular and multicellular organisms. By comparing selected cell preparations, you will be able to identify different cell structures and propose explanations for their functions.

**Materials** (per team of 2)
- 2 pairs of safety goggles
- 2 lab aprons
- coverslip

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Procedure

1. Separate one layer from an onion quarter, and hold it so that the concave (curved inward) surface faces you. Snap it backward (Figure 6A.1a) to separate the transparent, paper-thin layer of cells from the outer curve of the scale.

2. Use forceps to peel off a small section of the thin layer, and lay it flat on a microscope slide.

3. Add 1 or 2 drops of Lugol’s iodine solution and a coverslip. WARNING: Lugol’s iodine solution is a poison if ingested, is a strong irritant, and can stain clothing. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher immediately.

4. Examine the slide first with low power and then with high power.

5. Sketch a few cells as they appear under high power. How many dimensions do the cells appear to have when viewed through high power? Sketch a single cell as it would appear if you could see three dimensions. Using the procedure from Investigation PC, estimate and record cell size.

6. Identify the cell wall, nucleus, and cytosol. Sketch each in a diagram. You may be able to see a nucleolus within a nucleus. If so, sketch it also. Add a drop of salt solution (Figure 6A.1b)
at one edge of the coverslip. The paper will act as a wick, pulling the salt solution across the slide and into contact with the cells. Continue observing the cells until the cytosol appears to pull away from the cell walls. The boundary of the cytosol is the plasma membrane.

7. Obtain a prepared slide of Gram-stained bacteria. The slide has three sections, each of which contains a different species of bacteria. Examine each section with the low-power objective and then the high-power. Sketch in your logbook a few bacteria of each species under high power. Note the color and shape of each species. Estimate and record the cell sizes.

8. Obtain a prepared slide containing human and frog blood. Examine each blood sample under low power, then high. Find a field where the cells are separate and distinct, and sketch a few cells of each type under high power. Estimate and record the cell sizes.

9. Obtain a prepared slide containing plant and animal cells. Examine the plant and animal cells under low power, then high. Find a field where the cells are separate and distinct, and sketch a few cells of each type under high power. Estimate and record the cell sizes.

10. Obtain a prepared slide of paramecia. Examine the cells under low power, then high. Find a field where the cells are separate and distinct, and sketch a few under high power. Estimate and record cell sizes. Paramecia are mobile organisms. Try to identify cell structures that play a role in locomotion.

11. Wash your hands before leaving the laboratory.

Analysis

1. Are the plasma membranes of plant cells difficult to see? Explain your answer.

2. Did the Lugol’s iodine solution aid in your observation of cells? Why do biologists use stains to study cells?

3. What is the significance of the observation that some bacteria stain purple with Gram’s stain and others stain red?

4. What differences did you observe between the human and frog blood samples? Do these observations suggest that humans and frogs have different ways of maintaining their blood cells?

5. Construct a summary table comparing the common organelles of plant and animal cells. What parts of the plant cell were not present in the animal cell? What are the functions of these plant-specific cell structures?

6. What cell structures are used by the paramecia for locomotion? Are there similar structures in any human cells, and if so, what might be their functions?

Investigation 6B ◆ From One Cell to Many

Single-celled organisms come in many different shapes and sizes. Despite their varied appearance, these organisms all must carry out certain essential life processes. To that end, they have evolved specific cell structures to meet each of those needs. Among multicellular organisms, some protists are colonial, where each member of the colony is nearly identical to the next. A true multicellular organism, such as a plant or an animal, requires that cells exhibit a division of labor.

In this investigation, with the aim of identifying the life processes that all cells share, you first will observe microorganisms found in pond water. Next, you will examine prepared slides of various mammalian tissue types. Your task is to observe them carefully and, based on their appearance, form a hypothesis as to which life process that cell type contributes.

Materials (per team of 2)

2 pairs of safety goggles
2 lab aprons
coverslip
dropping pipet
microscope slide
compound microscope
key to pond-water organisms
pond-water culture
Detain™ in dropping bottle (slowing agent)
prepared slide A
prepared slide B
prepared slide C
prepared slide D
prepared slide E
Procedure
1. Place 1 or 2 drops of the pond-water culture on a microscope slide, add a coverslip, and examine under low power with your microscope.
2. Try making observations through the high-power lens, keeping in mind that most of the organisms are transparent or almost transparent. Decrease the amount of light by adjusting the diaphragm. Many of the organisms move rapidly and are hard to find under high power. The coverslip will slow some of them, but you can also add a drop of Detain™, a slowing agent.
3. Use the key to pond-water organisms to identify some of the organisms. Roundworms, daphnia, cyclops, rotifers, and immature forms (larvae) of insects are among the multicellular creatures that could be in your pond water. In your logbook, list as many life processes shared by the microorganisms as you can. For each process in your list, describe a tissue or cell type in a human that is involved in performing that function.
4. The five prepared slides are all from mammals and are associated with the functions of reproduction, movement, protection, food storage, and sensory communication. Obtain prepared slide A, and observe first under low power, then high. Sketch a few cells under high power.
5. Obtain prepared slides B through E in turn, and observe each under low power, then high. For each slide, sketch a few distinct cells under high power.
6. Use your observations to match each of the five prepared slides with one of the five functions: reproduction, movement, protection, food storage, and sensory communication.

Analysis
1. How many different organisms did you observe in the pond water? Describe some of the differences among them.

2. What limits the size of single-celled organisms?
3. At the genetic level, how does a lung cell differ from a brain cell?
4. Which prepared slides correspond to which functions? Explain your answers.
5. List other functions carried out by single-celled organisms and the cell types that correspond to them in multicellular organisms.

Investigations for Chapter 7
Transport Systems

Investigation 7A  Water Movement in Plants

The normal pathway of moving water in a living plant is first into the roots, then through the stem, and finally into the leaves. Environmental influences, the chemical properties of water, and structures in the plant are involved in the movement. This laboratory investigation deals with the following questions:
1. What plant structures—roots, stems, or leaves—are most important in the movement of water?
2. What are the types of cells that transport water in a plant? What are their characteristics?
3. Is all the water that is delivered to the leaves used, or is some lost?
4. How would you describe the source and direction of the force that moves water upward in a plant, against the force of gravity?

Hypothesis: Before beginning the investigation, read the procedure for Part A. Write a prediction that describes what you think will happen to each test tube.

Materials (per team of 3)
- 3 pairs of safety goggles
- 6 18-mm × 150-mm test tubes
- test-tube rack
- glass-marking pencil
- heavy-duty aluminum foil
- scalpel
- petroleum jelly
- cotton swab
- compound microscope
- prepared slide of woody-stem cross section
- prepared slide of leaf cross section
PART A  Measuring Water Uptake

Procedure
1. Fill six test tubes with water to within 2 cm of the top, and cover the tops with aluminum foil. Label the test tubes 1 through 6. Treat each test tube as follows, using Figure 7A.1 as a guide.

Tube 1: Mark the water line.

Tube 2: With a scalpel, remove the roots of one bean or sunflower seedling 6 cm below the cotyledons. Pierce the aluminum foil with a pencil point, and ease the plant through the hole into the water. Mark the water line after the plant is in place.

Tube 3: Remove all leaf blades from another seedling, leaving only the leaf stems. Then repeat the procedure for test tube 2.

Tube 4: Remove the aluminum foil from the test tube. Sink an intact plant’s roots into the water. Mark the water line. Mold the aluminum foil to the rim of the test tube so that it seals the tube and supports the plant stem.

Tube 5: Remove the leaf blades from another seedling. Only the leaf stems should remain. Then repeat the procedure described for test tube 4 for this leafless plant.

Tube 6: With a cotton swab, brush petroleum jelly on the upper and lower surfaces of a seedling’s leaves. Repeat the procedure described for test tube 2.

2. Allow the rack of treated plants to stand in indirect light overnight. After 24 hours, observe the test tubes for any changes. Record your observations and any measurements you take.

Analysis
1. What is the purpose of the first test tube?
2. Identify variables in this experiment and how they were controlled.
3. Do the results of the investigation support or disprove your prediction? Explain your answer.
4. Based on your results, what do you predict would happen if
   a. you used seedlings that had twice the number of leaves as those you actually used for test tubes 2, 4, and 6? Explain your answer.
   b. the seedling in test tube 5 had twice the number of roots as the one you actually used? Explain your answer.
   c. you put petroleum jelly on the stem cut of the plant in test tube 6, as well as on the leaves? Explain your answer.
5. Based on your observations alone, which of the following statements is most likely correct? Give the reasons for your choice.
   a. Water is pushed upward in a plant by a force created in the lower parts of the plant.
   b. Water is pulled upward in a plant by a force created in the upper parts of the plant.
6. Account for your observations and results in terms of any changes that occur in the experimental setup.

7. Do the results of this experiment support or disprove the cohesion-tension hypothesis? Explain your answer.

8. Using your experimental results and your reading, describe the path of a water molecule through a seedling. Where does it begin? Where does it end up?

9. Describe an experiment that would trace the path of water molecules through a plant. How would you track the water molecules? How would you set up the experiment? How would you control the variables? (Hint: See Appendix 1B, “Radioisotopes and Research in Biology.”)

**PART B Transport Structures in Plants**

**Procedure**

3. Study the cross section of a leaf first with low power of the microscope and then with high power. Identify the following structures using Figure 7A.2 as a reference:

- **Upper epidermis**: thick-walled, flat cells without chloroplasts, covered with a waxy layer
- **Palisade layer**: tightly packed and column-shaped cells containing many chloroplasts
- **Spongy layer**: rounded cells containing chloroplasts, with air spaces between them
- **Veins**: transport tissue composed of thick-walled xylem and phloem cells
- **Lower epidermis**: thick-walled, flat cells without chloroplasts but with openings at intervals
- **Stomates**: openings in the lower epidermis
- **Guard cells**: two small cells surrounding each stomate and containing chloroplasts

4. Observe a slide showing the lower epidermis of a leaf under high power. Locate the guard cells enclosing a small slit, the stomate. Sketch the stomate and guard cells.

5. Study a cross section of a woody stem under low power. Identify the regions of the pith, vascular bundles, cambium, and bark. Turn to high power, and observe a vascular bundle in more detail. Identify the xylem, heavy-walled cells toward the center area of the stem, and the phloem, small thin-walled cells toward the outside area of the stem. Xylem and phloem cells are separated by a layer of living cells called cambium.

6. Carefully place a radish or grass seedling in a drop of water on a microscope slide, and observe with low power. Notice the extent of absorptive area provided by the root hairs. The young root’s darker, denser center region...
includes developing vascular bundles, also composed of xylem, phloem, and cambium cells.

7. Wash your hands before leaving the laboratory.

Analysis
1. Which adaptations of the leaf for the prevention of water loss did you observe? Describe them. In what ways may water loss by leaves help the plant? In what ways might it harm the plant?

2. Describe the adaptations to absorb water from the soil that you observed.

3. How does water enter the root hairs and epidermal cells?

4. What structure in the stem connects the water-conducting vessels of the roots and the veins of the leaf? Describe the structure.

5. Illustrate a pathway showing the route followed by water molecules as they move from the soil, through a plant, and into the atmosphere. Include the specific structures of the root, stem, and leaf that are involved. Use the information and observations you gathered during this investigation to create your illustrations.

Investigation 7B ◆ Exercise and Pulse Rate

You probably have experienced the sensation of your heart beating strongly inside your chest when you participated in a physical activity such as running, aerobic exercise, or athletic competition. The heartbeat rate increases in response to signals from the nervous and endocrine systems, which are monitoring the entire body. Pulse rate, as measured manually at the wrist, is a measure of how fast the heart is beating.

Read the Analysis questions, and develop a hypothesis about how exercise (mild or vigorous) affects heart rate and recovery time. Recovery time is the length of time it takes for the heartbeat to return to the resting rate. Design an experiment to test your hypothesis using the materials listed below.

Materials (per team of 2)
stopwatch or clock with a second hand

Safety
Keep in mind that you must have plenty of room to safely perform your pulse-raising activity; avoid bumping into other persons or objects. Make sure your activity area is free of hazards.

Procedure
Measure pulse at the wrist to carry out the experiment that you designed and that your teacher has approved (Figure 7B.1). In your logbook, record the pulse rate you measure. Organize your data in a table, and construct the graphs at the end of the experiment to display the data.

Analysis
1. What was the control in your experiment?

2. What label did you put on the y-axis of your graph? on the x-axis?

3. Are there any differences in heartbeat rates and recovery times for the different types of exercise among the students in your class? Explain your answer.

4. Describe the effect of varying degrees of exercise—mild, moderate, and strenuous—on heartbeat.

5. Are there any differences in heartbeat rates and recovery times between males and females in your class? Explain your answer.

6. Note any other conclusions you can make based on your data.
INVESTIGATIONS

Investigations for Chapter 8
The Cell Cycle

Investigation 8A  DNA Replication

DNA replication is the process by which exact copies are made of the DNA in prokaryotes and in the chromosomes of eukaryotes. During replication, the genetic code contained within a sequence of nucleotide bases in DNA is preserved. How does replication take place? This investigation will give you an opportunity to observe some of the basic steps involved in the process of replication.

Materials (per team of 2)
pop-it beads: 40 black, 40 white, 32 red, 32 green
string or twist ties
4 tags with string

Procedure

PART A  Building the DNA Molecule
You will build a double-stranded segment of DNA using colored pop-it beads and the following key for the nucleotide bases:
- black = adenine (A)
- white = thymine (T)
- green = guanine (G)
- red = cytosine (C)

1. Construct the first DNA strand by linking the colored pop-it beads to represent the following sequence of nucleotide bases:
   A A A G G T C T C T C T A A T T G G T C T C
   C T T A G G T C T C C T T

2. Attach a tag to the AAA end of the strand, and label the strand roman numeral I by marking the tag.

3. Now construct the complementary strand of DNA that would pair with strand I. Remember that thymine (T) bonds with adenine (A) and that guanine (G) bonds with cytosine (C).

4. Attach a tag to the TTT end of the strand, and label the strand II by marking the tag.

5. Place strand II beside strand I, and check to make certain that you have constructed the proper sequence of nucleotide bases in strand II. Green pop-it beads should be opposite red pop-it beads, and black pop-it beads should be opposite white pop-it beads. Make any necessary corrections in strand II in order to have the proper sequence of base pairs.

6. Tie a simple overhand knot with a short piece of string, or use a twist tie to join the first pop-it beads in strand I and strand II, as shown in Figure 8A.1 (top).

7. Repeat step 6 for the pop-it beads on the other end of strands I and II.

PART B  DNA Replication

8. Place your double-stranded DNA molecule on your table so that both strands are in horizontal straight lines and the AAA end of strand I is on your left. (Do not unfasten the ends.)

9. Beginning at the AAA end of strand I, count 22 pop-it beads from left to right. Tie the strands together between the 22nd and 23rd pairs of beads, as shown in Figure 8A.1 (bottom).

10. Untie the string holding the two strands together at the AAA end of strand I. Separate strand I and strand II so they form a Y.

11. DNA replication on strand I begins with the action of DNA polymerase at the AAA end and proceeds toward the replication fork (the point at which the nucleotide bases are joined at the 22nd base pair). Construct the new complementary strand for strand I beginning at the AAA end and working toward the replication fork. When you have finished, tie another
overhand knot at the AAA end of strand I to join it to its new complementary strand.

12. Replication of strand II begins with the action of DNA polymerase at the replication fork and works outward toward the TTT end. (This is the lagging strand of DNA.) Build the complementary DNA strand for strand II, proceeding from right to left; then tie the two strands together at the TTT end.

13. Now untie the overhand knots that join strands I and II at the 22nd base pair and at the right-hand end of the original double strand.

14. Continue the replication of strand I from left to right until you complete the new complementary strand. Tie another overhand knot to join strand I and its complement at the right-hand end of the molecule.

15. Continue the replication of strand II from the right-hand end to the left. When you reach the 22nd base pair, use another knot to join the two segments of the newly formed strand (the complement of strand II).

16. Tie another overhand knot in the right-hand end of strand II to join it to its new complementary strand.

Analysis

1. Compare the two new double-stranded molecules you have just completed. How are they similar to the original DNA molecule containing strands I and II? How are they different?

2. In your own words, describe a replication fork.

3. Describe the differences in the way strands I and II are replicated.

4. Describe how DNA replication makes it possible to produce two identical cells from one parent.

Materials (per team of 2)

compound microscope
modeling clay
prepared slide of animal embryo cells (Ascaris or whitefish)
prepared slide of onion root-tip cells

Procedure

1. Place the slide of root-tip cells on the microscope stage, and examine it under low power. Scan the entire section. Observe that cells far from the tip and cells right at the tip are not actively dividing. Locate the region of active mitosis between these two regions.

2. Change to the high-power objective. As you observe the cells, focus up and down slowly with the fine-adjustment knob to bring different structures into sharp focus. Find cells at various stages of mitosis. When the slide was prepared, the cells were killed at different stages of a continuous process. The cells can be compared to scrambled single frames of film. Figure out how you would piece the frames of the film together. Refer to Figure 8.11 for help. Make sketches from the slide of cells in interphase, prophase, metaphase, anaphase, and telophase as described in Section 8.6. Identify each by stage.

3. Examine a slide of developing Ascaris. Find a cell in which the chromosomes are long and threadlike. Try to count the number of individual chromosomes.

4. Find a cell in which the chromosomes are at the equator of the spindle. Compare the poles of this spindle with those of the spindles in the dividing plant cells you studied in steps 1 and 2.

5. Find a cell in which the chromosomes are separating and the cell is beginning to pinch together in the middle. Compare this method of cytoplasmic division with the method you observed in plants.
6. With clay and four large sketches of a plant cell, model each stage of mitosis, from prophase to telophase, for a plant cell with three pairs of chromosomes. Make each pair of chromosomes a different length or color.

Analysis
1. How is the process of mitosis in plant and animal cells similar?
2. How does mitosis in plant and animal cells differ?
3. Refer to Figure 8B.1, and study cells numbered 2, 7, 9, 11, 12, 13, 16, and 33. Rewrite the order of these cells to reflect the sequence of stages you would see if only one cell were undergoing mitosis.

4. Compare the number and types of chromosomes in the two new nuclei of the clay model with the number and types from the original parent nucleus.
5. If mitosis occurs in a cell but cell division does not occur, what is the result?

Investigations for Chapter 9
Expressing Genetic Information

Investigation 9A ◆ Transcription

DNA is the molecule in which all of the genetic information for the cell is stored. The information is encoded as a triplet code in which each sequence of three nucleotide bases codes for a specific piece of information. The DNA is contained in the nucleus, but the cellular processes take place in the cytosol. How does the information from the DNA get into the cytosol where it can be used? This investigation will help you understand that process.

Materials (per team of 2)
pop-it beads: 40 black, 40 white, 32 green, 32 red, 20 pink
string
tags

Procedure

PART A DNA Transcription
You will build a double-stranded segment of DNA and a single-stranded segment of messenger RNA (mRNA) using colored pop-it beads and the following color code for the nucleotide bases:

- black = adenine (A)
- white = thymine (T)
- green = guanine (G)
- red = cytosine (C)
- pink = uracil (U)

1. Construct DNA strand I, as you did in Investigation 8A, by linking the colored pop-it beads together to represent the following sequence of nucleotide bases:

   A A A G G T C T C C T C T A A T T G G T C T C C T T A G G T C T C C T T

2. Attach a tag labeled roman numeral I to the AAA end of strand I.
3. Now construct the complementary strand of DNA that would pair with strand I. Remember that thymine (T) bonds with adenine (A) and that guanine (G) bonds with cytosine (C).
4. Attach a tag labeled II to the TTT end of the complementary strand.
5. Place strand II beside strand I, and check to make certain that you have constructed the proper sequence of nucleotide bases in strand II. Green pop-it beads should be opposite red pop-it beads, and black pop-it beads should be opposite white pop-it beads. Make any corrections necessary to have the proper sequence of base pairs.
6. Position the strands on your work surface so that the AAA end of strand I is to your left. Using two pieces of string, tie together nucleotide bases 22 and the right end of the double strands with an overhand knot, as you did in Investigation 8A.
7. Open the left-hand side of the DNA molecule to form the replication fork. Assume that a molecule of RNA polymerase has just attached to the left-hand end of strand I and that a molecule of DNA polymerase has just attached to strand II at the replication fork.
8. Strand I will produce a single-strand molecule of mRNA instead of a new double-stranded molecule of DNA. RNA is produced by the process of transcription from strand I. The rules for forming mRNA are the same as for DNA except that uracil (pink) is used in place of thymine (white). Beginning at the AAA end of strand I, construct the mRNA molecule according to the sequence of bases in strand I. Continue moving toward the replication fork.
9. Untie the overhand knot at base pair 22. Continue the mRNA transcription on strand I, moving from left to right toward the right-hand end of the molecule. When you have finished, attach a tag labeled mRNA at the left-hand end of the mRNA molecule.
10. Complete the DNA replication of strand II by moving to the right-hand end of the molecule and working back to the left. Join the two segments of the complementary strand to complete the new DNA molecule. Tie together both ends of the double-stranded DNA molecule with string, and set it aside.

11. Check the sequence of nucleotide bases on the mRNA molecule against strand I of the DNA. Remember that uracil (pink) is substituted for thymine (white) in RNA. The mRNA molecule is now ready to move from the nucleus into the cytosol, where its message will be translated. Save your mRNA molecule and the double-stranded DNA molecule to use in Investigation 9B.

