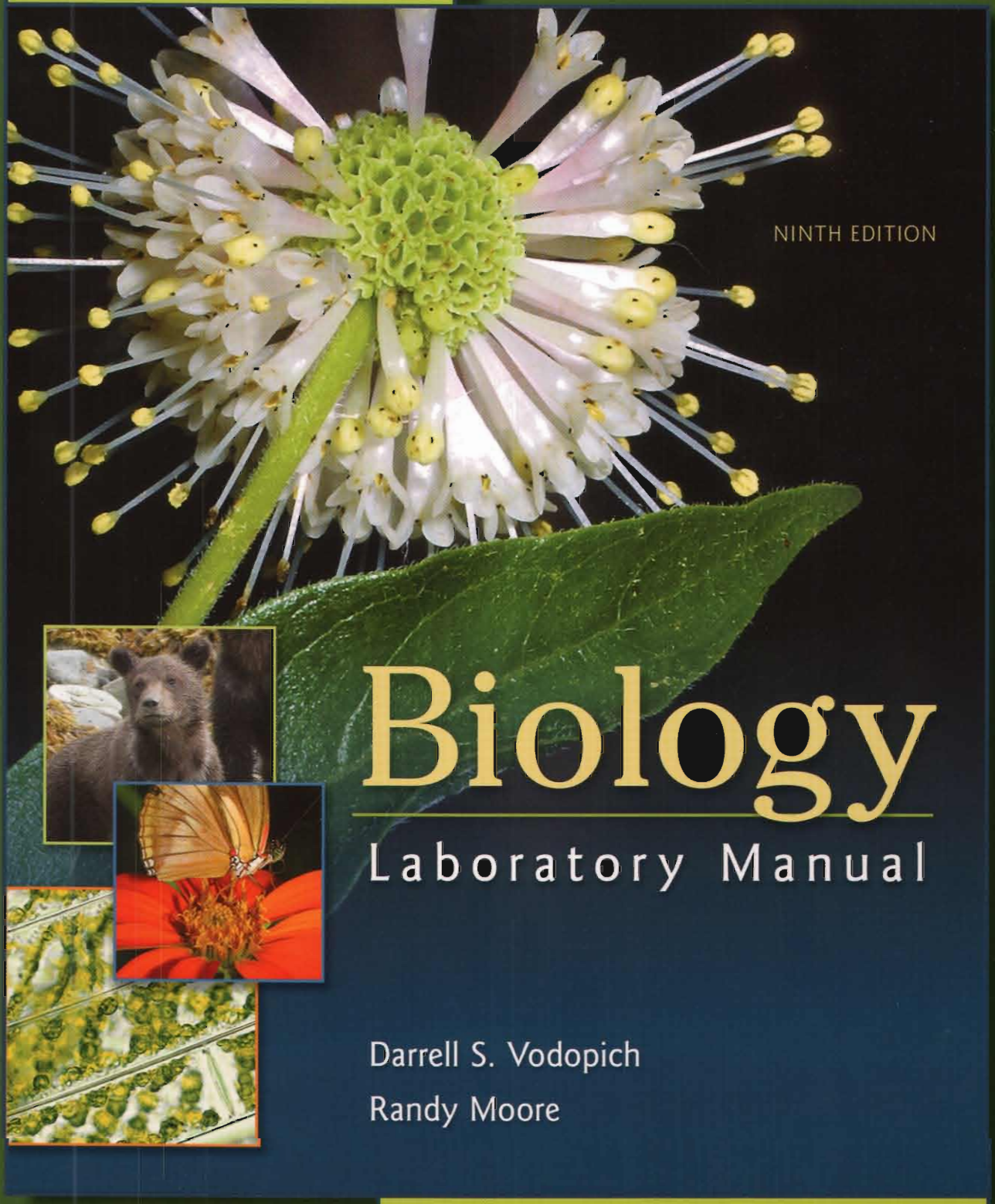
Selected Exercises from



NINTH EDITION

Biology

Laboratory Manual

Darrell S. Vodopich
Randy Moore

UNIVERSITY OF NORTH TEXAS

TABLE 26.2 Characteristics of the Six Kingdoms and Three Domains

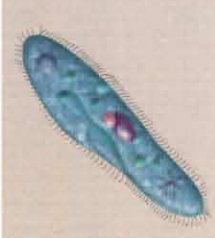
	 Archaea and Bacteria	 Protista	 Plantae	 Fungi	 Animalia
Cell Type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Nuclear Envelope	Absent	Present	Present	Present	Present
Transcription and Translation	Occur in same compartment	Occur in different compartments	Occur in different compartments	Occur in different compartments	Occur in different compartments
Histone Proteins Associated with DNA	Absent	Present	Present	Present	Present
Cytoskeleton	Absent	Present	Present	Present	Present
Mitochondria	Absent	Present (or absent)	Present	Present	Present
Chloroplasts	None (photosynthetic membranes in some types)	Present (some forms)	Present	Absent	Absent
Cell Wall	Noncellulose (polysaccharide plus amino acids)	Present in some forms, various types	Cellulose and other polysaccharides	Chitin and other noncellulose polysaccharides	Absent
Means of Genetic Recombination, If Present	Conjugation, transduction, transformation	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis
Mode of Nutrition	Autotrophic (chemosynthetic, photosynthetic) or heterotrophic	Photosynthetic or heterotrophic, or combination of both	Photosynthetic, chlorophylls *a* and *b*	Absorption	Ingestion
Motility	Bacterial flagella, gliding or nonmotile	9 + 2 cilia and flagella; amoeboid, contractile fibrils	None in most forms; 9 + 2 cilia and flagella in gametes of some forms	Both motile and nonmotile	9 + 2 cilia and flagella, contractile fibrils
Multicellularity	Absent	Absent in most forms	Present in all forms	Present in most forms	Present in all forms
Nervous System	None	Primitive mechanisms for conducting stimuli in some forms	A few have primitive mechanisms for conducting stimuli	None	Present (except sponges), often complex

TABLE 32.2 The Major Animal Phyla

Phylum	Typical Examples		Key Characteristics	Approximate Number of Named Species
Arthropoda (arthropods)	Beetles, other insects, crabs, spiders, scorpions, centipedes, millipedes		Most successful of all animal phyla; chitinous exoskeleton covering segmented bodies with paired, jointed appendages; many insect groups have wings.	1,000,000
Mollusca (mollusks)	Snails, oysters, octopuses, sea slugs		Soft-bodied animals whose bodies are divided into three parts: head-foot, visceral mass, and mantle; many have shells; almost all possess a unique rasping tongue, called a radula; 35,000 species are terrestrial.	110,000
Chordata (chordates)	Mammals, fish, reptiles, birds, amphibians		Segmented coelomates with a notochord; possess a dorsal nerve cord, pharyngeal slits, and a postanal tail at some stage of life; in vertebrates, the notochord is replaced during development by the spinal column; 20,000 species are terrestrial.	56,000
Platyhelminthes (flatworms)	Planarians, tapeworms, liver flukes		Compact, unsegmented, bilaterally symmetrical worms; no body cavity; digestive cavity has only one opening. Many species are parasites and can lose the digestive cavity.	20,000
Nematoda (roundworms)	*Ascaris*, pinworms, hookworms, *Filaria*		Pseudocoelomate or acoelomate, unsegmented, bilaterally symmetrical worms; complete tubular digestive tract with mouth and anus; live in great numbers in soil and aquatic sediments; some are important animal parasites.	25,000
Annelida (segmented worms)	Earthworms, polychaetes, tube worms, leeches		Coelomate, serially segmented, bilaterally symmetrical worms; complete digestive tract; most have bristles called setae on each segment that anchor them during crawling.	16,000
Cnidaria (cnidarians)	Jellyfish, *Hydra*, corals, sea anemones		Soft, gelatinous, radially symmetrical bodies whose digestive cavity has a single opening; possess tentacles armed with stinging cells called cnidocytes that shoot sharp harpoons called nematocysts; most species are marine.	10,000
Echinodermata (kchinoderms)	Sea stars, sea urchins, sand dollars, sea cucumbers		Deuterostomes with pentaradial symmetry in the adults; five-part body plan and unique water-vascular system with tube feet; able to regenerate lost body parts; marine. Endoskeleton of calcium plates.	7,000
Porifera (sponges)	Barrel sponges, boring sponges, basket sponges, vase sponges		Asymmetrical bodies without distinct tissues or organs; saclike body consists of two layers breached by many pores; internal cavity lined with food-filtering cells called choanocytes; most are marine (150 species live in fresh water).	5,150

Selected Exercises from

Biology
Laboratory Manual

UNIVERSITY OF NORTH TEXAS

Darrell S. Vodopich
Baylor University

Randy Moore
University of Minnesota

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Selected Exercises from
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Contents

Preface

We designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, safe, easy to perform, and especially appropriate for large classes. Few experiments require more than one class meeting to complete the procedure. Each exercise includes many photographs, traditional topics, and experiments that help students learn about life. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you read about these areas, you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined—your future success as a scientist and an informed citizen depends on your ability to seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions—their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTOR

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple, safe, and straightforward experiments are most effective if you interpret the work in depth. Most experiments can be done easily by a student in 2 to 3 hours. Terminology, structures, photographs, and concepts are limited to those the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance and is not an autotutorial system. We've tried to guide students from observations to conclusions, to help students make their own discoveries, and to make the transition from observation to biological principles. But discussions and interactions between student and instructor are major components of a successful laboratory experience. Be sure to examine the "Questions for Further Thought and Study" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad applications to their world.

THE NINTH EDITION

All exercises in this edition were critiqued by a review panel of current users, and their suggested revisions were carefully considered and incorporated.

KEY UPDATES

- NEW INVESTIGATIVE APPROACH—Every lab exercise now includes an "Investigation" in which students can use the ideas and skills they learned in the lab to address questions they pose. These exercises are meant not only to help students use what they've learned, but also for them to use their creativity to do biology. These exercises are described in more detail on page xii.
- The number of tables and figures has been extended with more than 70 new additions, revisions, and replacements.
- Additional Boxed Inserts to highlight applications of procedures.
- Increased references to the applications and relevance of specific procedures and content, especially applications to health science.
- Over 50 new or revised figures and photographs.
- An increased emphasis on safety, including more frequent and clearly defined icons, references, and warnings of potential safety problems.

Specific changes include:

Exercise 1: Scientific Method: The Process of Science

- New Scientific Theories
- New Investigation added: How temperature affects the production of CO_2 by yeast
- Added three new questions at the end of the exercise

Exercise 2: Measurements in Biology: The Metric System and Data Analysis

- New section: Significant Figures
- Added new box: Rounding Numbers
- New Investigation added: Investigation on Variation in the areas and shapes of leaves

Exercise 3: The Microscope: Basic Skills of Light Microscopy

- New section: Caring for Your Microscope
- New Investigation added: The shapes, surface areas and volumes of red blood cells

Exercise 4: The Cell: Structure and Function

- New Investigation added: The response of single-celled organisms to environmental stimuli

Exercise 5: Solutions, Acids and Bases: The pH Scale

- New investigation added: The properties of Phillips Milk of Magnesia, a popular antacid.
- Added a new question at the end of the Exercise

Exercise 6: Biologically Important Molecules: Carbohydrates, Proteins, Lipids, and Nucleic Acids

- New Investigation added: Variation in starch storage by roots versus leaves
- Added a new question at the end of the Exercise

Exercise 7: Separating Organic Compounds: Column Chromatography, Paper Chromatography, and Gel Electrophoresis

- Added new material to section on Interpreting a DNA-Sequencing Gel
- New Investigation added: The importance of the length of the column in column chromatography
- Added two new questions at the end of the Exercise

Exercise 8: Spectrophotometry: Identifying Solutes and Determining Their Concentration

- New Investigation added: The impact of contaminants on spectrophotometry

Exercise 9: Diffusion and Osmosis: Passive Movement of Molecules in Biological Systems

- New Investigation added: Determining the concentrations of solutes in plant tissue

Exercise 10: Cellular membranes: Effects of Physical and Chemical Stress

- New Investigation added: Effects of environmental stimuli on cellular membranes

Exercise 11: Enzymes: Factors Affecting the Rate of Activity

- New Investigation added: Factors affecting the rate of enzymatic activity
- Added three new questions at the end of the Exercise

Exercise 12: Respiration Aerobic and Anaerobic Oxidation of Organic Molecules

- New Investigation added: The effect of environmental stimuli on cellular respiration
- Added two new questions at the end of the Exercise

Exercise 13: Photosynthesis: Pigment Separation, Starch Production, and CO2 Uptake

- New investigation added: Relative uptake and production of CO2 during photosynthesis

Exercise 14: Mitosis: Replication of Eukaryotic Cells

- New Investigation added: The time elapsed during the various stages of mitosis

Exercise 15: Meiosis: Reduction Division and Gametogenesis

- New Investigation added: Variation in the morphology of vertebrate sperm cells

Exercise 16: Molecular Biology and Biotechnology: DNA Isolation and Genetic Transformation

- New Investigation added: Antibiotic resistance by transformed bacteria
- Added two new questions at the end of the Exercise

Exercise 17: Genetics: The Principles of Mendel

- Updated whole Exercise
- New Investigation added: The frequency of homozygous recessive traits in humans

Exercise 18: Evolution: Natural Selection and Morphological Change in Green Algae

- New Investigation added: The effect of selection against heterozygotes

Exercise 19: Human Evolution: Skull Examination

- New Investigation added: Migration of common ancestors during the evolution of humans

Exercise 20: Ecology: Diversity and Interaction in Plant Communities

- New Investigation added: Production of allelopathic chemicals by different plant organs

Exercise 21: Community Succession

- New Investigation added: Community succession and the "spoilage" of beverages

Exercise 22: Population Growth: Limitations of the Environment

- New Investigation added: The response of population growth to environmental conditions

Exercise 23: Pollution: The Effects of Chemical, Thermal, and Acid Pollution

- New Investigation added: Using the Allium to detect variation in water quality

Exercise 24: Survey of Prokaryotes: Kingdoms Archaebacteria and Bacteria

- New Investigation added: Bacterial sensitivity to inhibitors

Exercise 25: Survey of Protists: The Algae

- Added new table on Eukaryotic super groups
- New Investigation added: The response to algae to changing environmental stimuli
- Updated whole Exercise

Exercise 26: Survey of Protists: Protozoa and Slime Molds

- New investigation added: The sensitivity of protozoa to nutrients

Exercise 27: Survey of the Kingdom Fungi: Molds, Sac Fungi, Mushrooms, and Lichens

- New Investigation added: The antimicrobial properties of common fungi
- Added two new questions at the end of the Exercise

Exercise 28: Survey of the Plant Kingdom: Liverworts, Mosses, and Hornworts of Phyla Hepaticophyta, Bryophyta, and Anthocerophyta

- New Investigation added: The roles of bryophytes in the environment
- Added a new question at the end of the Exercise

Exercise 29: Survey of the Plant Kingdom: Seedless Vascular Plants of Phyla Pterophyta and Lycophyta

- New investigation added: The "resurrection" of a "resurrection plant".

Exercise 30: Survey of the Plant Kingdom: Gymnosperms of Phyla Cycadophyta, Ginkgophyta, Coniferophyta, and Gnetophyta

- New Investigation added: Release of pollen from pine cones
- Added two new questions at the end of the Exercise

Exercise 31: Survey of the Plant Kingdom: Angiosperms

- New Investigation added: Grocery store botany
- Added three new questions at the end of the Exercise

Exercise 32: Plant Anatomy: Vegetative Structure of Vascular Plants

- New Investigation added: How plants sense and respond to light and gravity
- Added two new questions at the end of the Exercise

Exercise 33: Plant Physiology: Transpiration

- New investigation added: Transpiration rates in different species of plants

Exercise 34: Plant Physiology: Tropisms, Nutrition, and Growth Regulators

- New investigation added: Influence of environmental stimuli on seed germination

Exercise 35: Bioassay: Measuring Physiologically Active Substances

- New investigation added: Influence of plant growth regulators on plant tissues and organs

Exercise 36: Survey of the Animal Kingdom: Phyla Porifera and Cnidaria

- New Investigation added: Prey detection by hydra
- Added a new question at the end of the Exercise

Exercise 37: Survey of the Animal Kingdom: Phyla Platyhelminthes and Nematoda

- New investigation added: Detection of macromolecules by planaria
- Added a new question at the end of the Exercise

Exercise 38: Survey of the Animal Kingdom: Phyla Mollusca and Annelida

- New Investigation added: The use of leeches in medicine

Exercise 39: Survey of the Animal Kingdom: Phylum Arthropoda

- New Investigation added: Variation in crustacean appendages
- Added two new questions at the end of the Exercise

Exercise 40: Survey of the Animal Kingdom: Phyla Echinodermata, Hemichordata, and Chordata

- New investigation added: Adaptations of vertebrate skeletons
- Added two new questions at the end of the Exercise

Exercise 41: Vertebrate Animal Tissues: Epithelial, Connective, Muscular, and Nervous Tissues

- New investigation added: Use of strains to enhance visibility of cellular structures

Exercise 42: Human Biology: The Human Skeletal System

- New investigation added: Assess skeletal morphology for functions of protection and strength
- Added two new boxes on Spinal Curvatures and Osteoporosis
- Added new Procedure 42.3 on Vocabulary of skeletal movement

Exercise 43: Human Biology: Muscles and Muscle Contractions

- New Investigation added: Determining minimum recovery time from muscle fatigue

Exercise 44: Human Biology: Breathing

- New investigation added: The relative contributions of diaphragm and intercoastal muscles to breathing

Exercise 45: Human Biology: Circulation and Blood Pressure

- New Investigation added: Variation in pulse and sensitivity to minor movement

Exercise 46: Human Biology: Sensory Perception

- New investigation added: The nervous system's accommodation to stimuli
- Added a new Procedure on Binocular vision

Exercise 47: Vertebrate Anatomy: External Features and Skeletal System of the Rat

- New investigation added: Skeletal modification for bipedal locomotion
- Added a question at the end of the Exercise

Exercise 48: Vertebrate Anatomy: Muscles and Internal Organs of the Rat

- New investigation added: Muscular system modification for bipedal locomotion
- Added new Procedure on Location and function of internal organs

Exercise 49: Vertebrate Anatomy: Urogenital and Circulatory Systems of the Rat

- New Investigation added: Vertebrate heart mass versus total body mass

Exercise 50: Embryology: Comparative Morphologies and Strategies of Development

- New investigation added: Permeability of egg shells

Exercise 51: Animal Behavior: Taxis, Kinesis, and Agonistic Behavior

- New Investigation added: Variation in flight initiation distance

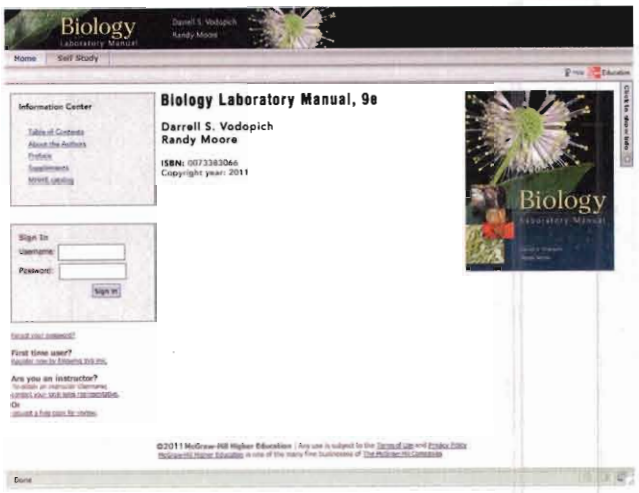
ONLINE LEARNING

Students can enhance their understanding of the concepts with the rich study materials available with McGraw-Hill Connect™ Biology. Connect Biology is an interactive learning platform that provides auto-graded study materials, animations, pre-lab assessments and more. Instructors can access quality instructional resources including images for presentation, lecture capture, and powerful reporting—all in an easy-to-use interface. Learn more at **www.mcgrawhillconnect.com.**

Also new to this edition is the open access website with self-study quizzes and printable pre-lab worksheets available at **www.mhhe.com/vodopich9e**

This website includes:

- Practice questions that allow students to familiarize themselves with the core concepts, offer them an alternative study path, and answer the frequent question "What will the tests be like?"
- Worksheets for each exercise that assist students' focus on the important concepts, can be completed and submitted by each student before leaving lab or as homework.
- Excellent animations that enrich the students understanding of key biological concepts.
- A repository for a growing collection of videos that demonstrate the "how to" parts of standard as well as new procedures.



Reviewers

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INVESTIGATIONS IN BIOLOGY

The best way to learn biology is to *do* biology. There are many ways to do this. For example, throughout this manual you'll find *directed labs* that use traditional skills and activities (e.g., how to use a microscope) to immerse you in the process of biology. Similarly, *thematic labs* will involve you in discovering the themes of biology (e.g., evolution, ecology). These activities will help you experience the biology you have learned from lectures, your textbook, and this manual.

We also want you to design your own experiments so that you can learn biology *your* way. These activities, which are part of every lab, are *investigative labs*. Some of these investigations are independent activities, whereas others are extensions of topics studied in directed labs and thematic labs. In investigative labs, you'll apply the skills you've learned to answer your own questions about biology. In doing so, you'll be challenged to create and develop *your* way of answering scientific questions.

Investigations in biology often go far beyond simply following the steps of the scientific method. Indeed, investigation is a broad pursuit that includes observations, experiments, analysis of the work of others, reliable procedures, and repetition. It's more of an approach to answering questions than it is a set of rigid procedures. Although investigation doesn't have to be complicated, it does require creativity, planning, patience, and attention to detail.

Investigations proceed along a variety of paths, depending on the investigator and the question being asked. But the steps we've described below can improve any investigation, including those suggested in this lab manual.

Establish a clear question. Investigations begin with observations and questions. Simple, straightforward questions are usually the best. When you've decided on your question, *write it down*. You will be surprised how much easier it is to recall and refine a written question than it is to develop a vague idea rattling around in your head. Make sure your question is stated clearly. And here's a tip for asking productive questions: Learn as much as you can about what you're proposing to do. The more background information you have, the better your questions will be, and the more likely your results will make sense.

Not all questions require controlled experiments. For example, some investigations are descriptive rather than experimental. Decide whether your question is best answered with experiments in controlled systems or with observations in natural systems. You may investigate the impact of pollutants

by administering them to controlled organisms, or you may choose to describe observations about a pollutant's effects in a natural community. Both approaches can lead to interesting and important results.

Design a reliable experiment. Outline what you are going to do, and write down the steps of your procedure in numerical order. The most reliable experiments are usually the simplest ones. Complicated procedures are often hard to repeat and are prone to error. Remember that a hallmark of good science is that it's repeatable. To keep things simple and repeatable it's best—whenever you can—to isolate a single variable and hold all other conditions constant. That way you can easily repeat your experiment and refine your ability to reliably measure the most important variable.

Simple, reliable procedures also make it easier to establish appropriate controls. If all conditions surrounding your experiment except one variable are held constant, then it is relatively easy to design a good control. A good control is a replicate procedure with the variable of interest either held constant or absent. For example, if you want to detect the effects of a pollutant on plant growth, then you need a control with the same growth conditions as the pollutant treatments, but without the pollutant you are studying.

Another good tip for designing successful investigations is to use readily available organisms and materials for procedures. Good science does not have to be complicated with expensive equipment or exotic organisms. There is no need to use a rat if a fruit fly will do.

If the experiment you're proposing requires materials other than the ones provided, ask your instructor if those materials are available. Also, get input from other people about your proposed work—investing time *before* you do the work can save much time later.

Work objectively. Decide beforehand what result will validate your hypothesis and answer your question, and what result will invalidate your hypothesis. If possible, use tables and graphs to show your results. Write down not just your data, but also what your data mean. Try not to think about what your results *should be*. Instead, accept what they *are*. Some of the most interesting results are those that we didn't predict, for unexpected results often lead to more questions. And that's a good thing!

Strengthen your conclusions. The best way to strengthen your conclusions is to repeat your work. Along with repetition, conclusions are stronger when they are supported by different kinds of evidence. For example, if you are investigating the effects of a nutrient on plant growth, then your conclusion is stronger if you investigated more than one species of plant. Similarly, conclusions based on highly controlled laboratory experiments are strengthened by corroborative data on plant growth in natural communities with various levels of that nutrient.

Be prepared to revise your questions and experimental design. You'd be surprised how many initial experiments in an investigation "don't work." The results make no sense, or you can't measure the variable you thought you were going to measure with the precision you expected. Or, the first experiment gives one result and the second experiment gives another. If this happens, do not be overly concerned—this is precisely how "real science" goes. Think about what might be the problem; perhaps it's arising from some source of variation in one replicate that's not in the other replicates. The cure for that problem is revision and repetition. It's worth saying again ... good science is reliable and repeatable.

Figure out what your data mean. Discuss your data in light of your original question or hypothesis. Do your results support or falsify your hypothesis? Use your data to explain your reasoning. What is the significance of your work? That is, what can you conclude from your investigation? Are there other interpretations from results? How do your results compare with those of others? Based on what you've learned, can you now ask different or more probing questions to learn even more? Remember that correlation does not necessarily indicate cause and effect. If you had problems with your investigation, discuss how these problems might have been avoided. If you could repeat or revise your work, what would you do differently?

Be prepared to report your work. Scientists often remark that "you haven't done science until you have published your work." Lab write-ups are the beginning of a publication. The topics discussed in "How to Write a Scientific Paper or Laboratory Report" (pp. xxi–xxiii) will help prepare you to present your ideas to others.

Welcome to the Biology Laboratory

Welcome to the biology laboratory! Although reading your textbook and attending lectures are important ways of learning about biology, nothing can replace the importance of the laboratory. In lab you'll get hands-on experience with what you've heard and read about biology—for example, you'll observe organisms, do experiments, test ideas, collect data, and make conclusions about what you've learned. You'll do biology.

You'll enjoy the exercises in this manual—they're interesting, informative, and can be completed within the time limits of your laboratory period. We've provided questions to test your understanding of what you've done; in some of the exercises, we've also asked you to devise your own experiments to answer questions that you've posed. To make these exercises most useful and enjoyable, follow these guidelines:

THE IMPORTANCE OF COMING TO CLASS

Biology labs are designed to help you experience biology firsthand. To do that, you must attend class. If you want to do well in your biology course, you'll need to attend class and pay attention. To appreciate the importance of class attendance for making a good grade in your biology course, examine figure 1, a graph showing how students' grades in an introductory biology course relate to their rates of class attendance. Data are from a general biology class at the University

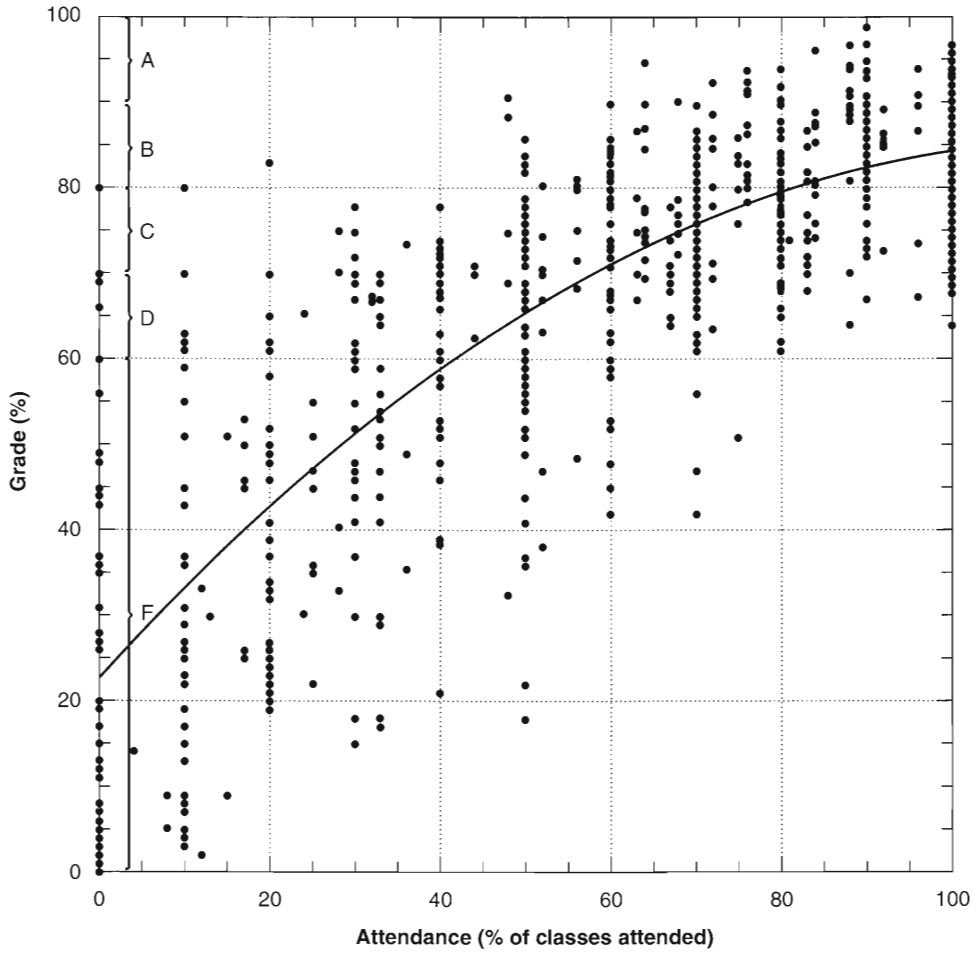


Figure 1
How students' grades in an introductory biology course relate to their rates of class attendance.

of Minnesota. On page xix, write an analysis of the data shown in figure 1. What do these data mean?

BEFORE COMING TO LAB

Read the exercise before coming to lab. This will give you a general idea about what you're going to do, as well as why you're going to do it. Knowing this will not only save time, it will also help you finish the experiments and make you aware of any safety-related issues associated with the lab. Before doing any procedures, you'll encounter a section of each exercise titled "SAFETY FIRST" that is marked with this icon:

This section of the lab will help ensure that you're aware of safety concerns (e.g., solvents, acids, bases, hotplates) associated with the work. If you have questions about these safety issues, contact your lab instructor before starting the lab work.

Notify your instructor if you are pregnant, color-blind, taking immunosuppressive drugs, have allergies, or have any other conditions that may require precautionary measures. Also, before coming to lab, cover any cuts or scrapes with a sterile, waterproof bandage.

WHEN IN LAB

1. Know what you are going to do. Read and understand the lab before coming to lab.
2. Don't start the exercise until you've discussed the exercise with your laboratory instructor. She/he will give you specific instructions about the lab and tell you how the exercise may have been modified.
3. Work carefully and thoughtfully, and stay focused as you work. You'll be able to finish each exercise within the allotted time if you are well prepared and stay busy. You'll not be able to finish the exercise if you spend your time talking about this weekend's party or last week's big game.
4. Discuss your observations, results, and conclusions with your instructor and lab partners. Perhaps their comments and ideas will help you better understand what you've observed.
5. Always follow instructions and follow safety guidelines presented by your instructor.
6. If you have questions, ask your instructor.

SAFETY IN THE LABORATORY

Laboratory accidents can affect individuals, classes, or the entire campus. To avoid such accidents, the exercises in this manual were designed with safety as a top priority. You'll be warned about any potentially hazardous situations or chemicals with this image:

When you see this image, pay special attention to the instructions.

The laboratory safety rules listed in table 1 will help make lab a safe place for everyone to learn biology. Remember, it is much easier to prevent an accident than to deal with its consequences.

Read the laboratory safety rules listed in table 1. If you do not understand them, or if you have questions, ask your instructor for an explanation. Then complete table 1 and sign the statement that is at the bottom of page xiii.

BEFORE YOU LEAVE LAB

Put away all equipment and glassware, and wipe clean your work area.

AFTER EACH LABORATORY

Soon after each lab, review what you did. What questions did you answer? What data did you gather? What conclusions did you make?

Also note any questions that remain. Try to answer these questions by using your textbook or visiting the library. If you can't answer the questions, discuss them with your instructor.

Welcome to the biology laboratory!

TABLE 1

LABORATORY SAFETY RULES

Rule	Why is this rule important? What could happen if this rule is not followed?
Behave responsibly. No horseplay or fooling around while in lab.	
Do not bring any food or beverages into lab, and do not eat, drink, smoke, chew gum, chew tobacco, or apply cosmetics when in lab. Never taste anything in lab. Do not put anything in lab into your mouth. Avoid touching your face, chewing on pens, and other similar behaviors while in lab. Always wear shoes in lab.	
Unless you are told otherwise by your instructor, assume that all chemicals and solutions in lab are poisonous, and act accordingly. Never pipette by mouth. Always use a mechanical pipetting device (e.g., a suction bulb) to pipette solutions. Clean up all spills immediately, and report all spills to your instructor.	
Wear safety goggles when working with chemicals. Carefully read the labels on bottles and know the chemical you are dealing with. Do not use chemicals from an unlabeled container, and do not return excess chemicals back to their container. Report all spills to your instructor immediately.	
Unless your instructor tells you to do otherwise, do not pour any solutions down the drain. Dispose of all materials as per instructions from your instructor.	
If you have long hair, tie it back. Don't wear dangling jewelry. If you are using open flames, roll up loose sleeves. Wear contact lenses at your own risk; contacts hold substances against the eye and make it difficult to wash your eyes thoroughly.	
Treat living organisms with care and respect.	
Your instructor will tell you the locations of lab safety equipment, including fire extinguishers, fire blanket, eyewash stations, and emergency showers. Familiarize yourself with the location and operation of this equipment.	
If anything is splashed into your eyes, wash your eyes thoroughly and immediately. Tell your lab instructor what happened.	
Notify your instructor of any allergies to latex, chemicals, stings, or other substances.	
If you break any glassware, do not pick up the pieces of broken glass with your hands. Instead, use a broom and dustpan to gather the broken glass. Ask your instructor how to dispose of the glass.	

TABLE 1

LABORATORY SAFETY RULES (CONTINUED)

Rule	Why is this rule important? What could happen if this rule is not followed?
Unless told by your instructor to do otherwise, work only during regular, assigned hours when the instructor is present. Do not conduct any unauthorized experiments; for example, do not mix any chemicals without your instructor's approval.	
Do not leave any experiments unattended unless you are authorized by your instructor to do so. If you leave your work area, slide your chair under the lab table. Keep walkways and desktops clean and clear by putting books, backpacks, and so on along the edge of the room, in the hall, in a locker, or in an adjacent room. Keep your work area as clean and uncluttered as possible.	
Don't touch or put anything on the surface of hotplates unless told to do so. Many types of hotplates have no visible sign that they are hot. Assume they *are* hot.	
Know how to use the equipment in lab. Most of the equipment is expensive; you may be required to pay all or part of its replacement cost. Keep water and solutions away from equipment and electrical outlets. Report malfunctioning equipment to your instructor. Leave equipment in the same place and condition that you found it. If you have any questions about or problems with equipment, contact your instructor.	
Know what to do and whom to contact if there is an emergency. Know the fastest way to get out of the lab. Immediately report all injuries—no matter how minor—to your instructor. Seek medical attention immediately if needed. If any injury appears to be life-threatening, call 911 immediately.	
At the end of each lab, clean your work area, wash your hands thoroughly with soap, slide your chair under the lab table, and return all equipment and supplies to their original locations. Do not remove any chemicals or equipment from the lab.	

Name _____

Lab Section _____

Your lab instructor may require that you submit this page at the end of today's lab.

1. In the space below, write an analysis of the data shown in figure 1.

After completing table 1, read and sign this statement:

2. I have read and I understand and agree to abide by the laboratory safety rules described in this exercise and discussed by my instructor. I know the locations of the safety equipment and materials. If I violate any of the laboratory safety rules, my instructor will lower my grade and/or remove me from the lab.

_____

Signature

_____

Name (printed)

_____

Date

How to Write a Scientific Paper or Laboratory Report

Your instructor may occasionally ask you to submit written reports describing the work you did in the lab. Although these reports will probably not be published in scientific magazines or journals, they are important because they will help you learn to write a scientific paper. A scientific paper is a written description of how the scientific method was used to study a problem.

Understanding how to write a scientific paper (such as a lab report) is important for several reasons. Scientists become known (or remain unknown) by their publications in books, magazines, and scientific journals. Regardless of the presumed importance of a scientist's discoveries, poor writing delays or prohibits publication because it makes it difficult to understand what the scientist did or the importance of the work. Poor writing usually indicates an inability or unwillingness of a scientist to think clearly.

Scientific papers are the vehicle for the transmission of scientific knowledge; they are available for others to read, test, refute, and build on. Few skills are more important to a scientist than learning how to write a scientific paper.

Before you finish reading this section, go to the library and browse through a few biological journals such as *American Journal of Botany*, *Ecology*, *Journal of Mammalogy*, or *Journal of Cell Biology*. Make photocopies of one or two of the articles that interest you. As you'll see, scientific papers follow a standard format that reflects the scientific method.

PARTS OF A SCIENTIFIC PAPER

Almost all scientific papers have these parts:

- Title
- List of authors
- Abstract
- Introduction
- Materials and methods
- Results
- Discussion
- References

Understanding this format eases the burden of writing a scientific paper, because writing is an exercise in organization. Refer to the journal articles you photocopied in the library as you read this text.

Title

The title of a paper is a short label (usually fewer than 10 words) that helps readers quickly determine their interest in the paper. The title should reflect the paper's content and contain the fewest number of words that adequately express the paper's content. The title should never contain abbreviations or jargon (jargon is overly specialized or technical language).

List of Authors

Only those people who actively contributed to the design, execution, or analysis of the experiment should be listed as authors.

Abstract

The abstract is a short paragraph (usually fewer than 250 words) that summarizes (1) the objectives and scope of the problem, (2) methodology, (3) data, and (4) conclusions. The abstract contains no references.

Introduction

The introduction concisely states why you did the work. Avoid exhaustive reviews of what has already been published; rather, limit the introduction to just enough pertinent information to orient the reader to your study.

The introduction of a scientific paper has two primary parts. The first part is a description of the nature and background of the problem. For example, what do we already know (or not know) about the problem? This description is developed by citing other scientists' work, to give a history of the study of the problem, and by pointing out gaps in our knowledge. The second part of the introduction states the objectives of the study.

Materials and Methods

The materials and methods section describes how, when, where, and what you did. It should contain enough detail to allow another scientist to repeat your experiment, but it should not be overwhelming.

Materials include items such as growth conditions, organisms, and the chemicals used in the experiment. Avoid trade names of chemicals and describe organisms with their scientific names (e.g., *Zea mays* rather than "corn"). Also describe growth conditions, diet, lighting, temperature, and so on.

Methods are usually presented chronologically, and this discussion is often subdivided with headings. Examples of methods include sampling techniques, types of microscopy, and statistical analyses. If possible, use references to describe methods.