Analysis
1. Compare the sequence of nucleotide bases in the mRNA molecule with the sequence of nucleotide bases in strand II. How are they the same? How are they different?
2. Compare the mRNA molecule with the DNA molecule you have set aside for use in Investigation 9B. How are they the same? How are they different?
3. Why do you think mRNA can leave the nucleus and DNA cannot?

Investigation 9B ◆ Translation

You have seen how DNA is replicated and how messenger RNA is formed from a DNA template. The mRNA can leave the nucleus and move into the cytosol, where the message it carries from the DNA can be translated into a sequence of amino acids in the formation of a protein. In this investigation, you will study another type of RNA, transfer RNA (tRNA), and the important role it plays in translation.

Materials (per team of 2)
- DNA molecule from Investigation 9A
- masking tape
- modeling clay
- mRNA molecule from Investigation 9A
- pop-it beads: 7 black, 10 red, 6 green, 13 pink tags
Procedures

Throughout this investigation, refer to Figure 9B.1, which shows what amino acids are coded for by each DNA triplet. The DNA triplets are in black type, and the mRNA complementary codons are in blue type.

The tRNA anticodons pair with the mRNA codons in the same way that mRNA complementary codons pair with DNA codons except for the substitution of uracil for thymine in tRNA. If you have difficulties decoding the tRNA codons, ask your teacher for help. Use the same color code for the pop-it beads that you used in Investigation 9A:
- black = adenine (A)
- white = thymine (T)
- green = guanine (G)
- red = cytosine (C)
- pink = uracil (U)

1. Place the double-stranded DNA molecule and the mRNA molecule across your work surface so that the mRNA lies next to strand I of the DNA and their complementary codons are side-by-side. Beginning at the AAA end, record the letters representing the first three nucleotide bases (the first codon) in strand I of your DNA molecule. Next, record the letters representing the first three bases (the first complementary codon) in your mRNA molecule. In the same way, record the letters representing the bases of the remaining 11 codons in the DNA and mRNA molecules.

2. Use Figure 9B.1 to determine which amino acid is coded for by each mRNA codon. (The DNA triplet and the mRNA complementary codon are adjacent in the figure.) List the appropriate amino acid opposite each mRNA codon.

3. Write the name of the first amino acid on a tag. Make a tag for each of the 11 other amino acids indicated by the codons in the mRNA strand.

4. Determine the anticodon sequence for the tRNA that would pair with the mRNA codon. Build the tRNA anticodon from pop-it beads using the code given above. Tie the tag showing the name of the appropriate amino acid near the middle pop-it bead, as shown in Figure 9B.2 (left). This model represents the tRNA anticodon with its attached amino acid that will take part in protein synthesis.

5. Repeat step 4 to build tRNAs for each of the remaining 11 triplet codons in the mRNA.

---

**FIGURE 9B.1**

The genetic code. The DNA codons appear in black type; the complementary mRNA codons are in blue. \( A = \text{adenine}, \ C = \text{cytosine}, \ G = \text{guanine}, \ T = \text{thymine}, \ U = \text{uracil}, \text{stop} = \text{chain termination or “nonsense” codon.} \)
6. To represent the large subunit of a ribosome, use one color of modeling clay to make a large oval the length of six pop-it beads. To represent the small subunit of a ribosome, make a narrower oval of a different color of modeling clay, also the length of six pop-it beads. Push the long sides of the two ovals together so their edges are joined and the small subunit lies on top of the large subunit, as shown in Figure 9B.2 (right). These two ovals represent a functional ribosome. When you finish you should have the following:
   1. double-stranded DNA molecule
   2. single-stranded mRNA molecule
   3. 12 tRNA molecules with amino acids attached
   4. 1 ribosome

7. Mark a small piece of masking tape A site and another piece P site. Press the A-site tape into the small ribosome subunit near the right side and the P-site tape near the left side.

8. Place the first codon of the mRNA molecule on the A site of the small subunit of the ribosome, and lightly press the three pop-it beads of the codon into the clay.

9. Select the tRNA anticodon that will pair with the mRNA codon. Place the tRNA on the larger subunit of the ribosome so that the amino acid points away from the mRNA molecule. Figure 9B.3 (top) shows these positions.

10. Move the mRNA molecule to the left so that the first codon is on the P site and the second codon is on the A site. Move the first tRNA anticodon to keep it paired with its mRNA codon.

11. Select the correct tRNA molecule to pair with the second mRNA codon that is now on the A site of the ribosome. Press both the mRNA and the tRNA molecules lightly into the clay.

12. Remove the tag representing the first amino acid from its tRNA anticodon, and tape it to the tag representing the second amino acid, as shown in Figure 9B.4 (top). You have just formed a peptide bond.
13. Move the mRNA molecule to the left by one codon so that the first codon with its tRNA anticodon is no longer on the ribosome. Put the first tRNA anticodon off to the side.

14. Select the appropriate tRNA anticodon to pair with the third mRNA codon, which is now on the A site of the ribosome, as shown in Figure 9B.4 (bottom). Attach the dipeptide formed in step 12 to this third amino acid.

15. Continue moving the mRNA molecule across the ribosome and pairing the tRNA anticodons with the mRNA codons. After each tRNA anticodon with its amino acid has arrived at the ribosome, remove the amino acid and attach it to the growing chain of amino acids.

16. When all 12 amino acids have been joined, remove the chain of amino acids from the tRNA and the ribosome. This model represents part of a protein molecule.

Analysis
1. Describe similarities and differences between the first DNA triplet and the first tRNA anticodon that you built.
2. What role does the ribosome play in protein synthesis?
3. What role does tRNA play in protein synthesis?
4. What role does mRNA play in protein synthesis?
5. Write a short paragraph that summarizes the roles of transcription and translation in protein synthesis.

Investigations for Chapter 10
Animal Growth and Development

Investigation 10A ◆ Development in Polychaete Worms

The marine polychaete worm *Chaetopterus variopedatus* lives in the sand near the low-tide level inside a leathery, U-shaped tube (Figure 10A.1). The sperm or eggs are visible inside the parapodia (leglike extensions from the body) near the posterior end of the worm. The sperm appear ivory white and give the parapodia a smooth, white appearance. The ovaries are in yellow coils and contain eggs that give the female parapodia a grainy appearance. You will observe the worm, remove eggs and sperm, fertilize the eggs, and observe the development of the embryos. The initial stages of development that you will observe look the same for nearly all sexually reproducing organisms.

Materials (per team of 2)
- 3 microscope slides
- 3 coverslips
- dropping pipette
- 3 finger bowls
- compound microscope
- forceps
- dissecting scissors
- 2 dissecting needles
- cheesecloth
- paper towels
- seawater
- male and female parapodia of *Chaetopterus*

SAFETY Spilled water can cause slippery footing and falls. Wipe up any spilled water immediately. Handle dissecting tools with care; immediately report any injuries to your teacher.

FIGURE 10A.1
Polychaete worm in a glass tube (top) and the worm’s natural tube (bottom).
Procedure
1. Rinse a small folded-over piece of cheesecloth in fresh water; then rinse it in seawater. Place the wet cheesecloth in the bottom of a finger bowl.
2. Using forceps and dissecting scissors, remove one parapodium with eggs from the female worm and place it on the wet cheesecloth in the finger bowl. Use the pipette to remove any eggs that spilled out of the parapodium, and squeeze the seawater and eggs out of the pipette onto the cheesecloth. Cut open the parapodium, and with the dissecting needle and forceps, tear the parapodium apart and release the eggs.
   \[\text{CAUTION: Dissecting tools are sharp; handle with care.}\]
3. Lift the cheesecloth, and pour about 1 cm of seawater into the finger bowl. Pick up the cheesecloth by the four corners; gently dip it up and down in the water, and move it around slowly. The movement should allow most of the eggs to filter into the seawater while sand and other debris stay behind. The eggs are tiny yellow or yellow orange dots. Note the time in your logbook. Discard the cheesecloth in a container designated by your teacher.
4. You must wait 15 minutes from the time you put the eggs in seawater before you add sperm. While you wait, you can obtain and study the sperm. Add 10 mL of fresh seawater to another finger bowl. Remove a parapodium with sperm from the male worm, and quickly place the parapodium into the fresh seawater. With a dropping pipette, pick up any sperm that were released in the bowl with the worm. The sperm will appear as a small cloud in the water. Add these sperm to those already in the 10 mL of water.
5. Place one drop of the sperm and seawater mixture onto a clean microscope slide, and add a coverslip. Examine the sperm using the low-power objective of a microscope. They will appear as small dots moving around. Switch to the high-power objective, and observe the sperm.
6. If you do not have many moving sperm, take another sample from the 10 mL of seawater. If moving sperm still are not evident, repeat steps 4 and 5.
7. After the eggs have been in the seawater for 15 minutes, add one drop of the sperm and seawater mixture to the eggs. The eggs should all be fertilized within 30 minutes. After 30 minutes, remove the eggs from the finger bowl and place them in another finger bowl with fresh seawater. The development of the embryos will take place in this bowl and can be observed after about 24 hours.
8. While you are waiting for 30 minutes, obtain fertilized eggs from bowls designated by your teacher and examine them under the microscope. Look for embryos at the 4-, 8-, and 16-cell stages, and note any other developmental stages you observe.
9. Wash your hands before leaving the laboratory.

Analysis
1. Examine photographs of human sperm in Figures 10.2 and 12.17. How do the sperm of the polychaete worm compare with the picture of human sperm? Describe any similarities or differences you observe.
2. Describe the development process you observed in the polychaete worm embryos.
3. The polychaete worm larvae usually develop within 24 hours after fertilization. Based on what you observed and your knowledge of cleavage and cell division, approximately how many cell divisions have occurred between the one-cell stage and the swimming larvae?
4. Describe any similarities between the development of the polychaete worm embryos and the development of human embryos.

Investigations for Chapter 11
Plant Growth and Development

Investigation 11A Seeds and Seedlings

A seed is a packaged plant—a complete set of instructions for growing a plant such as a maple tree or a geranium. The seed contains everything needed to produce a young plant. How does the seed change to a plant? What are the functions of the structures of the seed and the growing plant? What changes can
you observe? In this investigation, you will have the opportunity to find out.

**Hypothesis:** After reading the procedures, construct a hypothesis that predicts how petri dishes C and D will differ.

**Materials** (per team of 2)
- 2 pairs of safety goggles
- 2 lab aprons
- 10× hand lens
- scalpel
- petri dish with starch agar and germinating corn grains—dish C
- petri dish with starch agar and boiled corn grains—dish D
- Lugol's iodine solution in dropping bottle
- soaked germinating corn grains
- soaked bean seeds
- bean seeds germinated 1, 2, 3, and 10 days

**SAFETY** Put on your safety goggles and lab apron.

**PART A The Seed**

**Procedure**
1. Examine the external features of a bean seed. Notice that the seed is covered by a tough, leathery coat. Look along the concave inner edge of the seed, and find a scar. This scar marks the place where the seed was attached to the pod.
2. Remove the seed coat, and examine the two fleshy halves, the cotyledons, which are part of the embryo.
3. Using a scalpel, cut a small sliver from one of the cotyledons. Test the sliver with a drop of Lugol's iodine solution. Record the results in your logbook.

**CAUTION:** Scalpels are sharp; handle with care.

**PART B The Seedling**

**Procedure**
5. Examine bean seedlings that are 1, 2, and 3 days old.
6. Compare the 10-day-old seedling with the 3-day-old seedling.

**Analysis**
1. What part of the plant becomes established first?
2. Where are the first true leaves of the 3-day-old seedling?
3. What has happened to the cotyledons in the 10-day-old seedling?
4. Where is the seed coat in this plant?
5. Which part or parts of the embryo developed into the stem?
6. How are the first two tiny true leaves arranged on the stem?

PART C  Corn Seeds

Procedure
7. Cut a soaked, germinated corn grain lengthwise with the scalpel.
8. Test the cut surfaces with a few drops of Lugol’s iodine solution. Record your observations and conclusion.
9. The starch agar and plain agar in petri dishes A and B were tested with Lugol’s iodine solution as a demonstration. Observe the dishes. Record your observations and conclusion in your logbook.
10. On petri dish C, two or three corn grains have started to germinate on starch agar. Each grain was cut lengthwise, and the cut surfaces were placed on the starch agar for about 2 days. Petri dish D contains starch agar and boiled corn grains.
11. Cover the surface of the starch agar in petri dishes C and D with Lugol’s iodine solution.
12. After a few seconds, pour off the excess.
13. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. When you tested the cut surface of corn grains, what food was present?
2. What other nutrients might be present in the corn that were not demonstrated by the test of the cut surfaces?
3. What difference did you observe in the test of the agars in petri dishes A and B?
4. What difference did you observe when you tested petri dishes C and D? Suggest hypotheses that might account for what you observed.
5. What food substance would you expect to find in the areas where the germinating corn grains were?

Investigation 11B  Tropisms

This investigation allows you to observe tropisms. One-half of each team will conduct either Part A or Part B of the investigation. Observe all your team’s work so you can discuss all the results as a class.

Hypothesis: After reading the procedures, construct two hypotheses—one predicting how the germinating corn plants will be affected and the other predicting the patterns of growth of the radish seeds.

Materials (per team of 4; one-half of each team performing Part A or Part B)

PART A
petri dish
scissors
glass-marking pencil
nonabsorbent cotton
heavy blotting paper
transparent tape
modeling clay
4 soaked corn grains

PART B
4 flowerpots, about 8 cm in diameter
4 cardboard boxes, at least 5 cm higher than the flowerpots
red, blue, and clear cellophane
scissors
transparent tape
40 radish seeds
soil

PART A  Orientation of Shoots and Roots in Germinating Corn

Procedure
1. Place four soaked corn grains in the bottom half of a petri dish. Arrange them cotyledon side down, as shown in Figure 11B.1.
2. Fill the spaces between the corn grains with wads of nonabsorbent cotton to a depth slightly greater than the thickness of the grains.
3. Cut a piece of blotting paper slightly larger than the bottom of the petri dish, wet it thoroughly, and fit it snugly over the grains and the cotton.

\textbf{CAUTION: Scissors are sharp; handle with care.}

4. Hold the dish on its edge, and observe the grains through the bottom. If they do not stay in place, pack them with more cotton.
5. When the grains are secure in the dish, seal the two halves of the petri dish together with tape.
6. Rotate the dish until one of the grains is at the top. With the glass-marking pencil, write an \textit{a} on the petri dish beside the topmost grain. Then proceeding clockwise, label the other grains \textit{b}, \textit{c}, and \textit{d}. Also label the petri dish with a team symbol.
7. Use modeling clay to support the dish on edge, as shown in Figure 11B.1, and place it in dim light.
8. When the grains begin to germinate, make sketches every day for 5 days, showing the direction in which the root and the shoot grow from each grain.
9. Wash your hands thoroughly before leaving the laboratory.

\textbf{Analysis}

1. From which end of the corn grains did the roots grow? From which end of the grains did the shoots grow?

2. Did the roots eventually turn toward one direction? If so, what direction?
3. Did the shoots eventually turn toward one direction? If so, what direction?
4. To what stimulus did the roots and shoots seem to be responding?
5. In each case, were the responses positive (toward the stimulus) or negative (away from the stimulus)?
6. Why was it important to have the seeds oriented in four different directions?

\textbf{PART B Orientation of Radish Seedlings}

\textbf{Procedure}

10. Turn the four cardboard boxes upside down. Number them 1 to 4. Label each box with your team symbol.
11. Cut a rectangular hole in one side of boxes 1, 2, and 3. (Use the dimensions shown in Figure 11B.2.) Do not cut a hole in box 4.
12. Tape a piece of red cellophane over the hole in box 1, blue cellophane over the hole in box 2, and clear cellophane over the hole in box 3.
13. Number four flowerpots 1 to 4. Label each with your team symbol. Fill the pots to 1 cm below the top with soil.
14. In each pot, plant 10 radish seeds about 0.5 cm deep and 2 cm apart. Press the soil down firmly over the seeds, and water them gently. Place the pots in a location that receives strong light but not direct sunlight.

\textbf{FIGURE 11B.1}

\textbf{Petri dish setup.}

\textbf{FIGURE 11B.2}

\textbf{Flowerpot setup.}
15. Cover each pot with the box labeled with its number. Turn the boxes so the sides with holes face the light.

16. Once each day remove the boxes and water the soil. Do not move the pots; replace the boxes in their original positions.

17. When most of the radish seedlings have been above the ground for 2 or 3 days, record the height (length) of each seedling and calculate an average seedling height for each pot. Record the direction of stem growth in each pot—upright, curved slightly, or curved greatly. If curved, record in what direction with respect to the hole in the box.

18. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. Construct a data table to organize your observations. Include box number, average seedling height, direction of curving, and amount of curving.
2. In which flowerpot were the radish stems most nearly upright?
3. In which pot were the radish stems most curved? In what direction were they curved? Were the stems curved in any of the other pots? Which ones? In what direction did they curve?
4. To what stimulus do you think the radish stems responded?
5. What effect, if any, did the red and blue cellophane have on the direction of the radish stem growth?
6. Speculate about possible biological mechanisms that could account for your observations.

Investigations for Chapter 12
Reproduction

Investigation 12A ☠ A Model of Meiosis

Many biological events are easier to understand when they are modeled. In this investigation, you will use a model to simulate the events of meiosis.

Materials (per team of 2)
- modeling clay, red and blue, or red and blue pop-it beads
- 4 2-cm pieces of pipe cleaner
- large piece of paper

PART A Basic Meiosis

Procedure
1. Use the clay to form two blue and two red chromatids, each 6 cm long and about as thick as a pencil. Alternatively, use pop-it beads.
2. Place the pairs of similar chromatids side by side. Use pipe cleaners to represent centromeres. Press a piece of pipe cleaner across the centers of the two red 6-cm chromatids (made of clay or beads). This represents a chromosome that has replicated itself at the start of meiosis. Do the same for the blue replicated chromosomes. (Figure 12A.1 shows an example using clay.)
3. Form four more chromatids, two of each color, 10 cm long. Again, press a piece of pipe cleaner across the centers of the two pairs of red and blue chromatids.
4. On a sheet of paper, draw a spindle large enough to contain the chromosomes you have made. Assume that the spindle and chromatids have been formed and the nuclear membrane has disappeared.

FIGURE 12A.1
Chromosome models.
5. Pair the two 6-cm chromosomes so that the centromeres touch. Pair the two 10-cm chromosomes. Assume that the red chromosome of each pair came from the female parent. Its matching chromosome, the blue one, came from the male parent.

6. Arrange the two chromosome pairs along the equator (middle) of the spindle so that the red chromosomes are on one side and the blue on the other.

7. Holding on to the centromeres, pull the chromosomes of each matching pair toward opposite poles of the spindle. Once the chromosomes have been moved to the two poles, you have modeled the first meiotic division.

8. Draw two more spindles on the paper. These new spindles should be centered on each pole of the first meiotic division. Both spindles should be perpendicular to the first spindle. Your model cells are now ready for the second division of meiosis.

9. Place the chromosomes from each pole along the equator of each of the two new spindles. Unfasten the centromere of each chromosome. Grasp each chromatid at the point where the centromere was attached. Pull the chromatids to opposite poles of their spindles. Try to move each spindle’s chromatids simultaneously, as occurs in a living cell. Draw a circle around each group of chromosomes.

**Analysis**

1. How many cells were there at the start of meiosis? How many cells are formed at the end of meiosis?
2. How many chromosomes were in the cell at the beginning of meiosis? How many chromosomes were in each of the cells formed by meiosis?
3. What types of cells does meiosis produce?
4. How many of your cells at the end of meiosis had only red chromosomes in them? How many had only blue chromosomes in them?

**PART B Effects of Chromosome Position on Sorting**

10. A real cell is three-dimensional. Although the red chromosomes (from the female) may be on one side of the equator and the blue (from the male) may be on the other when they line up on the spindle, there is an equal chance that one red and one blue chromosome will be on the same side. Attach the chromatids as they were at the beginning of the investigation. Go back to step 6, and arrange the chromosomes so that one blue and one red chromosome are on each side of the equator. Complete meiosis I and II.

**Analysis**

1. How do these gametes compare with those you made earlier?
2. What difference does this change in position make in terms of genetic variation in the offspring?
3. How many different types of gametes could be made if there were three sets of chromosomes instead of just two?

**PART C Effects of Crossing-Over**

11. Reassemble your chromosome models. To show crossing-over (see Figure 13.19), exchange a small part of the clay from a chromatid making up one chromosome with an equal part from a chromatid of its homologous pair. The colors make the exchange visible throughout the rest of the investigation.

12. Place your chromosome pairs along the equator of the spindle as in step 6, and complete meiosis I and II.

**Analysis**

1. How many different types of gametes did you form? Did you form any gametes different from those formed by others in your class?
2. In general, how do you think crossing-over affects the number of different types of gametes that are formed?
3. In crossing-over, what actually is exchanged between the chromatids?
4. What are some of the advantages of using a model to visualize a process?
5. How did this model improve your understanding of the process of meiosis?
6. What are some disadvantages of this model?
Investigation 12B ◆ The Yeast Life Cycle

Baker’s yeast (*Saccharomyces cerevisiae*) is a unicellular organism that reproduces both sexually and asexually. Because the cells have a characteristic shape at each stage, it is possible to distinguish all the major stages of the life cycle under the microscope.

Yeast cells may be either haploid or diploid. Haploid cells occur in two mating types (sexes): mating type α (HAR) and mating type α (HBT). When α and α cells come in contact, they secrete hormonelike substances called mating pheromones, which cause them to develop into gametes. The α and α gametes pair and then fuse, forming a diploid zygote. The fusion of α and α gametes is similar to fertilization in animals except that both parents contribute cytoplasm and nuclei. Yeast zygotes reproduce asexually by budding. When cultured on a solid growth medium, a yeast zygote may grow into a visible colony that contains up to 100 million cells.

Diploid yeast do not mate, but in times of stress, such as when they have an unbalanced food supply, the diploid cells may sporulate, or form spores. The spores remain together, looking like ball bearings, in a transparent saclike structure called an ascus.

In this investigation, you will start with two haploid yeast strains of opposite mating types, mate them to form a diploid strain, and try to complete an entire yeast life cycle.

**Materials** (per team of 2)
- microscope slide
- coverslip
- dropping pipette
- glass-marking pencil
- compound microscope
- container of clean, flat toothpicks
- 6 self-sealing plastic bags labeled *waste* (1 for each day)
- 2 YED medium agar plates
- MV medium agar plate
- unknown medium agar plate
- agar slant cultures of HAR and HBT yeast strains

**Procedure**

**Day 0**
1. Prepare fresh cultures of both mating types. Colonies of the HAR strain of mating type α are red; those of the HBT strain of mating type α are cream-colored. Touch the flat end of a clean toothpick to the HAR strain; then gently drag it across the surface of a YED medium agar plate to make a streak about 1 cm long and 1 cm from the edge (Figure 12B.1). Discard the toothpick in the self-sealing plastic waste bag, being careful not to touch anything with it. Use a glass-marking pencil to label the bottom of the plate near the streak with an α. *Use a new clean toothpick from the container for each streak you do. Be careful not to touch the ends of the toothpicks to anything except yeast or the sterile agar. Discard used toothpicks in the plastic waste bag. Keep the lid on the agar plate except when transferring yeast.* Label this plate I, and add the date and your names. Incubate upside down for 1 day, or 2 days if your room is very cool.
2. Wash your hands thoroughly before leaving the laboratory.

![FIGURE 12B.1](https://via.placeholder.com/727-754_SWP2618_IV_C7-C13)

**FIGURE 12B.1**
Procedure for making a mating mixture from HAR and HBT yeast strains (a), for subculturing the mating mixture (b), and for inoculating the unknown medium plate (c).
Day 1

3. Use a microscope to examine some yeast cells of either mating type. To prepare a slide, touch a toothpick to the streak of either mating type $a$ or mating type $\alpha$ in plate I, mix it with a small drop of water on the slide, and place a coverslip over the drop. Discard the toothpick in the waste bag. Examine the cells with the high-power objective. In your logbook, sketch the cells.

4. Use a clean toothpick to transfer a small amount of mating type $a$ from the streak in plate I to the middle of the agar. Use another clean toothpick to transfer an equal amount of mating type $\alpha$ to the same place. Being careful not to tear the agar surface, thoroughly mix these two dots of yeast to make a mating mixture. Discard used toothpicks in the waste bag. Invert and incubate plate I at room temperature for 3 or 4 hours; then refrigerate until the next lab period. (Or refrigerate immediately, and then incubate at room temperature for 3 or 4 hours before the next step.)

5. Wash your hands thoroughly before leaving the laboratory.

Day 2

6. Remove plate I from the refrigerator. What color is the mating mixture colony? Use the microscope to examine the mating mixture, as in step 3. Discard used toothpicks in the waste bag. Sketch what you see and compare with your earlier drawings. Describe any differences in the types of cells you see. When haploid cells of opposite mating types ($a$ and $\alpha$) are mixed, they develop into pear-shaped haploid gametes. Do you see any gametes? A diploid zygote forms when two gametes fuse. Growing diploid cells are slightly larger and more oval than haploid cells. Do you see any evidence that gametes may be fusing into zygotes? Compared to day 1, are there more or fewer diploid cells?

7. Make a subculture by transferring some of the mating mixture with a clean toothpick to an MV agar plate. Discard used toothpicks in the waste bag. Label this plate II. Invert and incubate at least overnight, but not more than 2 nights.

8. Wash your hands thoroughly before leaving the laboratory.

Day 3

9. Use the microscope to examine the freshly grown subculture in plate II. Discard used toothpicks in the waste bag. What types of cells are present? Sketch each type. If any of the types seen in step 6 have disappeared, explain what happened to them.

10. On a plate of unknown medium, make several thick streaks of the freshly grown subculture. Discard used toothpicks in the waste bag. Label this plate $U$. Invert and incubate at room temperature at least 4 days.

11. Wash your hands thoroughly before leaving the laboratory.

Day 7

12. Use the microscope to examine yeast from plate U. Discard used toothpicks in the waste bag. You may need to use the fine adjustment on the microscope to distinguish cells at different levels. What cell types are present today that were not present before? Sketch these cell types, and compare them with the cell types you saw at other stages.

13. Refer to the introduction. How do you think the unknown medium differs from the YED medium? If the cells in the sacs are more frequently found in groups of four, do you think they were formed by meiosis or mitosis? Explain your answer. Are the cells in the sacs haploid or diploid? Explain your answer. What part of the life cycle seen on day 1 do these cells most resemble?

14. Transfer some yeast from plate U to a fresh YED medium agar plate. Discard used toothpicks in the waste bag. Label this plate III, invert and incubate at room temperature for about 5 hours, and then refrigerate until the next lab period. (Or refrigerate immediately and then incubate at room temperature for 5 hours before the next step.)

15. Wash your hands thoroughly before leaving the laboratory.
Day 8

16. Use the microscope to examine the growth from plate III. Discard used toothpicks in the waste bag. What life-cycle stages are present? Sketch the cells, and compare them with the stages you observed before. What evidence is there that a new life cycle has started?

17. Discard used culture plates as directed by your teacher, and wash your hands thoroughly before leaving the laboratory.

Analysis

1. In this investigation, you have observed the major events of a sexual life cycle. You could readily observe these events in yeast because it is a unicellular organism. In plants and animals, including humans, similar cellular events occur, but they are difficult to see. Although the changes were occurring too slowly for you to see, your sketches provide a record of the sequences. Think of them as pauses in a tape of a continually changing process. Notice in particular how the cycle repeats. On one page, draw a life-cycle diagram showing the different shapes of cells you observed in the order in which they appeared. Indicate where you first saw each type and when it disappeared, if it did.

2. Compare your sketches with the life-cycle diagram in Figure 12B.2, and try to identify each of the forms you saw.

3. For each of the cell forms you observed, indicate whether it was haploid or diploid.

4. Mark the points in your diagram where cells changed from haploid to diploid and from diploid to haploid.

5. Why do you think the two different mating types are not called female and male?

6. Can you think of a good argument for calling any particular point in the cycle the beginning or the end? Why or why not?

7. What would you expect to happen if you allowed the yeast in step 14 to grow for another day and then put them on the unknown medium again?

8. Table 12B.1 summarizes the similarities between stages in the yeast life cycle and the events in

![Yeast life cycle diagram](image-url)}
TABLE 12B.1
Comparison of the Yeast Life Cycle and Sexual Reproduction in Animals

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating pheromones</td>
<td>Sex hormones</td>
</tr>
<tr>
<td>Mating-type a and α gametes</td>
<td>Gametes (ova and sperm)</td>
</tr>
<tr>
<td>Fusion</td>
<td>Fertilization</td>
</tr>
<tr>
<td>Zygote</td>
<td>Zygote</td>
</tr>
<tr>
<td>Asexually reproducing diploid cells</td>
<td>Diploid body cells</td>
</tr>
<tr>
<td>Meiosis and formation of spores</td>
<td>Meiosis and formation of gametes</td>
</tr>
</tbody>
</table>

sexual reproduction in animals. For each change you observed in the yeast life cycle, indicate the step in the human sexual reproduction cycle that is most similar.

Investigation 12C ◆ Reproduction in Mosses and Flowering Plants

Although their reproductive organs differ as much as the environments in which they live and reproduce, the basic principles of sexual reproduction are the same in a moss, a flower, a bee, and a human. In this investigation, you will learn how the structures of a moss and a flower serve reproductive functions in their respective environments.

Mosses form mats on logs and on the forest floor, growing best in damp, shaded environments. Sporophytes, or diploid spore-producing structures, grow out of the tops of haploid gamete-producing structures, called gametophytes. Sporophytes often look like brownish hairs growing out of the mat of moss. Mosses cannot reproduce unless they are moist. Flowering plants, on the other hand, are found in many different environments and climates. They need water to live but not to reproduce.

Materials (per team of 2)
3 microscope slides
3 coverslips
dissecting needle
scalpel
forceps
dropping pipette
petri dish
cotton

compound microscope
dissecting microscope or 10× hand lens
modeling clay
prepared slide of filamentous stage of moss
prepared slide of moss male and female reproductive organs
sucrose solution (15%)
moss plant with sporophyte
fresh moss
gladiolus flower
other simple flowers
fresh bean or pea pods

Procedure

PART A  Moss
1. Examine a moss plant with a sporophyte attached. The sporophyte consists of a smooth, brownish stalk terminated by a small capsule. Separate the two generations by pulling the sporophyte stalk out of the leafy gametophyte shoot.
2. Using a dissecting needle, break open the sporophyte capsule into a drop of water on a slide. Add a coverslip and examine under the low power of a compound microscope. What are the structures you observe? How are these structures dispersed in nature? How are they adapted for life on land?

CAUTION: Needles are sharp; handle with care.
3. Most moss spores germinate on damp soil and produce a filamentous stage that looks like a branching green alga. Examine a prepared slide of this stage.
4. The filamentous stage gives rise to the leafy shoot of the gametophyte. Using forceps, carefully remove a leafy shoot from the fresh moss. How does this shoot obtain water for growth?

5. The reproductive organs of the gametophyte are at the upper end of the leafy shoot. Examine a prepared slide of these organs under the low power of a compound microscope. The male sex organs are saclike structures that produce large numbers of sperm cells. The female sex organs are flask-shaped and have long, twisted necks. An egg forms within the base of the female organ. How does a sperm reach the egg? Would you expect to find moss plants growing where there was little or no water? Explain your answer. The union of the egg and sperm results in a cell called the zygote. Where is the zygote formed? What grows from the zygote?

PART B Flowers

6. Examine the outside parts of a gladiolus flower. The outermost whorl of floral parts may be green and leaflike. These green sepals protected the flower bud when it was young. In some flowers, such as lilies, the sepals look like an outer whorl of petals. Petals are usually large and colorful and lie just inside the sepals. Both sepals and petals are attached to the enlarged end of a branch. These parts of the flower are not directly involved in sexual reproduction. What functions might petals have?

7. Strip away the sepals and petals to examine the reproductive structures. Around a central stalklike body are five to ten delicate stalks, each ending in a small sac, or anther. These are the male reproductive organs, or stamens. Thousands of pollen grains are produced in the anther. The number of stamens varies according to the type of flower. How many stamens are present in the flower you are using? How may pollen be carried from the anthers to the female part of the flower?

8. If the anthers are mature, shake some of the pollen into a drop of sucrose solution on a clean slide. Add a coverslip and examine with the low power of a compound microscope. What is the appearance of the pollen? How is the pollen adapted for dispersal?

9. Make another pollen preparation on a clean coverslip. Use modeling clay to make a 5-mm high chamber, slightly smaller than the coverslip, on a clean slide. Add a small drop of water to the chamber, and invert the pollen preparation over it. Examine after 15 minutes and again at the end of the lab period. What, if any, changes have occurred? (If no changes have occurred, store the slide in a covered petri dish containing a piece of cotton moistened with water, and examine it the next day.)

10. The central stalk surrounded by the stamens is the female reproductive organ, or carpel. It is composed of a large basal part, the ovary, above which is an elongated part, the style, ending in a stigma. How is the stigma adapted to trap the pollen grains and to provide a place for them to grow?

11. Use a scalpel to cut the ovary lengthwise. Using a hand lens or dissecting microscope, look at the cut surface. How many ovules can you see? Each ovule contains one egg. To what stage of the moss life cycle is the ovule comparable? Where is the pollen grain deposited? How does the sperm in the pollen grain reach the egg? To what stage of the moss life cycle is a pollen grain comparable?

**CAUTION: Scalpels are sharp; handle with care.**

12. The union of egg and sperm causes extensive changes in the female reproductive parts. Fertilization of the egg stimulates the growth of the ovary and the enclosed ovules. Carefully examine a fresh bean or pea pod. Open the pod to find the seeds. Which of the female reproductive structures is the pod of a bean or pea? What is the origin of a seed? If you plant ripe bean or pea seeds and water them, what will they produce? What can you conclude develops within a seed as a result of fertilization?

13. If time permits, examine other types of flowers. Compare the numbers of various parts and the ways the parts are arranged with respect to each other.
14. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. In alternation of generations in a moss, which is the predominant independent generation? Which is the less conspicuous generation?
2. Compare the life cycle of a moss (with alternation of generations) with your life cycle (with no alternation of generations).
3. Would you expect more variation in flowering plants or in those that reproduce asexually? Explain your answer.
4. Compare and contrast the sporophyte and gametophyte stages of a moss to those of a flowering plant.
5. Do flowering plants demonstrate more or less adaptation to a land environment than mosses? Explain your answer.

Investigations for Chapter 13
Patterns of Inheritance

Investigation 13A ◆ Probability

The probability of a chance event can be calculated mathematically using the following formula:

\[
\text{probability} = \frac{\text{number of events of choice}}{\text{number of possible events}}
\]

What is the probability that you will draw a spade from a shuffled deck of cards like that shown in Figure 13.13? There are 52 cards in the deck (52 possible events). Of these, 13 cards are spades (13 events of choice). Therefore, the probability of choosing a spade from this deck is \( \frac{13}{52} \) (or 1/4, or 0.25, or 25%). To determine the probability that you will draw the ace of diamonds, you again have 52 possible events, but this time there is only 1 event of choice. The probability is \( \frac{1}{52} \), or approximately 2%. In this investigation, you will determine the probability for the results of a coin toss.

Materials (per team of 2)
2 pennies (1 shiny, 1 dull)
cardboard box

Procedure
1. Work in teams of two. One person will be student A and the other will be student B.
2. Student A will prepare a score sheet with two columns—one labeled \( H \) (heads) and the other \( T \) (tails). Student B will toss a penny ten times. Toss it into a cardboard box to prevent the coin from rolling away.
3. Student A will use a slash mark (/) to indicate the results of each toss. Tally the tosses in the appropriate column on the score sheet. After ten tosses, draw a line across the two columns and pass the sheet to student B. Student A then will make ten tosses, and student B will tally the results.
4. Continue reversing roles until the results of 100 (ten series of ten) tosses have been tallied.
5. Prepare a score sheet with four columns labeled \( H/H \), \( Dull H/Shiny T \), \( Dull T/Shiny H \), and \( T/T \) (\( H = \text{heads, T = tails} \)). Obtain two pennies—one dull and one shiny. Toss both pennies together 20 times while your partner tallies each result in the appropriate column of the score sheet.
6. Reverse roles once so that you have a total of 40 tosses.

Analysis
1. How many heads are probable in a series of ten tosses? How many did you actually observe in the first ten tosses?
2. Deviation is a measure of the difference between the expected and observed results. It is not the difference itself. It is the ratio of the sum of the differences between expected and observed results to the total number of observations. Thus,

\[
\text{deviation} = \frac{\text{difference between heads expected and heads observed} + \text{difference between tails expected and tails observed}}{\text{number of tosses}}
\]

Calculate the deviation for each of the ten sets of tosses.
3. Calculate the deviation for your team’s total (100 tosses).

4. Add the data of all teams in your class. Calculate the class deviation.

5. If your school has more than one biology class, combine the data of all classes. Calculate the deviation for all classes.

6. How does increasing the number of tosses affect the average size of the deviation? These results demonstrate an important principle of probability. State what it is.

7. On the chalkboard, record the data for tossing two pennies together. Add each column of the chart. In how many columns do data concerning heads of a dull penny appear?

8. In what fraction of the total number of tosses did heads of dull pennies occur?

9. In how many columns do data concerning heads of a shiny penny occur?

10. In what fraction of the total number of tosses did heads of the shiny pennies occur?

11. In how many columns do heads of both dull and shiny pennies appear?

12. In what fraction of the total number of tosses did heads of both the pennies appear at the same time?

13. To which of the following is this fraction closest: the sum, the difference, or the product of the two fractions for heads of one penny at a time?

14. Your answer suggests a second important principle of probability that concerns the relationship between the probabilities of separate events and the probability of a combination of events. State this relationship.

15. When you toss two coins together, there are only three possibilities—H/H, T/T, or H/T. These three combinations will occur 100% of the time. The rules of probability predict that H/H and T/T each will occur 25% of the time. What is the expected probability for the combination of heads on one coin and tails on the other?

16. When you toss a dull penny and a shiny penny together, what is the probability that heads will occur on the dull penny? What is the probability that tails will occur on the shiny penny? Calculate the probability that the dull penny will be heads and the shiny penny will be tails if you toss the two pennies together. Compare this answer to the answer in question 15. How do you account for the different answers? Are there other ways than Dull H/Shiny T to get the H/T combination?

17. How many different ways can you get the H/T combination on two coins tossed together? What is the probability of each of those different ways occurring? Is the probability of getting heads and tails in any combination of pennies closest to the sum, the difference, or the product of the probabilities for getting heads and tails in each of the different ways?

18. Your answer suggests a third important principle of probability that concerns the relationship between (1) the probability of either one of two mutually exclusive events occurring and (2) the individual probabilities of those events. State this relationship.

---

Investigation 13B ♦ Seedling Phenotypes

Albinism is a rare condition found in both plants and animals. The cells of albino animals or plants lack certain pigments. Albino plants, for example, have no chlorophyll (Figure 13B.1). In this investigation, you will observe the influence of both heredity and environment on a plant’s ability to produce chlorophyll.

**FIGURE 13B.1**

Corn seedlings. Growing among the green plants, the albino corn seedlings lack the pigment chlorophyll.
Materials (per team of 2)
- petri dish
- filter or blotting paper
- light-proof box or aluminum foil
- about 50 tobacco seeds

Procedure

1. Evenly sprinkle tobacco seeds over moistened filter paper in the bottom of a petri dish. The seeds should be separated by at least twice their length. **CAUTION: Wash hands after handling tobacco seeds. They may be treated with a fungicide.**

2. Put the cover over the dish. Put the dish in a lighted area for about 4 days. Record observations daily in your logbook.

3. After 4 or 5 days, wrap the dish in foil or cover it with a light-proof box. Put the setup in a dark place where it will not be disturbed for 3 or 4 more days. Be sure the paper is moist at all times, and add water if necessary. Do not expose the seeds to light when checking them.

4. After the seeds have germinated, usually in about a week to 10 days, remove the light-proof cover and examine the seedlings. Observe especially the color of the tiny leaves. Record your observations.

5. Replace the cover on the petri dish, and put it in a well-lighted place for a few days. Be sure the paper is moist at all times. Observe the seedlings each day as they are exposed to the light.

6. After being in the light for a few days, count the number of plants of each color. In your logbook, record your observations and the numbers of each type of plant.

7. Wash your hands thoroughly before leaving the laboratory.

Analysis

1. What was the color of the tobacco plants while they were growing in the dark?

2. Did light have the same effect on all of the plants? Explain your answer.

3. The seeds used in this investigation came from a specially bred tobacco plant. Do you suppose the parent plants were both green, one green and one albino, or both albino? (Hint: Consider the role of chlorophyll in the life of a plant.) Explain your answer.

4. Does light have any effect on a tobacco plant’s ability to produce chlorophyll? Explain your answer.

### TABLE 13B.1

Mendel's Results from Crossing Pea Plants with Single Contrasting Traits

<table>
<thead>
<tr>
<th>P, cross</th>
<th>F₁ plants (self-pollinated)</th>
<th>F₂ plants</th>
<th>Actual ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round × wrinkled seeds</td>
<td>all round</td>
<td>round × round</td>
<td>5,474 round 1,850 wrinkled 7,324 total</td>
</tr>
<tr>
<td>Yellow × green seed (cotyledons)</td>
<td>all yellow</td>
<td>yellow × yellow</td>
<td>6,022 yellow 2,001 green 8,023 total</td>
</tr>
<tr>
<td>Green x yellow pods</td>
<td>all green</td>
<td>green × green</td>
<td>428 green 152 yellow 580 total</td>
</tr>
<tr>
<td>Long x short stems</td>
<td>all long</td>
<td>long × long</td>
<td>787 long 277 short 1,064 total</td>
</tr>
</tbody>
</table>
5. Is light the only factor required for a tobacco plant to produce chlorophyll? Explain your answer.

6. Which seedlings showed the influence of heredity on chlorophyll development? Explain your answer.

7. What was the approximate ratio of green to albino plants that appeared when seedlings were grown in the light for several days? Compare this ratio with Mendel’s data for crossing garden peas shown in Table 13B.1.

**Investigation 13C ◆ A Dihybrid Cross**

Sexually reproducing organisms have haploid and diploid stages. In flowering plants and animals, only the gametes are haploid, and traits (the phenotype) can be observed only in the diploid stage. When Mendel made dihybrid crosses to study the inheritance of two different traits, such as seed shape and seed color, he could observe the traits only in the diploid cells of the parents and their offspring. He had to use probability to calculate the most likely genotypes of the gametes.

In the yeast *Saccharomyces cerevisiae*, however, phenotypes of some traits can be seen in the haploid cell colonies. In Investigation 12B, for example, you could observe the color trait red or cream in both haploid and diploid stages of the yeast life cycle. Not all yeast traits are visible, however, so geneticists use the methods Beadle and Tatum devised for studying mutations; that is, they isolate mutants that cannot make some essential substance. The inability of the mutant strain to make the substance and the ability of the normal strain to make it are two different forms of a trait.