Experiments described in a scientific paper must be reproducible. Thus, the quality of materials and methods is judged by the reader's ability to repeat the experiment. If a colleague can't repeat your experiment, the materials and methods section is probably poorly written.

For most lab reports, do not copy the experimental procedures word for word from the lab manual. Rather, summarize what you did in several sentences.

Results

The results section is the heart of a scientific paper. It should clearly summarize your findings and leave no doubt about the outcome of your study. For example, state that "All animals died 29 hours after eating cyanide" or "Table 1 shows the influence of 2,4-D on leaf growth." Keep it simple and to the point.

Tables and graphs are excellent ways to present results but shouldn't completely replace a written summary of results. Tables are ideal for presenting large amounts of numerical data, and graphs are an excellent way to summarize data and show relationships between independent and dependent variables. The variable that the scientist established and controlled during the experiment is the **independent variable**. It is presented on the *x*-axis of the graph. Protein content of a diet might be an independent variable in an experiment measuring weight gain by an animal (fig. 2). Similarly, time and temperature are often independent variables.

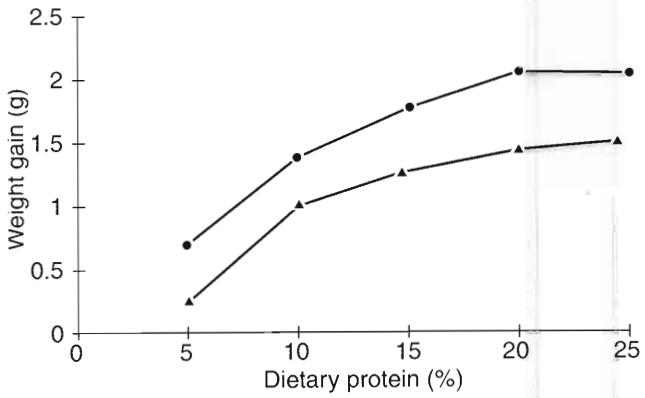


Figure 2
Sample graph from a scientific paper.

The **dependent variable** changes in response to changes in the independent variable and is presented on the *y*-axis of the graph. Weight and growth rate are examples of dependent variables that may change in response to light, temperature, diet, and so on. Graphs must also have a title (e.g., "Influence of Temperature on Root Elongation"), labeled axes (e.g., "Temperature," "Root Elongation"), and scaled units along each axis appropriate to each variable (e.g., °C, mm h^{-1}). Place tables and graphs on separate pages from the text.

Discussion

It's not enough to simply report your findings; you must also discuss what they mean and why they're important. This is the purpose of the discussion section of a scientific paper. This section should interpret your results relative to the objectives you described in the introduction and answer the question "So what?" or "What does it mean?" A good discussion section should do the following:

- Discuss your findings; that is, present relationships, principles, and generalizations. Point out exceptions and lack of correlations.
- Don't conceal anomalous results; rather, describe unsettled points. State how your results relate to existing knowledge.
- State the significance and implications of your data. What do your results mean? If your data are strong, don't hesitate to use statements beginning with "I conclude that . . . ."

References

Scientists rely heavily on information presented in papers written by their colleagues. Indeed, the introduction, materials and methods, and discussion sections of a paper often

contain citations of other publications. The format for these citations varies in different biological journals. The following citation for an article is in the format recommended by the Council of Biology Editors:*

White, H.B., III. Coenzymes as fossils of an earlier molecular state. *J. Mol. Evol.* 7:101–104; 1976.

A FEW SIMPLE RULES FOR WRITING EFFECTIVELY

Informative sentences and well-organized paragraphs are the foundation of a good scientific paper. Listed here are a few rules to help you write effectively. Following these rules won't necessarily make you a Hemingway, but it will probably improve your writing.

- *Write clearly and simply.* For example, "the biota exhibited a 100% mortality response" is a wordy and pretentious way of saying "all of the organisms died." Remember, keep it simple and straightforward.
- *Keep related words together.* Consider the following sentence taken from a scientific publication: "Lying on top of the intestine, you perhaps make out a small transparent thread." Do we really have to lie on top of the intestine to see the thread? The author meant that "a small transparent thread lies atop the intestine."
- *Use active voice.* Write "Good writers avoid passive voice," not "The passive voice is avoided by good writers." Here are some other examples of passive voice:

  Poor: My first lab report will always be remembered by me. (passive)

  Better: I'll always remember my first lab report. (active)

  Poor: Examination of patients was accomplished by me. (passive)

  Better: I examined patients. (active)
- *Write positively.* For example, write "The rats were always sick" instead of "The rats were never healthy."

  Use definite and specific sentences. For example, write "It rained every day for a week" instead of "A period of unfavorable growth conditions set in."
- *Be sure of the meaning of every word that you use, and write exactly what you mean.* Refer to a dictionary and thesaurus to ensure clarity and proper word usage. For example, you allude to a book, and elude a pursuer.
- *Delete unnecessary words.* For example:

Replace	*With*
The question as to whether	Whether
Advance notice	Notice
At this point in time	Now
Be that as it may	But
In the event that	If
General consensus	Consensus
Young juvenile	Juvenile
Student body	Students
Due to the fact that	Because
Chemotherapeutic agent	Drug

- *Use metric measurements* (see Exercise 2).
- *Be sure that each paragraph conveys a single major idea and has a topic sentence.* The topic sentence should state the main idea of the paragraph.
- *Have a friend or colleague read a draft of your writing and suggest improvements.*
- *Don't plagiarize.* Learn to summarize and be sure to cite all references from which you extracted information.

A neat and typed presentation is a must. If you use a word processor, remember to use the spell checker. Carefully proofread to catch mistakes. Put your work aside for at least 24 hours before you proofread. If you're interested in learning more about improving your writing, read *The Elements of Style* (4th ed.), by W. Strunk and E. B. White (New Jersey: Prentice Hall, 2000).

* Council of Biology Editors style manual: A guide for authors, editors, and publishers in the biological sciences. *5th ed. Council of Biology Editors;* 1983.

Measurements in Biology
The Metric System and Data Analysis

2

Objectives

By the end of this exercise you should be able to:

1. Identify the metric units used to measure length, volume, mass, and temperature.
2. Measure length, volume, mass, and temperature in metric units.
3. Convert one metric unit to another (e.g., grams to kilograms).
4. Use measures of volume and mass to calculate density.
5. Practice the use of simple statistical calculations such as mean, median, range, and standard deviation.
6. Analyze sample data using statistical tools.

Every day we're bombarded with numbers and measurements. They come at us from all directions, including while we're at the supermarket, gas station, golf course, and pharmacy, as well as while we're in our classrooms and kitchens. Virtually every package that we touch is described by a measurement.

Scientists use a standard method to collect data as well as use mathematics to analyze measurements. We must measure things before we can objectively describe what we are observing, before we can experiment with biological processes, and before we can predict how organisms respond, adjust to, and modify their world. Once we have made our measurements, we can analyze our data and look for variation and the sources of that variation. Then we can infer the causes and effects of the biological processes that interest us.

THE METRIC SYSTEM

Scientists throughout the world use the **metric system** to make measurements. The metric system is also used in everyday life virtually everywhere except the United States. With few exceptions (e.g., liter bottles of soda, 35-mm film), most measurements in the United States use the antiquated English system of pounds, inches, feet, and so on. Check with your instructor about bringing to class common grocery store items with volumes and weights in metric units, or examining those items on display.

Metric measurement is used worldwide in science to improve communication in the scientific community. Scientists make all of their measurements in the metric system; they do not routinely convert from one system to another. However, the following conversions will help give you a sense of how some common English units are related to their metric equivalents:

1 inch = 2.5 centimeters
1 foot = 30 centimeters
1 yard = 0.9 meter
1 mile = 1.6 kilometers
1 ounce = 28 grams
1 pound = 0.45 kilogram
1 fluid ounce = 30 milliliters
1 pint = 0.47 liter
1 quart = 0.95 liter
1 gallon = 3.8 liters
1 cup = 0.24 liters

If you want to know those conversions, see Appendix II.

This exercise will introduce you to making metric measurements of length, mass, volume, and temperature. During this lab, you should spend your time making measurements, not reading background information. Therefore, *before lab, read this exercise carefully to familiarize yourself with the basic units of the metric system.*

Metric units commonly used in biology include:

meter (m)—the basic unit of length
liter (L)—the basic unit of volume
kilogram (kg)—the basic unit of mass
degrees Celsius (°C)—the basic unit of temperature

Unlike the English system with which you are already familiar, the metric system is based on units of ten. This simplifies conversions from one metric unit to another (e.g., from kilometers to meters). This base-ten system is similar to our monetary system, in which 10 cents equals a dime, 10 dimes equals a dollar, and so forth. Units of ten in the metric system are indicated by Latin and Greek prefixes placed before the base units:

Prefix (Latin)		*Division of Metric Unit*	
deci	(d)	0.1	10^{-1}
centi	(c)	0.01	10^{-2}
milli	(m)	0.001	10^{-3}
micro	(μ)	0.000001	10^{-6}
nano	(n)	0.000000001	10^{-9}
pico	(p)	0.000000000001	10^{-12}

Prefix (Greek)		*Multiple of Metric Unit*	
deka	(da)	10	10^{1}
hecto	(h)	100	10^{2}
kilo	(k)	1000	10^{3}
mega	(M)	1000000	10^{6}
giga	(G)	1000000000	10^{9}

Thus, multiply by:

0.01 to convert centimeters to meters
0.001 to convert millimeters to meters
1000 to convert kilometers to meters
0.1 to convert millimeters to centimeters

For example, there are 10 millimeters per centimeter. Therefore,

$$62 \text{ cm} \times \frac{10 \text{ mm}}{\text{cm}} = 620 \text{ mm}$$

In these conversion equations, the units being converted *from* (in this case, centimeters) cancel out, leaving you with the desired units (in this case, millimeters). Also note that when units are converted to *smaller* units, the number associated with the new units will *increase*, and vice versa. For example, 620 meters = 0.620 kilometers = 620,000 millimeters = 62,000 centimeters.

Question 1
Make the following metric conversions:

1 meter = centimeters = millimeters
92.4 millimeters = meters = centimeters
10 kilometers = meters = decimeters
82 centimeters = meters = millimeters
3.1 kilograms = grams = milligrams

Length and Area

The **meter** (m) is the basic unit of length. Units of area are squared units (i.e., two-dimensional) of length.

1 m = 100 cm = 1000 mm = 0.001 km = 1×10^{-3} km
1 km = 1000 m = 10^{3} m
1 cm = 0.01 m = 10^{-2} m = 10 mm
470 m = 0.470 km
1 cm^2 = 100 mm^2 (i.e., 10 mm × 10 mm = 100 mm^2)

To help you appreciate the magnitudes of these units, here are the lengths and areas of some familiar objects:

Length

Housefly	0.5 cm
Mt. Everest	8848 m
Diameter of penny	1.9 cm
Toyota Camry	4.7 m

Area

Total skin area of adult human male	1.8 m^2
Football field (goal line to goal line)	4459 m^2
Surface area of human lungs	80 m^2
Central Park (New York City)	3.4 km^2
Ping-pong table	4.18 m^2
Credit card	46 cm^2

Procedure 2.1

Make metric measurements of length and area

Most biologists measure lengths with metric rulers or metersticks.

1. Examine intervals marked on the metric rulers and metersticks available in the lab.
2. Make the following measurements. Be sure to include units for each measurement.

Length of this page _____
Width of this page _____
Area of this page (Area = Length × Width) _____
Your height _____
Thickness of this manual _____
Height of a 200-mL beaker _____
Height of ceiling _____

Question 2
What are some potential sources of error in your measurements?

Volume

Volume is the space occupied by an object. Units of volume are cubed (i.e., three-dimensional) units of length. The liter (L) is the basic unit of volume.

1 L = 1000 cm^3 = 1000 mL
1 L = 0.1 m × 0.1 m × 0.1 m
1 cm^3 = 0.000001 m^3

To help you appreciate the magnitudes of these units, here are the volumes of some familiar objects:

Chicken egg	60 mL
One breath of air	500 cm^3
Coke can	355 mL

Scientists often measure volumes with pipets and graduated cylinders. Pipets are used to measure small volumes, typi-

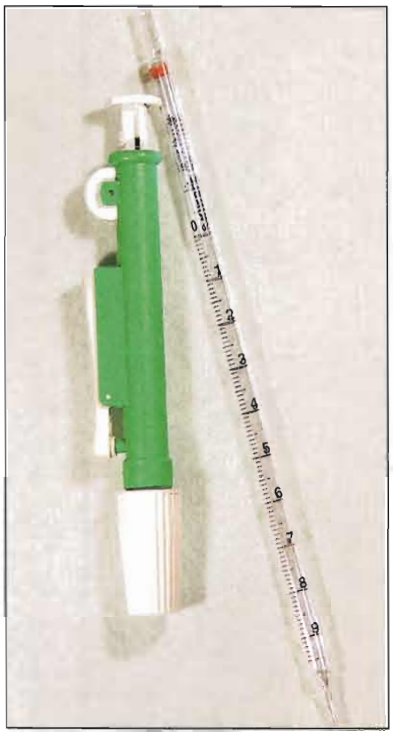


Figure 2.1
A pipet is used to extract and dispense volumes of liquid. A suction bulb (shown in green on the left) draws fluid into the pipet, and graduated markings on the pipet allow precise measurement of a fluid's volume. Never use your mouth to suck fluid into a pipet.

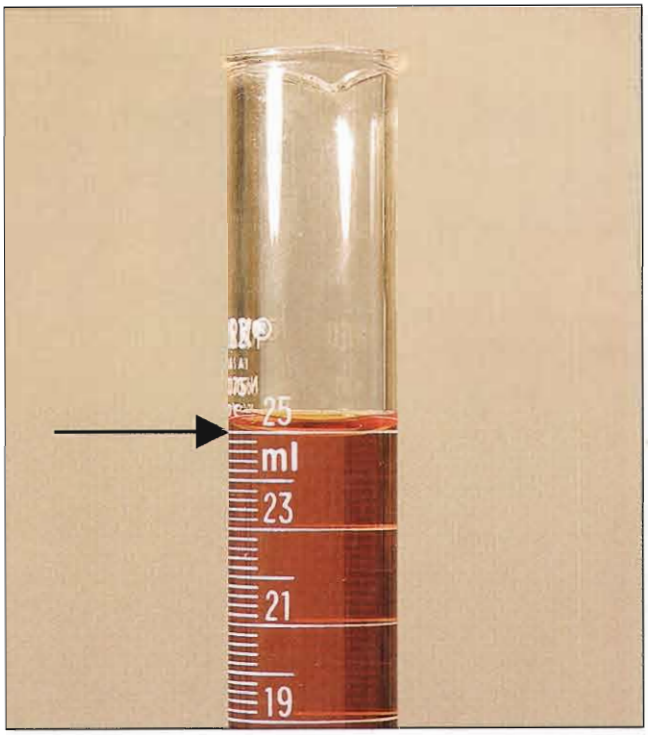


Figure 2.2
When measuring the volume of liquid in a graduated cylinder, always measure at the bottom of the meniscus. The bottom of the meniscus in this photograph is indicated by the arrow. The correct volume is 25 mL.

cally 25 mL or less. Liquid is drawn into a pipet using a bulb or pipet pump (fig. 2.1). Never pipet by mouth.

Graduated cylinders are used to measure larger volumes. To appreciate how to make a measurement accurately, pour 40–50 mL of water into a 100-mL graduated cylinder, and observe the interface between the water and air. This interface, called the **meniscus,** is curved because of surface tension and the adhesion of water to the sides of the cylinder. When measuring the liquid in a cylinder such as a graduated cylinder, always position your eyes level with the meniscus and read the volume at the lowest level (fig. 2.2).

Procedure 2.2

Make metric measurements of volume

1. Biologists often use graduated cylinders to measure volumes. Locate the graduated cylinders available in the lab to make the following measurements. Determine what measurements the markings on the graduated cylinder represent. Be sure to include units for each measurement.
2. Measure the milliliters needed to fill a cup (provided in the lab). _____
3. Measure the liters in a gallon. _____

Procedure 2.3

Measure the volume of a solid object by water displacement

1. Obtain a 100-mL graduated cylinder, a thumb-sized rock, and a glass marble.
2. Fill the graduated cylinder with 70 mL of water.
3. Gently submerge the rock in the graduated cylinder and notice that the volume of the contents rises.
4. Carefully observe the meniscus of the fluid and record its volume.
5. Calculate and record the volume of the rock by subtracting the original volume (70 mL) from the new volume.

   Rock volume _____
6. Repeat steps 2–5 to measure and record the volume of the marble.

   Marble volume _____

Biologists use pipets to measure and transfer small volumes of liquid from one container to another. The following procedure will help you appreciate the usefulness of pipets.

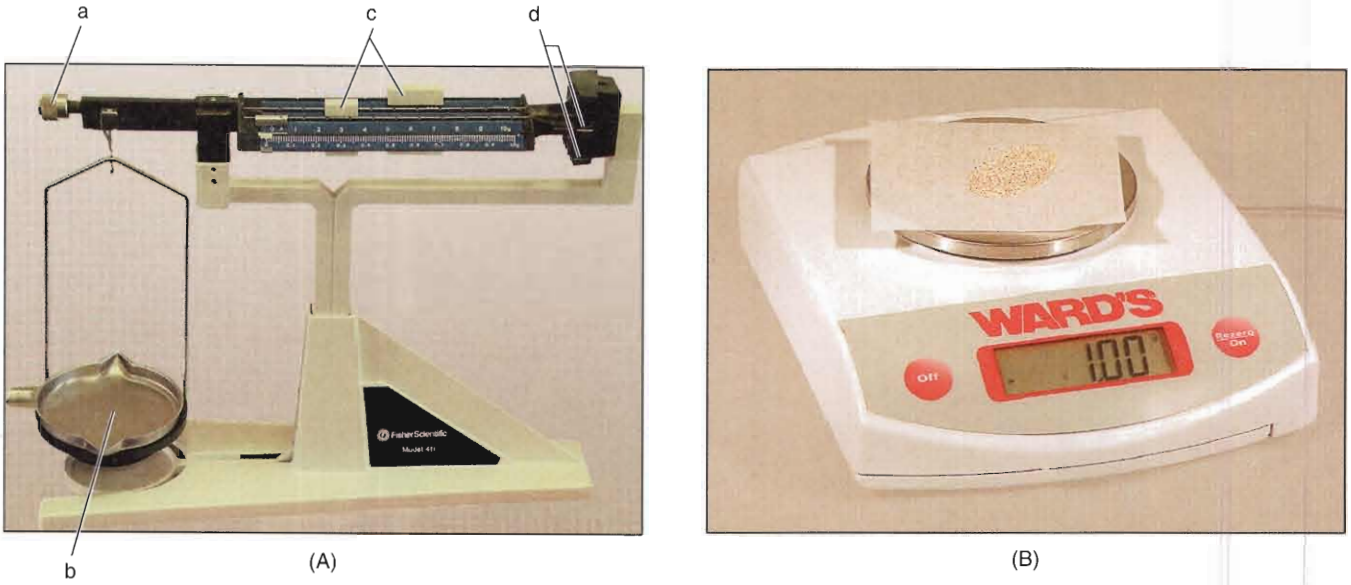


Figure 2.3

Biologists use balances to measure mass. (A) The parts of a triple-beam balance include (*a*) the zero-adjustment knob, (*b*) measuring pan, (*c*) movable masses on horizontal beams, and (*d*) balance marks. (B) A top-loading balance has a measuring pan, a power switch, and a zero calibration button.

Procedure 2.4

Learn to use a pipet

1. Add approximately 100 mL of water to a 100-mL beaker.
2. Use a 5-mL pipet with a bulb or another filling device provided by your instructor to remove some water from the beaker.
3. Fill the pipet to the zero mark.
4. To read the liquid level correctly, your eye must be directly in line with the bottom of the meniscus.
5. Release the liquid into another container.

Question 3

What volume of liquid did you measure?

Mass

The **kilogram** (kg) is the basic unit of mass.[1] A kilogram equals the mass of 1000 cubic centimeters (cm^3) of water at 4°C. Similarly,

$$1\ kg = 1000\ g = 10^3\ g$$
$$1\ mg = 0.001\ g = 10^{-3}\ g$$

Here are the masses of some familiar objects:

9V battery	40 g
Ping-pong ball	2.45 g
Basketball	0.62 kg
Quarter	6.25 g

[1] *Remember that mass is not necessarily synonymous with weight. Mass measures an object's* potential *to interact with gravity, whereas weight is the force exerted by gravity on an object. Thus, a weightless object in outer space has the same mass as it has on earth.*

Biologists often measure mass with a triple-beam balance (fig. 2.3), which gets its name from its three horizontal beams. Suspended from each of the three beams are movable masses. Each of the three beams of the balance is marked with graduations: the closest beam has 0.1-g graduations, the middle beam has 100-g graduations, and the farthest beam has 10-g graduations.

Before making any measurements, clean the weighing pan and move all of the suspended weights to the far left. The balance marks should line up to indicate zero grams; if they do not, turn the adjustment knob until they do. Measure the mass of an object by placing it in the center of the weighing pan and moving the suspended masses until the beams balance. The mass of the object is the sum of the masses indicated by the weights on the three beams.

Procedure 2.5

Make metric measurements of mass

1. Biologists often use a triple-beam balance or a top loading scale to measure mass. Locate the triple-beam balances or scales in the lab.
2. Measure the masses of the following items. Be sure to include units for each measurement.

   Penny _____

   Paper clip _____

   Pencil _____

   Rock (used in procedure 2.3) _____

   100-mL beaker (empty) _____

   100-mL beaker containing 50 mL of water _____

Question 4

a. **Density** is mass per unit volume. Use data that you've gathered to determine the density of water at room temperature.

Density of water = (mass/volume) = _____

b. What is the density of the wooden pencil? Does it float? Why?

c. What is the density of the rock? Does it sink? Why?

Temperature

Temperature is the measure of the kinetic energy of molecules—that is, the amount of heat in a system. Biologists measure temperature with a thermometer calibrated in degrees Celsius (°C). The Celsius scale is based on water freezing at 0°C and boiling at 100°C. You can interconvert °C and degrees Fahrenheit (°F) using the formula 5(°F) = 9(°C) + 160. Here are some typical temperatures:

40°C	a very hot summer day
30.6°C	butter melts
75°C	hot coffee
−20°C	temperature in a freezer
37°C	human body temperature

Procedure 2.6

Make metric measurements of temperature

1. Obtain a thermometer in the lab. Handle a thermometer with care. If it breaks, notify your instructor immediately.
2. Determine the range of the temperatures that can be measured with your thermometer by examining the scale imprinted along the barrel of the thermometer.
3. Measure the following temperatures:

   Room temperature _____ °C

   Cold tap water _____ °C

   Hot tap water _____ °C

   Inside refrigerator _____ °C

UNDERSTANDING NUMERICAL DATA

Statistics offer a way to organize, summarize, and describe data—the data are usually samples of information from a much larger population of values. Statistics and statistical tests allow us to analyze the sample and draw inferences about the entire population. Consequently, the use of statistics enables us to make decisions even though we have incomplete data about a population. Although this may seem unscientific, we do it all the time; for example, we diagnose diseases with a drop of blood. Decisions are based on statistics when it is impossible or unrealistic to analyze an entire population.

Let's say that you want to know the mass of a typical apple in your orchard. To obtain this information, you could analyze one apple, but how would you know that you'd picked a "typical" sample? After all, the batch from which you chose the apple may contain many others, each a little different. You'd get a better estimate of "typical" if you increased your sample size to a few hundred apples, or even to 10,000. Or, better yet, to 1,000,000.

The only way to be certain of your conclusions would be to measure all the apples in your orchard. This is impossible, so you must choose apples that *represent* all of the other apples—that is, you must be working with a *representative sample*. A statistical analysis of those sample apples reduces the sample values to a few characteristic measurements (e.g., mean mass). As you increase the size of the sample, these characteristic measurements provide an ever-improving estimation of what is "typical."

There are a variety of software programs that perform statistical analyses of data; all you have to do is enter your data into a spreadsheet, select the data that you want to analyze, and perform the analysis. Although these software packages save time and can increase accuracy, you still need to understand a few of the basic variables that you'll use to understand your numerical data. We'll start with the mean and median:

The **mean** is the arithmetic average of a group of measurements. Chance errors in measurements tend to cancel themselves when means are calculated for relatively large samples; a value that is too high because of random error is often balanced by a value that is too low for the same reason.

Hints for Using the Metric System

1. Use decimals, not fractions (e.g., 2.5 m, not $2\frac{1}{2}$ m).
2. Express measurements in units requiring only a few decimal places. For example, 0.3 m is more easily manipulated and understood than 300000000 nm.
3. When measuring pure water, the metric system offers an easy and common conversion from volume measured in liters to volume measured in cubic meters to mass measured in grams: 1 mL = 1 cm^3 = 1 g.
4. The metric system uses symbols rather than abbreviations. Therefore, do not place a period after metric symbols (e.g., 1 g, not 1 g.). Use a period after a symbol only at the end of a sentence.
5. Do not mix units or symbols (e.g., 9.2 m, not 9 m 200 mm).
6. Metric symbols are always singular (e.g., 10 km, not 10 kms).
7. Except for degrees Celsius, always leave a space between a number and a metric symbol (e.g., 20 mm, not 20mm; 10°C, not 10° C).
8. Use a zero before a decimal point when the number is less than one (e.g., 0.42 m, not .42 m).

The **median** is the middle value of a group of measurements.

The median is less sensitive to extreme values than is the mean. To appreciate this, consider a sample consisting of 14 leaves having the following lengths (all in mm):

80 69 62 74 69 51 45 40 9 64 65 64 61 67

The mean length is 58.6 mm. However, none of the leaves are that length, and most of the leaves are longer than 60 mm. In biology, the mean is usually preferred to the median when reporting descriptive statistics.

Question 5

a. Does the mean always describe the "typical" measurement? Why or why not?

b. What information about a sample does a mean *not* provide?

Determine the median by arranging the measurements in numerical order:

9 40 45 51 61 63 64 64 65 67 69 69 73 80

The median is between the seventh and eighth measurement: 64 mm. In this sample, the mean differs from the median.

Question 6

a. What is responsible for this difference between the mean and median?

b. How would the median change if the 9-mm-long leaf was not in the sample?

c. How would the mean change if the 9-mm-long leaf was not in the sample?

d. Consider these samples:

Sample 1: 25 35 32 28

Sample 2: 15 75 10 20

What is the mean for Sample 1? _____

What is the mean for Sample 2? _____

In most of the exercises in this manual, you'll have time to make only one or two measurements of a biological structure or phenomenon. In these instances, a mean may be the only descriptor of the sample. However, if your class combines its data so that there are many measurements, you'll need to know how to do a couple of other calculations so that you understand the variation within your sample.

Significant Figures

Let's suppose that you're measuring the length of a bone, as shown in figure 2.4. How would you record this length—as 8 cm? 8.3 cm? 8.33 cm? 8.33333 cm? To answer this question, you need to know something about significant figures.

Significant figures are the number of figures required to record a measurement so that only the last digit in the number is in doubt. For example, if the ruler you're using is calibrated only in centimeters and you find that the object you're measuring is between 8 and 9 cm long (fig. 2.4), then you should estimate your measurement only to a tenth of a centimeter. That is, a measurement of 8.3 cm is acceptable, but 8.33 is not because it implies a precision that did not exist in the equipment you used to make the measurement. If, however, your ruler was calibrated in millimeters, then 8.33 cm would be acceptable. Remember this: When recording measurements, include all of the digits you are sure of plus an estimate to the nearest one-tenth of the next smaller digit.

Here are some other guidelines for using the correct number of significant figures in your measurements:

When adding or subtracting measurements, the answer should have no more precision than the measurement having the least number of significant figures. For example, suppose the air temperature in an incubator drops from 8.663°C to 8.2°C. This is a difference of 8.663°C – 8.2°C = 0.5°C, not 0.463°C. If the second temperature reading had been 8.200°C, then the correct answer would have been 0.463°C.

When converting measurements from one set of units to another, do not introduce precision that is not present in the first number. For example, 8.3 cm = 83 mm, not 83.0 mm.

When manipulating two measurements simultaneously, the precision of the final measurement should not exceed that of the least number of significant figures. For example, the calculation for the mass of 17.2 mL of water is 17.2 mL × 0.997821 g mL^{-1} = 17.2 g, not 17.162521 g.

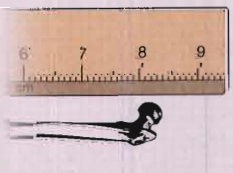


Figure 2.4
How long is this bone? 8 cm? 8.3 cm? 8.33 cm?

Variability

As you can see, the samples in Question 6d are different, but their means are the same. Thus, the mean does not reveal all there is to know about these samples. To understand how these samples are different, you need other statistics: the range and standard deviation.

The **range** is the difference between the extreme measurements (i.e., smallest and largest) of the sample. In Sample 1, the range is $35 - 25 = 10$; in Sample 2 the range is $75 - 10 = 65$. The range provides a sense of the variation of the sample, but the range can be artificially inflated by one or two extreme values. Notice the extreme values in the sample of leaf measurements previously discussed. Moreover, ranges do not tell us anything about the measurements between the extremes.

Question 7

a. Could two samples have the same mean but different ranges? Explain.

b. Could two samples have the same range but different means? Explain.

The **standard deviation** indicates how measurements vary about the mean. The standard deviation is easy to calculate. Begin by calculating the mean, measuring the deviation of each sample from the mean, squaring each deviation, and then summing the deviations. This summation results in the **sum of squared deviations.** For example, consider a group of shrimp that are 22, 19, 18, and 21 cm long. The mean length of these shrimp is 20 cm.

Sample Value	*Mean*	*Deviation*	*(Deviation)²*
22	20	2	4
19	20	−1	1
21	20	1	1
18	20	−2	4

Sum of Squared Deviations = 10

Rounding Numbers

Do not change the value of the last significant digit if that digit is followed by a number that is less than 5. For example, if two significant figures are required, 6.449 rounds to 6.4, 66.449 rounds to 66, 66.641 rounds to 67, and 6.591 rounds to 6.6. Here is how an original measurement of 49.5149 rounds to various numbers of significant figures:

Five significant figures:	49.515
Four significant figures:	49.51
Three significant figures:	49.5
Two significant figures:	50
One significant figure:	50

Statisticians disagree on what to do when the number following the last significant figure is exactly 5, as in 89.5 (and, in this case, the precision is limited to two significant figures). Some round the measurement to the higher number, while others claim that doing so introduces bias into the data. You can decide which approach to take, but be consistent.

The summary equation for the sum of squared deviations is:

$$\text{Sum of squared deviations} = \sum_{i=1}^{N}(x_i - \bar{x})^2$$

where

N = **total number of samples**

$\bar{x}$ = **the sample mean**

x_i = **measurement of an individual sample**

This formula is simple. The summation sign $(\sum_{i=1}^{N})$ means to add up all the squared deviations from the first one ($i = 1$) to the last one ($i = N$). The sum of squared deviations (10) divided by the number of samples minus one ($4 - 1 = 3$) produces a value of $10/3 = 3.3$ cm² (note that the units are centimeters squared). This is the **variance:**

$$\text{Variance} = \frac{\text{sum of squared deviations}}{N - 1}$$

The square root of the variance, 1.8 cm, equals the **standard deviation (SD):**

$$\text{SD} = \sqrt{\text{Variance}} = \sqrt{3.3} = 1.8$$

The standard deviation is usually reported with the mean in statements such as, "The mean length of the leaf was 20 ± 1.8 cm."

The standard deviation helps us understand the spread or variation of a sample. For many distributions of measurements, the mean ± 1 SD includes 68% of the measurements, whereas the mean ± 2 SD includes 95% of the measurements.

Procedure 2.7

Gather and analyze data statistically

1. Use a meterstick or tape measure to measure your height in centimeters. Record your height here: _____ cm
2. Record your height and gender (male or female) on the board in the lab.
3. After all of your classmates have reported their heights, calculate the following:

Size of sample

- All classmates _____
- Male classmates _____
- Female classmates _____

Mean height

- All classmates _____
- Male classmates _____
- Female classmates _____

Median height

- All classmates _____
- Male classmates _____
- Female classmates _____

Range

- All classmates _____ to _____
- Male classmates _____ to _____
- Female classmates _____ to _____

Standard deviation

- All classmates ± _____
- Male classmates ± _____
- Female classmates ± _____

If there is sufficient time, obtain a newspaper that advertises cars, groceries, or other common commodities. Choose one example (e.g., new cars) and determine its average price (e.g., determine the average price of a new car).

Question 8

a. What does your calculation tell you?

b. What are the limitations of your sample?

Your instructor may ask you to do other statistical tests, such as Student's *t*, chi-square, and analysis of variance (ANOVA). The type of test you'll do will depend on the amount and type of data you analyze, as well as the hypotheses you are trying to test.

INVESTIGATION

Variation in the Areas and Shapes of Leaves

Observation: Leaves, which are the primary photosynthetic organ of most plants, are adapted for absorbing light. This involves exposing large surface areas to the environment.

Question: How does the surface area and shape of leaves vary on different parts of plants?

a. Establish a working lab group and obtain Investigation Worksheet 2 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. If leaves are not available from outdoor plants (e.g., during winter), use the plants provided by your instructor that were grown indoors. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 2 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What are the advantages and disadvantages of using the metric system of measurements?

2. Why is it important for all scientists to use a standard system of measures rather than the system that may be most popular in their home country or region?

3. Do you lose or gain information when you use statistics to reduce a population to a few characteristic numbers? Explain your answer.

4. Suppose that you made repeated measurements of your height. If you used good technique, would you expect the range to be large or small? Explain your answer.

5. Suppose that a biologist states that the average height of undergraduate students at your university is 205 cm plus or minus a standard deviation of 17 cm. What does this mean?

6. What does a small standard deviation signify? What does a large standard deviation signify?

The Microscope
Basic Skills of Light Microscopy

3

Objectives

By the end of this exercise you should be able to:

1. Identify and explain the functions of the primary parts of a compound microscope and dissecting (stereoscopic) microscope.
2. Practice carrying and focusing a microscope properly.
3. Use a compound microscope and dissecting microscope to examine biological specimens.
4. Prepare a wet mount, determine the magnification and size of the field of view, and determine the depth of field.

Many organisms and biological structures are too small to be seen with the unaided eye (fig. 3.1). Biologists often use a light microscope to observe such specimens. A **light microscope** is a coordinated system of lenses arranged to produce an enlarged, focusable image of a specimen. A light microscope **magnifies** a specimen, meaning that it increases its apparent size. Magnification with a light microscope is usually accompanied by improved **resolution,** the ability to distinguish two points as separate points. Thus, the better the resolution, the sharper or crisper the image appears. The resolving power of the unaided eye is approximately 0.1 mm (1 in = 25.4 mm), meaning that our eyes can distinguish two points 0.1 mm apart. A light microscope, used properly, can improve resolution as much as 1000-fold (i.e., to 0.1 μm).

The ability to discern detail also depends on **contrast,** the amount of difference between the lightest and darkest parts of an image. Therefore, many specimens examined with a light microscope are stained with artificial dyes that increase contrast and make the specimen more visible.

The invention of the light microscope was profoundly important to biology, because it was used to formulate the cell theory and study biological structure at the cellular level. Light microscopy has revealed a vast new world to the human eye and mind (fig. 3.2). Today, the light microscope is the most fundamental tool of many biologists.

THE COMPOUND LIGHT MICROSCOPE

Study and learn the parts of the typical compound light microscope shown in figure 3.3. A light microscope has two, sometimes three, systems: an illuminating system, an imaging system, and possibly a viewing and recording system.

Illuminating System

The illuminating system, which concentrates light on the specimen, usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a lightbulb located at the base of the microscope. The light source illuminates the specimen by passing light through a thin, almost transparent part of the specimen. The **condenser lens,** located immediately below the specimen, focuses light from the light source onto the specimen. Just below the condenser is the **condenser iris diaphragm,** a knurled ring or lever that can be opened and closed to regulate the amount of light reaching the specimen. When the condenser iris diaphragm is open, the image will be bright; when closed, the image will be dim.

Caring for Your Microscope

Microscopes are powerful tools for understanding biology. However, they're also expensive and fragile and require special care. When you use your microscope, always do the following to ensure optimal performance and care:

- Always carry your microscope upright with both hands—one hand under the base and the other around the microscope's arm (fig. 3.3).
- Always begin by cleaning the ocular and objective lenses with lens paper.
- Always start your examinations with the low-power objective in place.
- If you shift to the high-power objective, rotate the objective into place carefully. Never force the objective lens into place. If the objective lens contacts the slide, stop and restart your examination with the low-power objective lens.
- After shifting to the high-power objective, always use only the fine adjustment to focus the image.
- When you've completed your work with the microscope, clean the lenses with lens paper, wrap the electrical cord securely around the microscope's arm, and return your microscope to its storage area.