In the yeast dihybrid cross (the outcomes of which are listed in Table 13C.1), you will follow two forms of each of two traits: red versus cream color and tryptophan-dependent (requiring this amino acid to grow) versus tryptophan-independent (not requiring this amino acid). In Investigation 12B, a red strain of yeast of one mating type is crossed with a cream-colored strain of the other type. The diploid strain is cream-colored. If there is a single gene for this trait, there must be one allele that determines cream color in the haploid strain and another allele that determines red color. In the diploid strain then, there must be one of each of these alleles. Which form of the color trait is dominant?

By observing the color of a colony that a haploid strain forms, you can predict with certainty which allele it carries. In the case of a cream-colored diploid, however, you cannot be sure of the genotype. It could carry either one allele for cream color and one for red or two alleles for cream color. The trait defined by tryptophan-dependence or independence works in much the same way. A tryptophan-dependent haploid must carry the allele for tryptophan-dependence (a defective form of the gene); a tryptophan-independent haploid must carry the allele for tryptophan-independence (the functional form of the gene), but a tryptophan-independent diploid could be either homozygous or heterozygous for the functional allele.

Using symbols for the traits, the allele for the dominant cream form is \( R \) and the recessive red

<table>
<thead>
<tr>
<th>TABLE 13C.1</th>
<th>Growth of Yeast on Two Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haploid</strong></td>
<td><strong>Diploid</strong></td>
</tr>
<tr>
<td>( RT )</td>
<td>( RR \quad TT )</td>
</tr>
<tr>
<td></td>
<td>( RR \quad Tt )</td>
</tr>
<tr>
<td></td>
<td>( Ar \quad TT )</td>
</tr>
<tr>
<td></td>
<td>( Ar \quad Tt )</td>
</tr>
<tr>
<td>( Rt )</td>
<td>( RR \quad tt )</td>
</tr>
<tr>
<td></td>
<td>( Rt \quad tt )</td>
</tr>
<tr>
<td>( rT )</td>
<td>( rr \quad TT )</td>
</tr>
<tr>
<td></td>
<td>( rr \quad Tt )</td>
</tr>
<tr>
<td>( rt )</td>
<td>( rr \quad tt )</td>
</tr>
</tbody>
</table>
form is \( r \); the normal tryptophan-independent form
of that gene is \( T \) and the tryptophan-dependent form
is \( t \). Whereas the color trait is visible, determining
tryptophan dependence or independence requires a
simple growth test.

Table 13C.1 shows how all possible combinations
of these two traits can be determined by testing the
yeast on two types of growth medium: a nutritionally
complete medium (COMP) and a medium lacking
tryptophan (MIN).

**Materials**
(per team of 2)
container of clean flat toothpicks
glass-marking pencil
3 self-sealing plastic bags labeled waste (1 for each day)
biohazard bag
complete growth medium agar plate (COMP)
minimal + adenine growth medium agar plate (MIN)
COMP plate with 24-hour cultures of yeast strains
HAO, HAR, HAT, HART, HBO, HBR, HBT, and HBRT

**Procedure**

**PART A Predicting the Gametes of the Parents**
The diploid parents of this dihybrid cross would
have been \( rr TT \) and \( RR tt \). To predict the \( F_2 \)
offspring, it is necessary to predict the different
types and relative numbers of gametes that can be
produced. The simplest way to make this prediction
is to diagram how the chromosomes separate at
meiosis. Figure 13C.1a shows the gametes predicted
from the pure-breeding diploid red parent \( (rr TT) \).

![Diagram of meiosis and gamete production](image)

Two pairs of chromosomes are represented, one
carrying the gene for color (in this case red, \( r \)) and
the other carrying the gene for the ability to make
tryptophan (in this case tryptophan-independent, \( T \)).
At the first meiotic division (M_I), chromosome pairs
segregate (separate). At the second meiotic division
(M_II), the two chromatids of each chromosome
segregate into the nuclei of the gametes. In this case,
segregation can occur in only one way because only
one allele of each gene is represented. All the
gametes from a pure-breeding diploid are the same
(\( rT \)). The probability of this parent producing a
gamete with the genotype of \( rT \) is 1/1, or 100%.

1. In your logbook, copy the diagram in
Figure 13C.1b (or use the diagram your teacher
provides) for the cream-colored, tryptophan-
dependent parent \( (RR tt) \). Fill in the missing
symbols. Each chromatid should have one letter.
2. Use the symbols to describe for each parent
strain the genotypes and phenotypes of all the
possible gametes and the relative probabilities
of their occurrence.
3. The haploid gametes produced from these two
pure-breeding parents mate to form the diploid
zygotes of the \( F_1 \) generation. Use the symbols to
describe the genotype and phenotype of the
diploid zygotes that could be formed from the
fusion of these gametes. At this point, your
diagrams should show that they all will have the
dihybrid genotype \( Rr Tt \).
PART B  Predicting the Gametes of the F₁ Diploids

Because there are two equally probable ways that the alleles of the two genes can separate at Meiosis I, the chromosome diagram for the segregation of the R, r; T, and t alleles is more complicated. These are shown in Figure 13C.2. Since these two patterns of segregation are equally probable, the two diagrams together illustrate the relative numbers of all possible genotypes.

4. Copy the diagrams in Figure 13C.2 into your logbook, or use the diagrams your teacher provides. Fill in the missing symbols. Each chromatid should have a symbol.

5. What is the total number of different gametes represented in the diagram?

6. How many different genotypes are represented? Give their symbols.

7. How many times is each genotype represented?

8. What is the probability of occurrence of each genotype among the total number of gametes shown? Compare this prediction with the gametes in Figure 13.15.

9. Draw a checkerboard diagram for all the possible crosses among these gametes. This diagram should illustrate the 16 different combinations predicted. Instead of male and female gametes, use mating-type a and mating-type α haploid strains. In each square of the diagram, construct the diploid genotype that would result from the fusion of the corresponding gametes. How many different genotypes are there? How many different phenotypes? Does this diagram predict a 9:3:3:1 ratio? What specific phenotypes would be represented in these ratios?

PART C  Testing the Predicted Phenotypes

Day 0

10. Make two templates for setting up crosses. Copy the pattern shown in Figure 13C.3 onto a piece of paper twice so that each fits the bottom of a petri dish. (Or use the templates your teacher provides.)

11. Tape one template to the bottom of a plate of complete growth medium (COMP) so that you can read it through the agar.

**FIGURE 13C.2**
Gametes that could be formed by yeast organisms heterozygous for two traits.

**FIGURE 13C.3**
Template for dihybrid yeast cross.
12. Transfer a small sample of each strain onto the agar directly over its corresponding label. To do this, touch the flat end of a clean toothpick to strain HAO on the plate your teacher provides. Gently drag the toothpick along the box labeled HAO on the COMP agar plate to make a streak about 1 cm long. Discard the toothpick in the self-closing waste bag, being careful not to touch anything with it. Repeat this procedure for all eight strains, using a new toothpick for each transfer. Be careful not to touch the ends of the toothpicks to anything except yeast or the sterile agar. Discard used toothpicks in the self-closing plastic waste bag. Keep the lid on the plate at all times except when transferring yeast.

13. Invert and incubate the plate at room temperature.

14. Wash your hands before leaving the laboratory.

Day 1

15. On the same COMP plate, make a mating mixture for each of the mating-type α strains with each of the mating-type γ strains. To do this, use the flat end of a clean toothpick to transfer a dot of the HAO strain of freshly grown cells to each of the boxes below it on the template. Discard the toothpick in the waste bag. Repeat this procedure for HAR, HAT, and HART, using a new toothpick for each strain. Using the same procedure, transfer a dot of the freshly grown cells of each HB strain to each of the boxes to the right of the strain on the template. Place these dots side-by-side but not touching. Use a clean toothpick to mix each pair of spots together. Be sure to use a clean toothpick each time you mix pairs together. Discard all toothpicks in the waste bag.

16. Invert and incubate the plate at room temperature.

17. Wash your hands before leaving the laboratory.

Day 2

18. Test each mating mixture and parent strain for its ability to grow on MIN agar. To keep track of the tests, tape a copy of the template to the bottom of the MIN plate. Use the flat end of a clean toothpick to transfer a small amount of each strain and mixture from the COMP plate to the corresponding position on the MIN plate. Be sure to use a clean toothpick each time you change strains and mixtures. Discard all toothpicks in the waste bag.

19. Invert and incubate both plates until the next day.

20. Wash your hands before leaving the laboratory.

Day 3

21. Copy the score sheet in Figure 13C.4 into your logbook. (Or tape in the copy your teacher provides.) Record the color and growth phenotypes of each parent haploid strain and F₂ diploid on the score sheet for both plates.

22. Tabulate the different phenotypes observed among the F₂ diploids and the number of times each one occurred among the 16 crosses.

23. Compare the F₂ phenotypes with the predictions you made in Part A. Explain how your actual results either support or contradict your predictions.

24. Discard all plates in the biohazard bag.

25. Wash your hands before leaving the laboratory.

Analysis

Because yeast exhibit most of the same traits in the haploid stage (gametes) and the diploid stage, you knew the precise genotypes of the gametes (haploid strains) that you mated to produce the F₂ diploids. This removed the element of chance at this step. In Part B, however, you had to deal with the role of chance to predict the numbers and genotypes of the gametes from the F₁ diploids. Throughout the entire

![Figure 13C.4](score_sheet_for_dihybrid_yeast_cross.png)

**Score sheet for dihybrid yeast cross.**
process, beginning with the parental cross (P) and going through to the F₂ offspring, there are some steps at which chance plays a role, so the results can only be expressed as a probability. In other steps, chance is not a factor, so you can predict the outcome exactly.

1. List the steps in which chance is a factor.
2. List the steps in which chance is not a factor.
3. Explain why the outcome of some steps involves chance, whereas the outcome of others does not.

Investigations for Chapter 14
Other Forms of Inheritance

Investigation 14A ♦ Jumping Genes

Our understanding of genetics has been greatly aided by studying organisms that display one or more unusual traits due to the impact of altered or mutated genes. Indeed, some of the fundamental behaviors of genes were discovered through careful observation and experimentation, even before the structure of DNA was worked out. In this investigation, you will use your powers of observation and critical thinking to describe mechanisms by which mutations change in the appearance of corn kernels. Remember that each kernel on an ear of corn represents a separate fertilization. This means that corn kernels on an ear can be thought of as siblings.

Materials (per team of 2)
paper and pencils

PART A Analysis of Corn Ears

Procedure
1. In this variety of corn, purple kernels are the dominant phenotype. The purple pigment is produced through the activity of an enzyme encoded by a gene at the C locus. Colorless (white) kernels result from a mutation in the C gene. The dominant allele is designated $C$ while the recessive allele is $c$.
2. Examine the two ears of corn pictured in Figure 14A.1, and count how many kernels of each color are on each ear. Count at least 150 kernels per ear of corn. Record the data in your logbook.

Analysis
1. What was the ratio of purple to white kernels on the ear of corn labeled $a$?
2. Using this data, what were the likely genotypes of the parent plants?
3. What was the ratio of purple to white kernels on the ear of corn labeled $b$?
4. Using this data, what were the likely genotypes of the parent plants?
5. Did either of the ears have kernels that were difficult to score as either purple or white?

PART B Analysis of Individual Corn Kernels

Procedure
3. Barbara McClintock studied the color patterns of kernels in Indian corn in the 1940s. She determined that the speckled appearance of some kernels could be explained by the presence of unstable mutations in the corn genome. Today we know that the mutations originally studied by McClintock are due to the insertion of a foreign DNA sequence, called a transposon or jumping gene, into the coding region of a gene.
4. Examine Figure 14A.2, and answer Analysis questions 1 and 2.
5. Figure 14A.2 illustrates how movement of the transposon results in speckled corn kernels (Figure 14A.1b). Using the diagram in Figure 14A.3a as a model, draw in your logbook corresponding diagrams for the corn kernels depicted in b, c, and d. Answer Analysis questions 3–7.

**Analysis**

1. What happens when a transposon inserts into the C locus?

2. What causes the appearance of the purple spots on the speckled corn kernels?

3. Why is the transposon in the C locus referred to as unstable?

4. Why does the kernel in Figure 14A.3b display fewer spots than the one in Figure 14A.3a?

5. Why are the spots on the kernel in Figure 14A.3c larger than those in a and b?

6. How can you account for the presence of the white spot in the dark sector of the kernel shown in Figure 14A.3d?

7. Does insertion of a transposon always stop expression of the affected gene?

8. What is the evolutionary significance of transposons?

---

**Investigations for Chapter 15**

**Advances in Molecular Genetics**

**Investigation 15A ◆ Determining Mutation Frequency in Bacteria**

In this investigation, you will attempt to determine the frequency of formation of white mutants from cultures of the red-pigmented bacterium *Serratia marcescens*, strain D1 (Figure 15A.1). Many factors contribute to mutation frequency (the proportion of mutants that exists in a culture at any given moment). Two factors are especially important in increasing the proportion of mutants as the population grows. The first is the reproduction of the mutants themselves, and the second is the mutation rate of the culture. The mutation rate is the probability that a mutation will occur in a generation of cells.

Consider two generations of 100 bacterial cells with a mutation rate of 1/10. (This rate is convenient for demonstration purposes but is unrealistically high.) Since the proportion of mutants is 1:10, of the original 100 cells, 90 are wild types and 10 are mutants. The mutants will divide to produce 20 cells of their own type. Among the wild type cells, however, 90% (or a theoretical 81 cells) will divide to produce 162 wild-type cells, while 10% (or 9 cells) will mutate and divide to produce 18 new mutant cells. These 18 new mutant cells plus the 20 from the reproduction of the old mutants yield 38 mutants in...
this generation of 200 cells. This same reasoning carried into the second generation yields a proportion of mutants in the population of about 108:400 as compared to 1:10 in our original population.

Of course, if this increase in the population of mutants were to continue, nearly the entire culture would soon be mutants. This condition is prevented, however, by back mutations (the reversion of the mutant genes to their original state). Back mutations too have their own characteristic mutation rates. When enough mutants have accumulated, back mutations begin; the forward and backward mutations should reach an equilibrium and just balance each other. At this point, the culture should reach and maintain a mutation equilibrium (a constant proportion of mutant cells).

The procedure for this investigation involves spreading the bacteria over the surface of a sterile agar plate, incubating the bacteria overnight, and analyzing the bacterial colonies that result. Your starting culture of S. marcescens is too concentrated to analyze directly, so you will prepare a series of tenfold dilutions (called serial dilutions) and spread them on separate agar plates. Ideally, one of the dilutions will result in about 200–400 colonies growing on the plate. Remember, we assume that each colony results from a single bacterium.

Materials (per team of 3)
3 lab aprons
3 pairs of plastic gloves
4 S. marcescens agar plates
2 mL starter culture of red S. marcescens, strain D1
glass-marking pencil
6 tubes of sterile water, 9 mL each
bacteria spreader
5 sterile graduated pipettes
rubber pipet bulb
250-mL beaker
50 mL isopropyl alcohol (70%)
incubator

Procedure
1. Use a sterile pipette to prepare your first tenfold dilution of the starter culture of S. marcescens. Pipet 1 mL of the bacterial culture into one of the test tubes containing 9 mL of sterile water. Mix the dilution by gently tapping the tube with your finger, and label it 1:10 or \(10^1\).
2. Use a sterile pipette to prepare a 1:100 dilution by adding 1 mL of the 1:10 dilution to a second tube containing 9 mL of sterile water. Mix the dilution by gently tapping the tube with your finger, and label it 1:100 or \(10^2\).
3. Repeat step 2 four more times to prepare dilutions of 1:1,000; 1:10,000; 1:100,000; 1:1,000,000 (or \(10^3\), \(10^4\), \(10^5\), \(10^6\)).
4. Obtain four S. marcescens agar plates, and label them \(10^3\), \(10^4\), \(10^5\), and \(10^6\).
5. Use a sterile pipette to add 0.1 mL (100 \(\mu\)L) of the \(10^5\) dilution to the surface of the S. marcescens agar plate labeled \(10^5\).
6. Sterilize the bacterial spreader by dipping it into a beaker of 70% isopropyl alcohol. Let it air dry (about 10 seconds) before spreading cells. Spread the cells evenly over the surface of the plate. Be careful not to press too hard, or you will break the agar surface.
7. Repeat steps 5 and 6 to spread the $10^4$, $10^5$, and $10^6$ dilutions on the appropriately labeled plates.

8. Invert the four plates, and incubate them for 24 hours in a 278°C incubator.

9. Following the incubation, select a plate containing 200 to 400 colonies for counting.

10. Count the total number of colonies on your plate; note the number that are red and the number that are white. Also note colonies of other colors.

Analysis

1. What was the viable count of red and white S. marcescens per milliliter in the original starter culture? Remember that each plate was inoculated with 0.1 mL rather than 1 mL. Thus, if 200 colonies were on the plate inoculated with the $10^6$ dilution, the number of viable organisms per milliliter would be $200 \times 10^4$, or 2,000,000,000 per milliliter.

2. What percentage of the total number of colonies represented white mutants? Pool the results of the class, and calculate the class average. This number will represent the mutation frequency for S. marcescens, strain D1.

3. Was the loss in pigmentation by S. marcescens accompanied by a change in morphology of the bacterial colonies? If such changes were noticed, how would they complicate the proper identification of the white colonies as mutants of the red forms?

4. Did you notice any single colonies with both red and white segments? How might such an occurrence be explained?

5. Can you suggest a way to allow your white mutants to regain their red pigment?

In the appropriate labeled plates.

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4. Did you notice any single colonies with both red and white segments? How might such an occurrence be explained?

5. Can you suggest a way to allow your white mutants to regain their red pigment?

Investigations for Chapter 16

Population Genetics

Investigation 16A: Sickle-Cell Disease

What is the significance of the variations we see from one individual to another? Does each variation provide some special advantage? Biologists assume that most variations within a species have survival value. Could this assumption include variations that cause diseases? For example, geneticists have described hundreds of variations in the structure of the hemoglobin molecule inside human red blood cells. Some of these variations cause disease, and others do not. Could a variation in hemoglobin structure that causes disease have survival value to a population? If so, under what circumstances?

Sickle-cell disease is a disorder in which the abnormal hemoglobin molecules inside red blood cells combine with each other when the oxygen supply is low, causing the cells to lose their flexibility and assume an abnormal sickle shape (see Figure 16.5). The sickle cells clump together and block small blood vessels, stopping the flow of nutrients and oxygen. Vital organs may be damaged, and the individual may die. In this investigation, you will examine the conditions that influence the inheritance of sickle-cell disease.

Materials (per team of 3)

- paper and pencils

PART A

Procedure

Read the following information about sickle-cell disease, and answer the Analysis questions.

Sickle-cell disease is due to the homozygous presence of the sickle-cell allele (symbol: $Hb^S$). Heterozygous ($Hb^S/Hb^A$) individuals have sickle-cell trait and can be identified by a blood test. ($Hb^A$ represents the allele for normal hemoglobin.) About 0.25% of African-Americans are homozygous ($Hb^S/Hb^S$) and have sickle-cell disease. In certain parts of Africa, about 4% of black Africans have sickle-cell disease.

The Hardy-Weinberg model (see Section 16.3) enables us to predict when the allele frequencies in a population will remain constant. Because people with sickle-cell disease frequently die in childhood, however, the frequency of the allele causing this disease should not remain constant. Each death removes a pair of the sickle-cell-causing alleles from the population.
Figure 16A.1 shows the locations in Africa where the allele frequency of the \( Hb^s \) form is highest. These locations are also where a fatal form of malaria is found. Studies reveal that people homozygous for the normal allele \((Hb^AHb^A)\) often die of malaria. However, people with the heterozygous genotype do not contract the fatal form of malaria.

**Analysis**

1. What are the three possible genotypes involving the sickle-cell allele and its normal allele?

2. What are the phenotypes that would be associated with each genotype?

3. The Hardy-Weinberg model was described in Section 16.3. The model is summarized by this equation: \( p^2 + 2pq + q^2 = 1 \). Based on this model, what percentage of the African-American population has the genotype \( Hb^sHb^A \)?

4. In some areas of Africa, the frequency of the \( Hb^s \) allele is very high. What percentage of the black African population in these areas has the genotype \( Hb^sHb^A \)?

5. The relatively high frequency of the \( Hb^s \) allele has tended to remain constant in these areas of Africa. Under usual conditions, would you expect the frequency of a harmful allele to remain constant? Explain your answer.

6. Propose three possible explanations for the observation that the frequency of the \( Hb^s \) and \( Hb^A \) alleles remains high in these areas, despite its lethal effect in the homozygous condition.

7. What are the advantages and disadvantages of having each of the three genotypes you determined in question 1? Which genotype(s) would tend to survive?

8. Assuming that \( Hb^A Hb^A \) individuals die from malaria, what would be the allele frequencies of \( Hb^s \) and \( Hb^A \) in a population made up of the surviving genotype(s)?

9. The allele frequencies in the African population under discussion are not 0.5 for each form. Considering that fact, do you think the assumption in question 8 (that an individual with \( Hb^sHb^A \) will die from malaria) is reasonable? Which genotype do you think is less likely to be fatal? Explain your answer.

10. How can you explain the lower frequency of the allele causing sickle-cell disease among people of African descent who now live in America?

**PART B**

**Procedure**

Read the following information about the differing chemical structures of normal hemoglobin and sickle-cell hemoglobin, and answer the Analysis questions.

The sickle-shaped red blood cell is caused by a mutation that affects the hemoglobin molecule in the cells. Hemoglobin is a protein and therefore is made of amino acids. Scientists have been able to compare the chemical structure of normal hemoglobin, called hemoglobin A, with the hemoglobin found in sickle cells, called hemoglobin S. They found that both types of hemoglobin molecules contain 560 amino acids of 19 different types but differ in the substitution of valine for glutamate at a specific position in hemoglobin S (see Section 16.4).
Analysis
1. What are the nucleotide codes for the two amino acids that are different in hemoglobin A and hemoglobin S? (Consult the genetic code in Figure 9.4.) What is the simplest error in coding that could have occurred to cause the mutation?
2. Write a few paragraphs to summarize sickle-cell disease. Emphasize the ideas of mutation, selection, survival value, and evolution.

Investigations for Chapter 17
The Origin of Life

Investigation 17A ◆ Molecular Evolution in the Test Tube

When most people think of biological evolution, they think of living organisms and the formation of new species in the wild. Biologists have recognized since the 1960s, however, that the most essential features of biological evolution—replication, variation, and selection—apply equally to molecules. It is possible, for example, to study nucleic-acid molecules reproducing in the test tube (in vitro) and to watch them evolve new properties.

In this investigation, you will simulate an experiment in RNA evolution. The bacteriophage Qβ, which normally infects Escherichia coli, has a genome that consists of a single RNA molecule 4,000 nucleotides long. During the infection cycle, the RNA must enter the bacterial cell. To accomplish this, three of the four genes encoded by the RNA's 4,000 nucleotides specify proteins that enable the RNA to enter the bacterial cell and the "progeny" RNAs to spread to new bacteria. The fourth gene encodes viral replicase, the protein enzyme that uses the viral RNA as a template on which to assemble monomers into new copies of the RNA. The replicase enzyme initiates copying of the RNA by binding to a small subset of bases within it, called the origin of replication. These few bases alone are all that any Qβ RNA molecule needs in order to be copied by the replicase. Any molecule with an intact sequence at the origin of replication will be either not copied at all or copied at an altered rate.

This natural system can be streamlined and simplified to study evolution in a test tube. This in vitro system consists of the Qβ RNA molecule plus the materials needed for RNA synthesis (the A, U, C, and G nucleotide building blocks plus the replicase enzyme). This RNA system also has a built-in mutation feature that ensures that the RNA progeny molecules exhibit molecular variation (base-sequence changes). Such mutations come about because the replicase enzyme sometimes makes mistakes. On average, for each Qβ RNA molecule copied, there are one or two random base changes (mutations). Furthermore, the replicase occasionally produces molecules shortened by random amounts. Such shortened RNA molecules cannot infect bacteria but can still be copied in the test tube, provided that they retain the sequence for origin of replication. This system therefore incorporates two of the three features essential for evolution: replication and variation.

The third essential feature of evolution, selection, can be introduced by applying some form of selective pressure to the system. This is accomplished by limiting the time available for the RNA molecules to be copied. Such a constraint confers a selective advantage on RNA molecules that can be copied more quickly. In this scenario, speed of replication becomes a "phenotype" of the molecules and a test of their "fitness" in the test-tube environment.

Materials (per person)
• paper and pencils

Procedure
1. The investigator begins the experiment by adding Qβ RNA to a test tube containing replicase enzyme and nucleotide monomers. Replication reactions proceed for just 15 minutes. Then a random sample of the progeny RNAs is transferred from the first tube into a second tube containing a fresh supply of replicase and nucleotides (but no RNA other than that transferred). The replication process
again proceeds for 15 minutes, and a sample from this second tube is transferred to a third tube containing more fresh raw materials. This serial-transfer process is repeated 72 times (Figure 17A.1). During the experiment, the investigator monitors the total amount of RNA that accumulates in each tube, as well as the size and nucleotide composition of each “generation” of RNA molecules.

2. Use what you know about the experimental setup and about evolution to formulate a hypothesis and make some general predictions about how the starting RNA population changes during the course of the experiment. Your hypothesis should make predictions about changes that might occur in three phenotypic traits of the RNA molecules: (1) the speed of replication; (2) the length of the molecules; (3) the ability of the RNA molecules to infect *E. coli* bacteria.

3. Examine the data in Figure 17A.2, and if necessary, revise your initial hypothesis.

**Analysis**

1. What can you conclude from the data in Figure 17A.2 about the total number of molecules produced in each generation? What does this imply about the average speed of replication of the molecules?

2. What was the reason for the change in the speed of RNA replication?

3. Two major changes occurred in the RNA molecules during the course of the experiment that account for their altered replication speed:
Copying errors that replaced one base with another were made randomly by the “sloppy” replicase. The second reason for altered replication speed is apparent from the general trend in the size of the molecules over time. How would you describe this trend? What was happening to the longer molecules?

4. Imposing a time limit on the population for replication effectively made the copying process a competition—a race. This competition was certainly not “intentional” on the part of the molecules, but it was nevertheless inevitable simply because of the way the experimental environment operated. Why did each generation of RNA replicate faster than the preceding one?

5. If you were to test the RNAs of successive test-tube generations for their ability to carry out natural infection-replication cycles in bacteria, what would you expect to find?

6. After going from a length of 4,000 bases to approximately 700 bases, the molecules became no shorter. Suggest a reason that the size of the RNA stabilized around 700 bases.

7. A change in environmental conditions will select for new traits in molecules, just as it selects for new phenotypic traits in populations of organisms evolving in nature. Suppose a chemical inhibitor of replication was added to the test-tube system. If the amount of inhibitor was enough to slow down but not completely prevent replication, predict what would happen to the speed of RNA replication over time.

**Investigation 17B  Coacervates**

Under certain conditions, the proteins, carbohydrates, and other materials in a solution may group together into organized droplets called coacervates. Because coacervates have some properties that resemble those of living things, droplets like them might have been an important step in the origin of life. In this investigation, you can produce coacervates and study the conditions under which they form. You also can compare the appearance of coacervates with the appearance of the one-celled organisms, amoebas.

**Materials** (per team of 2)

- 2 pairs of safety goggles
- 2 lab aprons
- 3 coverslips
- dropping pipette
- 10-mL graduated cylinder
- 3 microscope slides
- test tube
- rubber stopper for test tube
- compound microscope
- wide-range pH test paper
- gelatin suspension (1%)
- gum arabic suspension (1%)

**FIGURE 17A.2**

Simulated results from the in vitro evolution experiment.
**INVESTIGATIONS**

**HCl** (0.1\textit{M})

amoebas, living or prepared slides

---

**SAFETY** Put on your safety goggles and lab apron. Tie back long hair.

---

**Procedure**

1. Mix together 5 mL of the gelatin suspension and 3 mL of the gum arabic suspension in a test tube. Gelatin is a protein. Gum arabic is a carbohydrate and is related to sugars and starches. Measure and record in your logbook the pH of this mixture.

2. Place a drop of the gelatin–gum arabic mixture on a slide, and observe it under the low power of the microscope.

3. Slowly add dilute hydrochloric acid (0.1\textit{M}) to the test tube, one drop at a time. After each drop, mix well and then wait a few seconds to see if the mixture becomes uniformly cloudy. If the liquid in the test tube remains clear, add another drop of acid. Continue adding acid one drop at a time until the mixture remains uniformly cloudy.

   **CAUTION:** 0.1\textit{M} HCl is a mild irritant. Avoid skin/eye contact; do not ingest. If contact occurs, flush affected area with water for 15 minutes; rinse mouth with water; call your teacher.

4. When the material turns cloudy, take another pH reading. At this point, carefully observe a drop of the liquid under the microscope. Look for coacervates, structures resembling those in Figure 17.16. If you cannot see them, try adjusting the light and using high power. Add another drop of acid to the test tube, mix, and observe again. If you still do not observe coacervates, repeat the procedure from the beginning, for you may have added the acid too rapidly. When you are successful, record your observations and make sketches of the coacervate droplets.

5. Examine a wet-mount preparation of living amoebas or a prepared slide, and compare their structure and organization with the larger coacervates.

6. When you have finished your observations of the coacervates, add more dilute acid to the test tube, a drop at a time. Mix after adding each drop, and measure the pH after every third drop. Continue until the solution becomes clear again. Examine a drop under the microscope, and measure the new pH.

7. Wash your hands before leaving the laboratory.

**Analysis**

1. How do the materials you used to make coacervates compare with those that might have been present in the ancient oceans?

2. In what pH range did the coacervate droplets form?

3. Did the pH change as expected as a result of adding more acid to the solution between coacervate formation and clearing?

4. When dilute hydrochloric acid was added beyond a certain point, the coacervates disappeared. What might you add to the test tube to make the coacervates reappear?

5. How might the coacervate droplets be made more visible under the microscope?

6. How might coacervates have contributed to the formation of the first cells?

7. Do coacervates display the necessary criteria to be described as living? Explain your answer.

---

**Investigations for Chapter 18**

**Diversity and Variation**

**Investigation 18A** ◆ Using Cladistics to Construct Evolutionary Trees

The world’s biodiversity may seem overwhelming, but patterns emerge when you examine traits that organisms have in common. Biologists have devised a method of organizing living things into smaller and smaller nested groups based on the presence of
newly evolved traits. This method of analysis is called cladistics. The groups represent collections of organisms that belong to various taxonomic categories within Linnaeus’s updated taxonomic hierarchy. This hierarchy consists of seven or eight taxonomic categories, including domains (sometimes called empires or superkingdoms), kingdoms, phyla (animals) or divisions (bacteria, plants, and fungi), classes, orders, families, genera, and species.

In this investigation, you will use cladistic analysis to reconstruct the evolutionary history of different groups of organisms. The aim is to use the appearance of newly evolved traits to reconstruct common ancestry. These relationships can be used to sort the organisms to create a natural classification system that is based on evolutionary relatedness rather than an artificial classification that is based on overall superficial resemblances.

Materials (per person)
“IS-Clad Method of Cladistics” handout
“Mammals” handout
“Mammalian Cladogram” handout

Procedure

PART A  Plants
1. Examine the data table in the “IS-Clad Method of Cladistics” handout. Based on the information in the data table, write an appropriate column heading in the upper left portion of the table. Also supply a title for the data table.
2. Using the information in the data table, complete the diagram on your handout. Draw in the remaining plants, and identify their shared traits. Note: The moss doesn’t share features with the other plants and should not be included in the diagram. It is called an outgroup and is included in the cladistic analysis for comparison purposes.
3. Use your completed diagram to fill in the cladogram at the bottom of the handout. Look at the innermost circle on the diagram. This circle identifies the characteristic that the fewest types of plants have in common. Fill in the missing name and image of the plant sharing this trait at the top left side of the cladogram. Follow the lines downward, and fill in the trait in the open circle labeled node A.
4. Look at the next outer circle on the diagram. This circle identifies a trait that a larger group of plants share. Fill in the missing name and image of the plant, and write the trait in node B.
5. Repeat steps 3 and 4 to identify the trait that four plants have in common. Write this trait in the bottom node.

PART B  Mammals
6. Examine the “Mammals” handout. Using the information in the data table, complete the diagram on your handout. Draw in the remaining mammals, and identify their shared traits. Remember that one type of mammal represents the outgroup and is not represented in the diagram. As before, the innermost circle on the diagram identifies the characteristic that the fewest types of mammals have in common. The next outer circle on the diagram identifies a trait that a larger group of mammals share. Repeat the process until you have accounted for all the mammal types.
7. Use your completed diagram to fill in the “Mammalian Cladogram” handout.

Analysis
1. How can cladistic analysis be used in classifying organisms and reconstructing their evolutionary history?
2. What type of information does cladistics use?
3. Why is it important to know what traits are possessed by a common ancestor?
4. How might the discovery of an extinct group that had never been seen before affect the construction of a cladogram?
5. How would the addition of a maple tree affect the plant cladogram in Part A?

Investigation 18B  Structural Characteristics of Animals

In this investigation, you will observe selected external characteristics of animals, and you will use these characteristics to classify the animals. Careful
**TABLE 18B.1**
Classification Chart for Invertebrates

<table>
<thead>
<tr>
<th>Name of animal</th>
<th>Phylum</th>
<th>Phylum</th>
<th>Phylum</th>
<th>Phylum</th>
<th>Phylum</th>
<th>Phylum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exoskeleton¹</td>
<td>present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body symmetry</td>
<td>radial²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bilateral³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>part bilateral, part spiral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jointed walking legs</td>
<td>3 pairs present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 pairs present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>more than 4 pairs present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body segmentation⁴</td>
<td>present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tentacles⁵</td>
<td>more than 4 present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 or fewer present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antennae⁶</td>
<td>2 or more pairs present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 pair present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Exoskeleton: a skeleton on the outer surface of an animal, enclosing the animal.
² Radial symmetry: body parts arranged in a circular manner around a central part or region, as in a bicycle wheel.
³ Bilateral symmetry: matching body parts along the right and left sides of a line running from one end of the animal to the other, as in the body of a bus.
⁴ Body segmentation: a structural pattern in which the body is divided into a series of more or less similar sections, the boundaries of which are usually indicated by grooves encircling the body.
⁵ Tentacles: slender, flexible structures that can be lengthened or shortened; usually attached near the mouth.
⁶ Antennae: slender structures that can be waved about but cannot change length; usually attached to the head.
<table>
<thead>
<tr>
<th>Skin structures</th>
<th>Name of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>hair present</td>
<td></td>
</tr>
<tr>
<td>feathers present</td>
<td></td>
</tr>
<tr>
<td>scales present</td>
<td></td>
</tr>
<tr>
<td>none of above present</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appendages</th>
<th>Name of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>wings present</td>
<td></td>
</tr>
<tr>
<td>legs present</td>
<td></td>
</tr>
<tr>
<td>fins present</td>
<td></td>
</tr>
<tr>
<td>none of above present</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Skeleton</th>
<th>Name of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>bony¹</td>
<td></td>
</tr>
<tr>
<td>cartilaginous²</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Teeth</th>
<th>Name of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>present</td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td></td>
</tr>
</tbody>
</table>

¹Bony skeleton: a skeleton in which most of the parts are hard and relatively rigid because of the hard mineral matter they contain.

²Cartilaginous skeleton: a skeleton in which all the parts are tough but flexible because they are composed of cartilage, a substance that does not contain significant deposits of hard minerals.
observation and note taking are required. Two questions may help guide your observations: How are these animals similar? How are these animals different?

This investigation will be a field trip. Your teacher will describe the objectives, schedule, and place or places to be visited. Make careful observations of the animals you see, and attempt to work out a classification scheme based on your observations.

**Materials** (per student)
charts of animal characteristics to be observed and recorded

**Procedure**
1. Before the field trip, study Figure 18.11 and the illustrations of Kingdom Animalia in “A Brief Summary of Organisms.” Make a mental note of types of characteristics that could help in organizing and recording your data.
2. On the field trip, observe an animal carefully before you record any data. Note those features that can be used to distinguish it from other animals. Also note the more general characteristics that can be used to group the animal with others that appear to be related to it.
3. Record your observations on charts similar to Tables 18B.1 and 18B.2, or use other charts as directed by your teacher. Try to identify the phylum or class to which the animal belongs.
4. Make additional notes beyond the observations you record on the charts. Save these notes for discussion after the field trip.
5. Repeat steps 2–4 for each animal to be observed. If you have questions, try to ask them before making entries in the charts.

**Analysis**
1. Which of the external characteristics you observed were of the greatest value in grouping animals?
2. What features were of greatest value in distinguishing one species from another?
3. Which of the animals you observed seemed most closely related to one another?
4. Which of the animals you observed seemed least closely related to one another?
5. On the basis of your observations, write a short paragraph that distinguishes animals from organisms in other kingdoms.
6. Based on the animals you observed, what changes, if any, would you make in the classification charts you used?
7. What advantages and disadvantages did you find in studying living animals rather than preserved specimens or illustrations in magazines and books?
8. What types of characteristics that you were unable to observe would have proved helpful in distinguishing between species of some of the animals you saw?
9. What roles did type of food and characteristics of feeding play in your classification of similar and different groups of animals?

**Investigations for Chapter 19**
**Changes in Species**

**Investigation 19A  ♦  Geological Time**

Sedimentary rocks such as shale are found in areas that were once sea or lake beds. The fine layers seen in sedimentary rocks were produced by the compression of many layers of sediment that were deposited over thousands or millions of years (Figure 19A.1).

**FIGURE 19A.1**
Sedimentary rock strata from the Green River Formation shale deposit in Utah.
Assuming that geological forces have not fractured or folded the deposits, scientists can study the layers in sedimentary rocks to estimate the age of the deposit. Strata lying close to the surface are presumed to be deposited more recently than strata lying farther down in the deposit. This type of reasoning leads to relative dating, where samples or fossils can be placed easily in their chronological order. Other types of dating, such as counting tree rings, produce a numerical result and are referred to as absolute dating. Perhaps the most accurate absolute-dating method is radioisotopic dating. This technique measures the abundance of certain radioactive isotopes in a specimen. Knowing the rate of radioactive decay allows the age of the sample to be calculated.

In this investigation, you will examine a shale specimen to estimate its age and, by extrapolation, that of the entire deposit. The Green River Formation is a shale deposit averaging 600 m thick and covering parts of Colorado, Wyoming, and Utah. The strata in the Green River Formation are very thin layers that were deposited in annual sedimentation cycles called varves. Each varve consists of a pair of layers, one light and the other dark. The light layer is thick, coarse-grained, and rich in calcium carbonate; while the dark layer is thin, fine-grained, and rich in organic material.

Materials (per team of 2)
compound microscope
piece of shale
metric ruler
calculator (optional)

Procedure
1. Examine your piece of shale, and use a ruler to estimate its thickness. Record the data in millimeters (mm) in your logbook.
2. Estimate the total number of dark bands running through your sample, and record the prediction in your logbook.
3. Examine your specimen under the microscope, using low power. Be sure that the varved layers are facing toward you. Place the ruler on the surface of the shale so that the ruler markings are parallel to the varve layers. Record in your logbook the number of varves in a typical 1-mm section. Count the dark bands only.
4. Determine the total number of varves for your specimen by multiplying the thickness of your specimen (in millimeters) by the number of varves per millimeter determined in step 3. Record the result in your logbook.
5. When instructed by your teacher, report your varve count per millimeter. The class data will be used to construct a histogram.

Analysis
1. How thick is an average year’s deposit?
2. Why is it impossible that one layer would cross over into other layers?
3. How long would it take for 1 m of sediment to be deposited?
4. If the average thickness of the Green River Formation is 600 m, how long was this lake (actually a series of three different lakes) in existence before drying up?
5. What type of evidence would persuade scientists that these strata formed at the bottom of ancient lakes?
6. What causes the formation of varves in this sedimentary rock?
7. How does this investigation illustrate the principles of relative and absolute dating?
8. Since dark bands contain organic material, what is the explanation for the occasional unusually thick dark bands?
9. Use the class data to determine the average value for varves per millimeter.

Investigation 19B  A Model Gene Pool

In 1908, Godfrey Hardy, an English mathematician, wrote to the editor of Science with regard to some remarks of a Mr. Yule.

Mr. Yule is reported to have suggested, as a criticism of the Mendelian position, that if brachydactyly is dominant, “in the course of time one would expect, in the absence of counteracting factors, to get three brachydactyious persons to one normal.”

It is not difficult to prove, however, that such an expectation would be quite groundless [using] . . . a little mathematics of the multiplication-table type.
The letter addresses an early and common criticism of Gregor Mendel’s work on inheritance. Many scientists thought that Mendel’s explanations of dominance and recessiveness suggested that recessive traits ultimately would be eliminated from the population and only dominant traits would remain. This investigation allows you to explore the validity of that assumption, using brachydactyly as an example.

Brachydactyly is a dominant disorder of the hands in which the fingers are shortened because of shortening of the bones. The actual frequency of occurrence is about 1 in 1 million births. You will use smaller numbers in setting up a model of a gene pool from which to randomly select pairs of alleles that represent individuals of a new generation.

**Materials** (per team of 3)
- 2 containers with lids, one labeled *male* and one labeled *female*
- 38 white beans
- 82 red beans

**WARNING:** Do not eat the beans. Wash your hands immediately after handling the beans or seeds. They may be treated with a fungicide.

**Procedure**
1. In this activity, you will set up a model of a human population by using red beans to represent the allele (*B*) for the dominant trait—brachydactyly—and white beans to represent the allele (*b*) for the recessive trait—normal hands. Before you begin, review the Hardy-Weinberg model in Section 16.3. List the five assumptions made by the Hardy-Weinberg model for a population that maintains a genetic equilibrium.
2. Place 19 white beans in the container labeled *male* and 19 white beans in the container labeled *female*. Add 41 red beans to each of the containers. Place the lid on each container, and shake the beans. What do the beans in each container represent?
3. To represent fertilization and the resulting possible allele combinations that make up the genotype of a new individual (*F₁* generation), you will select one bean from each container. What genotypes are possible?
4. In your logbook, prepare a table similar to Table 19B.1.
5. Remove the lid, and without looking, select one bean from each container. Use tally marks to record the results of this first selection in the table. Return the beans to their respective containers, cover the containers, and shake them again. Make a total of 60 selections for the *F₁* generation, returning the beans to the containers each time. In the table, total the number of each genotype in the *F₁* generation. Why should you return the beans to their respective containers after each selection?
6. Examine the data in your table for the *F₁* generation. Each pair of beans represents an individual of the *F₁* generation having a certain genotype. Assume that half the bean pairs of one genotype represent the males of the population and that half the bean pairs of the genotype represent the females. For example, if you have 18 pairs of red-red (*BB*) combinations, 9 pairs are males and 9 pairs are females. Determine how many of each type (*BB, Bb, bb*) should be

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tally marks</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Tally marks</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>bb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 19B.1**
Genotypes for Two Generations
male and how many female. Record the numbers in your table.