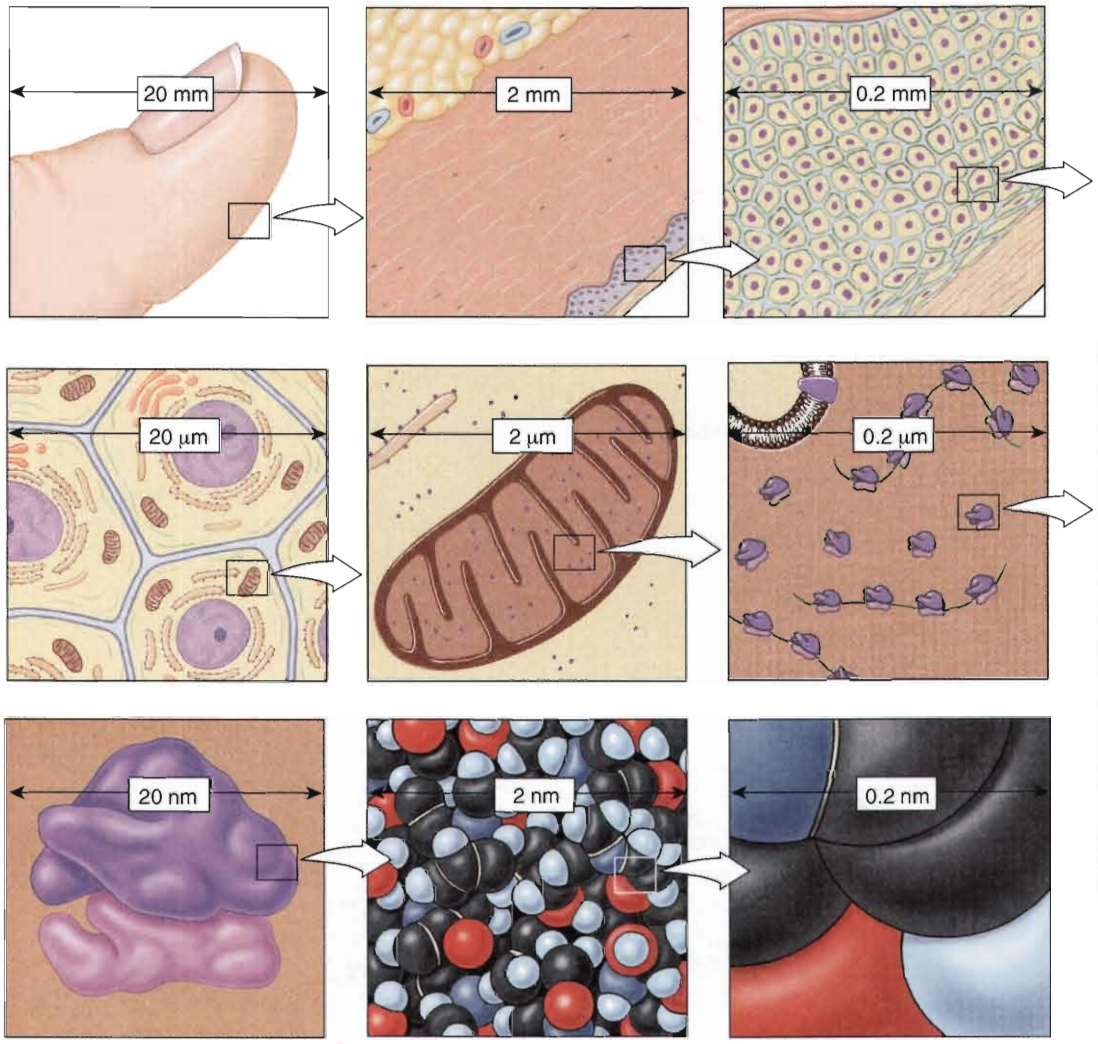


Figure 3.1

The size of cells and their contents. This diagram shows the size of human skin cells, organelles, and molecules. In general, the diameter of a human skin cell is about 20 micrometers (μm), of a mitochondrion is 2 μm, of a ribosome is 20 nanometers (nm), of a protein molecule is 2 nm, and of an atom is 0.2 nm.

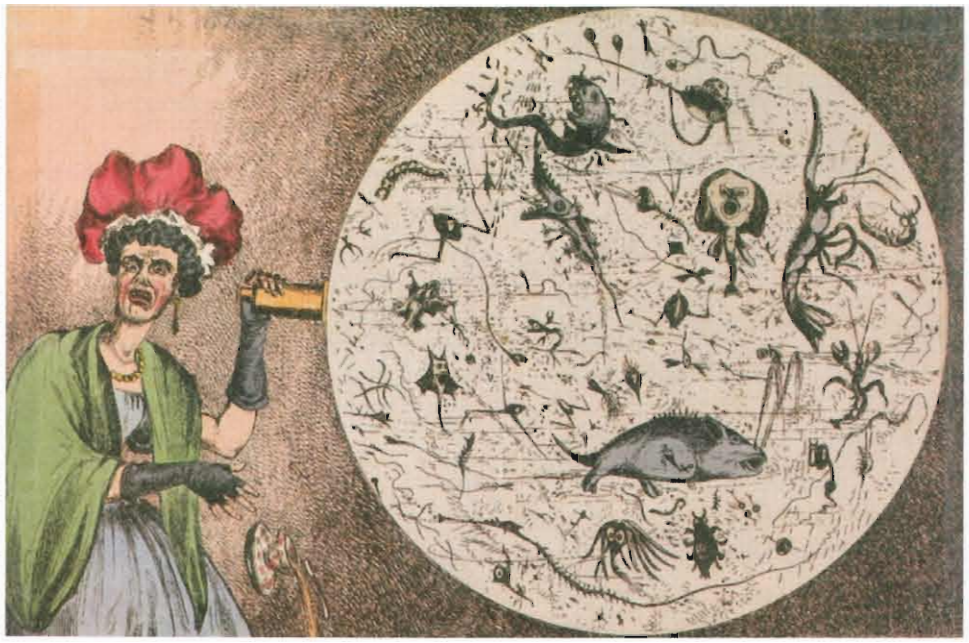
Imaging System

The imaging system improves resolution and magnifies the image. It consists of the objective and ocular (eyepiece) lenses and a body tube. The **objectives** are three or four lenses mounted on a revolving nosepiece. Each objective is a series of several lenses that magnify the image, improve resolution, and correct aberrations in the image. The most common configuration for student microscopes includes four objectives: low magnification (4×), medium magnification (10×), high magnification (40×), and oil immersion (100×). Using the oil immersion objective requires special instructions, as explained in Exercise 24 to study bacteria. To avoid damaging your microscope do not use the oil immersion objective during this exercise.

The magnifying power of each objective is etched on the side of the lens (e.g., 4×). The **ocular** is the lens that you look through. Microscopes with one ocular are **monocular** microscopes, and those with two are **binocular** microscopes. Oculars usually magnify the image ten times. The **body tube** is a metal casing through which light passes to the oculars. In microscopes with bent body tubes and inclined oculars, the body tube contains mirrors and a prism that redirects light to the oculars. The **stage** secures the glass slide on which the specimen is mounted.

Viewing and Recording System

The viewing and recording system, if present, converts radiation to a viewable and/or permanent image. The viewing and recording system usually consists of a camera or video screen. Most student microscopes do not have viewing and recording systems.



Heath, William. *Monster Soup commonly called Thames Water...*, date unknown. Art Gallery of Ontario, Toronto. Gift of the Trier-Fodor Foundation, 1980.

Figure 3.2

"Egad, I thought it was tea, but I see I've been drinking a blooming micro-zoo!" says this horrified, proper nineteenth-century London woman when she used a microscope to examine her tea. People were shocked to learn that there is an active, living world too small for us to see.

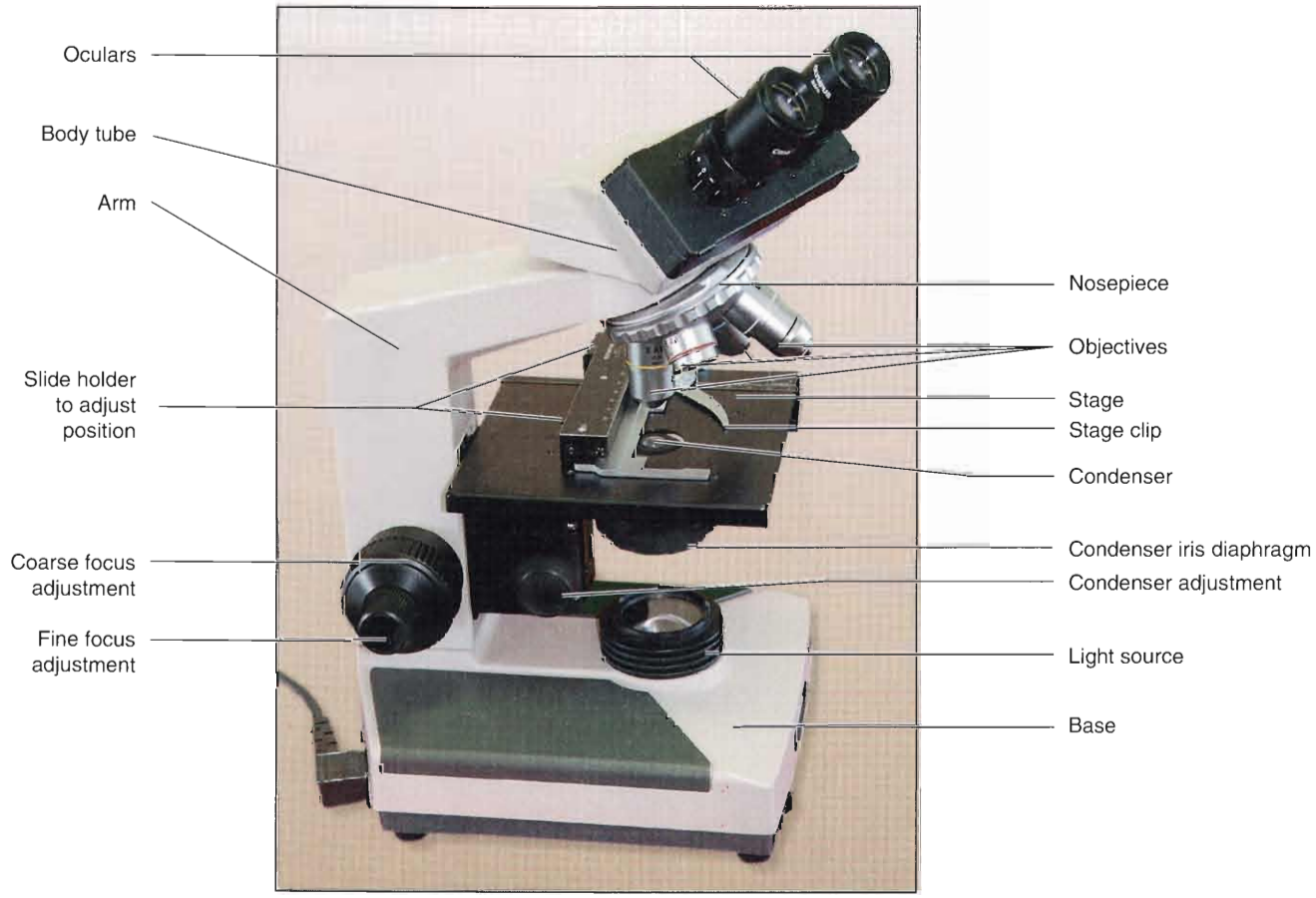


Figure 3.3

Major parts of a compound light microscope.

A Summary of How to Use a Compound Light Microscope

1. Place the specimen on the microscope's stage.
2. Rotate the low-power objective into place. Center the specimen below the objective.
3. Look through the oculars while using the coarse adjustment to focus on the specimen. Center the area of the specimen that you want to examine.
4. Slowly rotate the high-power objective into place. Look through the oculars while you use the fine-adjustment to focus on the specimen.
5. If you "lose" your specimen when you switch from low power to high power, retrace the previous steps, paying special attention to placing the specimen in the center of the field of view.

USING A COMPOUND MICROSCOPE

Although the maximum magnification of light microscopes has not increased significantly during the last century, the construction and design of light microscopes have improved the resolution of newer models. For example, built-in light sources have replaced adjustable mirrors in the illuminating system, and lenses are made of better glass than they were in the past.

Your lab instructor will review with you the parts of the microscopes (and their functions) you will use in the lab. After familiarizing yourself with the parts of a microscope, you're now ready for some hands-on experience with the instrument.

Procedure 3.1

Use a compound microscope

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base. Place your microscope on the table in front of you.

Do not use paper towels or Kimwipes to clean the lenses of your microscope; they can scratch the lenses. Clean the lenses only with lens paper.

2. Plug in the microscope and turn on the light source.
3. If it isn't already in position, rotate the nosepiece until the low-power (4×) objective is in line with the light source. (The 4× objective is sometimes called the "scanning objective" because it enables users to scan large areas of a specimen.) You'll feel the objective click into place when it is positioned properly. *Always begin examining slides with the low-power objective.*
4. Locate the coarse adjustment knob on the side of the microscope. Depending on the type of microscope that you're using, the coarse adjustment knob moves either the nosepiece (with its objectives) or the stage to focus the lenses on the specimen. Only a partial turn of the coarse adjustment knob moves the stage or nosepiece a relatively large distance. *The coarse adjustment should only be used when you're viewing a specimen with the 4× or 10× objective lens.*
5. If your microscope is binocular, adjust the distance between the oculars to match the distance between your pupils. If your microscope is monocular, keep both eyes open when using the microscope. After a little practice you will ignore the image received by the eye not looking through the ocular.
6. Focus a specimen by using the following steps:
   - *a.* Place a microscope slide of newsprint of the letter *e* on the horizontal stage so that the *e* is directly below the low-power objective lens and is right side up. It should be centered over the hole in the stage.
   - *b.* Rotate the coarse adjustment knob to move the objective within 1 cm of the stage (1 cm = 0.4 in).
   - *c.* Look through the oculars with both eyes open.
   - *d.* Rotate the coarse adjustment knob (i.e., raising the objective lens or lowering the stage) until the *e* comes into focus. If you don't see an image, the *e* is probably off center. Be sure that the *e* is directly below the objective lens and that you can see a spot of light surrounding the *e*.
   - *e.* Focus up and down to achieve the crispest image.
   - *f.* Adjust the condenser iris diaphragm so that the brightness of the transmitted light provides the best view.
   - *g.* Observe the letter, then rotate the nosepiece to align the 10× objective to finish your observation. Do not use the oil immersion objective.

Question 1

a. As you view the letter *e*, how is it oriented? Upside down or right side up?

b. How does the image move when the slide is moved to the right or left? Toward you or away from you?

c. What happens to the brightness of the view when you go from 4× to 10×?

Magnification

Procedure 3.2

Determine magnification

1. Estimate the magnification of the *e* by looking at the magnified image on lowest magnification (4×), and then at the *e* without using the microscope.

2. Examine each objective and record the magnifications of the objectives and oculars of your microscope in table 3.1.
3. Calculate and record in table 3.1 the total magnification for each objective following this formula:

$$Mag_{Tot} = Mag_{Obj} \times Mag_{Ocu}$$

where

Mag_{Tot} = **total magnification of the image**

Mag_{Obj} = **magnification of the objective lens**

Mag_{Ocu} = **magnification of the ocular lens**

For example, if you're viewing the specimen with a 4× objective lens and a 10× ocular, the total magnification of the image is 4 × 10 = 40×. That is, the specimen appears 40 times larger than it is.

4. Slowly rotate the high-power (i.e., 40×) objective into place. *Be sure that the objective does not touch the slide!* If the objective does not rotate into place without touching the slide, do not force it; ask your lab instructor to help you. After the 40× objective is in place, you should notice that the image remains near focus. Most light microscopes are **parfocal,** meaning that the image will remain nearly focused after the 40× objective lens is moved into place. Most light microscopes are also **parcentered,** meaning that the image will remain centered in the field of view after the 40× objective lens is in place.
5. You may need to readjust the iris diaphragm because the high-magnification objective allows less light to pass through to the ocular.
6. To fine-focus the image, locate the **fine adjustment knob** on the side of the microscope. Turning this knob changes the specimen-to-objective distance slightly and therefore makes it easy to fine-focus the image.

Never use the coarse adjustment knob to fine-focus an image on high power.

7. Compare the size of the image under high magnification with the image under low magnification.

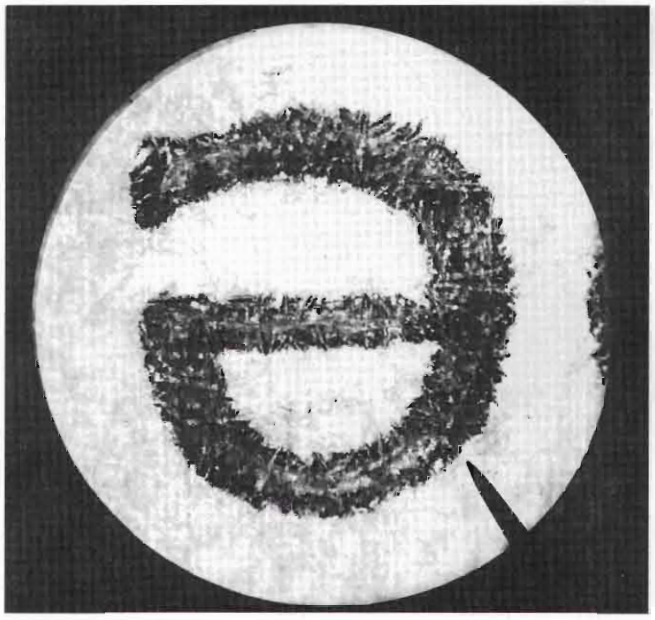


Figure 3.4
The circular, illuminated field of view of a compound light microscope. Shown here is the letter *e* from newsprint that is magnified 40 times.

Question 2

a. How many times is the image of the *e* magnified when viewed through the high-power objective?

b. If you didn't already know what you were looking at, could you determine at this magnification that you were looking at a letter *e*? How?

Determine the Size of the Field of View

The **field of view** is the area that you can see through the ocular and objective (fig. 3.4). Knowing the size of the field of view is important because you can use it to determine the approximate size of an object you are examining. The field of view can be measured with ruled **micrometers** (fig. 3.5). An **ocular micrometer** is a small glass disk with thin lines numbered and etched in a row. It was put into an ocular on your microscope so that the lines superimpose on the image

TABLE 3.1

TOTAL MAGNIFICATIONS AND AREAS OF FIELD OF VIEW (FOV) FOR THREE OBJECTIVES

Objective Power	Objective Magnification	×	Ocular Magnification	=	Total Magnification	FOV Diameter (mm)	FOV Area (mm^2)	Measurement (mm) for 1 Ocular Space
4×	_____	×	_____	=	_____	_____	_____	_____
10×	_____	×	_____	=	_____	_____	_____	_____
40×	_____	×	_____	=	_____	_____	_____	_____

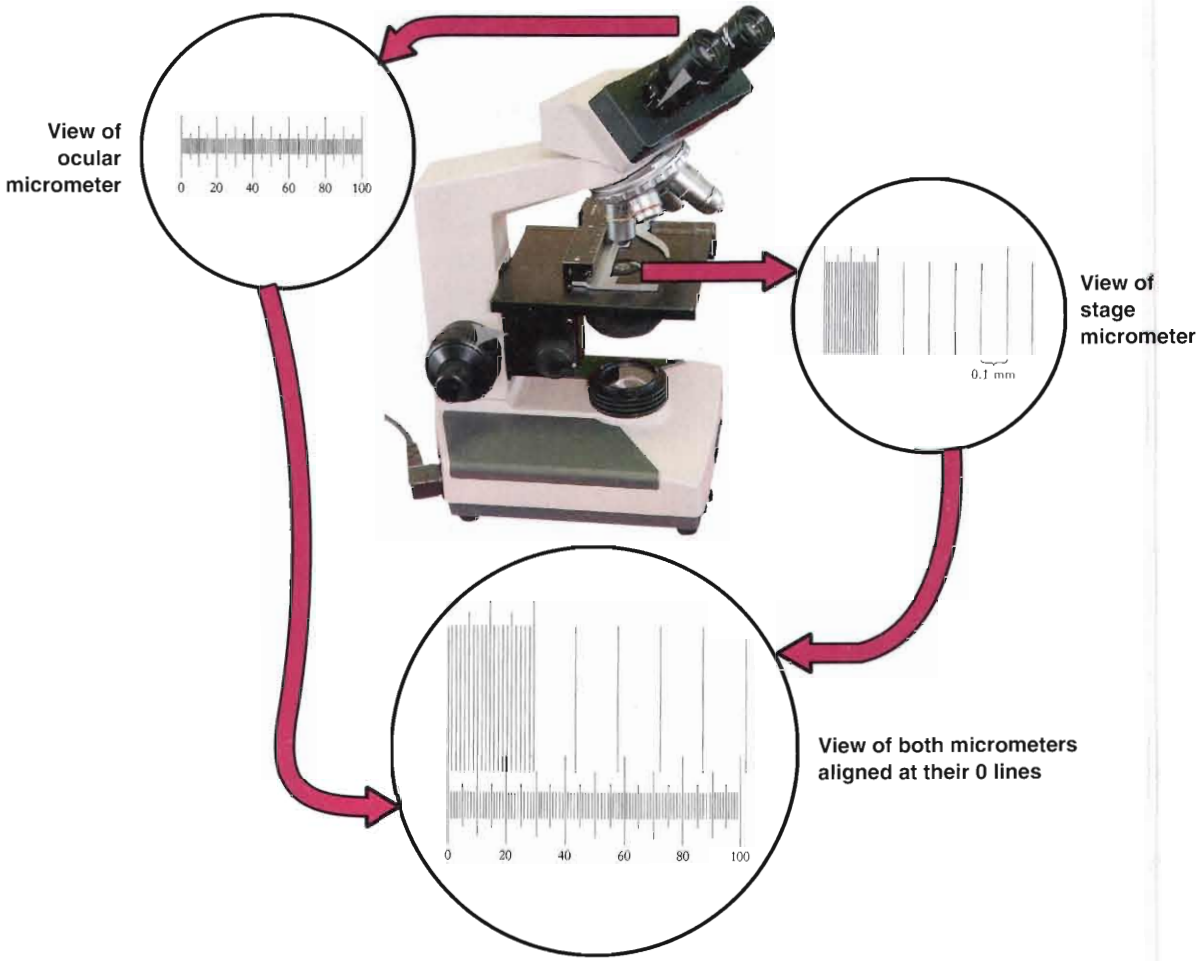


Figure 3.5

Stage and ocular micrometers. Micrometers are used to calibrate microscopes and measure the size of specimens.

and allow you to measure the specimen. Before you can use the micrometer you must determine for each magnification the apparent distance between the lines on the ocular micrometer. This means that you must calibrate the ocular micrometer by comparing its lines to those lines on a standard ruler called a **stage micrometer.** A stage micrometer is a glass slide having precisely spaced lines etched at known intervals.

Procedure 3.3

Use a stage micrometer to calibrate the ocular micrometer, and determine the size of the field of view

1. Rotate the ocular until the lines of the ocular micrometer parallel those of the stage micrometer (fig. 3.5).
2. Align lines at the left edges (0 lines) of the two micrometers by moving the stage micrometer (fig. 3.5).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. Record the values here.

$$y \text{ ocular spaces} = x \text{ stage spaces}$$

$$y =$$

$$x =$$

The smallest space on a stage micrometer = 0.01 mm, so

$$y \text{ ocular spaces (mm)} = x \text{ stage spaces} \times 0.01$$

$$1 \text{ ocular space (mm)} = (x/y) \times 0.01$$

4. Calculate the distance in millimeters between lines of the ocular micrometer. For example, if the length of 10 spaces on the ocular micrometer equals the length of seven spaces on the stage micrometer, then

$$y = 10$$

$$x = 7$$

$$10 \text{ ocular spaces (mm)} = 7 \text{ stage spaces} \times 0.01 \text{ mm}$$

$$1 \text{ ocular space (mm)} = (7 \times 0.01 \text{ mm})/10$$

$$1 \text{ ocular space (mm)} = 0.007 \text{ mm}$$

$$1 \text{ ocular space} = 7\ \mu\text{m}$$

Therefore, if a specimen spans eight spaces on your ocular micrometer with that objective in place, that specimen is 56 μm long.

5. Calibrate the ocular micrometer for each objective on your microscope. Record in table 3.1 the diameter of the field of view (FOV) for each objective. Also record for each objective lens in table 3.1 the measurement (mm) for 1 ocular space. You can use this information in future labs as you measure the sizes of organisms and their parts.
6. Calculate the radius, which is half the diameter.
7. Use this information to determine the area of the circular field of view with the following formula:

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

8. Record your calculated FOV areas in table 3.1.

Alternate Procedure 3.3

Use a transparent ruler to determine the size of the field of view

1. Obtain a clear plastic ruler with a metric scale.
2. Place the ruler on the stage and under the stage clips of your microscope. If your microscope has a mechanical stage, ask your instructor how to place the ruler to avoid damage. Carefully rotate the nosepiece to the objective of lowest magnification.
3. Slowly focus with the coarse adjustment and then the fine adjustment until the metric markings on the ruler are clear.
4. Align the ruler to measure the diameter of the circular field of view. The space between each line on the ruler should represent a 1-mm interval.
5. Record in table 3.1 the diameter of this low-magnification field of view. Also calculate the radius, which is half the diameter.
6. The ruler cannot be used to measure the diameters of the field of view at medium and high magnifications because the markings are too far apart. Therefore, these diameters must be calculated using the following formula:

$$\text{FOV}_{low} \times \text{Mag}_{low} = \text{FOV}_{high} \times \text{Mag}_{high}$$

where

FOV_{low} = diameter of the field of view of the low-power objective

Mag_{low} = magnification of the low-power objective (Be consistent and use the magnification of the objective, not total magnification.)

FOV_{high} = diameter of the field of view of the high-power objective

Mag_{high} = magnification of the high-power objective

For example, if 3.0 mm is the diameter of the field of view for a 4× low-power objective, then what is the diameter of the field of view of the 40× high-power objective?

$$3.0 \text{ mm} \times 4 = \text{FOV}_{high} \times 40$$
$$0.30 \text{ mm} = \text{FOV}_{high}$$

7. Calculate and record in table 3.1 the diameters of the field of view for the 10× and 40× magnifications.
8. Calculate and record in table 3.1 the circular area of the field of view for the three magnifications by using the following formula.

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

Question 3

a. Which provides the largest field of view, the 10× or 40× objective?

b. How much more area can you see with the 4× objective than with the 40× objective?

c. Why is it more difficult to locate an object starting with the high-power objective than with the low-power objective?

d. Which objective should you use to initially locate the specimen? Why?

Determine the Depth of Field

Depth of field is the thickness of the object in sharp focus (fig. 3.6). Depth of field varies with different objectives and magnifications.

Procedure 3.4

Determine the depth of the field of view

1. Using the low-power objective, examine a prepared slide of three colored threads mounted on top of each other.
2. Focus up and down and try to determine the order of the threads from top to bottom. The order of the threads will not be the same on all slides.
3. Re-examine the threads using the high-power objective lens.

Figure 3.6
A thin depth of field is apparent in this 100× image of cells of *Closterium*, a green alga. The upper and lower layers of cells are out of focus, while the midlayer of cells is within the thin depth of field and is clearly focused.

Question 4

a. Are all three colored threads in focus at low power?

b. Can all three threads be in focus at the same time using the high-power objective?

c. Which objective, high- or low-power, provides the greatest depth of field?

Preparing a Wet Mount of a Biological Specimen

Procedure 3.5

Prepare a wet mount of a biological specimen

1. Place a drop of water containing algal cells from a culture labeled "algae" on a clean microscope slide.
2. Place the edge of a clean coverslip at an edge of the drop at a 45° angle; then slowly lower the coverslip onto the drop so that no air bubbles are trapped (fig. 3.7). (Your instructor will demonstrate this technique.) This fresh preparation is called a **wet mount** and can be viewed with your microscope.
3. Experiment with various intensities of illumination. To do this, rotate the 4× objective into place and adjust the condenser iris diaphragm to produce the least illumination. Observe the image; note its clarity, contrast, and color. Repeat these observations with at least four different levels of illumination. The fourth level should have the diaphragm completely open.
4. Repeat step 3 for the 10× and 40× objectives.

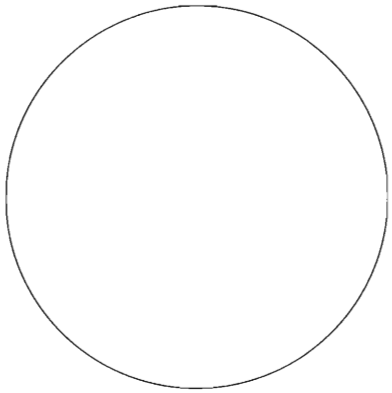
Question 5

a. Is the image always best with highest illumination?

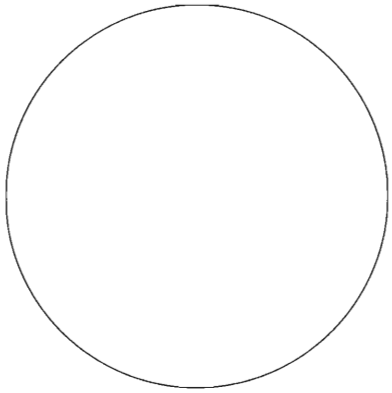
b. Is the same level of illumination best for all magnifications?

c. Which magnifications require the most illumination for best clarity and contrast?

5. Examine your preparation of algae, and sketch in the following field of view the organisms that you see. Don't mistake air bubbles for organisms! Air bubbles appear as uniformly round structures with dark, thick borders.



6. Prepare a wet mount of some newly hatched brine shrimp (*Artemia*, which are popularly referred to as "sea monkeys") and their eggs. Sketch in the following field of view what you see. Use your calculations for the diameter of the field of view to estimate the length of the shrimp.



Approximate length of the shrimp: _____

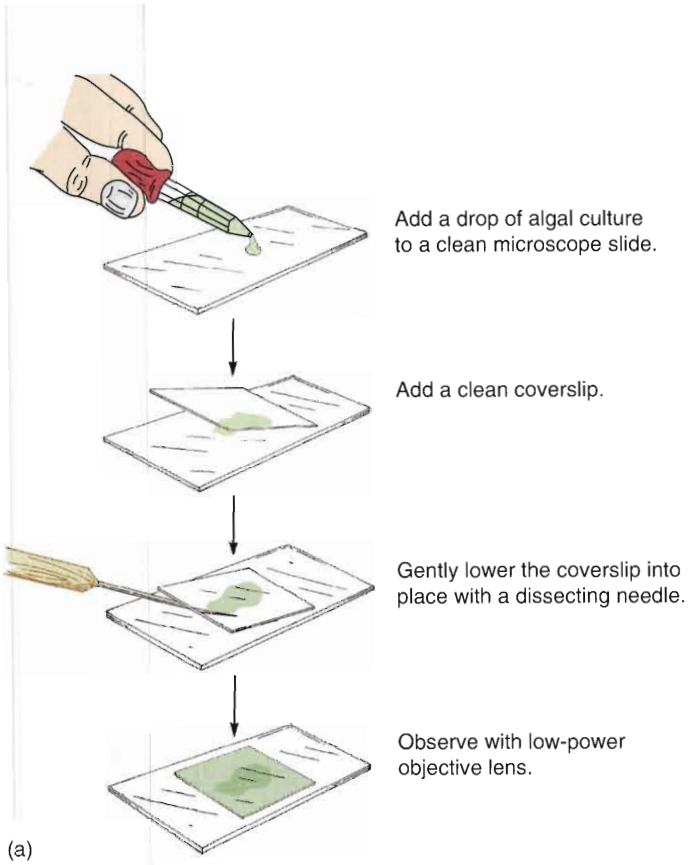


Figure 3.7

(*a*) Preparing a wet mount of a biological specimen. (*b*) A wet mount might include the common algae *Spirogyra*, 800×. See also figures 3.6, 24.9, and 25.1–25.4.

Question 6

a. Why is it important to put a coverslip over the drop of water when you prepare a wet mount?

b. Approximately how long and wide is a brine shrimp?

Practice

For practice using your microscope, prepare some wet mounts of pond water or a hay infusion to view the diversity of protozoa and algae (fig. 3.8). If the protozoa are moving too fast for you to examine carefully, add a drop of methylcellulose (often sold commercially as Proto-Slo) to your sample. (The methylcellulose will slow the movement of the protozoa.) Also examine the prepared slides available in the lab. You'll examine these slides in more detail in the coming weeks, so don't worry about their contents. Rather, use this exercise to familiarize yourself with the microscope. Also prepare wet mounts of the cultures available in the lab and sketch the organisms that you see. When you've finished, turn off the light source, cover your microscope, and store the microscope in its cabinet.

Figure 3.8

The diversity of organisms in pond water (200×).

THE DISSECTING (STEREOSCOPIC) MICROSCOPE

A **dissecting (stereoscopic) microscope** offers some advantages over a compound microscope. Although a compound microscope can produce high magnifications and excellent resolution, it has a small **working distance,** the distance between the objective lens and specimen. Therefore, it is difficult to manipulate a specimen while observing it with a compound microscope. Specimens that can be observed with a compound microscope are limited to those thin enough for light to pass through them. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope.

A dissecting microscope provides a much larger working distance than does a compound microscope. This distance is usually several centimeters (compared to a centimeter or less for a compound microscope), making it possible to dissect and manipulate most specimens. Also, most specimens for dissection are too thick to observe with transmitted light from a light source below the specimen. Therefore, many dissecting microscopes use a light source above the specimen; the image is formed from reflected light.

Dissecting microscopes are always binocular (fig. 3.9). Each ocular views the specimen at different angles through one or more objective lenses. This arrangement provides a three-dimensional image with a large depth of field. This is in contrast to the image in a compound microscope, which is basically two-dimensional. However, the advantages of a stereoscopic microscope are often offset by lower resolution and magnification than a compound microscope. Most dissecting microscopes have magnifications of 4× to 50×.

Procedure 3.6

Use a dissecting microscope

1. Carry the dissecting microscope to your desk.
2. Use figure 3.9 to familiarize yourself with the parts of your microscope.
3. Use your dissecting microscope to examine the organisms available in the lab. Sketch some of these organisms.
4. Use a ruler to measure the diameter of the field of view with your dissecting microscope at several levels of magnification.

Question 7

a. What is the area of the field of view when you use the lowest magnification of your dissecting microscope?

b. What is the area when you use the highest magnification?

c. Place a microscope slide of the letter *e* on the stage. As you view the letter *e* how is it oriented?

d. How does the image through a dissecting microscope move when the specimen is moved to the right or left? Toward you or away from you?

e. How does the direction of illumination differ in dissecting as opposed to compound microscopes?

A COMPARISON OF COMPOUND AND DISSECTING MICROSCOPES

Complete table 3.2 comparing magnification, resolution, size of the field of view, and depth of field of a dissecting microscope and a compound microscope. Use the terms *high*, *low*, or *same* to describe your comparisons.

Question 8

What other differences are there between compound and dissecting microscopes?

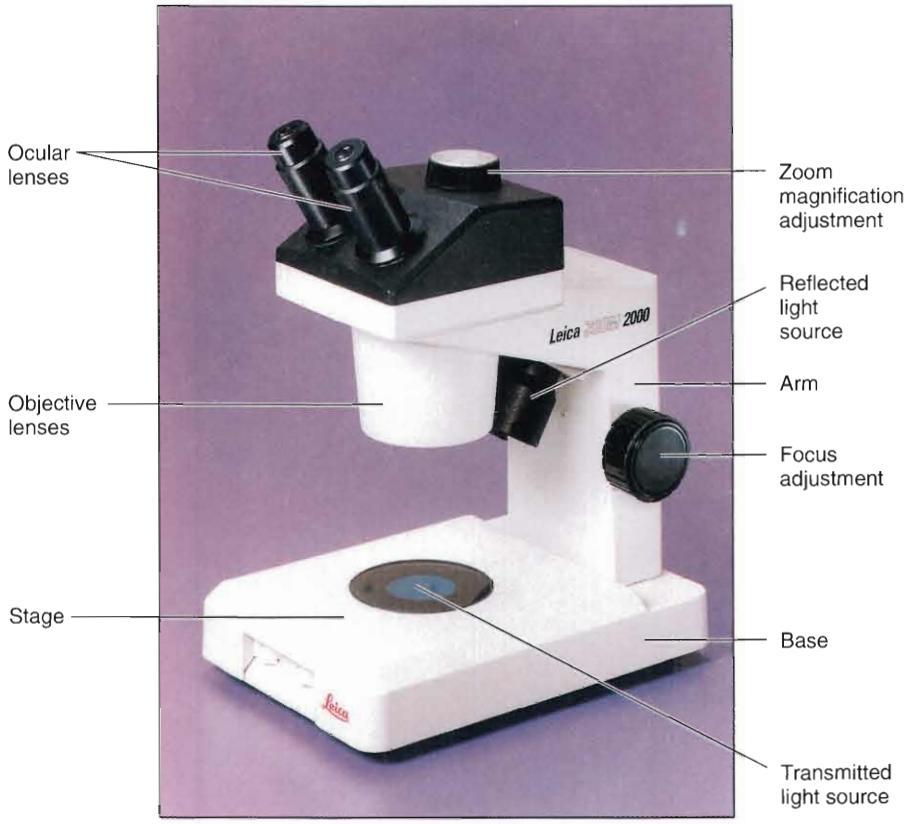


Figure 3.9
Dissecting (stereoscopic) microscope.

TABLE 3.2

A COMPARISON OF DISSECTING AND COMPOUND MICROSCOPES

Characteristic	Dissecting Microscope	Compound Microscope
Magnification	_____	_____
Resolution	_____	_____
Size of field of view	_____	_____
Depth of field	_____	_____

INVESTIGATION

The Shapes, Surface Areas, and Volumes of Red Blood Cells

Observation: Red blood cells, which are the most common type of blood cell, are used by vertebrates to deliver oxygen to body tissues. Red blood cells are filled with hemoglobin, which gives them their characteristic color.

Question: What are the shapes, surface areas, and volumes of red blood cells?

a. Establish a working lab group and obtain Investigation Worksheet 3 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 3 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What are the advantages of knowing the diameter of the field of view at a given magnification?

2. Why must specimens viewed with a compound microscope be thin? Why are they sometimes stained with dyes?

3. Why is depth of field important in studying biological structures? How can it affect your ability to find and examine a specimen?

4. What is the importance of adjusting the light intensity when viewing specimens with a compound microscope?

5. What is the function of each of the following parts of a compound and dissecting microscope?
   - Oculars
   - Objectives
   - Condenser
   - Iris diaphragm
   - Stage
   - Coarse adjustment
   - Fine adjustment

6. Examine the micrograph of the letter *e* shown in figure 3.4. This letter is magnified 40×. What is the actual height of the letter?

WRITING TO LEARN BIOLOGY

The smallest structures of cells are best seen with a transmission electron microscope. Refer to your textbook or other book and describe how an electron microscope can resolve such small structures. Write a short essay about the advantages and limitations of a **transmission electron microscope.**

The Cell
Structure and Function

4

Objectives

By the end of this exercise you should be able to:

1. Understand the differences between prokaryotes and eukaryotes and identify structures characteristic of each.
2. Prepare a wet mount to view cells with a compound microscope.
3. Understand the function of organelles visible with a light microscope.
4. Examine a cell's structure and determine whether it is from a plant, animal, or protist.

Cells are considered the basic unit of living organisms because they perform all of the processes we collectively call "life." All organisms are made of cells. Although most individual cells are visible only with the aid of a microscope, some may be a meter long (e.g., nerve cells) or as large as a small orange (e.g., the yolk of an ostrich egg). Despite these differences, all cells are designed similarly and share fundamental features.

Cytology is the study of cellular structure and function. The major tools of cytologists are light microscopy, electron microscopy, and cell chemistry. By studying the anatomy of a cell, we can find clues to how the cell works.

To understand the life processes of organisms, in today's lab you will study some of the features and variations among living cells. Prior to this exercise, review in your textbook the general features of cellular structure and function.

PROKARYOTIC CELLS

Bacteria and cyanobacteria are **prokaryotes** (fig. 4.1), and their diversity is considerable (>5000 species). Prokaryotes do not contain a membrane-bound nucleus or any other membrane-bound **organelles.** Organelles are organized structures of macromolecules having a specialized function and are suspended in the **cytoplasm.** The cytoplasm of prokaryotes is enclosed in a **plasma membrane** (cellular membrane) and is surrounded by a supporting **cell wall** covered by a gelatinous **capsule. Flagella** and hairlike

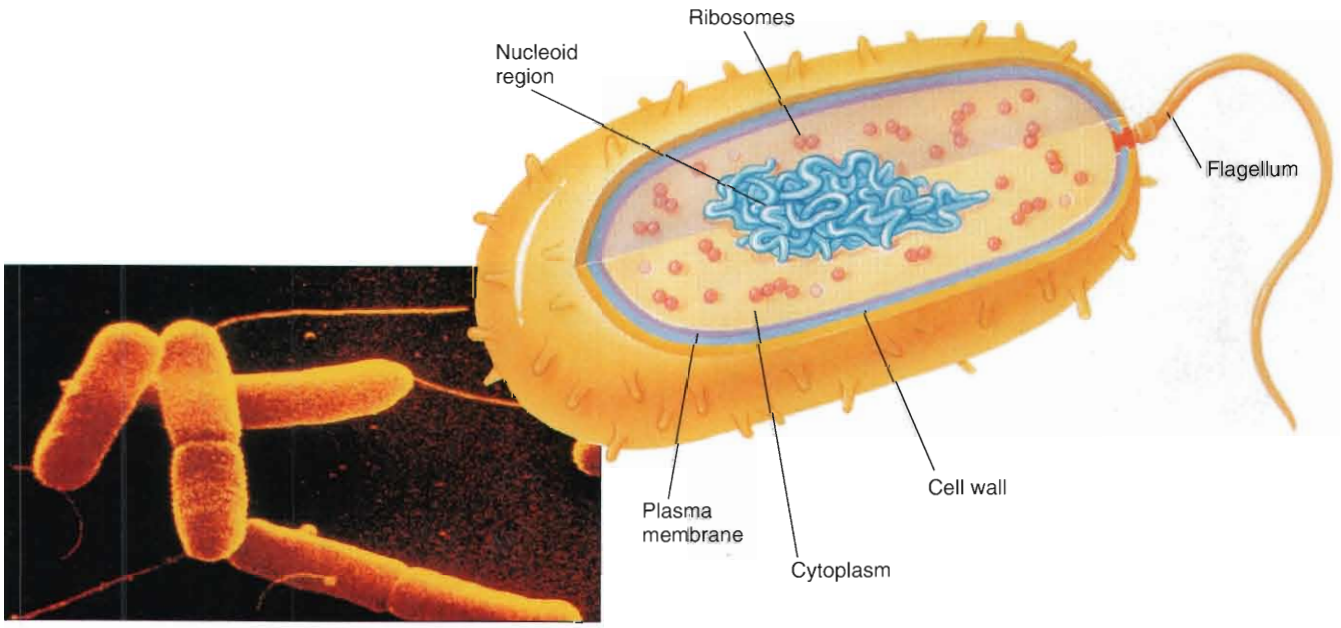


Figure 4.1
The structure of a bacterial cell. Bacteria lack a nuclear membrane. All prokaryotic (bacterial) cells have a nucleoid region, ribosomes, plasma membrane, cytoplasm, and cell wall, but not all have flagella (1500×).

outgrowths called **pili** are common in prokaryotes; flagella are used for movement, and pili are used to attach some types of bacteria to surfaces or to exchange genetic material with other bacteria. Within the cytoplasm of prokaryotes are **ribosomes** (small particles involved in protein synthesis) and **nucleoid regions** (concentrations of DNA). Prokaryotes do not reproduce sexually, but they have mechanisms for genetic recombination (see Exercise 16).

Cyanobacteria

The largest prokaryotes are **cyanobacteria,** also called blue-green algae. They contain chlorophyll *a* and accessory pigments for photosynthesis, but these pigments are not contained in membrane-bound chloroplasts. Instead, the pigments are held in photosynthetic membranes called **thylakoids** (fig. 4.2). Cyanobacteria are often surrounded by a **mucilaginous sheath.** Their ability to photosynthesize made them the primary contributors to the early oxygenation of the ancient earth's atmosphere.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

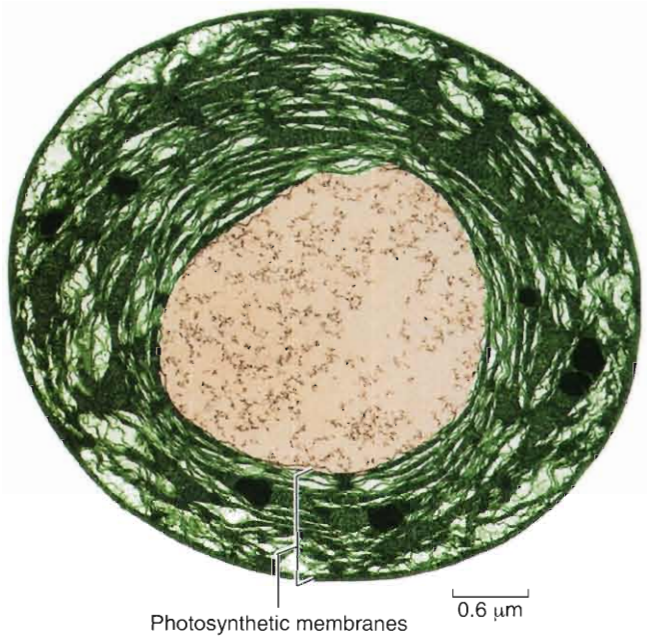


Figure 4.2

Electron micrograph of a photosynthetic bacterial cell, *Prochloron*, showing extensively folded photosynthetic membranes. The DNA is in the clear area in the central region of the cell; it is not membrane-bound (5200×).

Procedure 4.1

Examine cyanobacteria

1. Examine a prepared slide of *Oscillatoria*, a filament of cells, and one of *Gloeocapsa*, a loosely arranged colony (fig 4.3). Review Exercise 3 for the correct steps to use the microscope.
2. Focus with the low-power objective.
3. Rotate the high-power objective into place to see filaments and masses of cells.
4. Prepare a wet mount of *Oscillatoria* and one of *Gloeocapsa*. Review procedure 3.5 in Exercise 3 for preparing a wet mount.
5. Observe the cellular structures and draw the cellular shapes and relative sizes of *Oscillatoria* and *Gloeocapsa* in the following space. Use an ocular micrometer to measure their dimensions.

Oscillatoria *Gloeocapsa*

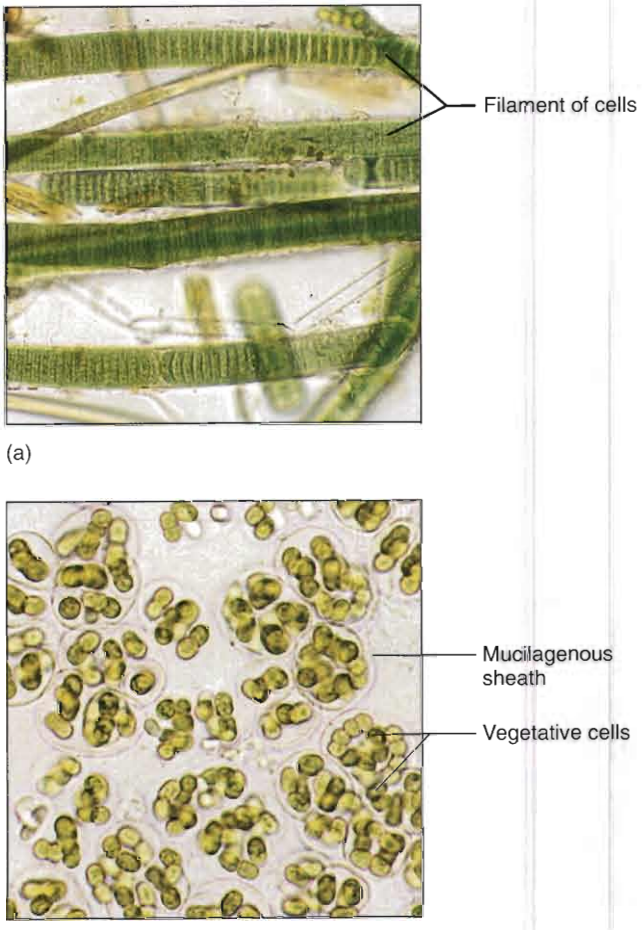


Figure 4.3

Common cyanobacteria. (*a*) *Oscillatoria* (40×). (*b*) *Gloeocapsa* (400×).

Question 1

a. Where are the pigments located in these cyanobacteria?

b. Are nuclei visible in cyanobacterial cells?

c. Which of these two genera has the most prominent mucilaginous sheath?

d. How many cells are held within one sheath of *Gloeocapsa?*

Bacteria

Most bacteria are much smaller than cyanobacteria and do not contain chlorophyll. Yogurt is a nutrient-rich culture of bacteria. The bacterial cells composing most of the yogurt are *Lactobacillus*, a bacterium adapted to live on milk sugar (lactose). *Lactobacillus* converts milk to yogurt. Yogurt is acidic and keeps longer than milk. Historically, *Lactobacillus* has been used in many parts of the world by peoples deficient in lactase, an enzyme that breaks down lactose. Many Middle Eastern and African cultures use the more digestible yogurt in their diets instead of milk.

Procedure 4.2

Examine bacteria

1. Place a tiny dab of yogurt on a microscope slide.
2. Mix this small amount of yogurt in a drop of water, add a coverslip, and examine the yogurt with a compound microscope. Review Exercise 3.
3. Focus with the low-power objective.
4. Rotate the high-power objective (40×) into place to see masses of rod-shaped cells.
5. Observe the simple, external structure of the bacteria and draw their cellular shapes in the following space:

Question 2

How does the size of *Lactobacillus* compare with that of *Oscillatoria* and *Gloeocapsa?*

EUKARYOTIC CELLS

Eukaryotic cells contain membrane-bound **nuclei** and other organelles (figs. 4.4, 4.5). Nuclei contain genetic material of a cell and control metabolism. **Cytoplasm** forms the matrix of the cell and is contained by the plasma membrane. Within the cytoplasm are a variety of organelles. **Chloroplasts** are elliptical green organelles in plant cells. Chloroplasts are the site of photosynthesis in plant cells and are green because they contain chlorophyll, a photosynthetic pigment capable of capturing light energy. **Mitochondria** are organelles found in plant and animal cells. These organelles are where aerobic respiration occurs. When viewed with a conventional light microscope, mitochondria are small, dark, and often difficult to see. All of the material and organelles contained by the plasma membrane are collectively called the **protoplast.**

Eukaryotic cells are structurally more complex than prokaryotic cells. Although some features of prokaryotic cells are in eukaryotic cells (e.g., ribosomes, cell membrane), eukaryotic cells also contain several organelles not found in prokaryotic cells (table 4.1). In the following exercise you will investigate some of these organelles.

PLANT CELLS

Procedure 4.3

Examine living *Elodea* cells and chloroplasts

1. Remove a young leaf from the tip of a sprig of *Elodea*. *Elodea* is a common pond weed used frequently in studies of photosynthesis, cellular structure, and cytoplasmic streaming.
2. Place this leaf, with the top surface facing up, in a drop of water on a microscope slide. The cells on the upper surface are larger and more easily examined. Add a coverslip, but do not let the leaf dry. Add another drop of water if necessary.
3. Examine the leaf with your microscope. Review Exercise 3. First use low, then high, magnification to bring the upper layer of cells into focus (fig. 4.6). Each of the small, regularly shaped units you see are cells surrounded by cell walls made primarily of **cellulose** (fig. 4.7). Cellulose is a complex carbohydrate made of glucose molecules attached end-to-end. The plasma membrane lies just inside the cell wall. Sketch what you see.

Question 3

a. What three-dimensional shape are *Elodea* cells?

b. Examine various layers of cells by focusing up and down through the layers. About how many cells thick is the leaf that you are observing?

c. What are the functions of the cell wall?

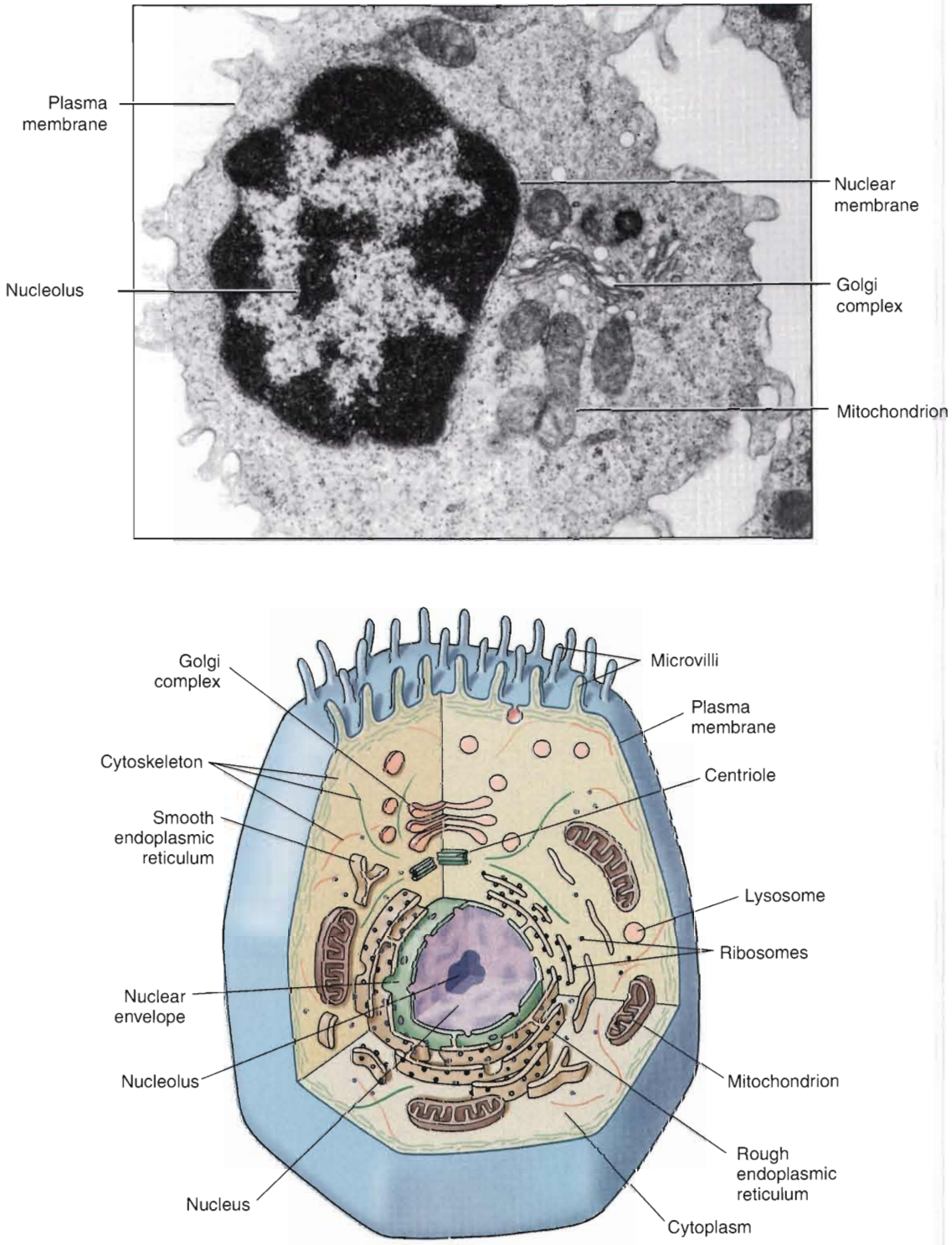
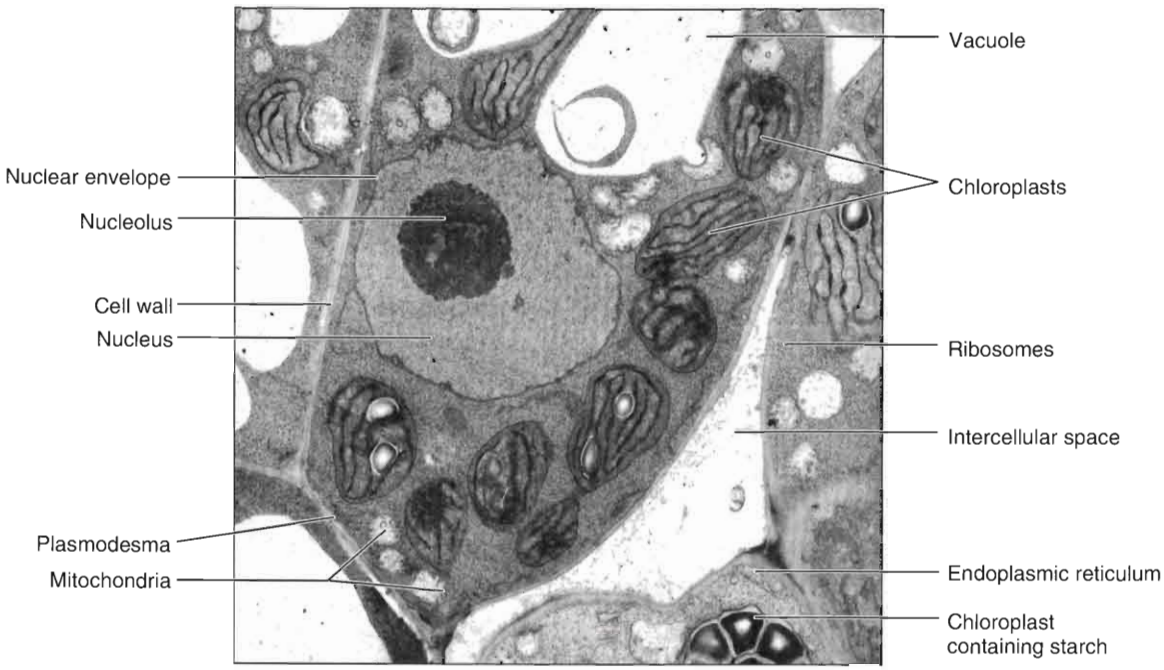


Figure 4.4

Structure of animal cells. Most organelles of animal cells are not visible with common light microscopes—therefore, our understanding of cellular structures is based mainly on research using electron micrographs, as shown with the upper photo and diagram. Cells are surrounded by a bilayered plasma membrane containing phospholipids and proteins. The nucleus houses chromosomal DNA and is surrounded by a double-membraned nuclear envelope. Centrioles organize spindle fibers during cell division. Endoplasmic reticulum (ER) is a system of membranes inside the cell. Rough ER has many ribosomes, and smooth ER has fewer ribosomes. Mitochrondria are sites of oxidative respiration and ATP synthesis. Microvilli are cytoplasmic projections that increase the surface area of some specialized animal cells. Golgi complexes are flat sacs and vesicles that collect and package substances made in the cell. Ribosomes are aggregations of proteins that conduct protein synthesis. Lysosomes contain enzymes important in recycling cellular debris (17,500×).



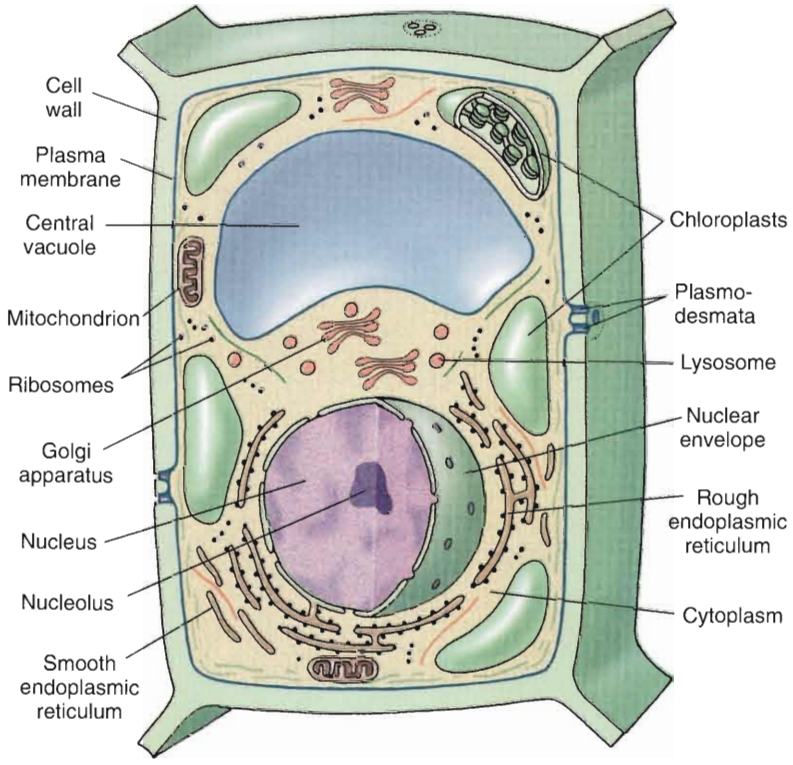


Figure 4.5

Structure of plant cells. This illustration shows relative proportions of the different parts of a plant cell. Most mature plant cells contain large central vacuoles, which occupy most of the volume of the cell. Cytoplasm is often a thin layer between the vacuole and the plasma membrane. Cytoplasm contains the cell's organelles (17,500×).

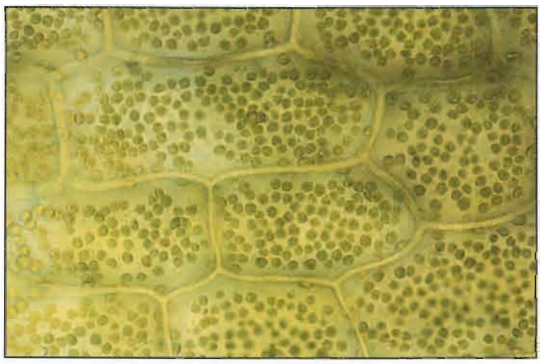
TABLE 4.1

SOME OF THE MAJOR DIFFERENCES BETWEEN PROKARYOTIC AND EUKARYOTIC CELLS AND BETWEEN PLANT AND ANIMAL CELLS

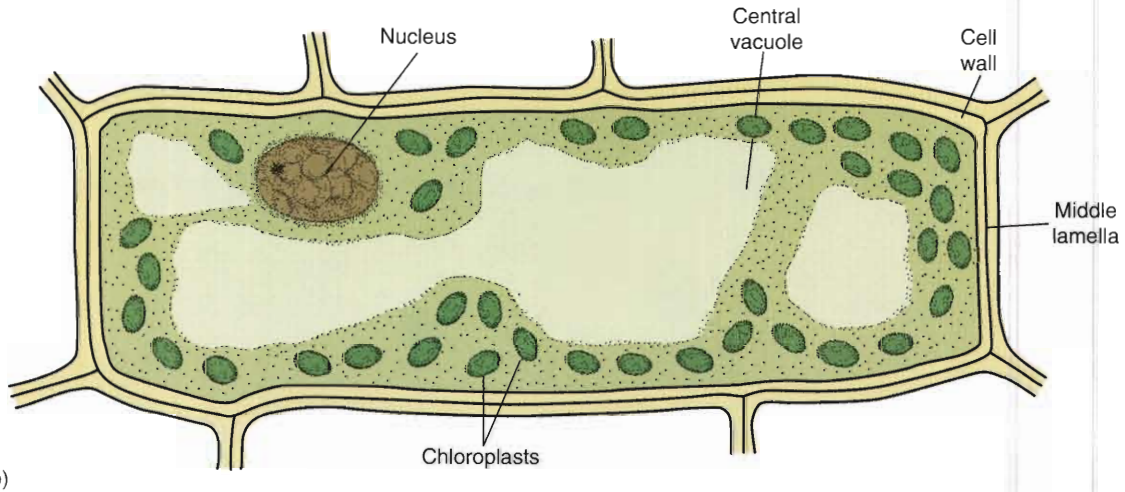
	Prokaryote	Eukaryote	
		Animal	Plant
EXTERIOR STRUCTURES			
Cell wall	Present (protein-polysaccharide)	Absent	Present (cellulose)
Cell membrane	Present	Present	Present
Flagella	May be present (single strand)	May be present	Absent except in sperm of a few species
INTERIOR STRUCTURES			
ER	Absent	Usually present	Usually present
Ribosomes	Present	Present	Present
Microtubules	Absent	Present	Present
Centrioles	Absent	Present	Absent
Golgi complex	Absent	Present	Present
OTHER ORGANELLES			
Nucleus	Absent	Present	Present
Mitochondria	Absent	Present	Present
Chloroplasts	Absent	Absent	Present
Chromosomes	A single circle of naked DNA	Multiple; DNA-protein complex	Multiple; DNA-protein complex
Vacuoles	Absent	Absent or small	Usually a large single vacuole

Figure 4.6

(*a*) *Elodea* cells showing abundant chloroplasts (400×). (*b*) The cellular structure of *Elodea* (150×).



(a)



(b)

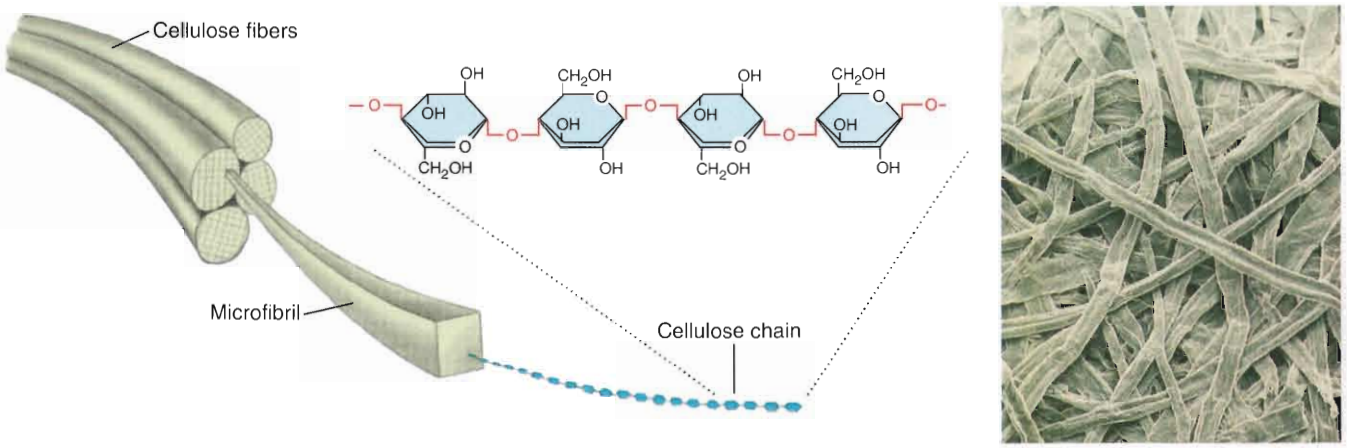


Figure 4.7

Cellulose is the most abundant organic compound on earth and is a polymer of glucose molecules. Free hydroxyl (OH^-) groups of the glucose molecules form hydrogen bonds between adjacent cellulose molecules to form cohesive microfibrils. Microfibrils align to form strong cellulose fibers that resist metabolic breakdown. Because humans cannot hydrolyze the bonds between glucose molecules of cellulose, cellulose is indigestible and its energy is unavailable. Cellulose passes through the human digestive tract as bulk fiber (20×).

d. Use an ocular micrometer or refer to the dimensions of the field of view calculated in Exercise 3 to measure the dimensions of an *Elodea* cell. What are the cell's approximate dimensions?

4. Chloroplasts appear as moderately sized green spheres within the cells (figs. 4.6, 4.8). Locate and sketch cells having many chloroplasts; estimate the number of chloroplasts in a healthy cell. Remember that a cell is three-dimensional, and some chloroplasts may obscure others.

Question 4

a. What shape are the chloroplasts? What is their function?

b. Where are the chloroplasts located within the *Elodea* cell—toward the perimeter or centrally located?

5. Determine the spatial distribution of chloroplasts within a cell. They may be pushed against the margins of the cell by the large **central vacuole** containing mostly water and bounded by a **vacuolar membrane.** The vacuole occupies about 90% of the volume of a mature cell. Its many functions include storage of organic and inorganic molecules, ions, water, enzymes, and waste products.

6. Search for a **nucleus;** it may or may not be readily visible. Nuclei usually are appressed to the cell wall as a faint gray sphere the size of a chloroplast or larger. Staining the cells with a drop of iodine may enhance the nucleus. If your preparation is particularly good, a **nucleolus** may be visible as a dense spot in the nucleus.

7. Search for some cells that may appear pink due to water-soluble pigments called anthocyanins. These pigments give many flowers and fruits their bright reddish color.

8. Warm the slide with intense light for about 10 min and search for movement of the chloroplasts. You may need to search many cells or make a new preparation. This movement is called **cytoplasmic streaming,** or **cyclosis.** Chloroplasts are not motile; instead, they are being moved by the activity of the cytoplasm. Add water if the cells appear to be drying out.

9. In the following space sketch a few cells of *Elodea;* compare the cells with those shown in figure 4.6.

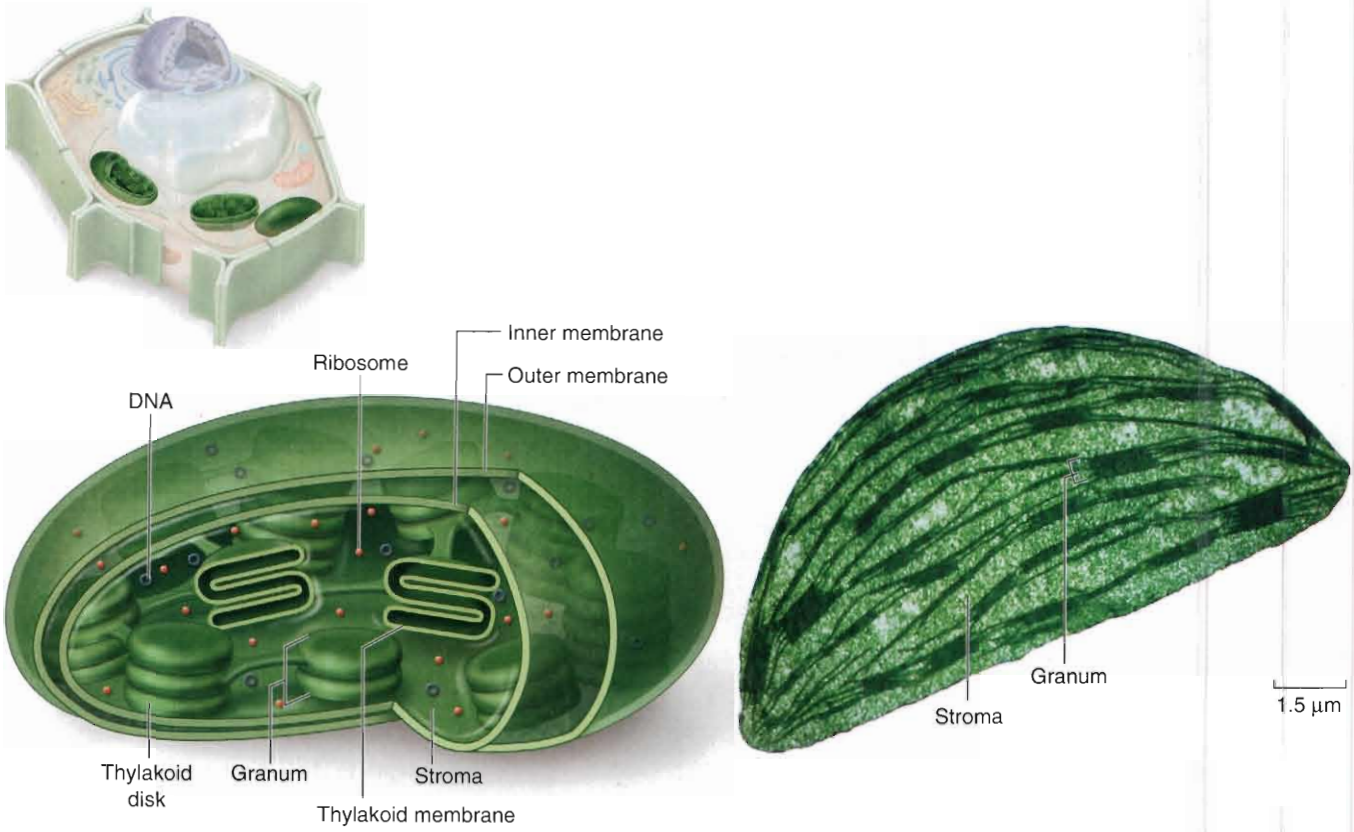


Figure 4.8

Chloroplast structure. The inner membrane of a chloroplast is fused to form stacks of closed vesicles called thylakoids. Photosynthesis occurs within these thylakoids. Thylakoids are typically stacked one on top of the other in columns called grana.

10. When you are finished examining *Elodea*, dispose of the *Elodea* as specified by your instructor.

Question 5

a. Can you see nuclei in *Elodea* cells?

b. What are the functions of nuclei?

c. Which are larger, chloroplasts or nuclei?

d. What is the approximate size of a nucleus?

e. Why is the granular-appearing cytoplasm more apparent at the sides of a cell rather than in the middle?

Question 6

a. Are all cellular components moving in the same direction and rate during cytoplasmic streaming?

b. What do you conclude about the uniformity of cytoplasmic streaming?

Cell Walls

Cell walls include an outer **primary cell wall** deposited during growth of the cell and a **middle lamella,** the substance holding walls of two adjacent cells together. The protoplasm of adjacent cells is connected by cytoplasmic strands called **plasmodesmata** that penetrate the cell walls (fig. 4.9).

Procedure 4.4

Examine cell walls and plasmodesmata

1. Prepare a wet mount of *Elodea* and examine the cell walls. Always begin your examination at the lowest magnification and cautiously move to higher magnifications. The middle lamella may be visible as a faint line between cells.
2. Obtain a prepared slide of tissue showing plasmodesmata. This tissue may be persimmon (*Diospyros*) endosperm, which has highly thickened primary walls. Sketch what you see.

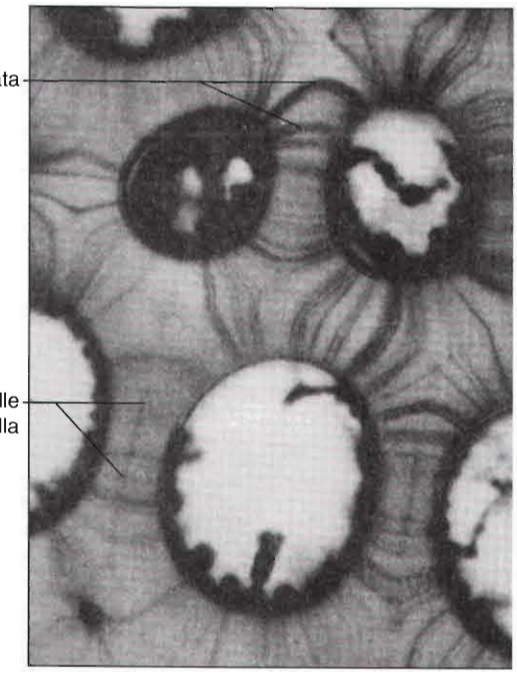


Figure 4.9
The thickened primary cell walls of persimmon endosperm show plasmodesmata connecting adjacent cells. Middle lamella appear as faint lines parallel to the cell surface (950×).

3. Locate the middle lamella as a faint line between cell walls.
4. Locate the plasmodesmata appearing as darkened lines perpendicular to the middle lamella and connecting the protoplasts of adjacent cells (fig. 4.9).

Question 7

a. What are the functions of plasmodesmata?

b. Why do you suspect that there are so many plasmodesmata connecting the cells in this fruit?

Onion Cells

Staining often reveals the structure of cells and cell organelles more clearly. A specimen is **stained** by adding a dye that preferentially colors some parts of the specimen but not others. Neutral red is a common stain that accumulates in the cytoplasm of the cell, leaving the cell walls clear. Nuclei appear as dense bodies in the translucent cytoplasm of the cells.

Procedure 4.5

Examine stained onion cells

1. Cut a red onion into eighths and remove a fleshy leaf.
2. Snap the leaf backward and remove the thin piece of the inner epidermis formed at the break point (fig. 4.10), as demonstrated by your lab instructor.
3. Place this epidermal tissue in a drop of water on a microscope slide, add a coverslip, and examine the tissue. This preparation should be one cell thick. Always begin your examination with the lowest magnification.
4. Stain the onion cells by placing a small drop of 0.1% neutral red at the edge of the coverslip. Draw the neutral red across the specimen by wicking. To wick the solution, hold the edge of a small piece of paper towel at the opposite edge of the coverslip and it will withdraw some fluid. This will cause the neutral red to flow over the onion and will not disturb the tissue under the coverslip.
5. Stain the tissue for 5–10 min.
6. Carefully focus to distinguish the vacuole surrounded by the stained cytoplasm.
7. Search for the nucleus of a cell (fig. 4.11). The nucleus may appear circular in the central part of the cell. In other cells it may appear flattened.

Question 8

How do you explain the differences in the apparent shapes and positions of the nuclei in different cells?

8. Repeat steps 1–7 and stain a new preparation of onion cells with other available stains, such as methylene blue.
9. In the following space sketch a few of the stained onion cells.

Question 9

a. What cellular structures of onion are more easily seen in stained as compared to unstained preparations?

b. Which of the available stains enhanced your observations the most?

c. Do onion cells have chloroplasts? Explain.

d. Use an ocular micrometer or the dimensions of the field of view (FOV) calculated in Exercise 3 to measure the dimensions of an onion epidermal cell. Are these cells larger or smaller than the *Elodea* cells you examined in procedure 4.3?

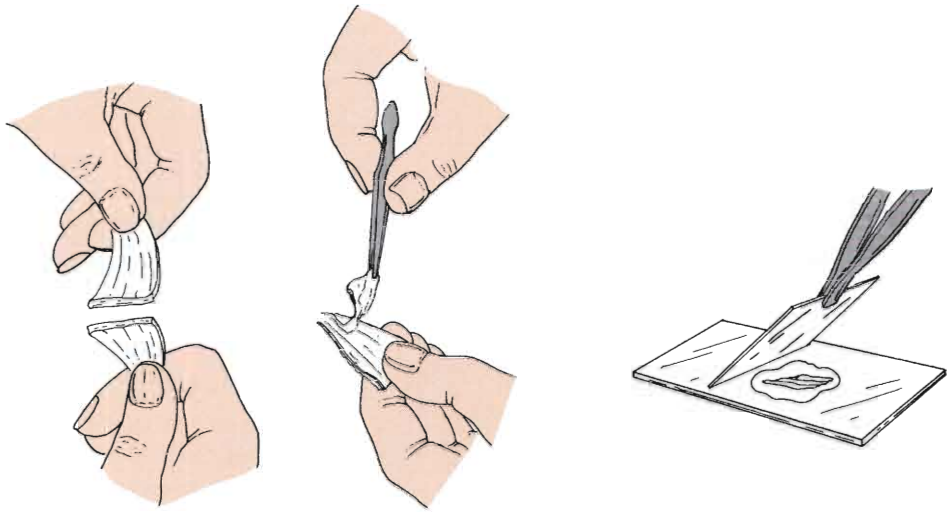


Figure 4.10
Preparing a wet mount of an onion epidermis.