7. Use the information in your table to determine how many red beans (B) and how many white beans (b) should be in each container to represent the parents of the next generation. Correct the bean counts in each container. Mix the beans thoroughly.

8. Make 60 more selections from the containers of beans, as you did in step 5. This will be the F2 generation. Tally and record the totals for each genotype.

9. Wash your hands before leaving the laboratory.

Analysis
1. What is the total population of individuals in the F1 generation?
2. In genetics, frequency refers to the probability that a particular event will occur in a population, or

\[
\frac{\text{number of individuals of one genotype}}{\text{number of individuals of all genotypes}} = \text{frequency}
\]

Determine the frequency of homozygous recessive individuals (bb) for the F1 generation.

3. According to the Hardy-Weinberg model, the frequency of homozygous recessive individuals (bb) in a population is expressed as \(q^2\). Therefore, the frequency of the b allele = \(\sqrt{q^2}\), or \(q\). What is the frequency of the b allele for the F1 population?

4. The Hardy-Weinberg model states that the frequencies for two alleles of a trait add up to 1, or 100%. The frequency of the allele for the dominant trait (in this case, B) is represented by \(p\). Therefore, \(p + q = 1\), or 100% of alleles for the trait in the population. To calculate the frequency of the allele for the dominant trait, use the formula \(p = 1 - q\) (remember, \(p = B\) and \(q = b\)).

5. Prepare a population gene-analysis table like the one in Figure 19B.1 to show the expected frequency of each genotype in the next generation (F2). Cross two heterozygous individuals, and show the frequencies you have determined for each allele. Multiply to show the frequency of each F2 genotype.

6. Review question 2. To determine the number of individuals of a particular genotype, you can multiply the frequency of the genotype by the number of individuals of all genotypes in the population. Predict the number of each genotype (BB, Bb, bb) that would occur in an F2 generation of 60 individuals.

7. How do the results of your bean selections for the F2 generation compare with your predictions from question 6?

8. Based on this model, respond to Mr. Yule’s 1908 critique of Mendelian genetics.

9. Suppose the BB and Bb genotypes represented a lethal condition in the population you have been studying. Can the Hardy-Weinberg model still be applied in this situation? Explain your answer.

10. Hardy made the following statement in his 1908 paper: “There is not the slightest foundation for the idea that a dominant character should show a tendency to spread over a whole population, or that a recessive should tend to die out.” Do your results support this statement?

11. What was the primary mistake in Mr. Yule’s suggestion?

Investigations for Chapter 20

Human Evolution

Investigation 20A ◆ Interpretation of Fossils

How do anthropologists learn about evolution? Fossil remains form a record of the evolution of early humans, hominids, and other primates. Even
though humans and other primates share many similar features, humans did not evolve from apes. Although primate brains have increased in both size and complexity during the course of evolution, the relationships between modern humans and other primates and fossil hominids cannot be determined only by an examination of braincase, or cranial, casts. Anthropologists often compare the skulls, jaws and teeth, pelvises, and femurs of fossil hominids and apes with those of modern primates. This investigation simulates some of the comparisons anthropologists make when studying fossil hominid remains. The activities include examination of several hominid cranial casts and comparison of some skeletal measurements for a human, an early hominid, and a gorilla.

**Materials** (per team of 2)
- paper and pencils
- metric ruler
- protractor
- graph paper

**PART A  Comparison of Cranial Casts**

**Procedure**
1. Examine the five cranial casts shown in Figure 20A.1. What is the volume of each? Which species do you predict to have the largest brain volume compared with body weight?
2. Using the information in Table 20A.1 and a sheet of graph paper, plot the ratio of brain volume to body mass for each of the five species.
3. In Figure 20A.1, which species have Broca’s area? On the *Homo sapiens* cranial cast, you can see that Broca’s area is an enlargement of part of the cerebellum. Broca’s area and two other areas of the brain are important in language and speech. Broca’s area sends signals to a part of the brain that controls the muscles

| TABLE 20A.1  
**Body Weight of Selected Species** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td><em>Tarsius</em> (tarsier)</td>
</tr>
<tr>
<td><em>Australopithecus</em></td>
</tr>
<tr>
<td><em>Homo erectus</em></td>
</tr>
<tr>
<td><em>Pan troglodytes</em> (chimpanzee)</td>
</tr>
<tr>
<td><em>Homo sapiens</em></td>
</tr>
</tbody>
</table>
of the face, jaw, tongue, and upper part of the throat. If a person is injured in Broca’s area, normal speech is impossible. What might the presence of Broca’s area indicate?

4. Convert the ratios of brain mass to body mass shown in Table 20A.2 to decimal fractions. Draw a bar graph of the fractions. On your graph, the x-axis should represent the five species; the y-axis, the ratio of brain mass to body mass.

Analysis
1. Based on your graph, what could you infer is the relationship between evolution in primates and the ratio of brain mass to body mass?

2. What major portion of the brain has enlarged most noticeably during the course of primate evolution?

3. Do you think the cranial cast of Australopithecus indicates that this hominid could have had a Broca’s area in its brain? Explain your answer.

4. Does the presence or absence of Broca’s area alone determine the language capabilities of a hominid? Explain your answer.

5. How does your bar graph affect your answer to question 1? Are brain size and ratios of brain mass to body mass reliable indicators of the course of primate evolution? Why or why not?

PART A Skeletal Comparisons

Procedure
5. Examine the three primate skulls in Figure 20A.2 and the drawings of lower jaws and the pelvises in Figure 20A.3. Imagine you are an anthropologist and the fossils have been placed before you for identification. Complete this hypothesis in your logbook: “If the skulls, jaws, and pelvises are significantly different, then . . .”

6. In your logbook, prepare a table similar to Table 20A.3, or tape in the table your teacher provides. Your task is to determine which skull, jaw, and pelvis belong to a human, which belong to an

---

**TABLE 20A.2**

<table>
<thead>
<tr>
<th>Mammal</th>
<th>Ratio of brain mass to body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree shrew</td>
<td>1:40</td>
</tr>
<tr>
<td>Macaque</td>
<td>1:170</td>
</tr>
<tr>
<td>Blue whale</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Human</td>
<td>1:45</td>
</tr>
<tr>
<td>Squirrel monkey</td>
<td>1:12</td>
</tr>
<tr>
<td>House mouse</td>
<td>1:40</td>
</tr>
<tr>
<td>Elephant</td>
<td>1:600</td>
</tr>
<tr>
<td>Porpoise</td>
<td>1:38</td>
</tr>
<tr>
<td>Gorilla</td>
<td>1:200</td>
</tr>
</tbody>
</table>

**FIGURE 20A.2**

A variety of primate skulls.
early hominid, and which belong to a gorilla. Record all observations and measurements in your table.

7. **Cranial volume:** The straight line drawn on each skull represents the brain volume for each primate. Measure in centimeters the distance from point A to point B for each skull. Multiply by 175 to approximate the cranial volume in cubic centimeters.

8. **Facial area:** Measure in centimeters from points C to D and from E to F on each skull. Multiply the measurements of each skull to determine the approximate area of the lower face. What might you infer about how the size of the facial area has changed through primate evolution?

9. **Facial projection:** Use a protractor to determine the angles created by the colored lines. What can you infer from these measurements?

10. **Brow ridge:** This is the bony ridge above the eye sockets. Record the presence or absence of this feature for each skull. Also note the relative sizes.

11. **Teeth:** Study the drawings of the jaws that are shown in Figure 20A.3. Record the number of teeth and the number of each type of tooth. Also look at the relative sizes of the different types of teeth.

12. **Pelvis:** Study the drawings of the pelvises in Figure 20A.3. Notice their relative sizes and whether the flange, or lower portion, projects to the rear. Measure in millimeters the diameter of the pelvic opening at the widest point.

13. Read these additional data.
   a. A larger cranial volume is characteristic of humans.
   b. A smaller lower facial area is characteristic of humans.
   c. A facial projection of about 90 degree is characteristic of humans.
   d. Modern humans have lost most of the brow ridge.
   e. All primates have the same number of teeth and the same number of each type.

---

**FIGURE 20A.3**
Primate lower jaws and pelvises.
f. Humans and other hominids have smaller canine teeth than do gorillas.
g. A smaller pelvis, with a broad blade, a flange extending to the rear, and a wider opening, is characteristic of primates that walk on two feet.
h. As brain size increased, the width of the pelvic opening increased to accommodate the birth of offspring with a larger head-to-body ratio.

Analysis
1. Compare the data you have assembled for each specimen. Which ones are hominid? Which characteristics are similar in all three primates? Which characteristics are similar in a and b?
2. Compare your data with the additional data. On the basis of your observations and measurements and the additional data, which fossil remains would you say are human, which are gorilla, and which are early hominid?
3. What might an anthropologist infer from the size of the pelvic opening?

4. Write a few paragraphs discussing the methods anthropologists use to determine human ancestry. Which of the characteristics you examined were most helpful? Which were least helpful? List any other additional observations or measurements that could be made for these specimens. Would having a more complete skeleton be helpful? Why?

Investigation 20B  Archaeological Interpretation

Because we cannot travel through time to see how people worked and lived thousands of years ago, we can never be sure that we understand the details of earlier cultures. Archaeologists search for clues among the remains of ancient peoples and civilizations. When they have found, dated, and studied the evidence, they formulate hypotheses to explain their findings. What emerges is a picture of life in a particular place hundreds or thousands of years ago. It is impossible to create a complete, detailed picture, but many reasonable and logical conclusions can be drawn. Some findings, however, can be interpreted in a variety of ways, and archaeologists may disagree about which interpretation is correct.

This investigation requires analysis of data found in archaeological digs. In nearly every case, several interpretations are possible. Think of as many interpretations as you can. Remember that your interpretations should account for all, not just some, of the existing data. In the final section of this investigation, you will predict how archaeologists far in the future might interpret evidence of today’s cultures and lifestyles.

Materials (per team of 3)
- paper and pencils

PART A  A Native American Cemetery in Newfoundland

Procedure
Read the following paragraphs, and answer the Analysis questions.
A scientist investigated a Native American burial site located near Port aux Choix, a small village on the west coast of Newfoundland (Figure 20B.1a). The burial ground appeared to have been used between 4,000 and 5,000 years ago. The graves were located in strips of very fine sand and were often covered with boulders or slabs of rock. One of the sand strips was almost 1.6 km long, varying from about 9 m to 22 m in width. It lay about 6 m above and parallel to the present high-water mark of the ocean shore. The estimated ages of the skeletons found at the site are in Table 20B.1. What do these data suggest about the lives of the Native Americans?

The teeth of many of the adults were worn; often the inner nerve was exposed. Some skeletons also had teeth missing, but there was no evidence of tooth decay.

Figures 20B.1b–e show some artifacts that were found in the graves. A great number of woodworking tools were also found.

Analysis
Answer the following questions. Give reasonable answers that are supported by the evidence found at the site or from your own knowledge and past experience.

1. What type of area might the Newfoundland burial site have been at the time the Native Americans lived there (for example, forest, mountains, beach)? Explain your answer.

2. List some possible reasons that the sandy strip was chosen as a burial site.

3. Boulders or slabs of rock covered many graves. Why might that practice have been common?

4. From the data on teeth, what can you infer about Native American diet and lifestyle?

5. What do you think the objects shown in Figure 20B.1b were used for? What purpose might the holes have served?

6. What might the objects in Figure 20B.1c and d have been used for?

7. What modern implement do the objects in Figure 20B.1e resemble? What might their function have been?

8. Considering the location of the site and its objects, what might the Native Americans have built from wood?
PART B Ancient People of Greece

Procedure
Read the following paragraphs, and answer the Analysis questions.

Stone Age people inhabited Greece long before the dawn of Classical Greek civilization. Until recently, little was known about the culture of these people who lived between 5,000 and 20,000 years ago. Excavations in and around a particular cave on the east coast of Greece began in 1967. Since then, archaeologists have gathered much evidence of the changes that occurred in the culture and lifestyles of Stone Age Greeks living there.

The oldest remains (20,000 years old) in the cave primarily are the bones of a single species of horse and some tools made from flint (rock that forms sharp edges when it breaks). What might explain the presence of the horse bones mixed with flint tools?

Newer remains (10,000 years old) include bones of red deer, bison, horses, and a species of wild goat, as well as remains of wild plants such as vetch and lentils (pea-like plants), shells of land snails, marine mollusks, and some small fish bones. These newer remains date from the time when a great ice age had come to an end. In later finds, from about 9,250 years ago, very large fish bones were found—the fish might have weighed 100 kg or more. At about the same time, tools made of obsidian, a type of volcanic glass, were found. The nearest source of obsidian is 150 km away from the excavation site and across a body of water. The site also yielded the oldest complete human skeleton found in Greece. A male of about 25 years of age was buried in a shallow grave covered with stones. Certain bone abnormalities indicated that he may have suffered from malaria, a tropical disease spread by mosquitoes.

Remains dated about 8,000 years old are dominated by the bones of goats and sheep found in and around the cave. These bones were very different from the goat and sheep bones found away from the cave. Evidence of wheat and barley seeds also was found. Among the tools found were axes and millstones. Only a few of the human graves at the site contained objects such as tools or jewelry. A 40-year-old woman, who probably died about 6,500 years ago, was buried with some bone tools and some obsidian blades. One infant was found near a marble vessel and a broken clay pot.

Analysis
1. In what ways did the diet of the cave’s inhabitants change over the centuries? Explain your answer.
2. What does the evidence from about 9,250 years ago suggest about a change in the people’s lifestyle?
3. How do you think the ancient people obtained the obsidian?
4. From the 8,000-year-old remains, what can you infer about the cave inhabitants’ lifestyle?
5. What might be the significance of finding items buried along with people?

PART C Excavating the Present

Procedure
Imagine that you are an archaeologist living in the year 4000. A catastrophe has destroyed the records of the past. You have only the remains that have survived the last 2,000 years as clues to what life was like in the 1990s. Imagine, furthermore, that you are excavating a site that was a school 2,000 years ago—your own school.

Analysis
1. What objects do you think you would find at the site? Make drawings of or list descriptions of the objects. Exchange them with your teammates.
2. For each object, suggest several interpretations. (Remember, you know nothing about the time
Investigations for Chapter 21
Nervous Systems

Investigation 21A  Sensory Receptors

How do you know and learn about the world in which you live? Information is received through receptor cells that function as part of the nervous system. Specialized receptors make possible the senses of touch, sight, hearing, smell, and taste. In this investigation, you will test some touch receptors.

Materials (per team of 2)
- nonmercury thermometer
- 2 round toothpicks
- 2 10d nails with blunt points
- pen with water-soluble ink
- paper towels
- large container of hot water
- large container of lukewarm water
- large container of ice water

Procedure

PART A  Skin and Temperature Sensations
1. Check the hot water with a thermometer to make sure it is more than comfortably warm (45° to 50°C), but not hot enough to burn your hand.

CAUTION: Hot water can cause serious burns. Do not let the water temperature exceed 50°C.
2. Put one of your hands in the hot water and the other in ice water. Leave them there 1 minute.
3. After 1 minute, remove your hands from the hot water and ice water. Immediately sink both hands in the lukewarm water.
4. Record in your logbook how the lukewarm water felt to each hand.

PART B  Sensory Receptors in Skin
5. Work in pairs for this part of the investigation. Determine who will be the experimenter and who will be the subject. Place one of the 10d nails in the ice-water container; place the other nail in the hot-water container.
6. Experimenter: Make a 5 cm × 7 cm grid of small points of ink on the inside of the subject’s wrist with the points 5 mm apart (Figure 21A.1). Make a larger copy of the grid in your logbook. Make it large enough to mark an H (hot) and a C (cold) by each grid point. If the sensation is felt by the subject, circle the letter. If it is not felt, put a line through the letter. This will allow you to test each grid point with a hot and cold probe and keep track of which points have been tested. Subject: Sit so your arm is resting comfortably on top of the desk with the wrist up. Look away from the experimenter.

FIGURE 21A.1
Grid on wrist.
7. **Experimenter:** Test hot and cold receptors on the subject’s wrist by touching the blunted point of a nail taken from the hot or cold water on each grid spot. Dry the nail quickly with the paper towel before touching the grid point. Alternate the hot and cold nails randomly.

**CAUTION:** Do not push the nails into the skin or in any way break the surface of the skin with the nails. Call your teacher in case of abrasion or cut. Do not put nails near your face or another’s face.

**Subject:** Tell the experimenter whether you have a hot or cold sensation.

**Experimenter:** Record the results of each touch by marking the appropriate letter in your logbook.

8. Reverse roles with your partner, and repeat the experiment.

**PART C Distance between Receptors**

9. **Subject:** Close your eyes, and turn your head away from the experimenter.

**Experimenter:** Gently touch the end of the subject’s index finger with the points of two toothpicks. Start with the points relatively far apart (1 cm), and then move them slowly together for each subsequent contact.

**CAUTION:** Do not push the toothpicks into the skin or in any way break the surface of the skin. Call your teacher in case of abrasion or cut. Do not put toothpicks near your face or another’s face.

**Subject:** Tell the experimenter whether you sense one or two toothpick points.

**Experimenter:** If the subject senses two points, lift the toothpicks and move them slowly together for the next test. Continue testing the subject until only one toothpick point is sensed.

10. **Experimenter:** Trace the outline of the subject’s finger in the logbook. Use two pencil dots to represent the closest points where the subject sensed both toothpick points.

11. Measure the distance, in millimeters, between the dots on the diagram. Measure the width of the finger in millimeters. Express these two measurements as the ratio \( \frac{\text{width of finger}}{\text{minimum distance}} \). Reduce the fraction to a small whole number.

12. Repeat steps 9 through 11, using the back of the hand. Start the touching technique with the points at least 6 cm apart. Record your results as a ratio and reduce as in step 11.

13. Switch roles and repeat the experiment.

**Analysis**

1. List the ratios you obtained for the fingertip and the back of the hand on the chalkboard under the correct heading *male* or *female*. How similar are the ratios for the people in your class? Are there any differences between the ratios for males and females?

2. Feeling the forehead is a common way to find out whether body temperature is above normal. On the basis of what you learned in Part A of this investigation, explain how a person with a slightly raised temperature of 40°C could feel cool to the touch.

3. Are the sensitive areas that are stimulated by cold the same sensitive areas that are simulated by heat? Explain your answer.

4. If a person heard a crackling fire and people talking about heat and then suddenly was touched with a piece of ice on the back of the neck, what sensation might he or she first experience? Explain your answer.

5. How does the distance between touch receptors on the fingers and those on the back of the hand compare? Explain the significance of the difference.

6. Develop a hypothesis that could account for the fact that when two toothpicks touch the skin, sometimes only one is felt.

**Investigation 21B Reaction Time**

Various sensory organs receive stimuli from the environment and send signals to the brain, where they are interpreted. Messages sent to muscles and glands cause specific reactions to occur. How fast can you react to sound stimuli? How fast can you react to visual stimuli? Are your reactions any faster if you use both sound and visual stimuli? Are your fingers faster than your arms in reacting to stimuli?
This investigation will give you a chance to answer those questions.

**Materials** (per team of 2)
- meterstick
- metric ruler

**Procedure**

**PART A  Finger Muscles**
1. Copy Table 21B.1 in your logbook to help organize your data collection.
2. Work in pairs for this experiment. Determine who will be the experimenter and who will be the subject.
   - **Subject:** Sit down with an arm resting on the desk so that your hand extends past the edge of the desk. Your thumb and forefinger should be parallel to the ground and 4 cm apart. Use the metric ruler to keep the fingers about 4 cm apart. If the distance between the fingers varies too much, the results will be affected.
3. **Experimenter:** Stand and hold a meterstick vertically between the thumb and forefinger of the subject so that the lowest number on the meterstick is between the subject’s thumb and forefinger.
4. **Experimenter:** Without warning, drop the stick.
5. **Subject:** Try to catch the meterstick with just your thumb and forefinger.
6. **Experimenter:** Look at the meterstick, and note the number of centimeters the stick dropped before the subject caught it. Record the distance in your logbook, but do not enter it in your table at this time.
7. Repeat the test four times, recording each distance the stick dropped. Determine the average for the five trials. Record the average in the data table under the heading “Sight only.”
8. Repeat the investigation with the subject’s eyes closed. The experimenter will snap his or her fingers or otherwise signal aloud when the stick is released. Calculate the average for five trials, and enter the figure in the data table under “Sound only.”
9. Repeat the investigation with the subject’s eyes open. The experimenter will use the same signal used in step 8 when the stick is released. Calculate the average for five trials, and enter the figure in the data table under “Sight and sound.”

**PART B  Arm Muscles**
10. **Subject:** Stand an arm’s length away from a classroom or hall wall, and place the palm of your hand, fingers up, flat against the wall. Lean slightly backward or move slightly backward so the palm of your hand is 4 cm away from the wall.
11. **Experimenter:** Stand and hold a meterstick against the wall so that the base of the subject’s hand is even with the lowest number on the meterstick.
12. **Experimenter:** Without warning, drop the stick.
13. **Subject:** Try to catch the stick by pinning it to the wall with the flat of your hand.
14. **Experimenter:** Look at the meterstick, and find the number of centimeters the stick dropped before the subject caught it (measure from the base of the hand). Record the distance in your logbook, but do not enter it in your table at this time.
15. Repeat the test four times, recording each distance the stick dropped. Determine the average for the five trials. Record the average in the data table under the heading “Sight only.”
16. Repeat the investigation with the subject’s eyes closed. The experimenter will snap his or her fingers or otherwise signal aloud when the stick is released. Calculate the average for five trials, and enter the figure in the data table under “Sound only.”
17. Repeat the investigation with the subject’s eyes open. The experimenter will use the same signal used in step 16 when the stick is released. Calculate the average for five trials, and enter

---

**TABLE 21B.1**
**Summary of Reaction Times**

<table>
<thead>
<tr>
<th>Method</th>
<th>Average distance meterstick travels (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sight only</td>
</tr>
<tr>
<td>Finger muscles</td>
<td></td>
</tr>
<tr>
<td>Arm muscles</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
</tr>
</tbody>
</table>
the figure in the data table under “Sight and sound.”