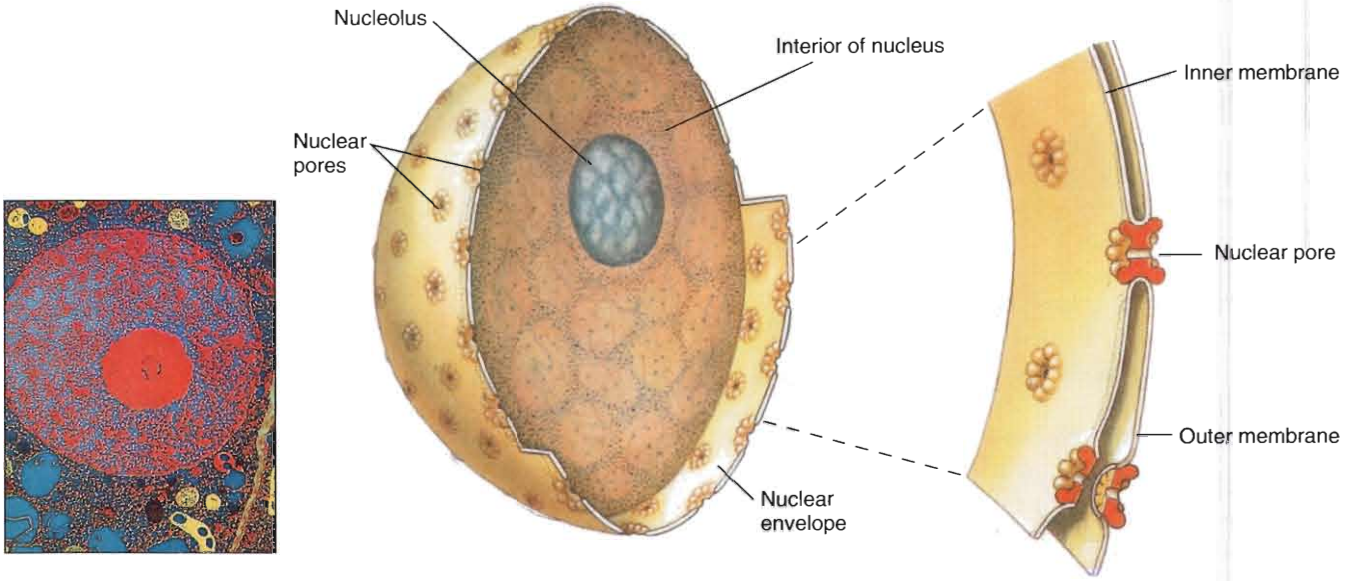


Figure 4.11
The nucleus. The nucleus consists of a double membrane, called a nuclear envelope, enclosing a fluid-filled interior containing the DNA. In the cross section, the individual nuclear pores extend through the two membrane layers of the envelope; the material within the pore is protein, which controls access through the pore (1765×).

Mitochondria

Mitochondria are surrounded by two membranes (fig 4.12). The inner membrane folds inward to form **cristae,** which hold respiratory enzymes and other large respiratory molecules in place. Some DNA also occurs in mitochondria. Chloroplasts also are double-membraned and contain DNA.

Procedure 4.6

Examine mitochondria in onion cells

1. On a clean glass slide mix two or three drops of the stain Janus Green B with one drop of 7% sucrose.
2. Prepare a thin piece of onion epidermis (as instructed in procedure 4.5) and mount it in the staining solution. The preparation should be one cell thick. For mitochondria to stain well, the onion cells must be healthy and metabolically active. Add a coverslip.
3. Search the periphery of cells to locate stained mitochondria. They are small blue spheres about 1 μm in diameter. The color will fade in 5–10 min, so examine your sample quickly and make a new preparation if needed.

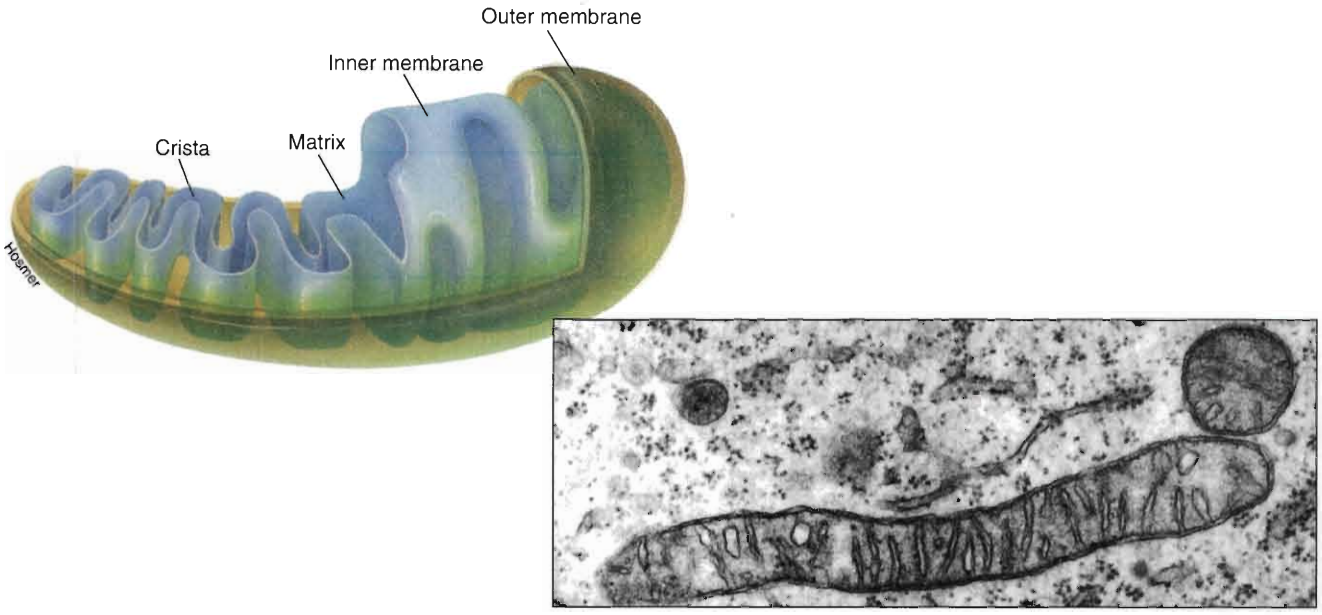


Figure 4.12
Mitochondrion in cross section. Mitochondria evolved from bacteria that long ago took up residence within the ancestors of present-day eukaryotes (80,000×).

Plastids

Plastids are organelles where food is made and stored. You have already examined chloroplasts, a type of plastid in which photosynthesis occurs. Other plastids have different functions. We will examine **amyloplasts,** plastids that store starch and therefore will stain darkly with iodine.

Procedure 4.7

Examine amyloplasts

1. Use a razor blade to make a thin section of a potato tuber. Make the section as thin as you can.
2. Place the section in a drop of water on a microscope slide and add a coverslip. Add another drop of water to the edge if needed.
3. Locate the small, clam-shaped amyloplasts within the cells. High magnification may reveal the eccentric lines distinguishing layers of deposited starch on the grains.
4. Stain the section by adding a drop of iodine to the edge of the coverslip. Iodine is a stain specific for starch (see Exercise 6, "Biologically Important Molecules"). If necessary, pull the stain under the coverslip by touching a paper towel to the water at the opposite edge of the coverslip.

Question 10

a. Are any cellular structures other than amyloplasts stained intensely by iodine?

b. What can you conclude about the location of starch in storage cells of potato?

c. What are the functions of amyloplasts in potatoes?

d. Why are potatoes a good source of carbohydrates?

ANIMAL CELLS

Animals, like plants, are eukaryotes. They share many similarities, and also have several differences (table 4.1).

Human Epithelial Cells

Human epithelial cells are sloughed from the inner surface of your mouth. They are flat cells with a readily visible nucleus.

Procedure 4.8

Examine human epithelial cells

1. Gently scrape the inside of your cheek with the broad end of a clean toothpick.
2. Stir the scrapings into a drop of water on a microscope slide, add a coverslip, and examine with your compound microscope. Dispose of used toothpicks in a container designated by your instructor.

3. Stain the cells by placing a small drop of methylene blue at one edge of the coverslip and drawing it under the coverslip with a piece of absorbent paper towel placed at the opposite side of the coverslip.
4. Prepare another slide and stain the cells with Janus Green B. Observe the mitochondria.
5. Use an ocular micrometer or the dimensions of the FOV calculated in Exercise 3 to measure the dimensions of a human epithelial cell.

Question 11

a. What structures visible in the stained preparation were invisible in the unstained preparation?

b. Were mitochondria as abundant in human epithelial cells as in onion epidermal cells (procedure 4.6)? Explain.

c. What similarities and differences are there between plant and animal cells?

d. How do the size and shape of a human epithelial cell differ from those of the *Elodea* and onion cells that you examined earlier?

e. Why do *Elodea* and onion cells have more consistent shapes than human epithelial cells?

6. After viewing the preparation, put the slides and coverslips in a container of 10% bleach.

PROTISTS

Amoeba and *Paramecium* are unicellular members of a large group of eukaryotic organisms called protists. You will learn more about protists in Exercises 24 and 25. In today's exercise, you'll examine *Amoeba* and *Paramecium*.

Amoeba

Amoeba is an irregularly shaped protist with many internal organelles (fig. 4.13). *Amoeba* move via amoeboid movement. **Amoeboid movement** occurs by means of **pseudopodia,** temporary protrusions of the cell. Pseudopodia also surround food particles and create food vacuoles, where food is digested. Another important structure in *Amoeba* is the **contractile vacuole** that accumulates and expels water and waste products.

Procedure 4.9

Examine *Amoeba*

1. Use an eyedropper to obtain a few drops from the bottom of an *Amoeba* culture. Examining the culture with a dissecting microscope may help you locate some organisms.
2. Place the organisms on a microscope slide.
3. Add a coverslip and use a compound microscope to locate a living *Amoeba*. Your instructor may allow you to view the *Amoeba* without using a coverslip, but view them *only* on 4× or 10× magnification.
4. Decrease the light intensity and observe an *Amoeba* for a few minutes.
5. Locate the structures shown in figure 4.13.
6. Examine a prepared slide of stained *Amoeba*; then observe a demonstration of *Amoeba* on a dark-field microscope if one is available.
7. Sketch an *Amoeba* in the following space.

Question 12

a. List the organelles found in plant cells, in *Amoeba*, and common to both.

b. Does *Amoeba* have a cell wall? How can you tell?

c. How do the appearances of *Amoeba* differ in live cells and preserved cells?

Paramecium

Like *Amoeba*, *Paramecium* is also a single-celled organism (fig. 4.14).

Procedure 4.10

Examine *Paramecium*

1. Place a small ring of methylcellulose on a microscope slide to slow the *Paramecium*.
2. Place a drop from a culture containing *Paramecium* inside the methylcellulose ring.
3. Use a toothpick to mix the methylcellulose with the drop of water from the culture of *Paramecium*.
4. Add a coverslip and examine *Paramecium* with your compound microscope. On the surface of *Paramecium* are cilia, short hairlike structures used for locomotion.

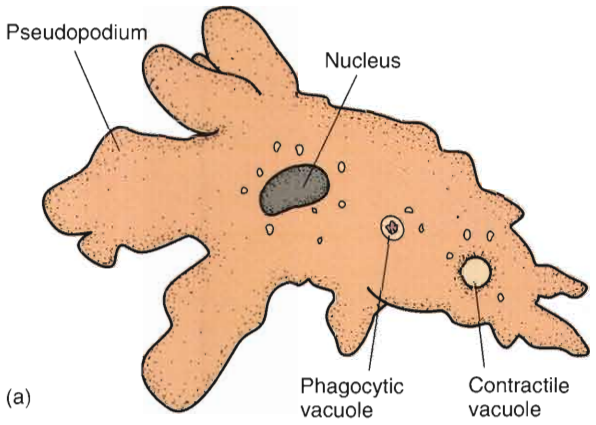
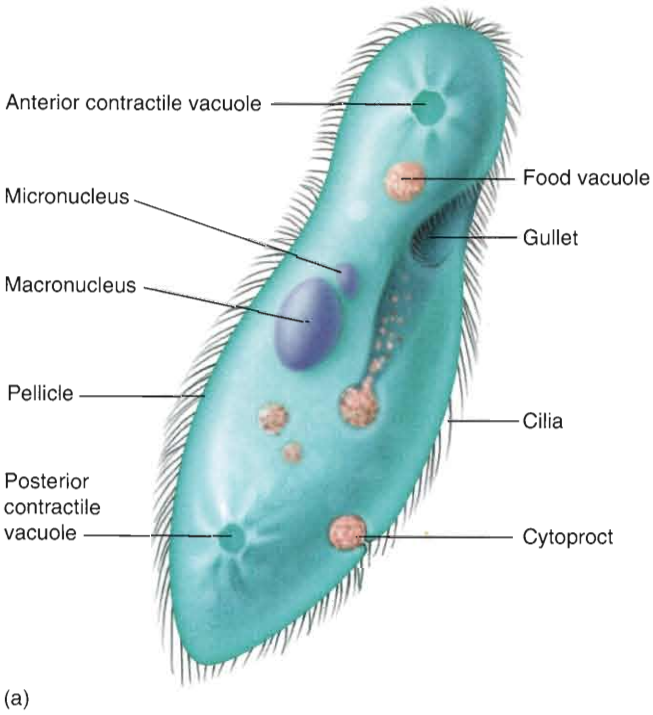


Figure 4.13
(*a*) Diagram of *Amoeba*. (*b*) Light micrograph of a living *Amoeba* (160×).



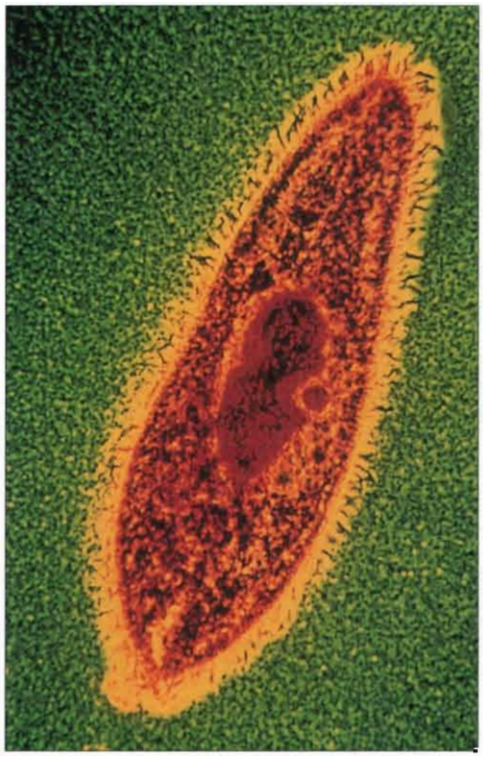


Figure 4.14
(*a*) Diagram of *Paramecium* (150×). (*b*) Light micrograph of a living *Paramecium*. Note the abundant cilia (150×).

5. Examine a prepared slide of stained *Paramecium*.
6. In the following space, sketch a *Paramecium*.

Question 13

a. How does movement of *Paramecium* compare to that of *Amoeba*?

b. How do shape and body consistency differ between *Amoeba* and *Paramecium*?

c. What structures in *Amoeba* and *Paramecium* also occur in plant cells? What structures in *Amoeba* and *Paramecium* do not occur in plant cells?

Procedure 4.11

You will be given a slide of an unknown organism. Use what you've learned in today's lab to identify the cells as prokaryotic or eukaryotic; if eukaryotic, identify the cells as plant, animal, or protist. Complete table 4.2 before leaving the lab. If instructed to do so, turn in table 4.2 before leaving the lab.

INVESTIGATION

The Responses of Single-Celled Organisms to Environmental Stimuli

Observation: Single-celled protists such as *Paramecium* and *Amoeba* live in water and are sensitive to environmental stimuli.

Question: How are the movements of single-celled protists affected by temperature?

a. Establish a working lab group and obtain Investigation Worksheet 4 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 4 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

TABLE 4.2

USING DISTINGUISHING FEATURES TO IDENTIFY AN UNKNOWN ORGANISM

OVERALL DESCRIPTION OF SPECIMEN:

NAME _____

UNKNOWN NO: _____

LAB SECTION: _____

BASED ON THE ABOVE, MY UNKNOWN ORGANISM IS A: (Circle One)	Prokaryote	Eukaryote	
IF THE SPECIMEN IS A EUKARYOTE, IT IS A(N): (Circle One)	Plant	Animal	Protist

Questions for Further Thought and Study

1. What is a cell?

2. Describe the structure and function of each cellular part that you observed with a light microscope.

3. Would you expect a cell of a multicellular organism to be more complex than the cell of a unicellular organism? Less complex? Why?

4. What is the purpose of using a biological stain when microscopically examining cellular components?

5. How are eukaryotic cells different from prokaryotic cells? How are they similar?

DOING BIOLOGY YOURSELF

Determine the total surface areas and volumes of the chloroplasts in a typical *Elodea* cell. Assume that each chloroplast is a sphere of 5 μm diameter. (The surface area of a sphere = πd^2; the volume of a sphere = $(\frac{4}{3})\pi r^3$.) What is the significance of these surface areas and volumes? Would it be advantageous for a cell to be filled with chloroplasts? Why or why not?

WRITING TO LEARN BIOLOGY

What criteria might you use to distinguish colonial organisms, such as many cyanobacteria, from truly multicellular organisms?

Solutions, Acids, and Bases
The pH Scale

5

Objectives

By the end of this exercise you should be able to:

1. Apply the concepts of mole and molarity to prepare solutions.
2. Measure the pH of various liquids.
3. Demonstrate that buffers stabilize the pH of a liquid.
4. Measure the ability of commercial antacids to buffer the pH of a liquid.

Chemicals in living systems are in solution. Biologists experiment with solutions because dissolved chemicals are more readily available to react than are solid, crystalline chemicals. A **solution** consists of a **solute**(s) dissolved in a **solvent.** For example, salt water is a solution in which salt (i.e., the solute) is dissolved in water (i.e., the solvent).

The concentration of a solute is often expressed as a percentage of the total solution (e.g., weight/volume or grams solute/100 mL solution). For example, a 3% (weight/volume) solution of sucrose is prepared by dissolving 30 g of sucrose in water for a total solution of 1 L (or 3 g of sucrose in water for a total volume of 100 mL).

Question 1

a. How many grams of sucrose would you dissolve in water for a total volume of 500 mL to make a 5% (weight/volume) solution?

b. How many grams of calcium chloride would you add to water for a total volume of 500 mL to make a 5% (weight/volume) solution?

c. How many grams of calcium chloride would you add to water for a total volume of 100 mL to make a 5% (weight/volume) solution?

Molarity is the most common measure of concentration. To understand how to prepare a molar solution you must first understand what is meant by a **mole** of a chemical. A mole is a standard measure of the amount of a chemical—one mole of any substance has 6.02×10^{23} molecules (Avogadro's number). One mole of NaCl and one mole of sucrose contain the same number of molecules. However, a mole of NaCl and a mole of sucrose weigh different amounts. This is because each chemical has a different **molecular weight,** and the weight of 1 mole of a chemical equals that chemical's molecular weight in grams. For example, the molecular weight of water (H_2O) is 18 g ($2H = 2 \times 1 = 2$; $O = 16$; $16 + 2 = 18$). A mole of water weighs 18 g. A mole of NaCl weighs 58.5 g (fig. 5.1). A chemical's molecular weight is the sum of the atomic weights of its component elements.

To further understand why biologists usually prepare solutions in molar concentrations rather than as percentages you must remember that chemicals react on a molecule by molecule basis—the number of molecules is more critical than the weight. It follows that expressing a solution's concentration in moles is a better measure of how much chemical is available to react. A solution that contains one mole of a chemical in 1 liter of solution has 6.02×10^{23} molecules available and is a 1-molar (1 M) solution. For example, a liter of solution containing 58.5 g of NaCl is a 1 M solution of NaCl (fig. 5.2).

To ensure that you have some practice with making basic chemical calculations, answer Questions 2 and 3 before coming to lab. Your instructor may want to check your answers during the lab period.

Question 2

a. How many grams of NaCl (molecular weight = 58.5 g $mole^{-1}$) would you dissolve in water to make a 0.5 M NaCl solution with 500 mL final volume?

_____ g

b. How many grams of NaCl (molecular weight = 58.5 g $mole^{-1}$) would you dissolve in water to make a 50 mM NaCl solution with 500 mL final volume?

_____ g

c. How many grams of sucrose (molecular weight = 342 g $mole^{-1}$) would you dissolve in water to make a 0.22 M sucrose solution with 1 L final volume?

_____ g

d. How many grams of sucrose (molecular weight = 342 g $mole^{-1}$) would you dissolve in water to make a 0.22 mM sucrose solution with 100 mL final volume?

_____ g

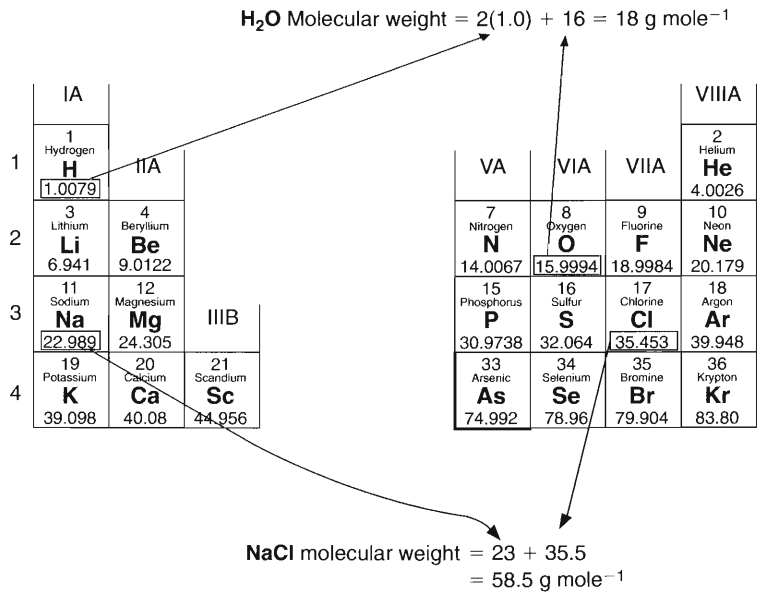


Figure 5.1

The atomic weights of elements are listed in the periodic table. Shown here are the portions of the periodic table that would be used to calculate the molecular weights of water (H_2O) and table salt (sodium chloride, NaCl). Note that g mole^{-1} = grams per mole.

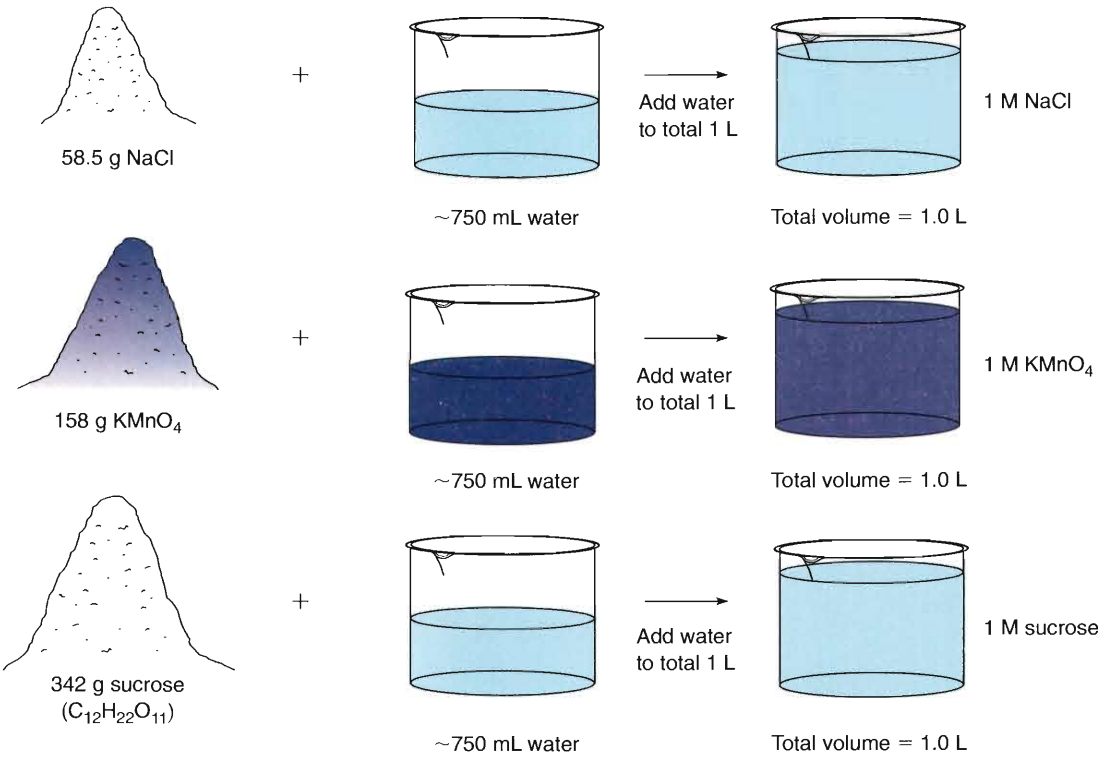


Figure 5.2

Preparing 1.0 M solutions of sodium chloride (NaCl; molecular weight = 58.5 g mole^{-1}), potassium permanganate ($KMnO_4$; molecular weight = 158 g mole^{-1}), and sucrose ($C_{12}H_{22}O_{11}$; molecular weight = 342 g mole^{-1}). Each of these solutions contains the same number of units of solutes (i.e., 6.02×10^{23} molecules).

e. How many grams of calcium chloride ($CaCl_2$; molecular weight = 111 g mole^{-1}) would you dissolve in water to make a total 0.111 M $CaCl_2$ solution with 1 L final volume?

_____ g

f. How many grams of calcium chloride ($CaCl_2$; molecular weight = 111 g mole^{-1}) would you dissolve in water to make a 0.2 M $CaCl_2$ solution with 200 mL final volume?

_____ g

g. If you were presented with 2 L of a 2 M sucrose stock solution, how many grams of sugar would be in a 100 mL aliquot?

_____g

h. To prepare the 5% sucrose solution called for in Question 1a how many moles of sugar did you add? What was the molarity of that solution?

i. To prepare the 5% calcium chloride solution called for in Question 1b how many moles of calcium chloride did you add? What was the molarity of that solution?

j. How many milliliters of a 2 M sucrose solution would contain 1 mole of sucrose?

Dilutions

To save time and space, biologists often prepare commonly used solutions in concentrated forms called **stock solutions.** These stock solutions are then diluted with water to make new solutions having a desired molarity. This process is called **dilution.**

Dilution involves spreading a given amount of solute throughout a larger solution. The number of moles of solute doesn't change when a solution is diluted but the volume of solution containing those moles increases. This means that the product of the initial volume (V_i) and initial molarity (M_i) must equal the product of the final volume (V_f) and final molarity (M_f):

$$V_iM_i = V_fM_f$$

where

V_i = initial volume
M_i = initial molarity
V_f = final volume
M_f = final molarity

Let's now use this simple equation to solve a dilution problem. Suppose we want to know how much water to add to 25 mL of a 0.50 M KOH solution to produce a solution having a KOH concentration of 0.35 M. In this case,

$M_i = 0.5$ M
$V_i = 25$ mL
$M_f = 0.35$ M
$V_f = ?$

We can now solve the problem:

$$V_iM_i = V_fM_f$$
$$(25 \text{ mL})(0.5 \text{ M}) = (V_f)(0.35 \text{ M})$$
$$V_f = 35.7 \text{ mL}$$

The initial volume (V_i) was 25 mL, so we must subtract 25 mL from 35.7 mL to get our answer: 35.7 mL − 25 mL = 10.7 mL of water to produce a KOH solution having a concentration of 0.35 M.

Question 3

a. How many mL of concentrated (18 M) sulfuric acid (H_2SO_4) are required to prepare 750 mL of 3 M sulfuric acid?

b. How would you prepare 100 mL of 0.4 M $MgSO_4$ from a stock solution of 2 M $MgSO_4$?

c. How many milliliters of water would you add to 100 mL of 1.0 M HCl to prepare a final solution of 0.25 M HCl?

ACIDS AND BASES

One of the most important applications of molarity involves the concentration of hydrogen ions (H^+) in a solution. Pure water is the standard by which all other solutions are compared, because water is an ionically neutral solution. This neutrality is not due to the absence of ions, but rather to the equal concentrations of positive and negative ions. When the oxygen of water pulls hard enough on an electron from one of its hydrogens, two ions form:

$$H_2O \longleftrightarrow H^+ + OH^-$$

This dissociation of water is rare and reversible, but it happens often enough for the concentration of H^+ in pure water to be 10^{-7} M. The solution is neutral because the concentration of OH^- is also 10^{-7} M. The sum of H^+ and OH^- ions will always equal 10^{-14}.

Acids are molecules that release hydrogen ions (H^+) when dissolved in water. Acids increase the concentration of H^+ in a solution. Bases are molecules that remove H^+ from solution. Bases decrease the concentration of H^+ in a solution. When the concentration of H^+ increases, the concentration of OH^- becomes proportionately less. For example, hydrocholoric acid (HCl) quickly ionizes in water and increases the concentration of H^+; therefore, HCl is an acid. In contrast, sodium hydroxide (NaOH) is a base because it ionizes and increases the concentration of OH^-, thereby lowering the relative proportion of H^+. Thus, if

enough acid is added to water to raise the H^+ concentration to 10^{-6} M, the OH^- concentration would decrease to 10^{-8} M.

By general agreement, the scale we use to measure acidity is the **pH scale** (*pH* stands for the *p*otential of *H*ydrogen ions). The pH is the negative logarithm of the concentration of H^+; that is,

$$\text{pH} = -\log [H^+]$$

As pH goes up, the concentration of H^+ goes down.The brackets indicate concentration of hydrogen ions. The pH scale ranges from 0 ($-\log 10^{\infty}$; most acidic) to 14 ($-\log 10^{-14}$; most basic). On this scale, pure water has a pH of 7 ($-\log 10^{-7}$); pH values less than 7 are acidic, whereas those above 7 are basic (fig. 5.3).

Figure 5.3 shows some pHs of some common (and a few not-so-common) substances. The pH scale is a logarithmic scale; each unit represents a change of tenfold. Thus, a lime with a pH of 2 is ten times more acidic than an apple with a pH of 3 and 100 times more acidic than a tomato having a pH of 4. Each decrease of 1.0 pH unit represents a tenfold increase in acidity. Each increase of 1.0 pH unit represents a tenfold decrease in acidity.

Question 4

a. Vinegar has a pH of 3 and household ammonia has a pH of 11. Is the concentration of H^+ greatest in the vinegar or ammonia?

b. How many times different is the concentration?

Measuring pH

A convenient way of measuring the pH of a solution is with **pH paper.** pH paper is treated with a chemical indicator that changes colors depending on the concentration of H^+ in the solution that it has contacted (fig. 5.4). The color chart on the container of pH paper relates the color of the pH paper to the pH of the solution. Here are some examples of pH indicators:

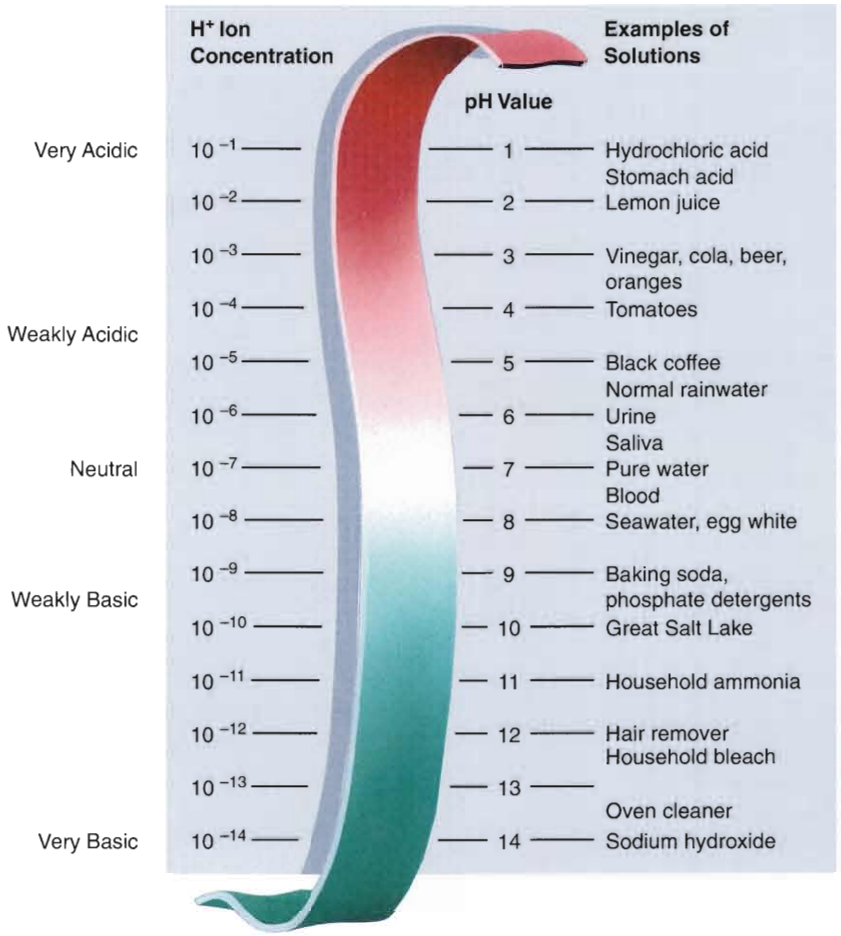


Figure 5.3

The pH scale. The pH value of a solution indicates its concentration of hydrogen ions. Solutions with a pH less than 7 are acidic, whereas those with a pH greater than 7 are basic. The pH scale is logarithmic: a pH change of 1 means a tenfold change in the concentration of hydrogen ions. Thus, lemon juice is 100 times more acidic than tomato juice, and seawater is 10 times more basic than pure water, which has a pH of 7.

Figure 5.4
Indicator pH paper is embedded with chemicals that change color according to the pH of a solution. According to the color chart provided on the container of pH paper, the lemon juice sampled with the paper strip on the left has a pH of 2. The pH test strip on the right indicates that the sodium hydroxide solution has a pH of 12.

Indicator	*Range*	*Color Change*
Methyl violet	0.2–3.0	yellow to blue-violet
Bromophenol blue	3.0–4.6	yellow to blue
Methyl red	4.4–6.2	red to yellow
Litmus	4.5–8.3	red to blue
Bromcresol purple	5.2–6.8	yellow to purple
Phenol red	6.8–8.0	yellow to red
Thymol blue	8.0–9.6	yellow to blue
Phenolphthalein	8.3–10.0	colorless to red

Procedure 5.1

Measure the pH of liquids

Use pH papers to measure the pH of the following liquids. Be as accurate as possible and use a fresh piece of pH paper or pH dipstick for each test.

SAFETY FIRST Before coming to lab you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Handle all of the solutions carefully. Although some are harmless (e.g., water, milk), others are caustic and can stain your clothes and burn your skin.

Vinegar _____
Skim milk _____
Apple juice _____
Grapefruit juice _____
Buttermilk _____
Black coffee _____
Sprite _____
Household bleach _____
Mixture of Sprite and baking soda _____
10 mM hydrochloric acid _____
1.0 mM hydrochloric acid _____
0.01 mM hydrochloric acid _____
Distilled water _____
Tap water _____
Dissolved aspirin _____
Soap solution _____
Shampoo _____
Mouthwash _____
Deodorant _____

Check your measurements of the hydrochloric acid solutions by comparing them with calculations using the following formula. For example,

$$\text{pH} = -\log[\text{H}^+]$$
$$10\ \text{mM HCl} = 10^{-2}\ \text{M HCl}$$
$$\text{pH} = -\log[10^{-2}]$$
$$\text{pH} = 2$$

Question 5
Are your measured pH values similar to the calculated pH values? What are possible sources of error?

Buffers

In most organisms, the pH is kept relatively constant by **buffers,** which are mixtures of a weak acid and a weak base that can combine with a strong acid or base to limit changes in pH. That is, buffers absorb excess H^+ as the pH decreases or release H^+ as the pH increases. Buffers minimize changes in pH (fig. 5.5). The addition of a small amount of acid to a buffered solution produces a small change in pH, whereas adding the same amount of acid to an unbuffered solution changes the pH drastically. Most biological fluids (e.g., milk, blood) contain buffers, the most important of which is bicarbonate:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

TABLE 5.1

TESTING THE BUFFERING CAPACITY OF VARIOUS SOLUTIONS

Procedure 5.2 Solution	Initial pH	pH after Adding Acid	Procedure 5.3 Solution	Drops of Acid
Water	_____	_____	*Alka-Seltzer*	_____
0.1 M NaCl	_____	_____	*Rolaids*	_____
Skim milk	_____	_____	*Tums*	_____
0.1 M phosphate buffer	_____	_____		

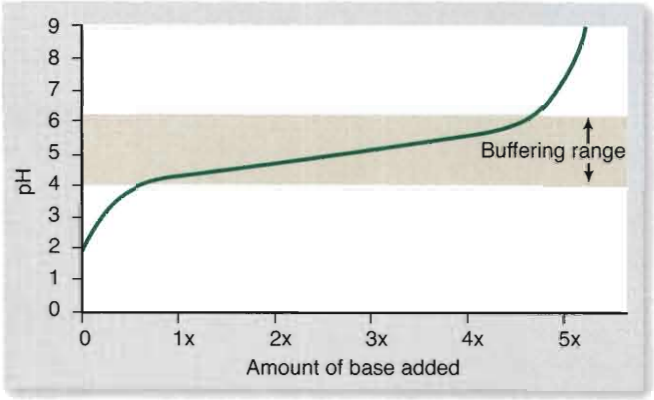


Figure 5.5

Buffers minimize changes in pH. Adding a base to a solution will raise the pH (neutralize some of the acid present). Thus, as more and more base is added, the pH continues to rise. However, a buffer makes the curve rise or fall very slowly over a portion of the pH scale, called the "buffering range" of that buffer.

For example, human blood contains buffers that maintain a pH of 7.3–7.5; Blood pH above 7.5 produces alkalosis, and blood pH below 7.3 produces acidosis. Both of these conditions can cause illness and even death.