18. Calculate the differences in the distances dropped by the meterstick for the two methods of catching it. Record the differences.

Analysis
1. In the sight-only test, what parts of your body are involved in catching the meterstick with your fingers? Which parts are involved when you catch the meterstick with your hand? Explain your answer.
2. In the sight-only test, what receptors are involved in both methods of catching the stick?
3. How do the results of the sound-only test compare with the results of the sight-only test?
4. Are there any differences between the reaction times of the finger muscles and those of the arm muscles? Explain this difference.
5. Compare the results of the sight-and-sound test with the sound-only and sight-only tests.
6. What can you conclude about your reactions to different stimuli?
7. What can you conclude about your reactions with different muscle groups?

Investigations for Chapter 22
Behavior

Investigation 22A ◆ A Lesson in Conditioning

Conditioning occurs when the patterns of innate behavior or reflexes change. In this investigation, you will determine if it is possible to condition a classmate to jerk his or her hand away when you make a noise.

Materials (per team of 2)
small rubber ball
scissors
cardboard box (book-box size)
noisemaker, such as an electric buzzer or a metal “cricket”

Procedure
1. Cut a hole near the bottom of one side of a cardboard box, similar to the one shown in Figure 22A.1. In the center of the top of the box, cut a small circular hole through which the ball can easily pass.

   Figure 22A.1. In the center of the top of the box, cut a small circular hole through which the ball can easily pass.

   CAUTION: Scissors are sharp. Handle with care.

2. Determine which person will be the experimenter and which person will be the subject. The subject should not read any further directions. In your logbook, prepare a table similar to Table 22A.1. Be sure you have room to record how the subject reacted and what combination of stimuli you used.

3. Place the subject in front of the box with his or her hand inserted through the hole, directly underneath the circular hole on the top. Make sure that the subject’s hand is mostly out of his or her sight.

4. Inform the subject that he or she will be attempting to keep the hand from being hit by the ball as it drops. Be sure the subject understands that the hand must remain in position at all times unless he or she removes it to keep from being hit by the ball.
INVESTIGATIONS 781

5. Stand behind the box with your noisemaker hidden from the subject’s view. Now drop the ball through the hole at the same time you make a noise with your noisemaker. Do this several times in a row. The subject probably will not be hit more than once or twice. Record the subject’s reaction and the stimulus used for each trial.

6. On the next trial, instead of dropping the ball, just make the noise. Record your results.

7. Randomly change the order of the stimuli used. Try some trials with both, several with just the noise alone, and several with just the ball. Record the results.

8. Wash your hands before leaving the laboratory.

Analysis
1. Compare your results with those from the rest of the class. Do you observe a pattern in the subjects’ reactions?
2. Are there any differences in the results? If so, can you suggest why?
3. Do you think another stimulus besides noise would work the same way? Why or why not?

Investigation 22B ◆ Trial-and-Error Learning

This investigation will allow you to experience how trial-and-error learning operates. You will do something differently from the way you are used to doing it and will examine the learning process that occurs.

Materials (per team of 2)
small mirror
stopwatch or clock with a second hand
20 star diagrams

Procedure
1. Decide who will begin as the experimenter and who will be the subject. You will change roles later.
2. Divide the 20 star diagrams into two sets of 10. Number each set from 1 through 10. On each diagram, choose one of the star points and label it start (Figure 22B.1). Do this for all 20 figures.
3. Construct a screen using the books. Make two piles of books, and lay the last book across the top of the two piles (Figure 22B.2). Leave enough room for your arm to fit through the space between the two piles of books.

4 pieces of graph paper
4 to 6 books (enough to make the screen in Figure 22B.2)
4. In your logbook, prepare a table with three headings: **Trial number**, **Number of errors**, and **Time in seconds**.

5. The subject will sit with his or her arm between the piles of books and will outline the star on the diagram without looking directly at the paper. The experimenter will hold a mirror in which the subject can see the reflected star diagram. The experimenter will time how long it takes the subject to outline the star.

6. The subject should begin at the point on the diagram labeled *start* and draw a line all the way around the star, trying to stay within the lines. The subject should look only at the reflection of the star diagram in the mirror held by the experimenter.

7. The experimenter should record in seconds how long it takes the subject to complete the outline of the star.

8. Repeat this procedure for the other nine figures. For each diagram, record the time to completion.

9. Change roles with your partner. Repeat steps 6 through 8.

10. When you and your partner have completed all 20 trials, count the number of errors in each trial. An error is counted every time the subject’s pencil line went outside the lines of the star diagram and returned. See Figure 22B.3 for an illustration of errors.

11. After you have counted the errors for each trial, graph the results for the ten trials you completed as the subject. Your partner will do the same for the other ten trials. Label the horizontal axis *time* and the vertical axis *number of errors*.

**Analysis**

1. Examine the number of errors for each of your ten trials, and compare those with the time it took you to complete the outline. What, if any, evidence indicates that learning occurred?

2. Compare your graph with those of your partner and with the other teams. Is there any pattern in the data? Explain your answer.

3. Summarize the results of this investigation in terms of trial-and-error learning. Use your data to support your conclusions.

**Investigation 22C ♦ A Field Study of Animal Behavior**

The study of an organism’s behaviors in an ecosystem is called ethology. These behaviors include its responses to the abiotic environment, to other species, and to other members of its own species. You can learn a great deal about an
organism by observing it under natural conditions, where its behavior is likely to be typical of the species. In this investigation, you will develop a systematic field study of an organism in its natural habitat and then relate its behaviors to its basic needs. Be careful not to attribute human values and emotions to the organism.

Procedure

1. Select a nondomesticated animal that is available for observation. Some of the following animals would make good subjects: insects and spiders; birds such as robins, blue jays, pigeons, sparrows, ducks, and geese; mammals such as deer, squirrels, chipmunks, muskrats, and raccoons; snails, slugs, and fish.

2. Most of these organisms live in fields, ponds, parks, or forests. Even an aquarium, though not the same as a natural habitat, provides a suitable environment for observation. The best times to observe are just before sunset and just after sunrise. Many animals feed at these times. You do not need to study the same individual each time because the behaviors you observe should be typical of the species.

3. Some behaviors on which to focus include orientation to external stimuli, such as wind, sun, and moisture; communication with other members of the species or with other species; feeding; courtship and mating; interaction with other members of the species or with members from other species, including protective measures for itself or its young; reactions to the presence of other species, including humans.

4. Once you have selected an organism to study, devise a procedure for your study and submit it to your teacher for approval. If possible, the organism should be observed several times. Your plan should include:
   a. The scientific and common names of your organism.
   b. Where and when you will make your observations.
   c. The question you plan to investigate.
   d. A hypothesis related to your question.

5. Conduct your observations. Your results will more accurately reflect natural behaviors if your subject is not aware of your presence.

6. Record your observations accurately and comprehensively in your logbook. You will use your data to prepare your report.

Analysis

Write a report relating the behaviors you observed to the organism’s basic needs. Remember not to attribute human motives to the animal. Your report should include:

a. Title, your name, and date.
b. The procedure approved by your teacher.
c. The actual procedure you followed if it differed from your proposal.
d. Your questions and hypothesis.
e. The data you collected: Organize your notes by quantifying as much data as possible (how many, how much, how often, and so on) using tables, charts, or graphs.
f. Conclusions: Interpretations or explanations of the behaviors observed in light of the basic needs of the animal.
g. Evaluation of your hypothesis: Did the data support or refute your hypothesis?
h. Recommendations for further study: What would you suggest if someone else were going to do the same study, or what would you do differently if you did the same study again?

Investigations for Chapter 23

Immune Systems

Investigation 23A  ♦  Antigen-Antibody Binding

One of the body’s most important defense mechanisms against infection is the production of antibodies, or immunoglobulins. These proteins circulate in the bloodstream, where they make up a part of the gamma globulin (IgG) fraction of blood plasma (see Appendix 23A, “Antibody Classes”). The production of antibodies can be stimulated by the antigens of an infecting agent such as a bacterium or
virus. An antibody binds to an antigen in a reaction that is highly specific—each type of antibody binds to a particular type of antigen and no other.

There are many laboratory procedures designed to detect the presence of antibodies and the interactions between antibodies and antigens. One such test is called ELISA—enzyme-linked immunosorbent assay—an immunological technique used to detect and quantify specific serum antibodies. (Serum is blood plasma without the clotting factors.)

In ELISA, serum to be tested is allowed to react with specific antigens. Serum antibodies that combine with the antigens are detected by treating the test system with a conjugate—another antibody linked to an enzyme. This second antibody binds to the antigen-antibody complex that formed earlier. The enzyme serves as a marker. When a substrate for the enzyme is added, a reaction between the substrate and the conjugate is indicated by a color change. (If no serum antibodies are present to bind with the conjugate, no color change will occur during the time of observation.) These reactions are diagrammed in Figure 23A.1. ELISA is used routinely in the screening of blood donors for antibody to HIV.

In this investigation, you will perform ELISA to observe a specific antigen-antibody reaction and to determine the amount of antibody present. You will use a microwell plate—a piece of plastic molded to form many small wells, somewhat like a miniature egg carton. Once applied, the antigen is absorbed onto the surface of the wells and can react with serum antibodies added later.

The test will span 3 days and will include the following steps:

- **Day 1**, application of antigens, blocking unbound sites
- **Day 2**, addition of primary antibodies
- **Day 3**, addition of secondary antibodies with conjugate, addition of substrate, checking for color

**Materials** (per team of 5)

- 5 pairs of safety goggles
- 5 lab aprons
- 15 pairs of disposable gloves
- paper towels
- white index card
- glass-marking pencil
- incubator
- 3 sheets of parafilm
- 96-well polyvinyl microwell plate
- 13 1-mL transfer pipettes
- 20 1.5-mL microfuge tubes
- distilled water

**Shared Materials**

- antigen 1 (bovine serum albumin; BSA) at antigen station 1
- antigen 2 (bovine serum transferrin) at antigen station 1
- 10× PBS-Tween at washing station 7

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**FIGURE 23A.1**

Steps in ELISA.
10× phosphate buffer saline (PBS) at washing station 7
1 mg gelatin mixed with distilled water at station 2
1 mL of antibody 1 at station 3
1 mL of antibody 2 at station 4
1 bottle of antibody 3 at station 5
substrate (ABTS) at station 6

**SAFETY** Put on your safety goggles, lab apron, and gloves. Tie back long hair.

### Procedure

#### Day 1

1. In your logbook, prepare a grid like the diagram in Figure 23A.2. The numbers identify the rows in the microwell plate, and the letters identify the wells. Along the sides and top of the grid, record what you add to each well as you follow the procedures. (On day 3, you will record ELISA results in each square of the grid.)

   ![Diagram of microwell plate for recording data.](image)

   **FIGURE 23A.2**
   Diagram of microwell plate for recording data.

   CAUTION: The chemicals you will be working with are irritants. Avoid skin/eye contact; do not ingest. Flush spills and splashes with water for 15 minutes. Call your teacher.

2. With a clean 1-mL transfer pipette, add 1 drop of antigen 1 (from station 1) to all 12 wells in rows A–D (top of plate).

3. With a clean 1-mL transfer pipette, add 1 drop of antigen 2 (from station 1) to all 12 wells in row E (bottom of plate).

4. Write your names on a piece of parafilm, and cover the plate with the parafilm. Place the plate in the 37°C incubator for 30 minutes.

5. Move your plate from the incubator to washing station 7, and remove the parafilm. Spray PBS-Tween across the plate, and recover it with the same parafilm. Gently shake the plate back and forth, but be careful not to spill the contents.

6. Remove the parafilm, and empty the plate into the sink. Spray PBS (at washing station 7) across the plate, and recover with the same parafilm. Gently shake the plate back and forth, but be careful not to spill the contents. Empty the contents into the sink.

7. With a clean 1-mL transfer pipette, add 1 drop of gelatin (from station 2) to every well (to block the unbound sites). Use the same piece of parafilm to recover your plate, and place it in the refrigerator.

8. Wash your hands before leaving the laboratory.

#### Day 2

9. Remove your plate from the refrigerator, and place it in the incubator for 5 minutes.

10. Take your plate to washing station 7, and remove the parafilm. Spray PBS across the plate, and recover the plate with the same parafilm. Gently shake the plate back and forth, but be careful not to spill the contents.

11. Go to station 3, and get 10 1.5-mL microfuge tubes. Label five tubes BSA 1, BSA 2, BSA 3, BSA 4, and BSA 5.
12. With a clean 1-mL transfer pipette, add 1 mL of antibody 1 to the tube labeled BSA 1. From tube BSA 1, use the transfer pipette to remove 500 μL (0.5 mL), and place this in tube BSA 2. With a clean transfer pipette, add 500 μL of distilled water to tube BSA 2. From tube BSA 2, remove 500 μL, and place this in tube BSA 3. With a clean transfer pipette, add 500 μL of distilled water to tube BSA 3. From tube BSA 3, remove 500 μL, and place this in tube BSA 4. With a clean transfer pipette, add 500 μL of distilled water to tube BSA 4. From tube BSA 4, remove 500 μL, and place this in tube BSA 5. With a clean transfer pipette, add 500 μL of distilled water to tube BSA 5.

13. Add BSA 1 to all the wells in column 2. Add BSA 2 to all the wells in column 3. Add BSA 3 to all the wells in column 4. Add BSA 4 to all the wells in column 5. Add BSA 5 to all the wells in column 6.

14. Go to station 4, and get 10 1.5-mL microfuge tubes. Label five tubes T1, T2, T3, T4, and T5.

15. With a clean transfer pipette, add 1 mL of antibody 2 to tube T1. Remove 500 μL, and place it in tube T2. With a clean transfer pipette, add 500 μL of distilled water to tube T2. From tube T2, remove 500 μL, and place it in tube T3. With a clean transfer pipette, add 500 μL of distilled water to tube T3. From tube T3, remove 500 μL, and place it in tube T4. With a clean transfer pipette, add 500 μL of distilled water to tube T4. From tube T4, remove 500 μL, and place it in tube T5. With a clean transfer pipette, add 500 μL of distilled water to tube T5.

16. Add T1 to all wells in column 8. Add T2 to all wells in column 9. Add T3 to all wells in column 10. Add T4 to all wells in column 11. Add T5 to all wells in column 12.

17. Get a new piece of parafilm, and write your names on it. Place it on the plate. Put your plate in the incubator for 30 minutes. Then place your plate in the refrigerator overnight.

18. Wash your hands thoroughly before leaving the laboratory.

Day 3

19. Remove your plate from the refrigerator, and take it to washing station 7. Remove the parafilm, and spray PBS-Tween across the plate; recover it with the same parafilm. Shake the plate gently back and forth, but be careful not to spill the contents of the plate.

20. Remove the parafilm, and empty the plate into the sink. Spray PBS across the plate, and recover it with the parafilm. Gently shake the plate back and forth, but be careful not to spill the contents of the plate. Remove the parafilm, and empty the plate into the sink.

21. Go to station 5 and with a clean 1-mL transfer pipette, add 1 drop of antibody 3 to each well. Cover the plate with a new piece of parafilm, and place the plate in the incubator for 30 minutes.

22. Take the plate to washing station 7, and remove the parafilm. Spray PBS-Tween across the plate, and recover it with the parafilm. Gently shake the plate back and forth, but be careful not to spill the contents.

23. Remove the parafilm, and empty the plate into the sink. Spray PBS across the plate, and recover it with the parafilm. Gently shake the plate back and forth, but be careful not to spill the contents. Remove the parafilm, and empty the plate into the sink.

24. Go to station 6 and with a clean 1-mL transfer pipette, add 1 drop of substrate (ABTS) to each well.

25. In your logbook or on a 3” × 5” card, use a plus sign (+) to record which wells have a color change. Put three plus signs for the strongly changed blocks, two plus signs for the moderately changed blocks, and one plus sign for the lightest blocks.
26. Wash your hands thoroughly before leaving the laboratory.

Analysis
1. Antigen 1 is bovine serum albumin (BSA)—a protein from cattle blood. Antigen 2 is bovine serum transferrin, another protein from cattle blood. What effect would these proteins have if they were injected into a different animal?
2. If the starting dilution of tube BSA 1 is 1:1,000, what are the final dilutions for tubes BSA 2, 3, 4, and 5? If the starting dilution of tube T1 is 1:1,000, are the end dilutions of tubes T2, T3, T4, and T5 the same as for the BSA tubes? Explain your answer.
3. Rabbit antibody 1 is from a rabbit that was previously injected with antigen from cattle (BSA). What should the rabbit serum contain as a result of that injection?
4. Rabbit antibody 2 is from a rabbit that was injected previously with antigen from cattle (transferrin). What should the rabbit serum contain as a result of that injection? How are these antibodies different?
5. Predict the ELISA results by completing the following hypotheses:
   a. If rabbit antiserum BSA is added to wells containing BSA, then . . .
   b. If rabbit antiserum BSA is added to wells containing transferrin, then . . .
   c. If rabbit antiserum BSA is added to wells containing nothing, then . . .
   d. If rabbit antiserum transferrin is added to wells containing BSA, then . . .
   e. If rabbit antiserum transferrin is added to wells containing transferrin, then . . .
   f. If rabbit antiserum transferrin is added to wells containing nothing, then . . .
6. The secondary antibody is goat antirabbit with a conjugate HRP (horseradish peroxidase). How is this antibody made?
7. Why is antibody added to the wells?
8. Based on your results, which well demonstrated an antigen-antibody reaction? Did these results confirm your hypotheses in question 5?
9. What is the highest dilution that gave a positive result? How does the serial dilution enable you to determine how much antibody is present?
10. Identify the controls used in this investigation, and explain the specific purpose of each.
11. Based on the steps in this investigation, describe how ELISA can be used to detect antibodies to HIV.
12. Does a positive ELISA test for the antibodies against HIV indicate that the individual has AIDS? Explain your answer.
13. ELISA also is the basis for the pregnancy-test kits that can be purchased at a pharmacy. How might ELISA work in a pregnancy test? (Hint: What substances might be present in the blood or urine of a woman only during pregnancy?)

Investigation 23B ☐ Antibody Diversity

We live in a microbe-filled world. There are numerous pathogens, or antigens, that have the potential to make us sick or even kill us. As you have read in Chapter 23, the immune system generally keeps these infectious agents in check through an organized set of responses.

One of the mechanisms the immune system uses to control infection and disease is the production of antibody proteins in response to the presence of antigens. Immunologists estimate that humans have the potential to produce approximately $10^6$ specific antibody proteins.

As Sections 9.1 and 9.2 explain, proteins are gene products. Therefore, the human genome (the complete complement of an organism’s genes) must contain enough genetic information to code for the production of $10^6$ antibody proteins in addition to all the other information an organism needs. But consider that there are approximately $3 \times 10^9$ base pairs in the human genome. Assuming that each gene is about $3 \times 10^4$ base pairs long on average, there are about $10^6$ genes in the human genome. Clearly, this number is not large enough to account for one gene devoted to the production of only one antibody. This investigation will help you explore the genetic rearrangement mechanism, discovered by Tonegawa and others, by which the human genome encodes information for such an incredible amount of
antibody diversity (see Biological Challenges: Susumu Tonegawa in Section 23.9).

**Materials (per team of 2)**
pop-it beads: 1 red, 1 pink, 1 orange, 1 yellow, 1 blue, 1 green, 1 white, 1 black, 13 lilac

**PART A**

**Procedure**
1. Assume that your body has been invaded by 12 different pathogens and must produce a different antibody light chain against each one. The complete gene for a light chain includes three classes of smaller genes: variable (V), joining (J), and constant (C).
2. Working as a team, use your pop-it beads to construct a DNA sequence that will code for the production of an antibody light chain. Each colored pop-it bead represents a gene segment. Snap the beads together in the sequence indicated in Figure 23B.1, using the following key: R = red, P = pink, O = orange, Y = yellow, Bl = blue, G = green, W = white, B = black, L = lilac. The gene segments are labeled J1–J3 for the joining region genes. There is only one C gene. The lilac pop-it beads represent introns. If necessary, review Section 9.4 on exons and introns.
3. Each complete light-chain gene is coded for by one V gene, one J gene, and the C gene. Construct a gene sequence that codes for a light chain by combining V1 (red), J1 (blue), and C (black).
4. Return V1, J1, and C to the original DNA strand. Now combine V2, J2, and C.

**Analysis**
1. How do the chains constructed in steps 3 and 4 differ?
2. Is there enough genetic information present in these two sequences (V1–J1–C and V2–J2–C) to produce ten different light chains? How many chains are possible?
3. How many light chains could you produce using V1 and each of the J genes?