Procedure 5.2

Test the ability of buffers to stabilize pH

1. Obtain and label four test tubes to receive the four solutions listed in table 5.1.
2. Place 5 mL of each solution into its appropriately labeled tube.
3. Measure the pH of each of the solutions in the tubes and record these initial values in table 5.1.
4. Add 5 drops of acid (0.1 M HCl) to the first tube. Cover the tube with parafilm and swirl the tube gently to mix the contents.
5. Measure the pH of the acidified solution and record it in table 5.1.
6. Repeat steps 4 and 5 for each of the remaining tubes. Record your results in table 5.1.

Question 6

a. Compare the initial pH and the pH after acid addition for each sample. Which is the most effective buffer? Least effective?

b. What accounts for the different buffering capacities of these fluids?

c. What is the biological importance of what you observed?

Procedure 5.3

Test the effectiveness of commercial antacids and other products

Commercial antacids such as *Alka-Seltzer*, *Rolaids*, and *Tums* claim to "neutralize stomach acid" by absorbing excess H^+ (produced as hydrochloric acid by the stomach; fig 5.6). To test the abilities of these products to absorb acids, do the following:

1. Use a mortar and pestle to pulverize the amount of antacid that is listed as one dose. Dissolve the crushed antacid in 100 mL of distilled water. Some of the products may require extensive stirring to get most or all of the powder to dissolve.
2. Using a pipet or 10-mL graduated cylinder add 5 mL of the antacid solution into a test tube. Add 4 drops of the indicator bromcresol purple to the tube. Cover the tube with Parafilm and invert the tube to mix the contents.
3. Add 0.1 M hydrochloric acid (HCl) dropwise to the tube; mix after each drop. Continue this process until the solution turns yellow, indicating an acidic solution.
4. Record in table 5.1 the number of drops of acid needed to generate the change of color. This number

Figure 5.6
A variety of over-the-counter remedies for heartburn and acid indigestion contain buffers that neutralize stomach acid.

Figure 5.7
These commercially available products soothe or prevent chronic stomach acid production. How do they do this?

of drops is an index to the amount of acid (H^+) that the solution neutralizes before the pH drops below the yellow endpoint of bromcresol purple (pH 5.2).

Question 7

a. Which antacid neutralizes the most acid? Which neutralizes the least acid?

b. What is the effect of dose (for example, the size of tablets or the amount of antacid per tablet) on your results and conclusions?

c. Examine the package of the products you tested. What are the active ingredients of each product? What does this tell you about how these products work?

Many people also use products such as *Zantac*, *Pepcid AC*, *Gaviscon*, *Prilosec*, *Tagamet*, *Pepcid AC Complete*, *Maalox*, and *Zantac 75* to soothe upset stomachs (fig. 5.7). Examine these products in lab, noting their claims and active ingredients. Based on your observations, write a hypothesis predicting each product's ability to absorb acid.

Now use procedure 5.3 to test each product's ability to absorb acid. List your results here.

Question 8

a. How accurate were your hypotheses?

b. How does each product work?

INVESTIGATION

The Properties of Phillips Milk of Magnesia, *a Popular Antacid*

Observation: *Phillips Milk of Magnesia* is a milky-white liquid that is a popular over-the-counter laxative and antacid. *Phillips Milk of Magnesia* is often taken by people suffering from "acid indigestion."

Question: How effective is *Phillips Milk of Magnesia* at neutralizing acid?

a. Establish a working lab group and obtain Investigation Worksheet 5 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 5 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your question, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What do buffers do and why are they important in biological systems?

2. Our stomachs secrete hydrochloric acid. Knowing the function of antacids, what do you think causes most "upset stomachs"?

3. The soft drink *Mr. Pibb* contains (among other things) 39 g of sucrose in 355 mL of solution. What is the molarity of this sucrose solution? What is the percentage (weight/volume) of sucrose in the solution?

4. Our stomachs secrete hydrochloric acid. What functions does this hydrochloric acid serve?

5. Suppose that the concentration of H^+ in Solution #1 is 10,000 times greater than Solution #2. What can you conclude about the difference in pH of these two solutions?

6. What is the active ingredient in *Phillips Milk of Magnesia*? How is this different from that of products such as *Tums*?

Biologically Important Molecules
Carbohydrates, Proteins, Lipids, and Nucleic Acids

6

Objectives

By the end of this exercise you should be able to:

1. Perform tests to detect the presence of carbohydrates, proteins, lipids, and nucleic acids.
2. Explain the importance of a positive and a negative control in biochemical tests.
3. Use biochemical tests to identify an unknown compound.

Most organic compounds in living organisms are **carbohydrates, proteins, lipids,** or **nucleic acids.** Each of these macromolecules is made of smaller subunits. These subunits are linked by **dehydration synthesis,** an energy-requiring process in which a molecule of water is removed and the two subunits are bonded covalently (fig. 6.1). Similarly, breaking the bond between the subunits requires the addition of a water molecule. This energy-releasing process is called **hydrolysis.**

The subunits of macromolecules are held together by covalent bonds and have different structures and properties. For example, lipids (made of fatty acids) have many C—H bonds and relatively little oxygen, while proteins (made of amino acids) have amino groups (—NH_2) and carboxyl (—COOH) groups. These characteristic subunits and groups impart different chemical properties to macromolecules—for example, monosaccharides such as glucose are polar and soluble in water, whereas lipids are nonpolar and insoluble in water.

CONTROLLED EXPERIMENTS TO IDENTIFY ORGANIC COMPOUNDS

Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments: (1) an **unknown solution** to be identified, and (2) **controls** to provide standards for comparison. As its name implies, an unknown solution may or may not contain the substance that the investigator is trying to detect. Only a carefully conducted experiment will reveal its contents. In contrast, controls are known solutions. We use controls to validate that our procedure is detecting what we expect it to detect and nothing more. During the experiment we compare the unknown solution's response to the experimental procedure with the control's response to that same procedure.

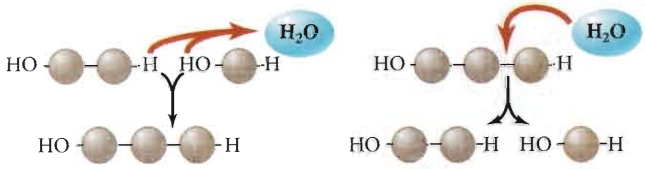
A **positive control** contains the variable for which you are testing; it reacts positively and demonstrates the test's ability to detect what you expect. For example, if you are testing for protein in unknown solutions, then an appropriate positive control is a solution known to contain protein. A positive reaction shows that your test reacts correctly; it also shows you what a positive test looks like.

A **negative control** does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

CARBOHYDRATES

Benedict's Test for Reducing Sugars

Carbohydrates are molecules made of C, H, and O in a ratio of 1:2:1 (e.g., the chemical formula for glucose is $C_6H_{12}O_6$). Carbohydrates are made of **monosaccharides,** or simple sugars (fig. 6.2). Paired monosaccharides form **disaccharides**—



(a) **Dehydration synthesis** (b) **Hydrolysis**

Figure 6.1

Making and breaking macromolecules. (*a*) **Dehydration synthesis.** Biological macromolecules are polymers formed by linking subunits together. The covalent bond between the subunits is formed by dehydration synthesis, an energy-requiring process that creates a water molecule for every bond formed. (*b*) **Hydrolysis.** Breaking the bond between subunits requires the returning of a water molecule with a subsequent release of energy, a process called hydrolysis.

for example, sucrose (table sugar) is a disaccharide of glucose linked to fructose. Similarly, linking three or more monosaccharides forms a **polysaccharide** such as starch, glycogen, or cellulose (fig. 6.3).

Question 1
Examine figure 6.2. Which groups of a glucose molecule are involved in forming a polysaccharide? Shade the groups with a pencil.

As already mentioned, the linkage of subunits in carbohydrates, as well as other macromolecules, involves the removal of a water molecule (dehydration). Figure 6.4 depicts how dehydration synthesis is used to make maltose and sucrose, two common disaccharides.

Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free aldehyde (−CHO) or ketone (−C=O) groups that reduce weak oxidizing agents such as the copper in Benedict's reagent. **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu^{2+}) ions to cuprous oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

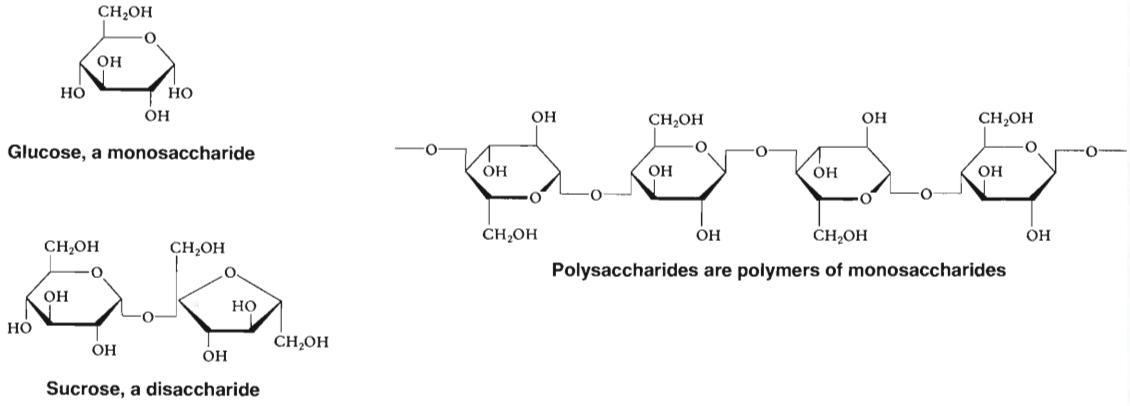


Figure 6.2
Carbohydrates consist of subunits of mono- or disaccharides. These subunits can be combined by dehydration synthesis (see fig. 6.4) to form polysaccharides.

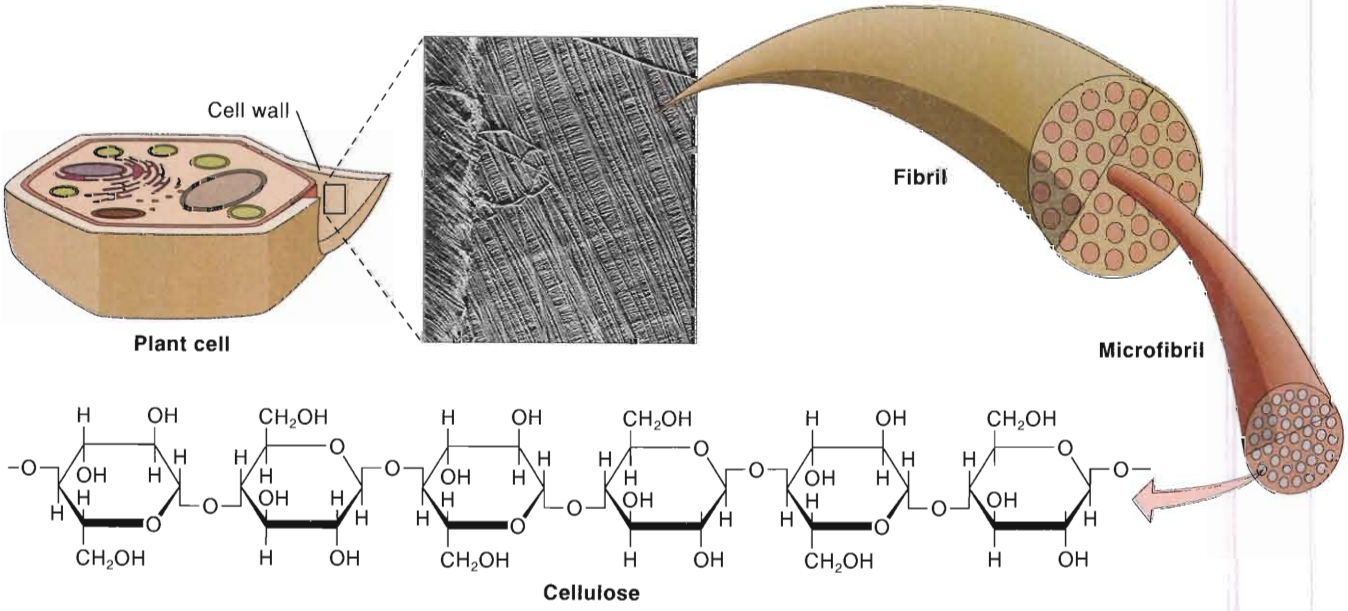


Figure 6.3
Plant cell walls are made of cellulose arranged in fibrils and microfibrils. The scanning electron micrograph shows the fibrils in a cell wall of the green alga *Chaetomorpha* (30,000×).

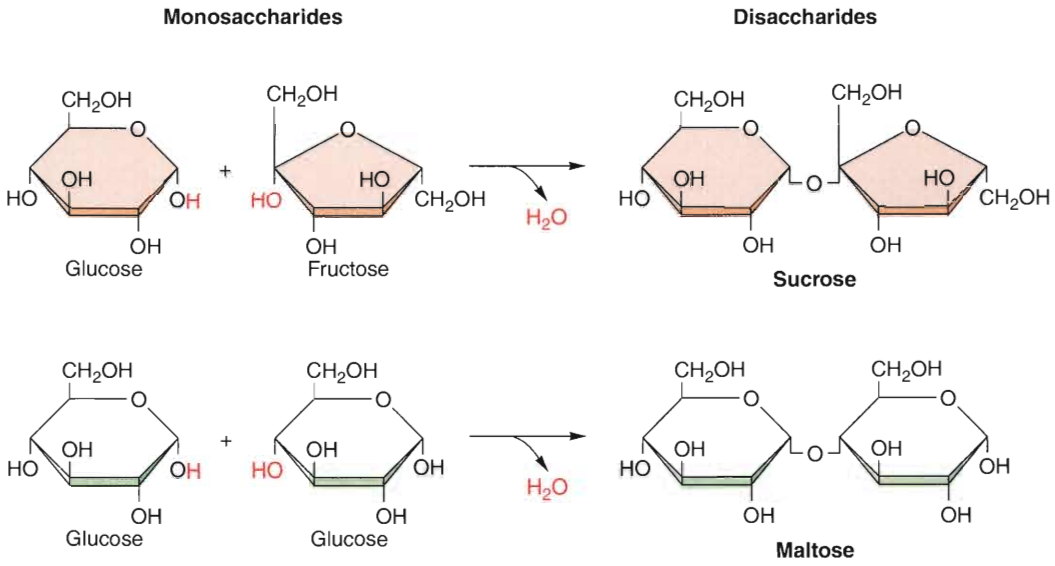


Figure 6.4

Dehydration synthesis is used to link monosaccharides (such as glucose and fructose) into disaccharides. The disaccharides shown here are maltose (malt sugar) and sucrose (table sugar).

Oxidized Benedict's reagent (Cu^{2+}) + Reducing sugar (R-COH)
(blue)

Heat
High pH

Reduced Benedict's reagent (Cu^{+}) + Oxidized sugar (R-COOH)
(green to reddish orange)

A green solution indicates a small amount of reducing sugars, and reddish orange indicates an abundance of reducing sugars. Nonreducing sugars such as sucrose produce no change in color (i.e., the solution remains blue).

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 6.1

Perform the Benedict's test for reducing sugars

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes. Add 2 mL of Benedict's solution to each tube.
3. Place all of the tubes in a gently boiling water-bath for 3 min and observe color changes during this time.
4. After 3 min, remove the tubes from the water-bath and give the tubes ample time to cool to room temperature. Record the color of their contents in table 6.1.
5. When you are finished, dispose of the contents of each tube as instructed by your instructor.

Question 2

a. Which of the solutions is a positive control? Negative control?

b. Which is a reducing sugar, sucrose or glucose? How do you know?

c. Which contains more reducing sugars, potato juice or onion juice? How do you know?

d. What does this tell you about how sugars are stored in onions and potatoes?

Iodine Test for Starch

Staining by iodine (iodine-potassium iodide, I_2KI) distinguishes starch from monosaccharides, disaccharides, and

TABLE 6.1

SOLUTIONS AND COLOR REACTIONS FOR (1) BENEDICT'S TEST FOR REDUCING SUGARS AND (2) IODINE TEST FOR STARCH

Tube	Solution	Benedict's Color Reaction	Iodine Color Reaction
1	10 drops onion juice		
2	10 drops potato juice		
3	10 drops sucrose solution		
4	10 drops glucose solution		
5	10 drops distilled water		
6	10 drops reducing-sugar solution		
7	10 drops starch solution		
8			
9			

other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose; iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with carbohydrates that are not coiled and remains yellowish brown. Therefore, a bluish-black color is a positive test for starch, and a yellowish-brown color (i.e., no color change) is a negative test for starch. Glycogen, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Procedure 6.2

Perform the iodine test for starch

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add seven to ten drops of iodine to each tube.
4. Record the color of the tubes' contents in table 6.1.

Question 3

a. Which of the solutions is a positive control? Which is a negative control?

b. Which colors more intensely, onion juice or potato juice? Why?

c. In what parts of a plant is the most starch typically stored?

PROTEINS

Proteins are remarkably versatile structural molecules found in all life forms (fig. 6.5). Proteins are made of amino acids (fig. 6.6), each of which has an amino group (—NH_2), a carboxyl (acid) group (—COOH), and a variable side chain (R). A **peptide bond** (fig. 6.7) forms between the amino group of one amino acid and the carboxyl group of an adjacent amino acid and is identified by a **Biuret test.** Specifically, peptide bonds (C—N bonds) in proteins complex with Cu^{2+} in Biuret reagent and produce a violet color. A Cu^{2+} must complex with four to six peptide bonds to produce a color; therefore, individual amino acids do not react positively. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

Biuret reagent is a 1% solution of $CuSO_4$ (copper sulfate). A violet color is a positive test for the presence of protein; the intensity of color relates to the number of peptide bonds that react.

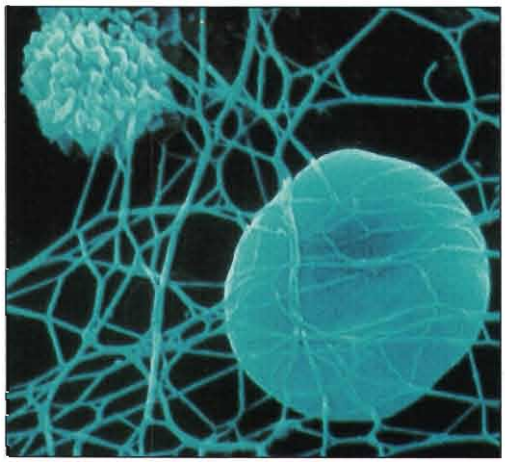
Question 4

Examine figure 6.6. Shade with a pencil the reactive amino and carboxyl groups on the three common amino acids shown.

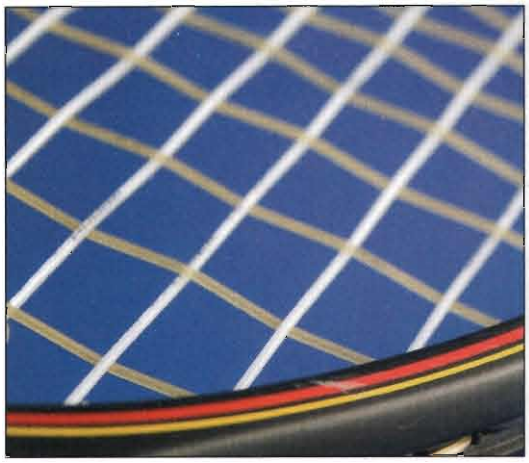
Procedure 6.3

Perform the Biuret test for protein

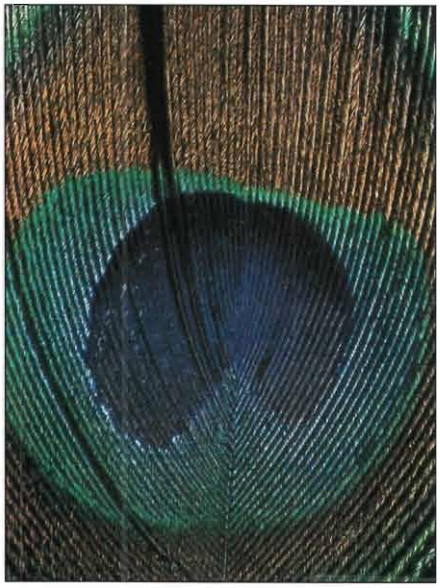
1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.2.
3. Add 2 mL of 2.5% sodium hydroxide (NaOH) to each tube.



(a) Fibrin

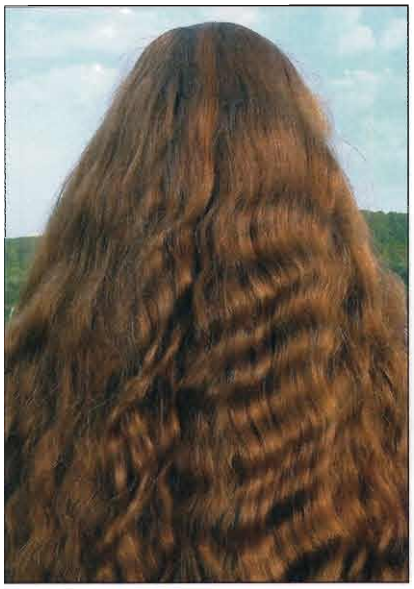


(b) Collagen



(c) Keratin

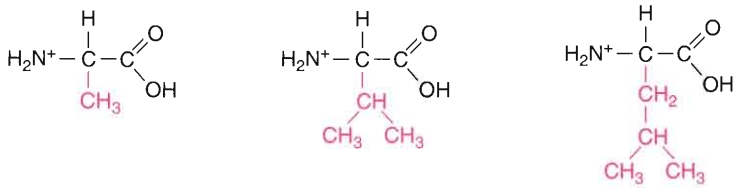
(d) Spider silk



(e) Hair

Figure 6.5

Common structural proteins. (*a*) Fibrin. This electron micrograph shows a red blood cell caught in threads of fibrin. Fibrin is important in the formation of blood clots. (*b*) Collagen. The so-called "cat-gut" strings of a tennis racket are made of collagen. (*c*) Keratin. This type of protein makes up bird feathers, such as this peacock feather. (*d*) Spider silk. The web spun by this agile spider is made of protein. (*e*) Hair. Hair is also a protein.



Alanine **Valine** **Leucine**

Figure 6.6

Structures of three amino acids common in proteins. Each amino acid has one carbon bonded to both an amine group (—NH_2) and a carboxyl group (—COOH). The side chains that make each amino acid unique are shown in red.

TABLE 6.2

SOLUTIONS AND COLOR REACTIONS FOR THE BIURET TEST FOR PROTEIN

Tube	Solution	Color
1	2 mL egg albumen	
2	2 mL honey	
3	2 mL amino acid solution	
4	2 mL distilled water	
5	2 mL protein solution	
6		
7		

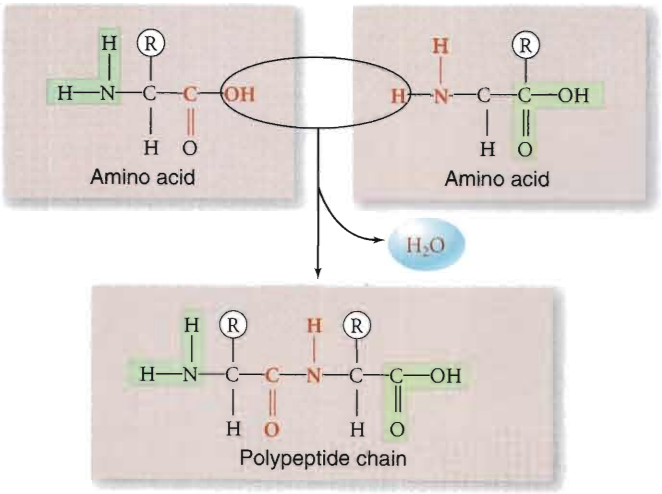


Figure 6.7

A peptide bond joins two amino acids, and peptide bonds link many amino acids to form polypeptides, or proteins. The formation of a peptide bond (i.e., between the carbon of one amino acid's carboxyl group and the nitrogen of another amino acid's amino group) liberates a water molecule. The R in these amino acids represents a variable side chain that characterizes each type of amino acid.

Do not spill the NaOH—it is extremely caustic. Rinse your skin if it comes in contact with NaOH.

4. Add three drops of Biuret reagent to each tube and mix.
5. Record the color of the tubes' contents in table 6.2.

Question 5

a. Which of the solutions is a positive control? Which is a negative control?

b. Which contains more protein (C—N bonds), egg albumen or honey? How can you tell?

c. Do free amino acids have peptide bonds?

LIPIDS

Lipids include a variety of molecules that dissolve in nonpolar solvents such as ether, acetone, methanol, or ethanol, but not as well in polar solvents such as water. Triglycerides (fats) are abundant lipids made of glycerol and three fatty acids (fig. 6.8). Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

Question 6

Examine figure 6.8. What are the reactive groups of the fatty acids?

Handle acetone carefully; it is toxic.

Procedure 6.4

Solubility of lipids in polar and nonpolar solvents

1. Obtain two test tubes. To one of the tubes, add 5 mL of water. To the other tube, add 5 mL of acetone.
2. Add a few drops of vegetable oil to each tube.

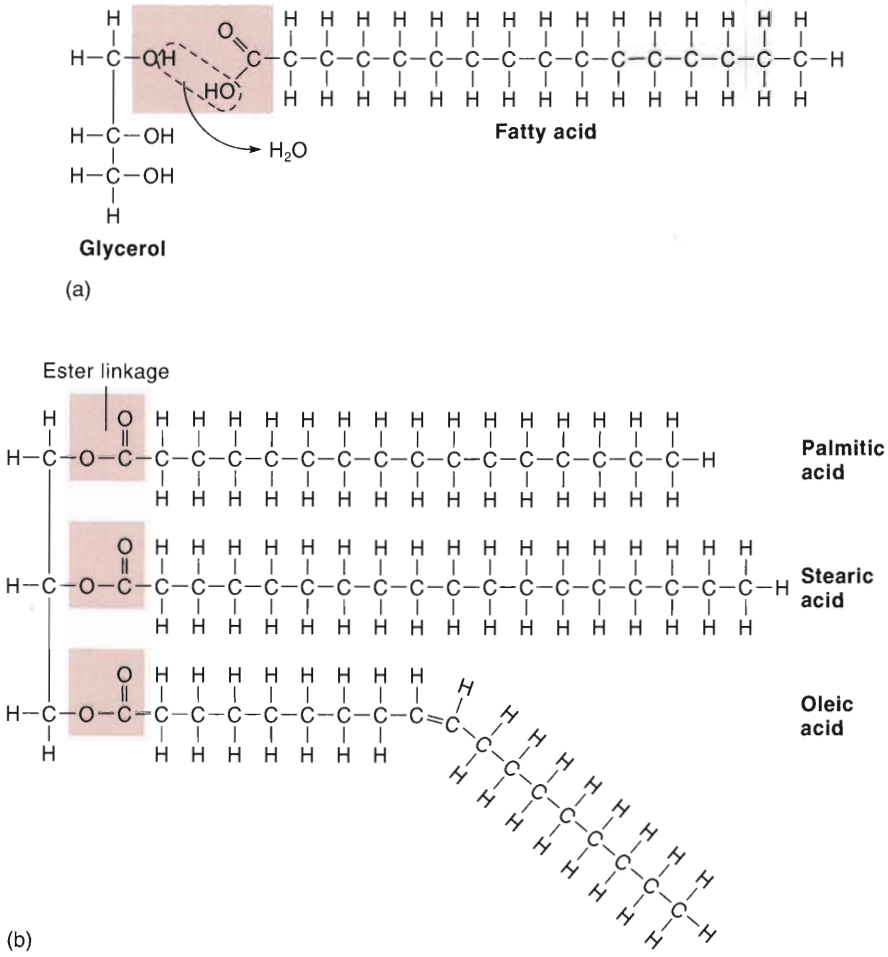


Figure 6.8

The structure of a fat includes glycerol and fatty acids. (*a*) An ester linkage forms when the carboxyl group of a fatty acid links to the hydroxyl group of glycerol, with the removal of a water molecule. (*b*) Fats are triacylglycerides whose fatty acids vary in length and vary in the presence and location of carbon–carbon double bonds.

Question 7

What do you conclude about the solubility of lipids in polar solvents such as water? In nonpolar solvents such as acetone?

Procedure 6.5

Perform the Sudan IV test for lipid

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.3.
3. Add five drops of water to tube 1 and five drops of Sudan IV to each of the remaining tubes. Mix the contents of each tube. Record the color of the tubes' contents in table 6.3.

Question 8

a. Is salad oil soluble in water?

b. Compare tubes 1 and 2. What is the distribution of the dye with respect to the separated water and oil?

TABLE 6.3

SOLUTIONS AND COLOR REACTIONS FOR THE SUDAN IV TEST FOR LIPIDS

Tube	Solution	Description of Reaction
1	1 mL salad oil + water	
2	1 mL salad oil + Sudan IV	
3	1 mL honey + Sudan IV	
4	1 mL distilled water + Sudan IV	
5	1 mL known lipid solution + Sudan IV	
6		
7		

TABLE 6.4

MATERIALS AND GREASE-SPOT REACTION AS A TEST FOR LIPID CONTENT

Food Product	Description of Grease-Spot Reaction
1	
2	
3	
4	
5	
6	

c. What observation indicates a positive test for lipid?

d. Does honey contain much lipid?

e. Lipids supply more than twice as many calories per gram as do carbohydrates. Based on your results, which contains more calories, oil or honey?

Grease-Spot Test for Lipids

A simpler test for lipids is based on their ability to produce translucent grease marks on unglazed paper.

Procedure 6.6

Perform the grease-spot test for lipids

1. Obtain a piece of brown wrapping paper or brown paper bag from your lab instructor.
2. Use an eyedropper to add a drop of salad oil near a corner of the piece of paper.
3. Add a drop of water near the opposite corner of the paper.
4. Let the fluids evaporate.
5. Look at the paper as you hold it up to a light.
6. Test other food products and solutions available in the lab in a similar way and record your results in table 6.4.

TABLE 6.5

SOLUTIONS AND COLOR REACTIONS FOR DISCHE DIPHENYLAMINE TEST FOR DNA

Tube	Solution	Color
1	2 mL DNA solution	
2	1 mL DNA solution, 1 mL water	
3	2 mL RNA solution	
4	2 mL distilled water	
5		
6		

Question 9
Which of the food products that you tested contain large amounts of lipid?

NUCLEIC ACIDS

DNA and RNA are nucleic acids made of nucleotide subunits (fig. 6.9). One major difference between DNA and RNA is their sugar: DNA contains deoxyribose, whereas RNA contains ribose. DNA can be identified chemically with the **Dische diphenylamine test.** Acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA.

Question 10
Examine figure 6.9*a*. Which groups on ribose and deoxyribose react when combining with the phosphate? Shade these groups. Also shade the reactive groups that combine with a nitrogenous base.

Procedure 6.7

Perform the Dische diphenylamine test for DNA

1. Obtain four test tubes and number them 1–4. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.5.
3. Add 2 mL of the Dische diphenylamine reagent to each tube and mix thoroughly.

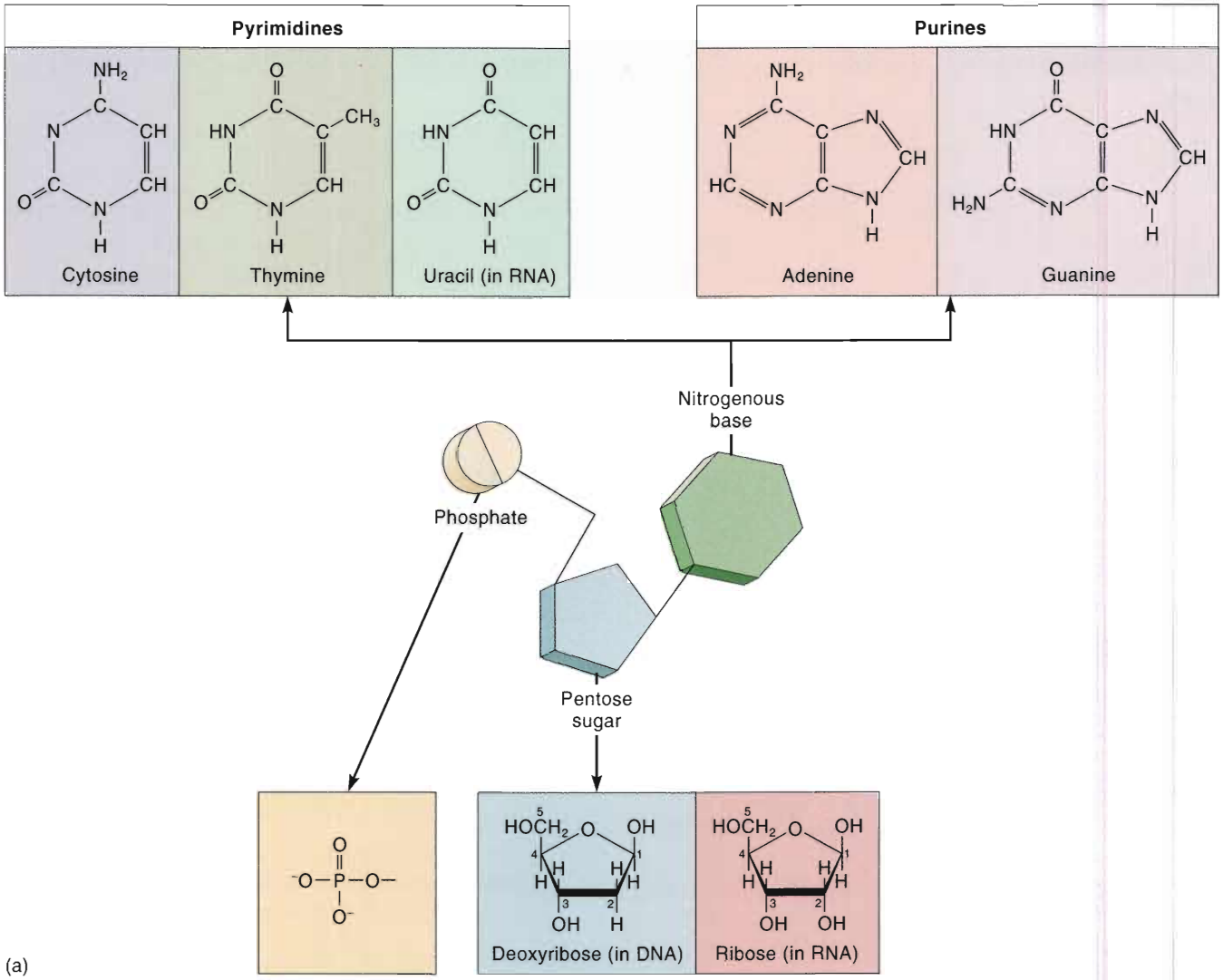
Handle the Dische diphenylamine reagent carefully; it is toxic. Wash your hands after the procedure.

4. Place the tubes in a gently boiling water-bath to speed the reaction.
5. After 10 min, transfer the tubes to an ice bath. Gently mix and observe the color of their contents as the tubes cool. Record your observations in table 6.5.

Question 11

a. How does the color compare between tubes 1 and 2? Why?

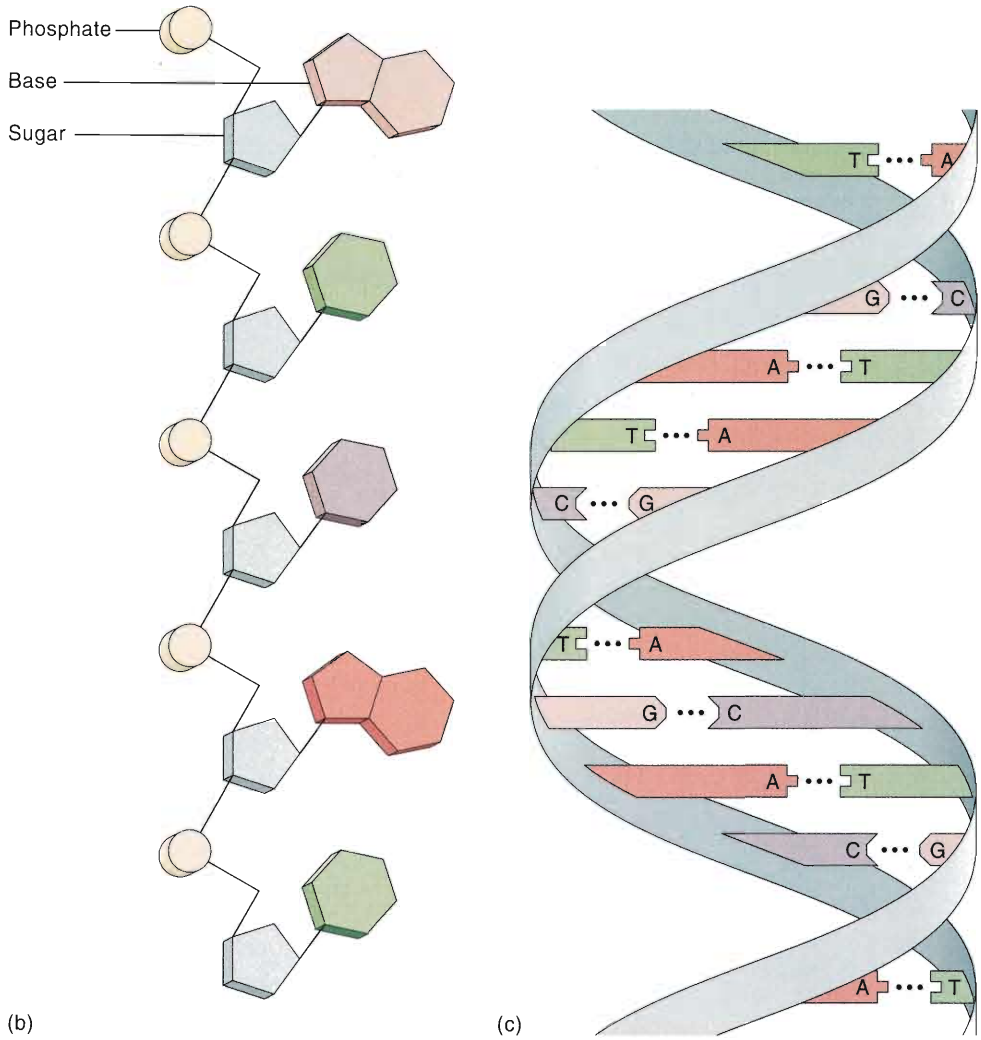
b. Do DNA and RNA react alike? Why or why not?



(a)

Figure 6.9

The structure of DNA and RNA. (*a*) Each nucleotide consists of three smaller building blocks: a nitrogenous base, a pentose sugar, and a phosphate group. (*b*) Nucleotides are bonded to each other by covalent bonds between the phosphate of one nucleotide and the sugar of the next nucleotide. (*c*) DNA is usually a double strand held together by hydrogen bonds between nitrogenous bases; A bonds only with T, and C bonds only with G. The double strand is twisted into a double helix.



(b) (c)

Figure 6.9 continued

INVESTIGATION I

Identify Unknowns

Each of the previously described tests is relatively specific; that is, iodine produces a bluish-black color with starch but not with other carbohydrates, protein, lipid, or nucleic acids. This specificity can be used to identify the contents of an unknown solution.

a. Obtain an unknown solution from your laboratory instructor. Record its number in table 6.6.
b. Obtain 10 clean test tubes.
c. Number five tubes for the sample as S1–S5. Number the other five tubes as controls C1–C5.
d. Place 2 mL of your unknown solution into each of tubes S1–S5.
e. Place 2 mL of distilled water into each of tubes C1–C5.
f. Use procedures 6.1–6.5 to detect reducing sugars, starch, protein, DNA, and lipids in your unknown. Your unknown may contain one, none, or several of these macromolecules. Record your results in table 6.6. Show table 6.6 and the following report (page 69) to your instructor before you leave the lab.

INVESTIGATION II

Variation in Starch Storage by Roots versus Leaves

Observation: Starch is the major storage product of photosynthesis in higher plants, and some plant organs more than others are specialized for storing starch. Iodine reacts with starch to produce a dark blue-black color.

Question: What are the relative amounts of starch stored in leaves versus roots of a flowering plant?

a. Establish a working lab group and obtain Investigation Worksheet 6 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 6 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Name _____ Lab Section _____

Unknown No. _____

TABLE 6.6

CHEMICAL TESTING TO IDENTIFY AN UNKNOWN

Biochemical Test	Color		Unknown Result
	Sample	Control	(+/−)
Benedict's test (reducing sugars)			
Iodine (starch)			
Biuret test (protein)			
Dische diphenylamine test (DNA)			
Sudan IV (lipid)			

Report: Identity of Unknown

Indicate which of the following are in your unknown:

Reducing sugars

Starch

Protein

DNA

Lipid

Comments:

Questions for Further Thought and Study

1. What is the importance of a positive control? What is the importance of a negative control?

2. What controls were used in each procedure that you performed in today's lab?

3. Why did you include controls in all of your tests?

4. Are controls always necessary? Why or why not?

5. What is a phospholipid? What functions do phospholipids have in cells?

WRITING TO LEARN BIOLOGY

What are the limitations of these common techniques in detecting the presence of a class of macromolecules? Do biologists who study plant cells commonly use the iodine test for starch? Why or why not?

DOING BIOLOGY YOURSELF

Design a procedure to indicate the amount of starch present in various plant tissue samples. How would you weigh your samples? How would you treat your samples? How would you quantify the iodine test?

7

Separating Organic Compounds

Column Chromatography, Paper Chromatography, and Gel Electrophoresis

Objectives

By the end of this exercise you should be able to:

1. Describe the basis for column chromatography, paper chromatography, and gel electrophoresis.
2. Use column chromatography, paper chromatography, and gel electrophoresis to separate organic compounds from mixtures.

Cells are a mixture of the types of organic compounds that you studied in Exercise 6: carbohydrates, proteins, lipids, and nucleic acids. Biologists characterize and study these compounds to understand how organisms function. This requires that biologists separate the compounds, such as amino acids and nucleotides, from mixtures. Three separation techniques that biologists use are column chromatography, paper chromatography, and gel electrophoresis.

In today's exercise you will use these common techniques to separate compounds from mixtures. The procedures are simple and model how these techniques are used by biologists in their research.

COLUMN CHROMATOGRAPHY

Column chromatography separates molecules according to their size and shape. The procedure is simple and involves placing a sample onto a column of beads having tiny pores. There are two ways that molecules can move through the column of beads: a fast route between the beads or a slower route through the tiny pores of the beads. Molecules too big to fit into the beads' pores move through the column quickly, whereas smaller molecules enter the beads' pores and move through the column more slowly (fig. 7.1). Movement of the molecules is analogous to going through or walking around a maze: It takes more time to walk through a maze than to walk around it.

The apparatus used for column chromatography is shown in figure 7.2 and consists of a chromatography column, a matrix, and a buffer.

- The **chromatography column** is a tube having a frit and a spout at its bottom. The frit is a membrane or porous disk that supports and keeps the matrix in the column but allows water and solutes to pass.
- The **matrix** is the material in the column that fractionates, or separates, the chemicals mixed in the sample. The matrix consists of beads having tiny pores and internal channels. The size of the beads' pores determines the matrix's **fractionation range,** the range of molecular weights the matrix can separate. These molecular weights are measured in units called daltons; 1 dalton $\approx$ 1 g mole^{-1}. Different kinds of matrices have different fractionation ranges. In today's exercise you'll use a matrix having a fractionation range of 1000 to 5000 daltons. As they move through the matrix, small molecules spend much time in the maze of channels and pores in the matrix. Large molecules do not.
- The **buffer** helps control the pH of the sample (see Exercise 5). A buffer is a solution with a known pH that resists changes in pH if other chemicals are added. The pH of a buffer remains relatively constant. This is important because the shapes of molecules such as proteins often vary according to their pH. The buffer carries the sample through the matrix, which separates the chemicals mixed in the sample.

Column chromatography can also separate compounds having the same molecular weight but different shapes. Compact, spherical molecules penetrate the pores and channels of the matrix more readily than do rod-shaped molecules. Thus, spherical molecules move through a column more slowly than do rod-shaped molecules.

During column chromatography, the buffer containing the sample mixture of chemicals moves through the column and is collected sequentially in test tubes from the bottom of the column. Biologists then assay the content of the tubes to determine which tubes contain the compounds in which they are interested.

Question 1

In today's exercise you'll isolate colored compounds from mixtures. However, most biological samples are colorless. How would you determine the contents of the test tubes if all of the samples were transparent?

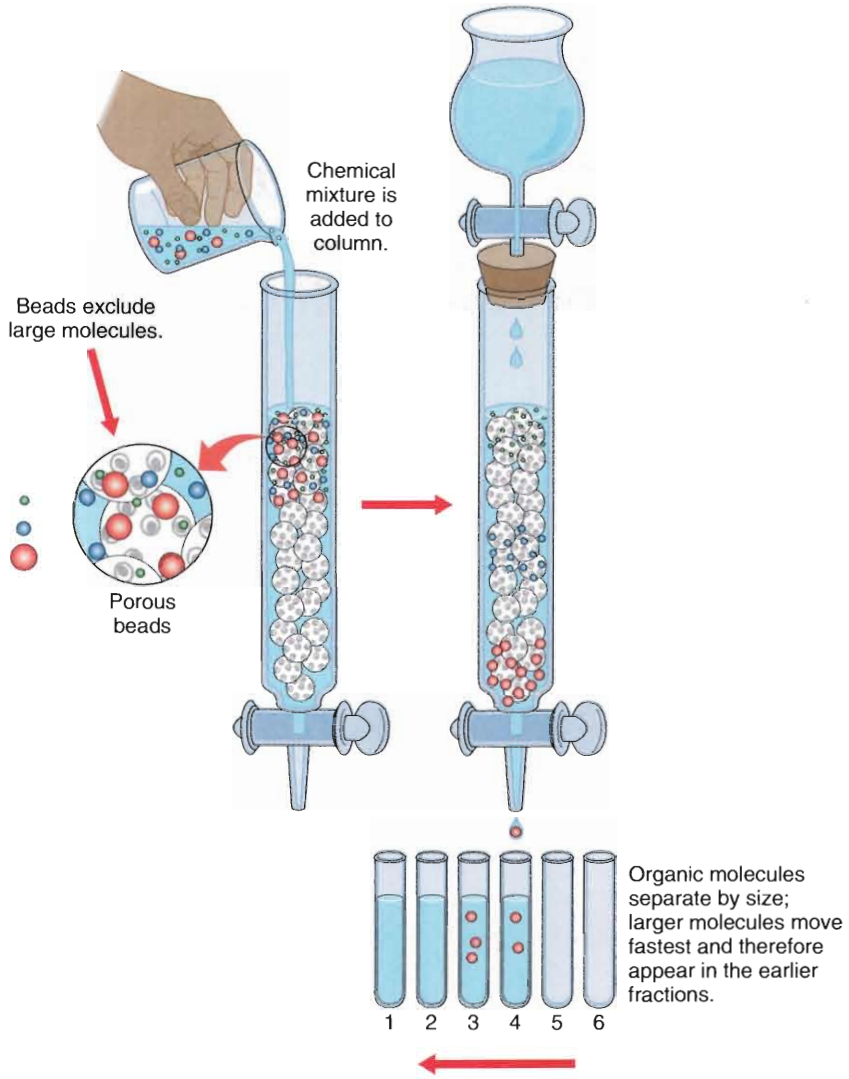


Figure 7.1

Separation of organic molecules by column chromatography. As the solution flows through the column, the smaller molecules are slowed down as they pass through the pores of the beads. Medium-sized molecules will pass through a bead with pores less frequently, and the largest molecules will quickly flow around all the beads. The exiting fluid is collected in fractions. The first fractions collected will contain the largest molecules.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 7.1

Separate compounds by column chromatography

1. Label nine microtubes 1–9.
2. Obtain an apparatus for column chromatography and carefully remove all of the buffer from above the beads with a transfer pipet. Do not remove any of the matrix.
3. Obtain a sample to be separated. The sample is a mixture of Orange G (molecular weight = 452 g $mole^{-1}$) and a rodlike polymer of glucose stained blue and having a molecular weight of about 2,000,000 g $mole^{-1}$.
4. Use a transfer pipet to slowly load 0.2 mL of the sample onto the top of the beads. Drip the sample down the inside walls of the column.

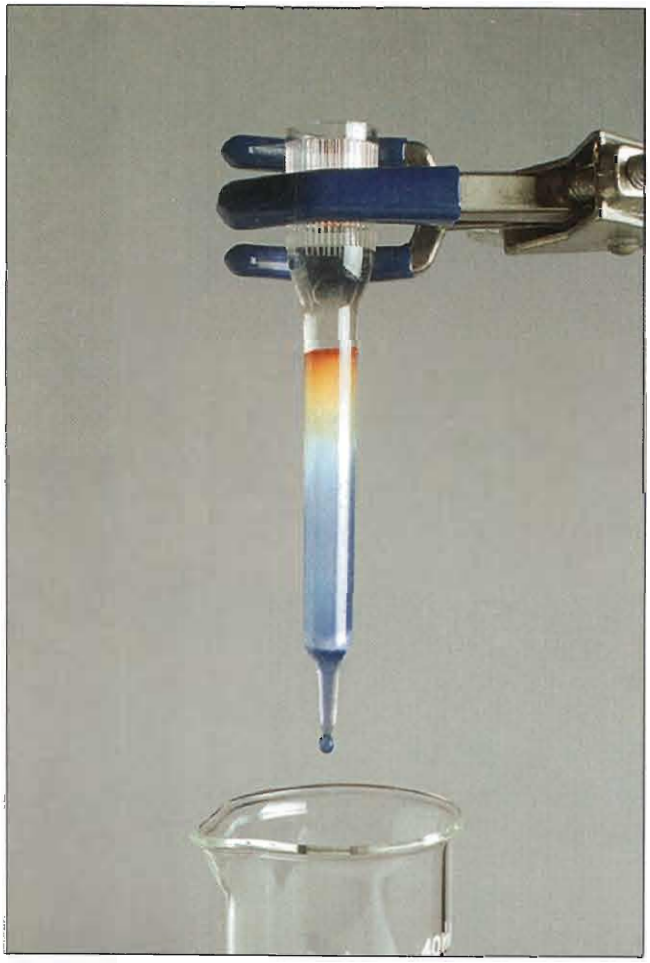


Figure 7.2
Apparatus for column chromatography. A fraction is being collected in the beaker.

5. Place a beaker under the column.
6. Slowly open the valve. This will cause the sample to enter the beads. Close the valve after the sample has completely entered the beads (i.e., when the top of the beads is exposed to air).
7. Use a transfer pipet to slowly cover the beads with buffer. Add buffer until the reservoir is almost full.
8. Hold microtube 1 under the tube and open the valve until you've collected about 1.0 mL of liquid.
9. Repeat step 8 for tubes 2–9. The sample will separate in the column.
10. Identify the tubes containing (1) the most orange dye, and (2) the most blue dye that eluted from the column.
11. Refill the reservoir with buffer and cover the reservoir with Parafilm.

Question 2

a. Was the color separation distinctive? Would you expect a longer column to more clearly separate the compounds? Why or why not?

b. Suppose your sample had consisted of a mixture of compounds having molecular weights of 50,000, 100,000, and 1,000,000 g mole^{-1}. What type of results would you predict? Explain your answer.

PAPER CHROMATOGRAPHY

Biologists often analyze the amino acid content of samples to determine protein sequences and enzyme structures. Amino acids can be separated by partitioning them between the stationary and mobile phases of paper chromatography. The **stationary phase** is the paper fibers, and the **mobile phase** is an organic solvent that moves along the paper.

Separation by paper chromatography begins by applying a liquid sample to a small spot on an origin line at one end of a piece of chromatography paper. The edge of the paper is then placed in a solvent. As the solvent moves up the paper, any sample molecules that are soluble in the solvent will move with the solvent. However, some molecules move faster than others based on their solubility in the mobile phase and their attraction to the stationary phase. These competing factors are different for different molecular structures, so each type of molecule moves at a different speed and occurs at a different position on the finished chromatogram.

Amino acids in solution have no color but react readily with molecules of ninhydrin to form a colored product. A completed chromatogram is sprayed with a ninhydrin solution and heated to detect the amino acids. The distance of these spots from the origin is measured and used to quantify the movement of a sample. The resulting R_f value (retardation factor) characterizes a known molecule in a known solvent under known conditions, and is calculated as follows:

$$R_f = \frac{\text{Distance moved by sample}}{\text{Distance from origin to solvent front}}$$

Procedure 7.2

Separate amino acids and identify unknowns by paper chromatography

1. Obtain a piece of chromatography paper 15 cm square. Avoid touching the paper with your fingers.

TABLE 7.1

CHROMATOGRAPHY DATA FOR DETERMINING AMINO ACID UNKNOWNS

Tick Mark Number	Amino Acid or Sample Number	Distance to Solvent Front	Distance Traveled by Sample	R_f	Identity of Unknown
1					
2					
3					
4					
5					

Use gloves, tissue, or some other means to handle the paper because oils from your skin will alter the migration of the molecules on the paper.

2. Lay the paper on a clean paper towel. Then use a pencil to draw a light line 2 cm from the bottom edge of the paper.
3. Draw five tick marks at 2.5 cm intervals from the left end of the line. Lightly label the marks 1–5 below the line.
4. Locate the five solutions available for the chromatography procedure. Three of the solutions are known amino acids. One solution is an unknown. The last solution is a plant extract or another unknown.
5. Use a wooden or glass applicator stick to "spot" one of the solutions on mark #1. To do this, dip the stick in the solution and touch it to the paper to apply a small drop (2–3 mm in diameter). Let the spot dry; then make three to five more applications on the same spot. Dry between each application. Record in table 7.1 the name of the solution next to the appropriate mark number.
6. Repeat step 5 for each of the other solutions.
7. Staple or paper clip the edges of the paper to form a cylinder with the spots on the outside and at the bottom.
8. Obtain a quart jar containing the chromatography solvent. The solvent should be 1 cm or less deep. The solvent consists of butanol, acetic acid, and water (2:1:1).
9. Place the cylinder upright in the jar (fig. 7.3). *The solvent must be below the pencil line and marks.* Close the lid to seal the jar.
10. Keep the jar out of direct light and heat. Allow the solvent to move up the paper for 2 hours (h) but not all the way to the top.
11. Open the jar and remove the chromatogram. Unclip and flatten the paper. Dry it with a fan or hair dryer. Work under a hood if possible to avoid breathing the solvent vapors.
12. Spray the chromatogram with ninhydrin. Carefully dry the chromatogram with warm air.
13. Circle with a pencil each of the spots. Measure the distance each of the spots has traveled and calculate the R_f for each spot. Record the values in table 7.1.
14. Determine the contents of the unknown solutions by comparing R_f values. Record the results in table 7.1.

GEL ELECTROPHORESIS

Gel electrophoresis separates molecules according to their charge, shape, and size (fig. 7.4). Buffered samples (mixtures of organic chemicals) are loaded into a Jello-like gel, after which an electrical current is placed across the gel. This current moves the charged molecules toward either the cathode or anode of the electrophoresis apparatus. The speed, direction, and distance that each molecule moves are related to its charge, shape, and size.

The apparatus for gel electrophoresis is shown in figure 7.5 and consists of an electrophoresis chamber, gel, buffer, samples, and a power supply.

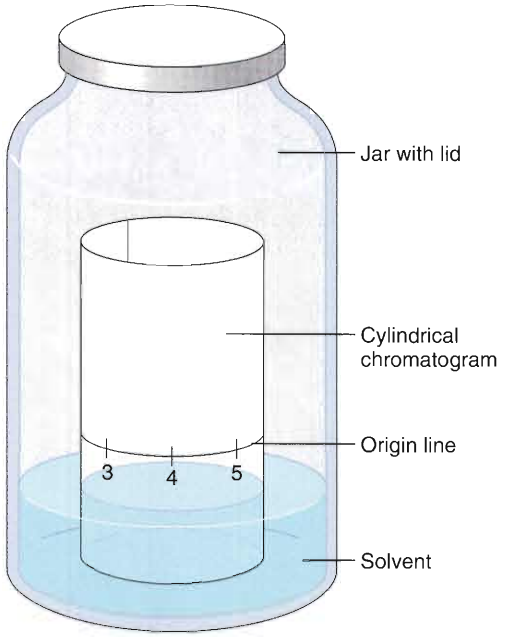


Figure 7.3

Apparatus for paper chromatography. Numbers on the chromatogram indicate the positions of multiple samples applied to the chromatogram. The samples will move up the chromatogram along with the solvent.

- The gel is made by dissolving agarose powder (a derivative of agar) in hot buffer. When the solution cools, it solidifies into a gel having many pores that function as a molecular sieve. The gel is submerged in a buffer-filled chamber containing electrodes.
- The buffer conducts electricity and helps control the pH. The pH affects the stability and charge of the samples.
- The samples are mixtures of chemicals loaded into wells in the gel. These samples move in the gel during electrophoresis. Samples are often mixed with glycerol or sucrose to make them denser than the buffer so that they will not mix with the buffer.
- The power supply provides a direct current across the gel. Charged molecules respond to the current by moving from the sample wells into the gel. Negatively charged molecules move through the gel toward the positive electrode (anode), whereas positively charged molecules move through the gel toward the negative electrode (cathode). The greater the voltage, the faster the molecules move.

The sieve properties of the gel affect the rate of movement of a sample through the gel. Small molecules move easier through the pores than do larger molecules. Consequently, small, compact (e.g., spherical) molecules move faster than do large, rodlike molecules. If molecules have similar shapes and molecular weights, the particles having the greatest charge move fastest and, therefore, the farthest.

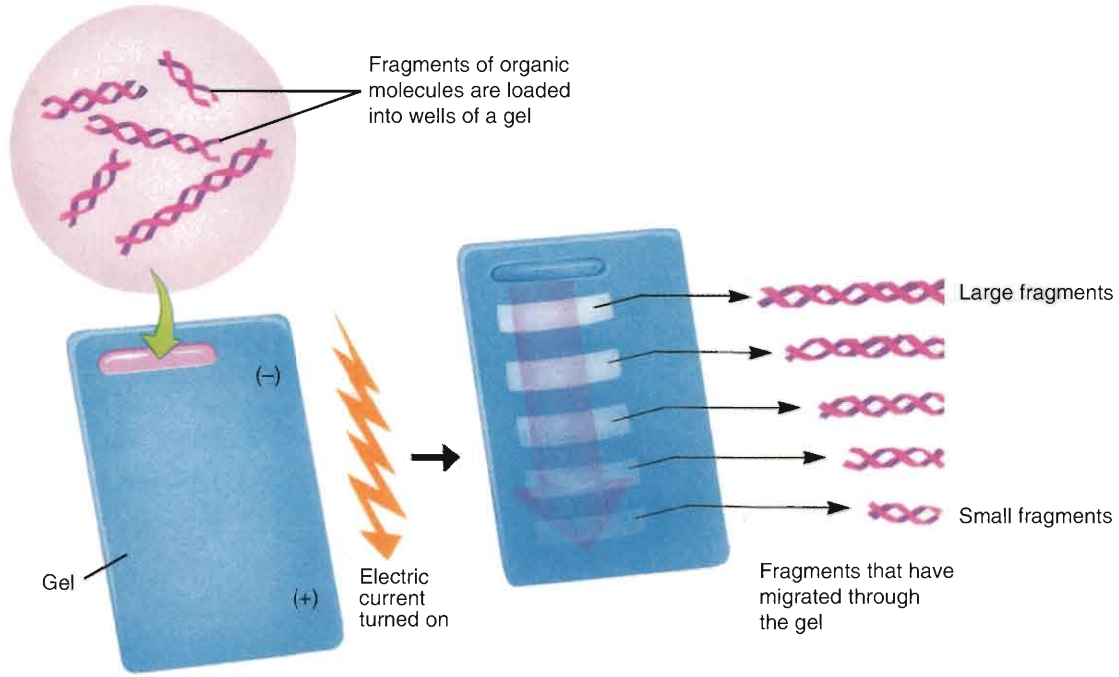
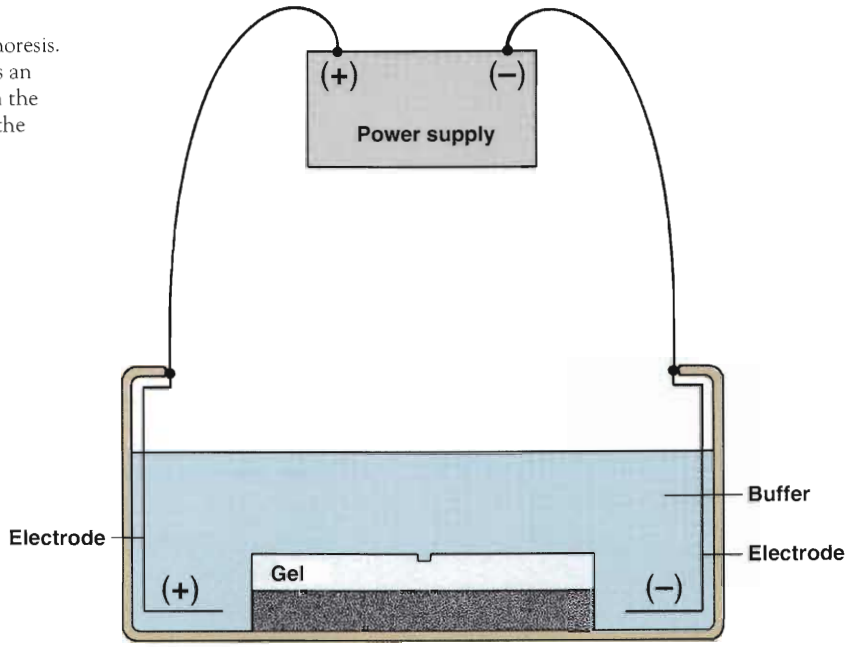


Figure 7.4

Gel electrophoresis. This process separates DNA fragments, protein fragments, and other organic compounds by causing them to move through an electrically charged gel. The fragments move according to their size, shape, and electrical charge; some fragments move slowly and some move quickly. When their migration is complete the fragments can be stained and visualized easily. In the example shown here, the DNA fragments were separated by size.

Figure 7.5
Apparatus for gel electrophoresis. The power supply produces an electrical gradient between the + and − poles and across the gel.



Procedure 7.3

Separate organic molecules by gel electrophoresis

1. Obtain an electrophoresis chamber. Cover the ends of the bed as shown in figure 7.6 and demonstrated by your instructor.
2. Place a six-tooth comb in or near the middle set of notches of the gel-cast bed. There should be a small space between the bottom of the teeth and the bed.
3. Mix a 0.8% (weight by volume) mixture of agarose powder in a sufficient volume of buffer to fill the gel chamber. Heat the mixture until the agarose dissolves.
4. When the hot agarose solution has cooled to 50°C, pour the agarose solution into the gel-cast bed (fig. 7.7).
5. After the gel has solidified, gently remove the comb by pulling it straight up (fig. 7.8). Use of a plastic spatula may help prevent tearing the gel. Use the sketch in figure 7.9 to label the wells formed in the gel by the comb.
6. Submerge the gel under the buffer in the electrophoresis chamber.
7. You will study six samples:
   Sample 1: Bromophenol blue (molecular weight = 670 g $mole^{-1}$)
   Sample 2: Methylene blue (molecular weight = 320 g $mole^{-1}$)
   Sample 3: Orange G (molecular weight = 452 g $mole^{-1}$)
   Sample 4: Xylene cyanol (molecular weight = 555 g $mole^{-1}$)
   Samples 5 and 6: Unknowns

Figure 7.6
Cover the ends of the removable gel bed with rubber end-caps or tape.

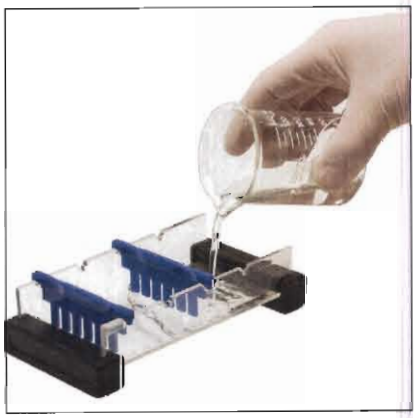


Figure 7.7
Place the comb near the center set of notches of the gel bed. Prepare the agarose solution and pour the gel.

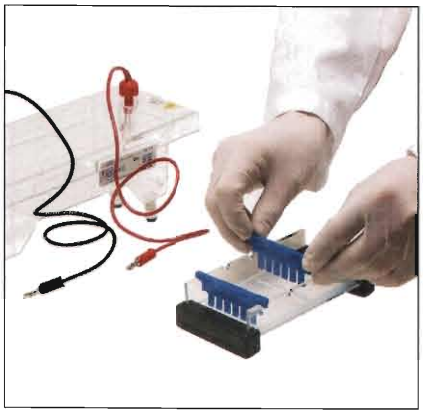


Figure 7.8
After the gel solidifies, gently remove the rubber end-caps (or tape) and pull the combs straight up from the gel.

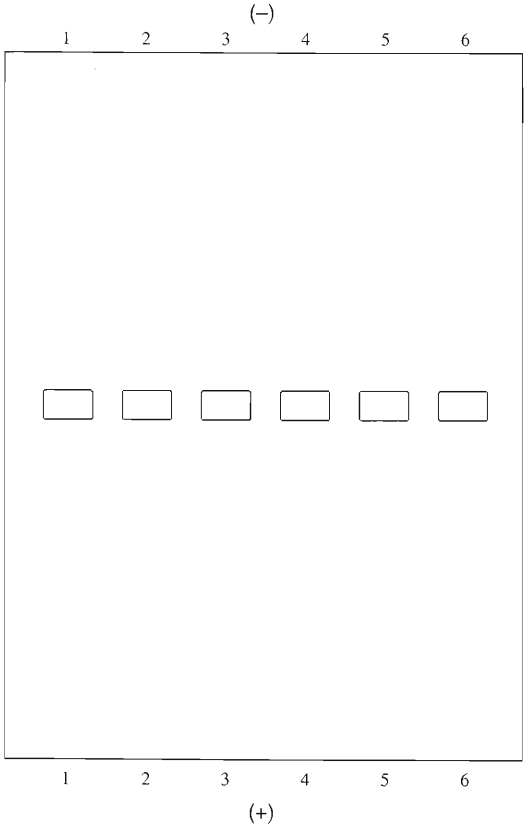


Figure 7.9
Sketch of the wells formed in the gel by the comb as viewed from above.

Use a micropipettor or a simple pipet and bulb to load the samples into the wells of the gel. If you use a micropipettor, your instructor will demonstrate its use. If you use a simple pipet and bulb, gently squeeze the pipet bulb to draw Sample 1 into the pipet. Be sure that the sample is in the lower part of the pipet. If the sample becomes lodged in the bulb, tap the pipet until the sample moves into the lower part.

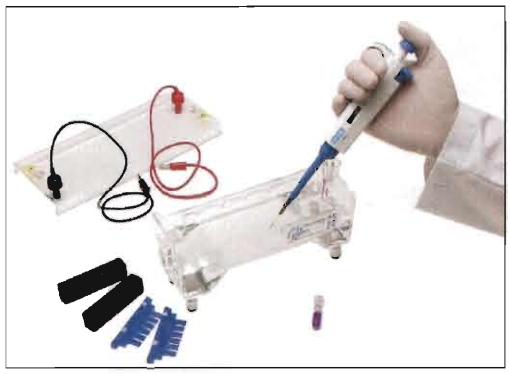


Figure 7.10
Submerge the gel in the buffer-filled electrophoresis chamber and load the samples into the wells of the gel.

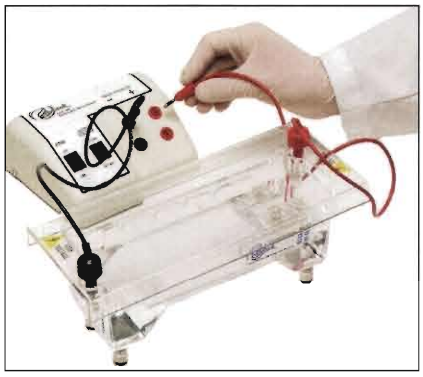


Figure 7.11
Attach the safety cover, connect the power source, and run the electrophoresis.

8. To eliminate excess air hold the pipet above the sample tube and slowly squeeze the bulb until the sample is near the pipet's opening.
9. Place the pipet tip into the electrophoresis buffer so it is barely inside sample well 1 (fig. 7.10). Do not touch the bottom of the sample well. Maintain pressure on the pipet bulb to avoid pulling buffer into the pipet.
10. Slowly inject the sample into the sample well. Stop squeezing the pipet when the well is full. Do not release the pressure on the bulb. Remove the pipet from the well.
11. Thoroughly rinse the pipet with distilled water.
12. Load the remaining five samples into the gel by repeating steps 6–10 (fig. 7.10). Load Sample 2 into the second well, Sample 3 into the third well, etc.
13. Carefully snap on the cover of the electrophoresis chamber (fig. 7.11). The red plug in the cover should be placed on the terminal indicated by the red dot. The black plug in the cover should be placed on the terminal indicated by the black dot.
14. Insert the plug of the black wire into the black (negative) input of the power supply. Insert the plug of

the red wire into the red (positive) input of the power supply.

15. Turn on the power and set the voltage at 90 V. You'll soon see bubbles forming on the electrodes. Examine the gel every 10 min.

16. After 30 min, turn off the power and disconnect the leads from the power source. Gently remove the cover from the chamber and sketch your results in figure 7.9.

Question 3

a. Bromophenol blue, Orange G, and xylene cyanol each have a negative charge at neutral pH, whereas methylene blue has a positive charge at neutral pH. How does this information relate to your results?

b. Did Orange G, bromophenol blue, and xylene cyanol move the same distance in the gel? Why or why not?

c. What compounds do you suspect are in Samples 5 and 6? Explain your answer.

INTERPRETING A DNA-SEQUENCING GEL

Examine figure 7.12, which includes a photograph of a gel used to determine the order, or sequence, of nucleotides in a strand of DNA. To prepare the sample for electrophoresis, samples of the DNA being investigated were put into each

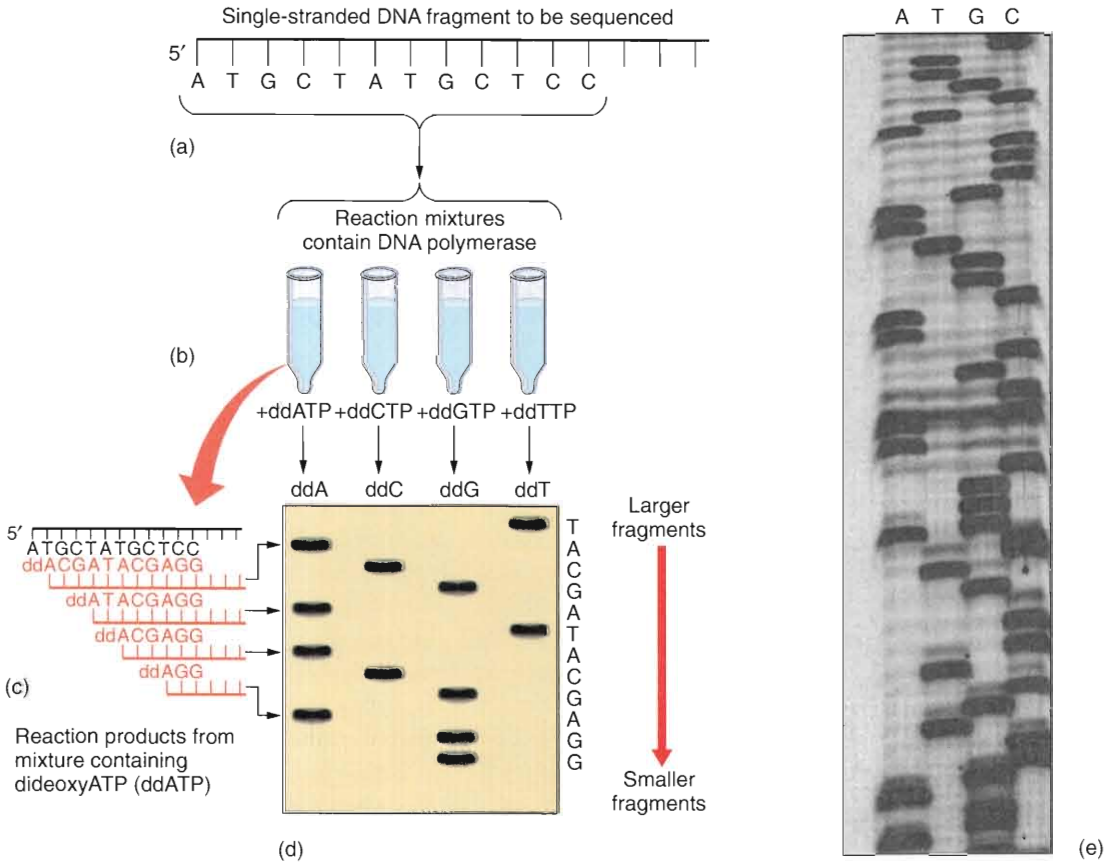


Figure 7.12

Determining the sequence of nucleotides in DNA. (*a*) Treating DNA with sodium hydroxide (NaOH) denatures double-stranded DNA into single-stranded DNA. One of the single strands of DNA to be sequenced is placed in each of four tubes. (*b*) The enzyme DNA polymerase is added to each tube along with a specific nucleotide-terminator. As polymerase replicates the DNA, the terminators are incorporated and will terminate various lengths of fragments of DNA. For example, the terminator ddATP will halt the reaction wherever adenosine occurs. The terminator ddATP (dideoxy adenosine triphosphate) will terminate a growing strand because it lacks a 3′ hydroxyl group and therefore cannot bond with the next deoxynucleotide. (*c*) Each tube will contain a sample of all possible replicated fragment lengths corresponding to the positions of that specific nucleotide. The sequences in red are the complement strands. (*d*) During electrophoresis, the fragments migrate at different rates according to their length. (*e*) The lanes of the resulting gel are labeled according to their base: A, adenine; T, thymine; G, guanine; and C, cytosine. This technique is usually referred to as "Sanger" sequencing in honor of Fred Sanger, a Nobel laureate who, in 1977, first sequenced a piece of DNA.

of four tubes and induced to replicate. Also, into the first tube, an adenine-terminator was added in addition to all the other nucleotides. As the complementary strand was being constructed the terminators were occasionally incorporated wherever an adenine nucleotide was used. This random incorporation resulted in all possible lengths of DNA pieces that had an adenine on the end. The same process was conducted in the other tubes with thymine-, guanine-, and cytosine-terminators; one treatment for each of the four lanes in the gel. Electrophoresis separated the replicated pieces of DNA by size. Staining the gel revealed which lengths of the complementary DNA were terminated by which nucleotide-terminators. Examine figure 7.12d.

The gel consists of four "lanes," labeled A, T, G, and C, indicating either adenine-, thymine-, guanine-, or cytosine-terminated pieces of DNA. By "reading" down the gel, you can determine the sequence of nucleotides in the DNA. For example, the uppermost band of the gel is in the T (thymine) lane. Therefore, the first base of the piece of DNA is thymine. Similarly, the next bands are in the A, C, G, and A lanes. Thus, the first five bases of the complementary strand DNA are T-A-C-G-A. List the next seven nucleotides of the DNA as indicated by the gel. Also list the sequence of the first 12 nucleotides in the original DNA being investigated.

Question 4

a. How did the sequence of nucleotides revealed on the gel differ from the sequence of the original strand of DNA?

b. Assume that the gel shown in Figure 7.12d is from blood collected at a murder scene. This blood does not match that of the victim. You have collected DNA from five people suspected of murder. Gels comparable to the one shown in Figure 7.12*d* read as follows for each of the suspects:

Suspect #1: T-A-C-G-A-T-A-C-G-A-C
Suspect #2: T-A-C-G-A-T-A-C-G-A-C
Suspect #3: T-A-C-G-A-C-A-C-G-C-G
Suspect #4: T-A-C-G-A-T-G-C-G-A-C
Suspect #5: T-A-C-G-A-T-C-C-G-T-C

What do you conclude from this evidence?

INVESTIGATION I

Refining the Paper Chromatography Procedure

Carefully planned and refined procedures are critical for laboratory techniques such as paper chromatography. The sensitivity of these techniques depends on a variety of factors, including the many parameters associated with timing, chemicals, measurements, and temperatures. In procedure 7.2 you were given a rather standardized protocol, but it can always be improved for specific experiments. For example, how would you modify the paper chromatography procedure to better resolve two amino acids having approximately the same R_f values? What parameter(s) of the experimental design might be tweaked to increase the technique's resolving power? We suggest that you begin your investigation in the following way:

a. List the parameters involved in paper chromatography. Think carefully; many factors are involved.

b. Choose one or two parameters that you can test for their impact on the chromatography results. Why did you choose these?

c. Choose two amino acids for experimentation. Why did you choose these two?

d. Choose your treatment levels for each parameter, and then do your experiment.

e. What did you conclude?

INVESTIGATION II

The Importance of the Length of the Column in Column Chromatography

Observation: Column chromatography is a common means of separating molecules according to their size and shape. The movement of molecules through a column is affected by several factors, including the column's matrix and the column's length.

Question: How does the length of a column affect the separation of molecules via column chromatography?

a. Establish a working lab group and obtain Investigation Worksheet 7 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 7 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. How are column chromatography, paper chromatography, and gel electrophoresis different? How are they similar?