**PART B**

**Procedure**
5. Assume that any V gene can combine with any J gene (and C) to produce a complete gene coding for an antibody light chain. Use the pop-it beads to construct complete genes that will code for ten distinct light chains. Record the sequences. You have already completed two:
   - RB l B V1 J1 C
   - PG B V2 J2 C

**Analysis**
1. How many complete light chains could you produce using the genetic information in the original strand?
2. If there were approximately 250 V genes, 5 J genes, and 1 C gene for the antibody light chain of the kappa type, how many different light chains could this information code for?
3. Using the symbols V, J, and C, write an equation for the number of light chains that can be produced by any given segment of DNA.
4. A gene may be defined as a sequence of nucleotides that codes for a functional product, such as tRNA, an enzyme, a structural protein, or a pigment. Does the model for production of

![FIGURE 23B.1](image-url)

**Pop-it-bead sequence.**
antibodies support that definition? Explain your answer.

5. The classical definition of a gene is “a hereditary unit that occupies a specific position (locus) within the genome or chromosome.”* Does the model for production of antibodies support that definition? Why or why not?


Investigations for Chapter 24
Ecosystem Structure and Function

Investigation 24A  Producers in an Ecosystem

In this investigation, you will study the producers in a small piece of the biosphere. It need not be a very large piece—only large enough to contain several types of producers that show interrelationships. Different schools have different opportunities for outdoor studies. Therefore, the study procedures used by you and your class will have to be designed to fit the piece of biosphere for your school. Read through the lab carefully to get an overview of what you might do.

Selecting a Study Area. A forest is a complex environment, providing opportunities to collect abundant data, but it is difficult to picture as a whole. A prairie is almost as complex but is somewhat easier to study. Cultivated areas, such as cornfields and pastures, are relatively simple to study. They are as important as forests and prairies since they cover a large portion of the land in the world.

Suitable areas also can be found in cities. Many schools have lawns with trees and shrubs. Here there are many fewer types of organisms than outside the city, but you can be more thorough in your study. You also can study vacant lots and spaces between buildings. Even such small places as cracks in the pavement, gutters, and the areas around trees often contain a surprising number of organisms.

Organizing the Work. Teamwork will be necessary for this study. Plan the work carefully, divide responsibilities, and take extensive notes.

Organizing the Data. It is convenient to divide producers into three groups for study: trees, shrubs, and saplings; herbaceous plants; and seedlings. In addition, organic litter, such as fallen leaves, or ground cover, such as lichen and mosses, may be present. Trees may be classified as either deciduous or coniferous. Canopy trees are at the top of the forest and receive direct sunlight. The trees below this top layer form the subcanopy. Shrubs are low woody plants between 0.5 and 3 m tall. A sapling is a young tree that is 2.5 to 4 cm in diameter and about 1.3 m high. Herbaceous plants are nonwoody plants that die down to the ground in the autumn. These include grasses, grains, and small flowering plants. A seedling is a very young tree with a stem less than 1 cm in diameter.

Materials (per team of 8)
The materials required depend on the methods used, but the following list is minimal for the field.

- strong twine, over 100 m long
- 4 stakes
- compass, magnetic
- metric ruler
- graph paper
- 2 metersticks
- hoop with area of 1 m²
- plastic bags
- rubber bands or twist ties
- can, 5-cm diameter
- 8 pairs of plastic gloves

Procedure
1. Choose your site.
   - In a forest, study areas should be approximately 10 m² and broken down into smaller areas, as shown in Figure 24A.1a.
   - In unforested areas, study areas should be 2 to 4 m on a side without internal divisions.
   - In vacant city lots, cultivated fields, and pastures, use smaller areas, about 1 m². (An area the size of a hula hoop works well.)

2. Each team should develop a hypothesis concerning the type of ecosystem of its site and what vegetation it expects to find.

3. Within the chosen site, measure a study area of appropriate size. Drive stakes into the ground at
the corner points, and connect the stakes with twine. The study area should be as square as possible. You may want to subdivide the area to make counting organisms easier.

4. Stand back and look at your study area. Walk around the outer boundary. Use symbols like the ones shown in Figure 24A.1b to sketch a profile of the ecosystem as you see it.

5. Indicate with numbers the various plant types that are recognizably different. For example, in Figure 24A.1b, the first plant observed was a deciduous (D) canopy tree (1D). Three of these were in the study area. The second species observed (labeled 2) was a conifer (C) subcanopy species. Species 3 also was a subcanopy conifer species, but different from species 2.

6. Once you have made a profile of your study area, you are ready for a closer look at its vegetation. So that the class as a whole gains a detailed picture of the vegetation, different teams can gather data on trees, shrubs, and saplings; herbaceous plants and seedlings; and litter.

7. Abundance refers to the number of individuals of one kind within a given area. Count the number of each type of producer in your study area. For example, count the number of each type of tree in the forest canopy and subcanopy layers in the main study area. Count shrubs and saplings in one of the smaller subdivided areas. Count the herbaceous plants and seedlings in an even smaller area. Special problems may arise. In a lawn, for example, there is no need to count blades of grass. However, a count of the weeds might be worthwhile, especially if comparisons will be made between well-trodden areas and protected ones. A frame (Figure 24A.1c) may be useful for this work.

8. Make a table to record your counts. Across the top, list the types of plants in your subdivided area. For example, if you are counting shrubs and seedlings, you may list evergreen shrubs, rosebushes, raspberry bushes, and so on. Under these headings, record your count of each type of plant. You may wish to gather a sample or characteristic part of the plant, such as a leaf, and place it in a plastic bag. Do not collect the whole plant, and be sure to wear plastic gloves. Some plants are rare or endangered species. Be sure to record where you collected the sample.

**CAUTION:** Do not collect any plants you cannot identify as harmless.

9. Teams that study organic litter and ground cover should collect samples in plastic bags (wear gloves). Take samples from the ground around trees, shrubs, and small plants in a small
subdivided area. Secure the bag with a rubber band or twist tie, and mark it to indicate where it was gathered. You will study these samples later in the laboratory. At the study site, examine a 15-cm² sample of litter in place. Carefully pick the sample apart. Record the layers and composition of each layer. Also try to determine its physical condition. These observations should include the state of decay, moisture level, odor, amount of shade at the location, and nearness to large plants.

**Analysis**

1. Calculate the number of each type of plant to determine its abundance. If several teams had study areas near one another, average the data for plants of the same type. List all of the plants on the study site in order of their abundance.

2. To determine density, divide the total number of individuals of each type by the area of the study site in square meters.

\[
\text{density} = \frac{\text{total number of each species}}{\text{total sample area (in m}^2\text{)}}
\]

3. List the plants in order of their densities. The teams analyzing the litter and ground cover also should calculate the abundance and density of each species in the samples from the study area.

4. With your classmates, make a summary of the important features of the plants in the ecosystems you studied. Include such factors as profile, abundance, density, and litter. Record this information in your logbook.

5. Ecosystems are usually named for the most obvious or important species of plants. For example, a deciduous forest might be called a beech-maple forest if those trees are dominant. After you have analyzed the data, determine the one or two species that appear to be the most abundant in the area you studied. What would you name this ecosystem? How does the data support or refute your hypothesis concerning the type of vegetation you expected to find?

6. How many layers of vegetation did you find in the entire study area? List the important species in each of these layers.

7. Some of the plants are in a layer because they are limited genetically to that maximum height. Others are young individuals that will some day grow higher. Review your data to determine how many of these young individuals were among the plants in each layer.

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**Investigation 24B  Relationships between a Plant and an Animal**

Plants and animals interact in a variety of ways. By setting up closed systems with an aquatic plant and an aquatic animal, you can study an interaction related to the carbon cycle. Carbon dioxide dissolves in water and forms a weak acid, which lowers the pH. This drop in pH indicates an increase in the concentration of carbon dioxide. Conversely, an increase in pH indicates a decrease in the concentration of carbon dioxide. Thus, you can measure any changes that occur in the system by measuring indirectly the concentration of carbon dioxide using a pH meter or wide-range pH paper.

Before beginning the experiment, read through the procedure and develop hypotheses that predict the changes that will occur in each test tube in the light and in the dark.

**Materials** (per team of 4)

- 4 25-mm × 200-mm test tubes
- glass-marking pencil
- test-tube rack
- aluminum foil
- light source
- pH meter or wide-range pH paper
- forceps
- 200 mL dechlorinated water
- 2 15-cm pieces of elodea
- 2 1- to 1 1/2-cm freshwater snails

**Procedure**

**Day 1**

1. Record all data in your logbook, or use the table your teacher provides.

2. Label the test tubes 1 through 4, and place them in the test-tube rack.
3. Pour dechlorinated water into each test tube to approximately 4 cm from the top. (Tap water becomes dechlorinated by standing for 24 hours—the chlorine escapes into the air.)

4. Add nothing more to test tube 1. To test tube 2, add a snail and a leafy stem of elodea. To test tube 3, add only a snail. To test tube 4, add only a leafy stem of elodea.

5. Determine the initial pH of each tube by using a pH meter or by dipping small strips of pH paper held with forceps into the test tubes and comparing the color change to a standard color chart.

6. When the readings have stabilized, record the current pH readings for test tubes 1 and 2 from the numerical data displayed, or record your readings from the pH paper and color chart.

7. To determine the pH of test tubes 3 and 4, remove the pH probe from test tube 2, rinse with dechlorinated water, and insert into test tube 3. When the readings have stabilized, record the current pH for test tube 3. Then remove the pH probe from test tube 3, rinse, and insert into test tube 4; record the current pH for test tube 4. Rinse the pH probe and replace in test tube 2. Alternatively, measure the initial pH values with pH paper as described.

8. Seal the top of each test tube with a double layer of aluminum foil. Press the foil tightly to the sides of the test tube. Place the test tubes in strong artificial light.

9. Repeat steps 6 and 7 every 10 minutes until the end of the period, taking care to work quickly and replace the foil covers. Record all readings in the data table.

10. Seal the test tubes, and leave in the light overnight.

11. Wash your hands thoroughly before leaving the laboratory.

Day 2

12. Observe the test tubes, and take pH readings as in steps 6 and 7. In the data table, record the pH readings and the condition of the organisms in each test tube.

13. Seal the test tubes, and wrap each one in aluminum foil to keep it dark.

14. Wash your hands thoroughly before leaving the laboratory.

Day 3

15. Repeat steps 12 and 13, but this time remove the aluminum foil and place the test tubes in the light.

16. Wash your hands before leaving the laboratory.

Day 4

17. Observe the test tubes again, record pH data, and stop the experiment.

18. Wash your hands thoroughly before leaving the laboratory.

Analysis

1. Construct graphs from your data, using a different color for each test tube.

2. Use information about photosynthesis, cellular respiration, and the carbon cycle to write a paragraph that explains the data.

3. What was the purpose of test tube 1?

4. Did the pH change in test tube 1? If so, how might you explain this change?

5. What effect would a pH change in test tube 1 have on the data for the other three test tubes?

6. What results might you expect if all the test tubes were kept in total darkness for the duration of the experiment?

7. Do the experimental data support your hypothesis? Explain your answer. If not, try to devise a general hypothesis that is consistent with all of your observations.

8. Suppose a snail were kept by itself in a sealed test tube for a week. Predict what is likely to happen, and explain your prediction.

Investigations for Chapter 25

Change in Ecosystems

Investigation 25A  ♦ Producing in an Aquatic Ecosystem

Photosynthetic organisms such as algae and cyanobacteria are producers essential to an aquatic ecosystem. The numbers and diversity of these
organisms provide a measure of the health of the ecosystem. How clean is the water in a lake, pond, or stream near your school? Technical tests can determine the exact identities and amounts of water pollutants, but even a simple survey of algae and cyanobacteria can be used as an index of water quality. Typically, clean water has a wide variety of algal and cyanobacterial species, with no species being especially dominant. Polluted water has fewer species present and greater numbers of each.

In this investigation, you will learn first to identify key genera of algae and cyanobacteria. Then you will test water samples known to be clean, moderately polluted, and polluted. Finally, you will use what you have learned to analyze a water sample from your area.

Materials
5 microscope slides
5 coverslips
dropping pipette
1-L beaker
plankton net
compound microscope
protist/cyanobacteria key
2-cm² piece of graph paper with 1-mm squares
5 flat toothpicks
Detain™ in a squeeze bottle
water sample with algae and cyanobacteria
water samples—1–3
water sample—from a local source

Procedure

PART A Field-of-View Calculations
1. Place 2-cm² piece of graph paper on a microscope slide, and add a coverslip. Examine the slide with the low power (100×) of your microscope. Align the squares so that one edge of a square just touches the bottom edge of the field of view. Count the number of squares from the bottom to the top of the field (Figure 25A.1a). The number of squares equals the diameter of the field of view in millimeters. Record the results and the magnification.
2. Repeat step 1 with the high power (400×) of your microscope. (Do not use the oil immersion lens.) The field of view will be less than 1 mm in diameter.
3. You will count individual organisms on a slide made from your water samples. To do so, you will move the slide across the microscope stage, the width of one field of view at a time, as illustrated in Figure 25A.1b. The area of the strip of fields equals the diameter of the field of view multiplied by the width of the coverslip (22 mm). Practice moving the slide one field of view at a time, going from one side of the coverslip to the other.

PART B Algae Identification
4. Place a drop of Detain™ (protist slowing agent) on a clean microscope slide. Using a dropping pipette, add one drop (0.1 mL) of the sample with algae and cyanobacteria, and use a toothpick to mix the two drops. Carefully add a coverslip.
5. Practice locating and identifying different organisms using the protist/cyanobacteria key.
Locate organisms under low power, and then use high power to make a positive identification.

6. Move the slide one field of view at a time, going from one side of the coverslip to the other. In each strip, count the number of individuals for each genus you can identify. Ask your teacher for help if you cannot identify some of the organisms.

7. Wash your hands thoroughly before leaving the laboratory.

PART C Identification of Water Samples

8. The various genera of algae display differing sensitivities to pollution. This means that the microbial diversity in a water sample is a reflection of the water's quality. You will examine water samples that are clean, intermediate, and polluted and determine which genera are associated with clean water and which with polluted water. This information will assist you in analyzing the quality of water obtained from a local aquatic ecosystem.

9. For each of the three water samples, you will count the number of organisms in each genus in three strips of fields of view. You will record in your logbook the genus and number of each type of algae observed in the water sample.

10. Put one drop of Detain™ and one drop (0.1 mL) of sample 1 on a slide. Mix with a clean toothpick. Add a coverslip. In your logbook, note the sample number.

11. Count and record in your logbook the number of individuals from each genus present in a strip of fields of view. Count units. (Colonies or filaments are counted as one unit.) Large filaments and colonies only partly lying in the strip should be counted as fractions. For example, if a filament of Stigeoclonium is only half in your field of view, record 0.5 instead of 1.

12. Repeat steps 10 and 11 two times.

13. Repeat steps 10–12 using samples 2 and 3.

14. Use your data and the fact that microbial diversity increases with water quality to identify the water samples as clean, intermediate, or polluted. Before proceeding to Part D, check with your teacher to determine if you correctly identified the quality of the three water samples.

PART D Investigation of Local Water Source

15. Using water from a local lake, pond, or stream, filter 1 L through a plankton net to trap the organisms. Rinse the organisms from the net into the 1-L beaker, and add clean water to a total of 100 mL. Repeat steps 10 and 11 to determine the relative water quality of your sample.

16. Wash your hands thoroughly before leaving the laboratory.

Analysis

1. Which algal genera can tolerate the highest levels of pollution?
2. Which algal genera are the most sensitive to pollution?
3. If your local water source has an intermediate or high pollution level, what might be the source of the pollution?
4. How can algae and cyanobacteria be helpful in polluted water?
5. How can algae and cyanobacteria be harmful in polluted water?

Investigation 25B Ecosystem Diversity within a Biome

The biosphere includes many different biomes, such as deserts, grasslands, and tropical rain forests. The purpose of this investigation is to study species diversity within a single type of biome. Species diversity is an expression of the community structure of an ecosystem. A biome with high species diversity will contain many equally or nearly equally abundant species. Biomes with low species diversity are characterized by few different species, even if they are plentiful.

The distribution of species within a biome varies depending on which area of the biome you examine. For example, grasses are more common in some areas, and shrubs or trees are common in others. The
distribution of animals and other species around tall grasses will be different from that among the trees or scrub. The biome you study will depend on your location. First you will observe the plants and animals in your biome. Then you will choose a question that can be addressed by sampling the species diversity from different locations within the biome.

**Materials** (per team of 6)
- 6 pairs of safety goggles
- 2 metal coat hangers or pieces of stiff wire
- 4 wooden stakes
- meterstick or metric tape
- trowel or small-bladed garden shovel
- white-enameled pan or large sheet of white paper
- 6 pairs of forceps
- wire screen of approximately 1-mm mesh curved into a bowl shape
- 2 30-cm pieces of PVC pipe, 1/2–1 inch diameter
- 50-cm wooden dowel that fits inside the pipe
- corks
- field guides

**Procedure**

**PART A  Field-Study Preparations**

1. As a class, select an area to study, such as a local park, forest, or grassland. Ideally, choose an area that is convenient to get to but is seldom disturbed by people. The more natural your biome, the greater the species diversity will be. Cultivated areas will exhibit fewer species but are easier to study. If studying natural biomes is inconvenient, suitable areas can be found in urban locations. Many schools have lawns and shrubs. You also can study vacant lots between buildings. These areas will contain fewer species but should allow you to be more thorough in your study.

2. Make and record observations of the plants and animals in your study area. Notice how the various plant species are distributed within the study area. Consider the types of relationships that exist between the plants and animals within the biome. Choose a question to study that relates to the distribution of species within the biome. For example, you might compare the distribution of species in different locations—under trees versus open areas or on top of a hill versus the bottom. Determine what type of data you will need to collect to answer the question. With your teacher, decide whether the entire class will study the same question or whether different teams will study different questions.

   **To study plants,** use quadrats—square or rectangular samples of the study areas. For trees, a quadrat of 10 m × 10 m is common; for shrubs, 4 m × 4 m is recommended. Where there are few trees or woody species, use quadrats made of stiff wire that are 0.5 m on each side. Note the size of the area inside the quadrat, and use this same size throughout your study.

   **To study organisms in the soil,** take soil cores of equal length from the appropriate locations in your study area. Push a length of pipe into soft soil approximately 25 cm deep. Gently twist the pipe in the ground, and pull it up with the soil core inside. Plug up the ends of the pipe with corks, and bring the pipes back to the lab for study. In the lab, carefully push the soil core out of the pipe with a wooden dowel. Count the numbers of earthworms, pill bugs, and other larger organisms you see, noting their depth in the core.

3. Select a team leader to assign tasks. For example, different team members can be responsible for collecting data on plants, insects, animals, and organisms in the soil. Don’t forget to include the physical aspects of the area, such as temperature, light intensity, and soil moisture. Prepare forms on which you can conveniently collect your data. Once you return to the classroom, paste the data forms into your logbook.

**CAUTION:** Never enter an area unless you have permission from the property owner. Be careful and aware that some plants and animals can be poisonous, harmful, or rare and endangered—make sure you know what you are touching.
4. Decide on a method for recording species-diversity data. Since it is not practical to identify the species of each organism observed and counted, you may want to use general terms, such as grasses, beetles, and spiders. When turning over logs and rocks to look for animals, be sure to return the sheltering objects to their original position once you have your data. Note the types, numbers, and activities of any animals you find.

PART B Conducting the Field Study

5. If your team is studying a small area, toss the quadrat on the ground at the first selected site. If you are studying a larger area, use stakes and a metric tape to define a quadrat.

6. Record all the different types of plants within your quadrat. If you don't know the names of the plants, describe them well enough so that you will be able to identify them in your other quadrats.

7. Estimate the cover of each plant species. This is the percentage of ground within the quadrat covered by the leaves and stems of that species. Suppose there are three different types of plants, as in Figure 25B.1. Estimate to the nearest 10% the amount of cover for each type of plant as well as the amount of bare area. The greater the amount of cover for a plant species, the greater its importance within the plant community.

8. To increase accuracy, have at least two members of your team estimate the cover of each plant species. Record both estimates and the average value in your logbook. Note that if some taller plants overshadow smaller plants, your estimated cover may exceed 100%.

9. To properly address your question, be sure to analyze at least three quadrats in appropriate locations of your study area. Note any trends in species diversity that relate to the question posed by your team.

10. Examine a sample of the surface soil and/or organic litter. Insert a trowel or small garden shovel into the ground to a depth of approximately 10 cm, and remove a cubic sample of approximately 10 cm × 10 cm × 10 cm. Using the wire screen that has been shaped into a bowl, sift the contents onto a white-enameled pan or a large sheet of white paper. Pick through the remaining litter with forceps. Organisms present probably will include a variety of insects (especially beetles), sow or pill bugs, millipedes and centipedes, spiders or other arachnids, worms, and fungi.

SAFETY Be sure to wear safety goggles when working with the soil samples.

11. Organize your team’s data in your logbook, and discuss whether it answers the question posed by the team at the beginning of the investigation.

Analysis

1. How did the species diversity change as a function of location within your study area?
2. What can you conclude from your data as to the question posed by your team?
3. What producers are found in your study area?
4. Which producer is dominant in your area? What effect does this species have on the rest of the study area?
5. Are the producers evenly divided in your study area? Can you explain the pattern of growth in terms of the question posed by your team?

6. Are there layers of producers? If so, what relationships can you find among the layers?

7. Does your study area produce all its own food, or is food carried in from beyond its boundaries? What evidence do you have for your answer?

8. What consumers are found in your study area?

9. Which consumers are herbivores, and which are carnivores? What evidence supports your answer?

10. What relationship can you find between the numbers of a particular herbivore and the numbers of a carnivore that eats it?

11. What is the evidence that one type of organism affects another in ways other than those involving food relationships?

12. What biome type best characterizes the area you studied? Explain your answer.