2. How would the results of electrophoresis vary if the voltage was increased? If the agarose was made more dense? Or if the migration was allowed to run twice as long?

3. How could knowing the nucleotide base sequence of a piece of DNA be important to a biologist?

4. How could knowing the nucleotide base sequence of a piece of DNA be important to someone trying to solve a crime?

5. How could knowing the nucleotide base sequence of a piece of DNA be important for someone studying a hereditary disease?

6. How could knowing the nucleotide base sequence of a piece of DNA be important for someone wanting to improve the yield of a crop such as corn?

WRITING TO LEARN BIOLOGY

Which of the methods discussed in this exercise would best quantify the relative amounts of the molecules being separated? Why?

9

Diffusion and Osmosis

Passive Movement of Molecules in Biological Systems

Objectives

By the end of this exercise you should be able to:

1. Observe Brownian movement and understand its relationship to molecular movement.
2. Explain the factors controlling a substance's direction and rate of diffusion.
3. Determine the direction and relative rates of diffusion of molecules of different sizes.
4. Determine the direction and rate of osmosis into and out of simulated cells in hypotonic, hypertonic, and isotonic environments.
5. Describe how hypotonic, hypertonic, and isotonic solutions affect the volume and integrity of blood cells.
6. Describe how a hypertonic solution affects the volume and integrity of plant cells.

All molecules display random thermal motion, or kinetic energy; this is why a dissolved molecule tends to move around in a solution. Kinetic energy causes molecules to diffuse outward from regions of high concentration to regions of lower concentrations. This random movement is constant, but the net movement of molecules from high to low concentration continues until the distribution of molecules becomes homogenous throughout the solution. For example, when a dye dissolves in a container of water, the dye disperses as the crystal dissolves. The rate of dispersal depends on the concentration of the dye, the size of the dye molecules, the temperature of the solution, and the density of the solvent. Regardless of this rate, the dye will eventually become uniformly distributed throughout the solution. This phenomenon is easily illustrated by placing a drop or crystal of dye into a glass of water (fig. 9.1).

In this exercise you will study the diffusion of molecules in artificial and living systems.

BROWNIAN MOVEMENT

Heat causes **random motion** of molecules and passively moves molecules in biological systems. Although we cannot directly see molecules move, we can see small particles move after collisions with moving molecules. This motion was originally described in 1827 by Robert Browning as he observed dead pollen grains in water and viewed them with a microscope. **Brownian movement** is visible using your microscope's high magnification. Carmine red dye mixed with soap produces a good suspension of small particles. The particles of red dye are small enough to vibrate when water molecules bump into them.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 9.1

Observe Brownian movement

1. Place a small drop of a carmine red suspension on a microscope slide and cover the drop with a coverslip.

Figure 9.1

Beakers of water before and after diffusion of a dye. Random movements of water and dye molecules drive diffusion, eventually resulting in a uniform distribution of the dye. Convection currents may also help distribute the dye in these solutions.

they have small pores that allow small molecules such as water molecules to pass but block large molecules such as glucose. However, remember that living cell membranes also discriminate among molecules based on charge and solubility whereas dialysis tubing does not. Dialysis tubing is only a physical model of a cell and its selectivity is based only on molecular size.

Examine some dialysis tubing. Although the dried material looks like a narrow sheet of cellophane, it is a flattened, open-ended tube.

In procedure 9.3 you will use two indicators: **phenolphthalein** and **iodine.** Phenolphthalein is a pH indicator that turns red in basic solutions (see Exercise 5). Iodine is a starch indicator that changes from yellow to dark blue in the presence of starch (see Exercise 6).

Procedure 9.3

Observe diffusion across a differentially permeable membrane

1. Obtain four pieces of string or dialysis clips and two pieces of water-soaked dialysis tubing approximately 15 cm long.
2. Seal one end of each bag by folding over 1–2 cm of the end. Then accordion-fold this end and tie it tightly with monofilament line or string (fig. 9.4). The ends of the tube must be sealed tightly to prevent leaks.
3. Roll the untied end of each tube between your thumb and finger to open it and form a bag.
4. Use either a graduated cylinder or pipet to fill one tube with 10 mL of water and add three drops of phenolphthalein. Seal the open end of the bag by folding the end and tying it securely.
5. Fill the other bag with 10 mL of starch suspension. Seal the open end of the bag by folding the end and tying it securely.
6. Gently rinse the outside of each bag in tap water.
7. Fill a beaker with 200 mL of tap water and add 10 drops of 1 M sodium hydroxide (NaOH). Submerge the dialysis bag containing phenolphthalein in the beaker.

Do not spill the NaOH. It is extremely caustic.

8. Fill a beaker with 200 mL of tap water and add 20–40 drops of iodine. Submerge the dialysis bag containing starch in the beaker.
9. Observe color changes in the two bags' contents and the surrounding solutions.
10. In this experiment some of the solutes can move through the membrane and some cannot. Water can freely move through the membrane, but the movement of water is not of interest in this experiment.
11. Record in figure 9.5 the color inside and outside the bags. Label the contents inside and outside the bags.

Question 4

a. Describe color changes in the two bags and their surrounding solutions.

b. For which molecules and ions (phenolphthalein, iodine, starch, Na^+, OH^-) does your experiment give evidence for passage through the semipermeable membrane?

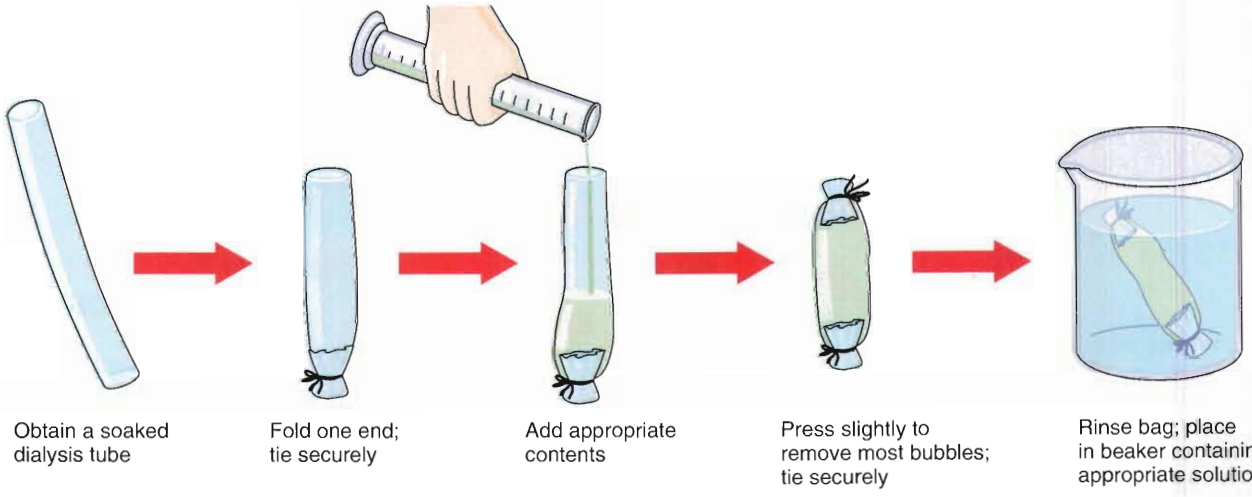


Figure 9.4
Preparation of dialysis tubing as a model of a cell surrounded by a differentially permeable membrane.

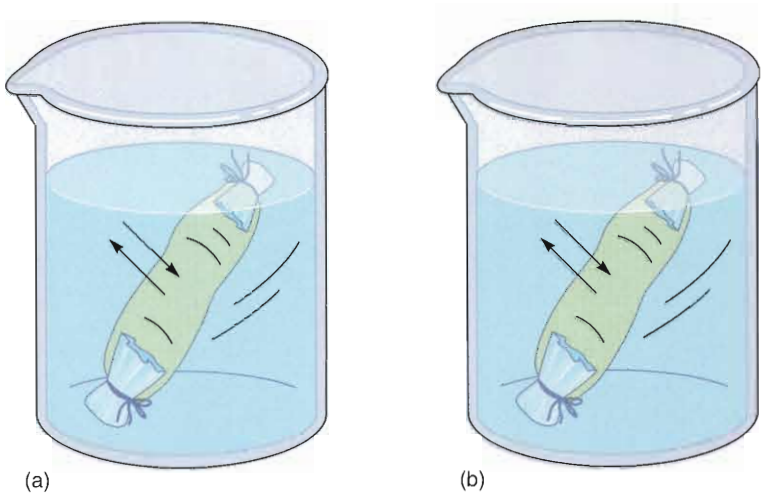


Figure 9.5

(*a*) Movements and reaction of sodium hydroxide and phenolphthalein through a differentially permeable membrane. (*b*) Movements and reaction of iodine and starch. Record the results of your experiment on this diagram.

c. What characteristic distinguishes those molecules and ions passing through the membrane from those that do not pass through the membrane?

OSMOSIS AND THE RATE OF DIFFUSION ALONG A CONCENTRATION GRADIENT

The speed at which a substance diffuses from one area to another depends primarily on the concentration gradient between those areas. For example, if concentrations of a diffusing substance at the two areas differ greatly, then diffusion is rapid. Conversely, when the concentration of a substance at the two areas is equal, the diffusion rate is zero and there is no net movement of the substance.

Osmosis is diffusion of water across a differentially permeable membrane. Osmosis follows the same laws as diffusion but always refers to water, the principal solvent in cells. A **solution** is a homogenous, liquid mixture of two or more kinds of molecules. A **solvent** is a fluid that dissolves substances, and a **solute** is a substance dissolved in a solution.

We can simulate osmosis by using dialysis bags to model cells under different conditions and measuring the direction and rate of osmosis. Each of the four dialysis bags in the following experiment is a model of a cell. Bag A simulates a cell with a solute concentration that is hypotonic relative to its environment. **Hypotonic** describes a solution with a lower concentration of solutes, especially those solutes that do not pass across the surrounding membrane. Water moves across semipermeable membranes out of hypotonic solutions. Conversely, the solution surrounding bag A is hypertonic relative to the cell. **Hypertonic** refers to a solution with a high concentration of solutes.

Bag B represents a cell whose solute concentration equals the concentration in the environment; that is, this cell (bag B) is isotonic to its environment. **Isotonic** refers to two solutions that have equal concentrations of solutes. Bags C and D are both hypertonic to their environment and have higher solute concentrations than the surrounding environment. Remember that the solute (sugar) does not pass through the membrane—only the water does.

NOTE

Start this experiment at the beginning of the lab period so that you'll have enough time to see results.

Procedure 9.4

Observe osmosis across a concentration gradient

1. Obtain eight pieces of string and four pieces of water-soaked dialysis tubing 15 cm long. Seal one end of each tube by folding and tying it tightly.
2. Open the other end of the tube by rolling it between your thumb and finger.
3. Fill the bags with the contents shown in figure 9.6. To label each bag, insert a small piece of paper with the appropriate letter (A, B, C, or D written on it in pencil).
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Gently blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 g.
6. Record these initial weights in table 9.1 in the first column.

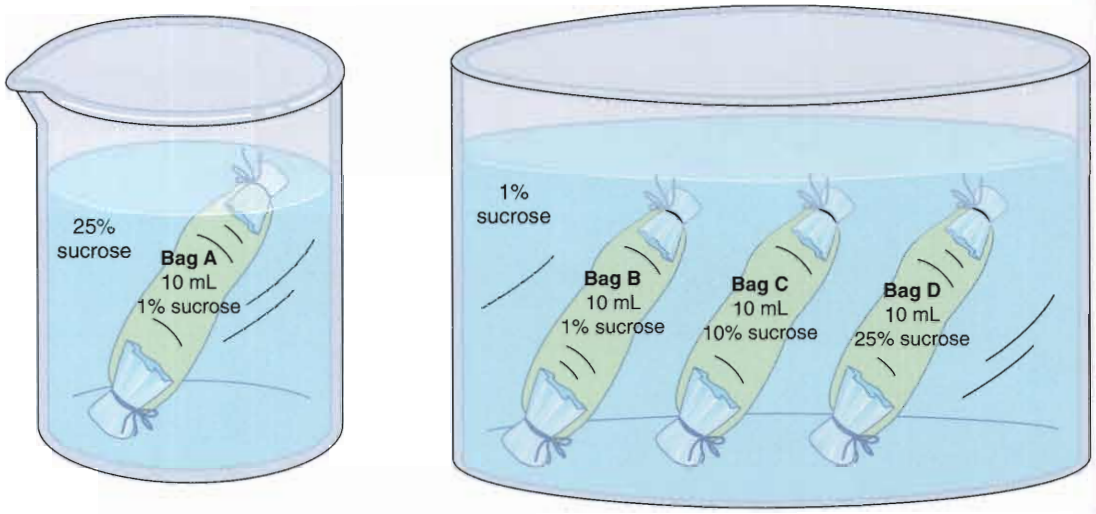


Figure 9.6

Experimental setup for four cellular models used to measure the rate of osmosis.

TABLE 9.1

CHANGES IN WEIGHT OF DIALYSIS BAGS USED AS CELLULAR MODELS*

	0 Min	15 Min		30 Min		45 Min		60 Min	
	Initial Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight
Bag A									
Bag B									
Bag C									
Bag D									

*Each change in weight is only for the previous 15-min interval.

7. Place bags B, C, and D in three individual beakers or one large bowl filled with 1% sucrose (fig. 9.6). Record the time.
8. Place bag A in a 250-mL beaker and fill the beaker with 150 mL of 25% sucrose. Record the time.
9. Remove the bags from the beakers at 15-min intervals for the next hour (or at intervals indicated by your instructor), gently blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers.
10. During the 15-min intervals, use your knowledge of osmosis to make hypotheses about the direction of water flow in each system (i.e., into or out of bag), and the extent of water flow in each system (i.e., in which system will osmosis be most rapid?).
11. For each 15-min interval record the total weight of each bag and its contents in table 9.1. Then calculate and record in table 9.1 the change in weight since the previous weighing.

Procedure 9.5

Graph osmosis

1. Use the graph paper at the end of this exercise to construct a graph with *Total Weight (g)* versus *Time (min)*. *Total Weight* changed in response to differences in the independent variable, so *Total Weight* is the **dependent variable.** The dependent variable is always graphed on the vertical axis. *Time* is the variable that you established and actively controlled and, therefore, is the **independent variable.** The independent variable is always graphed on the horizontal axis.
2. Graphs must have a title (e.g., Relationship between Time and Weight Gain), correctly labeled axes (e.g., *Total Weight, Time*), a label showing measurement units (e.g., g and min), and values along each axis (e.g., 0, 15, 30, 45, 60). Include these in your graph.
3. Plot the data for total weight at each time interval from table 9.1.
4. Include the data for all four bags as four separate curves on the same graph.

Question 5

a. Did water move across the membrane in all bags containing solutions of sugar?

b. In which bags did osmosis occur?

c. A concentration gradient for water must be present in cells for osmosis to occur. Which bag represented the steepest concentration gradient relative to its surrounding environment?

d. The steepest gradient should result in the highest rate of diffusion. Examine the data in table 9.1 for Change in Weight during the 15- and 30-min intervals. Did the greatest changes in weight occur in cells with the steepest concentration gradients? Why or why not?

Question 6

a. Refer to your graph. How does the slope of a segment of a curve relate to the rate of diffusion?

b. What influence on diffusion (i.e., temperature, pressure, concentration) causes the curves for bags C and D eventually to become horizontal (i.e., have a slope = 0)?

WATER POTENTIAL

Plants need to balance water uptake and loss as it moves from one part of a plant to another and in and out of cells by osmosis. However, the concentration gradient of water and solutes doesn't solely determine the direction and rate of water movement. Physical pressure influenced by cell walls and evaporation is also important. Plant physiologists refer to the combined effects of concentration and pressure such as that from cell walls as **water potential;** water will flow from an area of high water potential to an area of low potential. Both high water concentration (low solute concentration) and high pressure increase water potential. Similarly, high solutes and low pressure decrease water potential. In simple terms, water flows through a plant from the higher water potentials of the root tissues toward the lower water potentials of leaves. These lower potentials in leaves are created by their loss of water to the atmosphere (see Exercise 33). In the following procedure you will measure the concentration of solutes in potato cells and relate this concentration to water potential.

Procedure 9.6

Determine the concentration of solutes in living plant cells

1. Locate the five beakers prepared by your instructor with five concentrations of salt (NaCl) solution.
2. The cylinders of potato that you see in the solutions were all originally the same size (i.e., the same length or weight). Check the beaker labels to determine which measure of size (length or weight) you will be using as your data.
3. Record the initial values in table 9.2.
4. Carefully remove three of the potato cylinders from each solution and measure their size.
5. Record your data in table 9.2.
6. Calculate the mean change in size and record the data in table 9.2.
7. Your instructor may ask you to graph your data (see Question 7f). Follow his or her instructions.

Question 7

a. Which potato cylinders increased in size or weight? Why?

b. Which solution(s) contained a higher concentration of solutes and therefore a lower water potential than in the potato cells? Explain your answer.

c. Which salt solution best approximated the water potential in the potato cells?

d. For a growing potato plant what would you predict as the water potential of the potato relative to the soil? Relative to the leaves?

TABLE 9.2

CHANGE IN LENGTH OF POTATO CYLINDERS SURROUNDED BY DIFFERENT SALT CONCENTRATIONS

Concentration of Salt Solution (%)	Initial Size of Cylinders (millimeters or grams)	Changes in Size of Three Sample Cylinders			Mean Change in Size
0	_____	_____	_____	_____	_____
0.9	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____
10	_____	_____	_____	_____	_____
15	_____	_____	_____	_____	_____

e. What might be some sources of error in this experiment?

f. How could a graph of your data help you estimate the solute concentration of potato cells?

HEMOLYSIS OF BLOOD CELLS

Living red blood cells (erythrocytes) are good models for studying osmosis and diffusion in hypotonic, hypertonic, and isotonic solutions. Osmosis occurs when living cells are placed in a hypotonic or hypertonic environment and water diffuses into or out of the cell (fig. 9.7). For example, in the previous experiment water moved into cells toward the low concentration of water. However, osmosis into animal cells increases the hydrostatic (i.e., water) pressure and bursts the cells because they lack cell walls. This destruction of a cell by the influx of water (causing the cell to burst) is called **lysis.** Such destruction of a red blood cell is called **hemolysis.** If water flows out of a cell into a hypertonic solution, the cell will shrivel and become crenate.

Detect hemolysis and crenation in blood cells in three different solutions using the following procedure.

Procedure 9.7

Observe hemolysis

1. Obtain and label three test tubes and fill them with the solutions listed in table 9.3.
2. Add four drops of fresh sheep's blood to each tube.

Wash your hands thoroughly after working with blood products. Always handle sheep blood with caution and avoid skin contact.

3. Cover each tube with Parafilm and invert the tubes to mix the contents.
4. Hold each tube in front of a printed page and determine if you can read the print through the solution (fig. 9.8). Record your results in table 9.3.
5. Obtain a microscope, slide, and coverslip.
6. Use an eyedropper or pipet to obtain one drop from each tube. Make a wet mount and examine the blood cells. Use low magnification first and then higher magnification.
7. Record in table 9.3 the cell's condition as crenate, normal, or lysed.

Question 8

a. Through which test tubes could you read the printed page? Why?

b. Which concentration of NaCl lysed the cells?

c. Which of the three solutions most closely approximates the solute concentration in a red blood cell? How do you know?

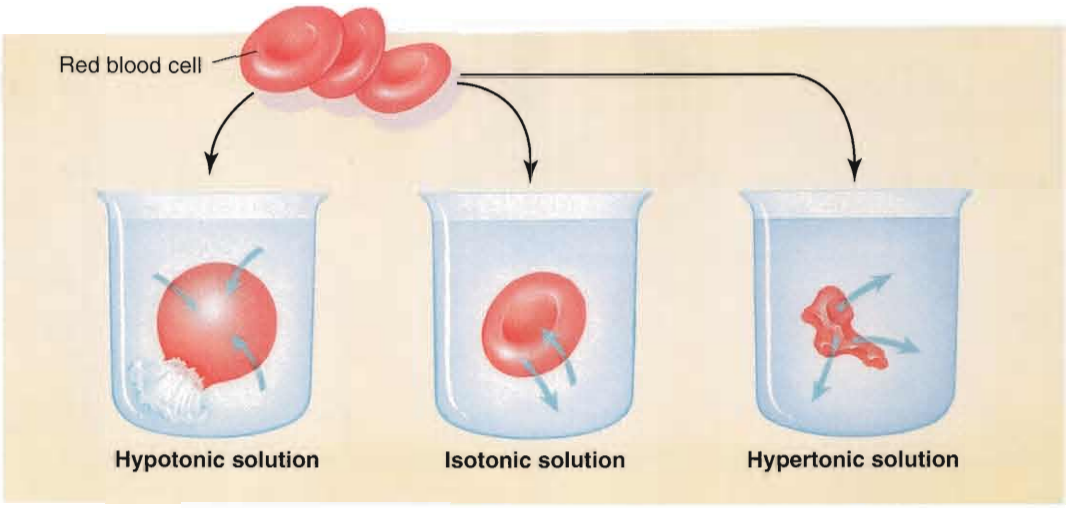


Figure 9.7

Osmosis of water surrounding animal cells. When the outer solution is hypotonic with respect to the cell, water will move into the cells and the cells will lyse; when it is hypertonic, water will move out of the cells and the cells will shrink (i.e., become crenate).

TABLE 9.3

HEMOLYSIS OF RED BLOOD CELLS EXPOSED TO THREE SOLUTIONS WITH DIFFERENT SOLUTE CONCENTRATIONS

Tube	Contents	Readable Print (yes/no)	Cell Condition (crenate/normal/lysed)
1	5 mL 10% NaCl	_____	_____
2	5 mL 0.9% NaCl	_____	_____
3	5 mL distilled water	_____	_____

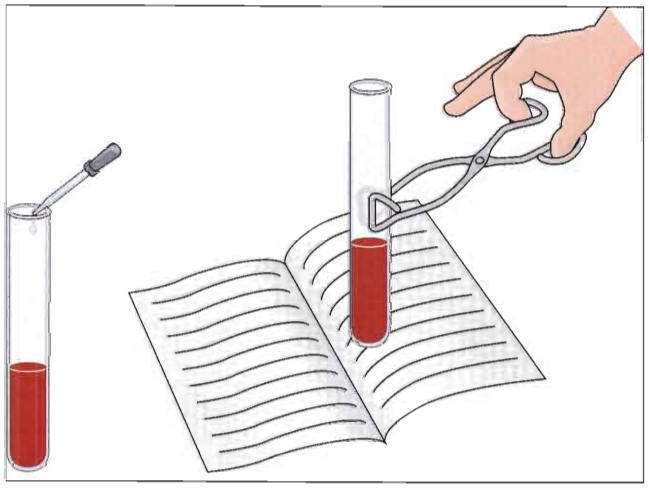


Figure 9.8

Experimental setup for determining hemolysis. Hypertonic solutions will hemolyze cells.

PLASMOLYSIS OF PLANT CELLS

Plasmolysis is the shrinking of the cytoplasm of a plant cell in response to diffusion of water out of the cell and into a hypertonic solution (high salt concentration) surrounding the cell (fig. 9.9). During plasmolysis the cellular membrane pulls away from the cell wall (fig. 9.10).

In procedure 9.8 you will examine the effects of highly concentrated solutions on osmosis and cellular contents.

Procedure 9.8

Observe plasmolysis

1. Prepare a wet mount of a thin layer of onion epidermis or *Elodea* leaf. Examine the cells.
2. Add two or three drops of 30% NaCl to one edge of the coverslip.
3. Wick this salt solution under the coverslip by touching a piece of absorbent paper towel to the fluid at the opposite edge of the coverslip.
4. Examine the cells. The cytoplasm is no longer pressed against the cell wall. This shrinkage is **plasmolysis.**

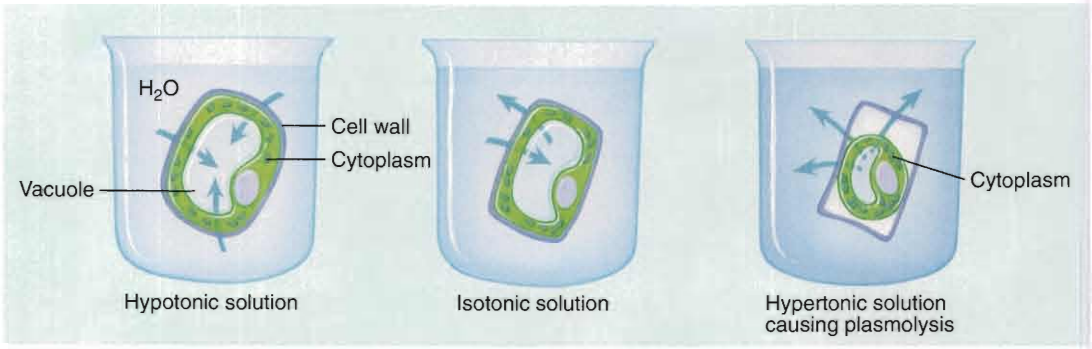
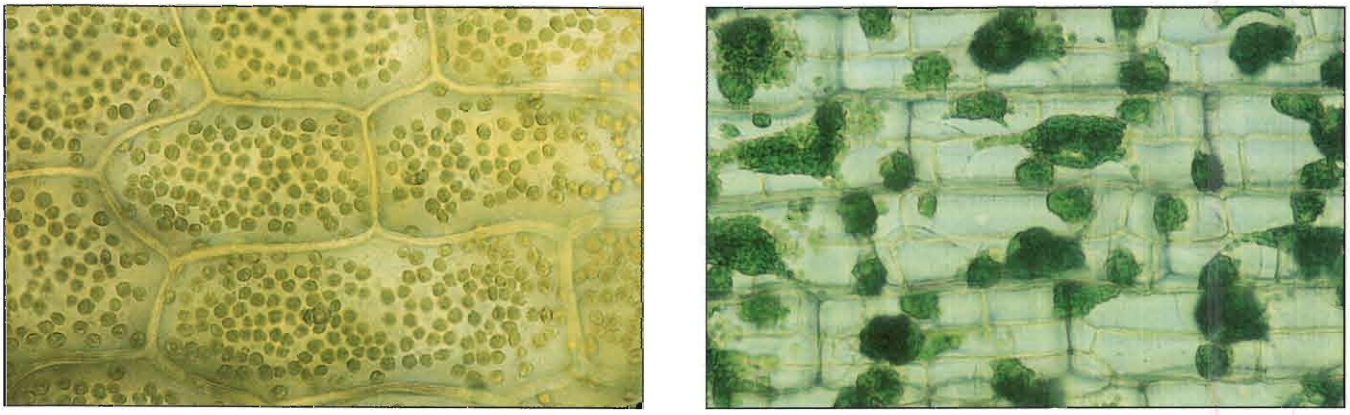


Figure 9.9

Osmosis of water into and out of plant cells. In most plant cells the large central vacuole contains a high concentration of solutes (i.e., the environment surrounding the cell is hypotonic to the cell), so water tends to diffuse into the cells, causing the cells to swell outward against their rigid cell walls. However, if a plant cell is immersed in a high-solute (hypertonic) solution, water will leave the cell, causing the cytoplasm to shrink and pull away from the cell wall.



(a) (b)

Figure 9.10

(*a*) Turgid *Elodea* cells. (*b*) Plasmolyzed *Elodea* cells showing the effects of exposure to a hypertonic solution.

Question 9

a. Why did the plant cells plasmolyze when immersed in a hypertonic solution?

b. What can you conclude about the permeability of the cell membrane (i.e., the membrane surrounding the cytoplasm) and vacuolar membrane (the membrane surrounding the vacuole) to water?

To observe the effects of cellular plasmolysis on a larger scale, compare petioles of celery that have been immersed overnight in distilled water or in a salt solution.

Question 10

What causes crispness (i.e., firmness, crunchiness) in celery?

INVESTIGATION

Determining the Concentrations of Solutes in Plant Tissue

Observation: Water moves into and out of cells along a concentration gradient. The more solutes that are present in cells, the greater the tendency for water to move into the cells.

Question: What is the approximate concentration of solutes in a piece of apple tissue?

a. Establish a working lab group and obtain Investigation Worksheet 9 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 9 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. Why must particles be extremely small to demonstrate Brownian movement?

2. What is the difference between molecular motion and diffusion?

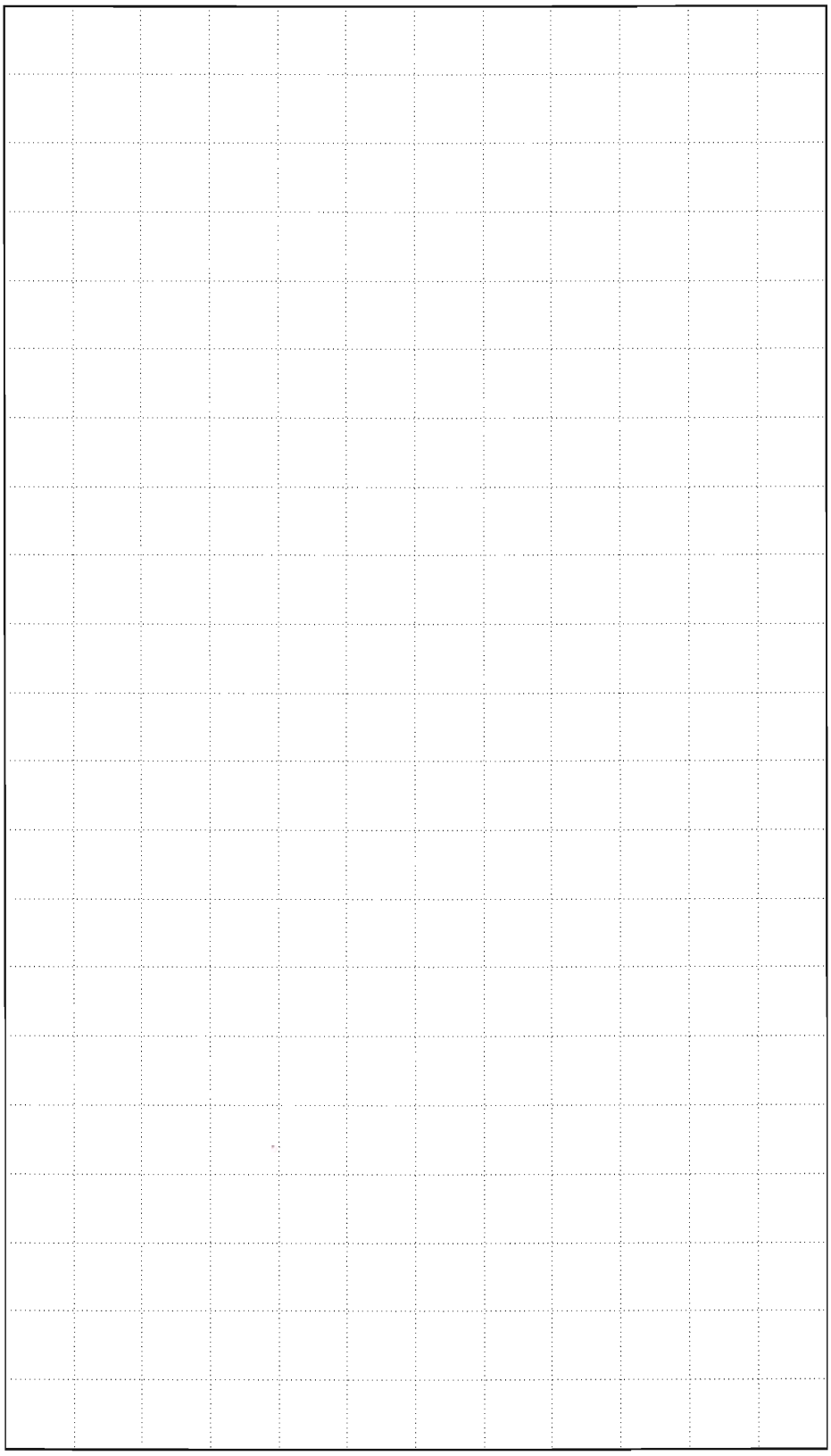
3. If you immerse your hand in distilled water for 15 min, will your cells lyse? Why or why not?

4. Your data for diffusion of water across a differentially permeable membrane in response to a sucrose gradient could be graphed with *Change in Weight* on the vertical axis rather than *Total Weight*. How would you interpret the slope of the curves produced when you do this?

5. How do cells such as algae and protists avoid lysis in fresh water?

WRITING TO LEARN BIOLOGY

Where in an animal might pressure affect diffusion of a substance?



Enzymes
Factors Affecting the Rate of Activity

11

Objectives

By the end of this exercise you should be able to:

1. Describe the structure and function of enzymes, and relate structure and function to active sites, modes of inhibition, and optimal conditions for enzymatic activity.
2. Predict how inhibitors and changes in temperature and pH affect enzymatic reaction rates.
3. Describe how some enzymatic reaction rates can be measured by color changes and gas liberation as products are formed.

Fortunately, not all chemical reactions within our cells occur spontaneously. If they did, our metabolism would be chaotic. Instead, most reactions in cells are controlled by proteins called **enzymes.** Enzymes are **biocatalysts,** meaning that they accelerate metabolic reactions to biologically useful rates. Specifically, enzymes catalyze (accelerate) reactions by lowering the activation energy needed for the reaction to occur (fig. 11.1).

Enzymes act by binding to reacting molecules, called the **substrate,** to form an **enzyme-substrate** complex. This complex stresses or distorts chemical bonds to form a **transition state** in which the substrate becomes more reactive and the metabolic reaction accelerates. The energy needed to form

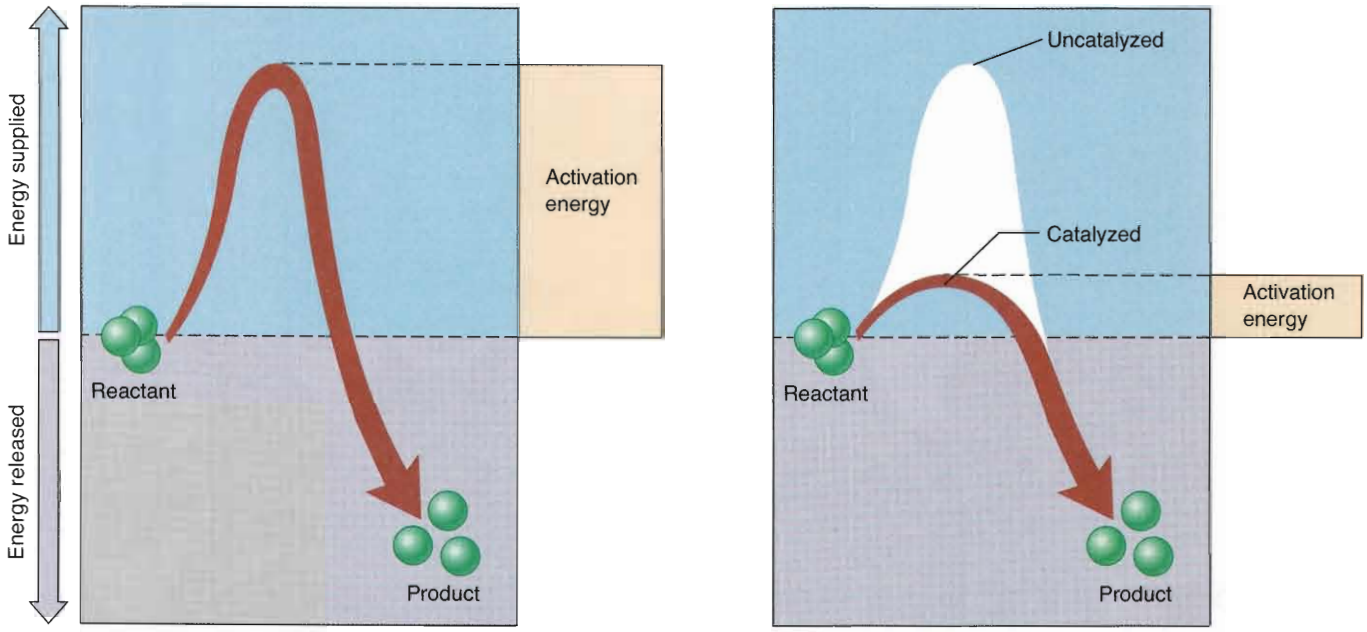
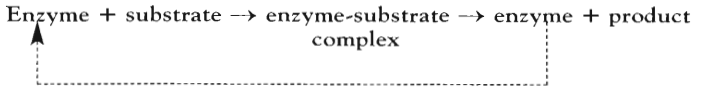


Figure 11.1

Activation energy and catalysis. (*a*) Exergonic reactions (those that release energy) do not necessarily proceed rapidly because energy must be supplied to destabilize existing chemical bonds. This extra energy is the activation energy for the reaction. (*b*) Catalysts accelerate particular reactions by lowering the amount of activation energy required to initiate the reaction.

the transition state is called **energy of activation** and is lowered by the enzyme. The site of attachment and the surrounding parts of the enzyme that stress the substrate's bonds constitute the enzyme's **active site.**



The reaction is complete when the **product** forms and the enzyme is released in its original condition. The enzyme then repeats the process with other molecules of substrate (fig. 11.2).

Enzymes are proteins made of long chains of amino acids that form complex shapes. Although cells contain many enzymes, each type of enzyme has a precise structure and function, and each enzyme catalyzes a specific reaction. This specificity results from an enzyme's unique structure and shape. For example, the shape of the active site on the enzyme's surface is complex and usually couples with only one type of substrate. Any structural change in an enzyme may **denature** or destroy its effectiveness by altering the active site and slowing down the reaction rate. Therefore, the rate of an enzymatic reaction depends on conditions in the immediate environment. These conditions affect the shape of the enzyme and modify the active site and precise fit of an enzyme and its substrate.

The range of values for environmental factors such as temperature and pH at which an enzyme functions best represents that enzyme's **optimal conditions.** The optimal conditions for the enzymes of an organism may be specific for that species and usually are adaptive for the environment of the organism. Other factors such as the amount of substrate or concentration of enzyme also affect the reaction rate.

In this exercise you will learn that environmental factors such as temperature and pH affect enzymatic reactions (fig. 11.3). You will also investigate how inhibitors affect enzymatic activity.

TEMPERATURE AFFECTS THE ACTIVITY OF ENZYMES

Heat increases the rate of most chemical reactions. During enzymatic reactions, faster molecular motion caused by heat increases the probability that enzyme molecules will contact substrate molecules. The rate of chemical reactions generally doubles with a 10°C rise in temperature. However, higher temperatures do not always accelerate enzymatic reactions; enzymatic reactions have an optimal range of temperatures. Temperatures above or below this range decrease the reaction rate. Extreme temperatures often denature enzymes.

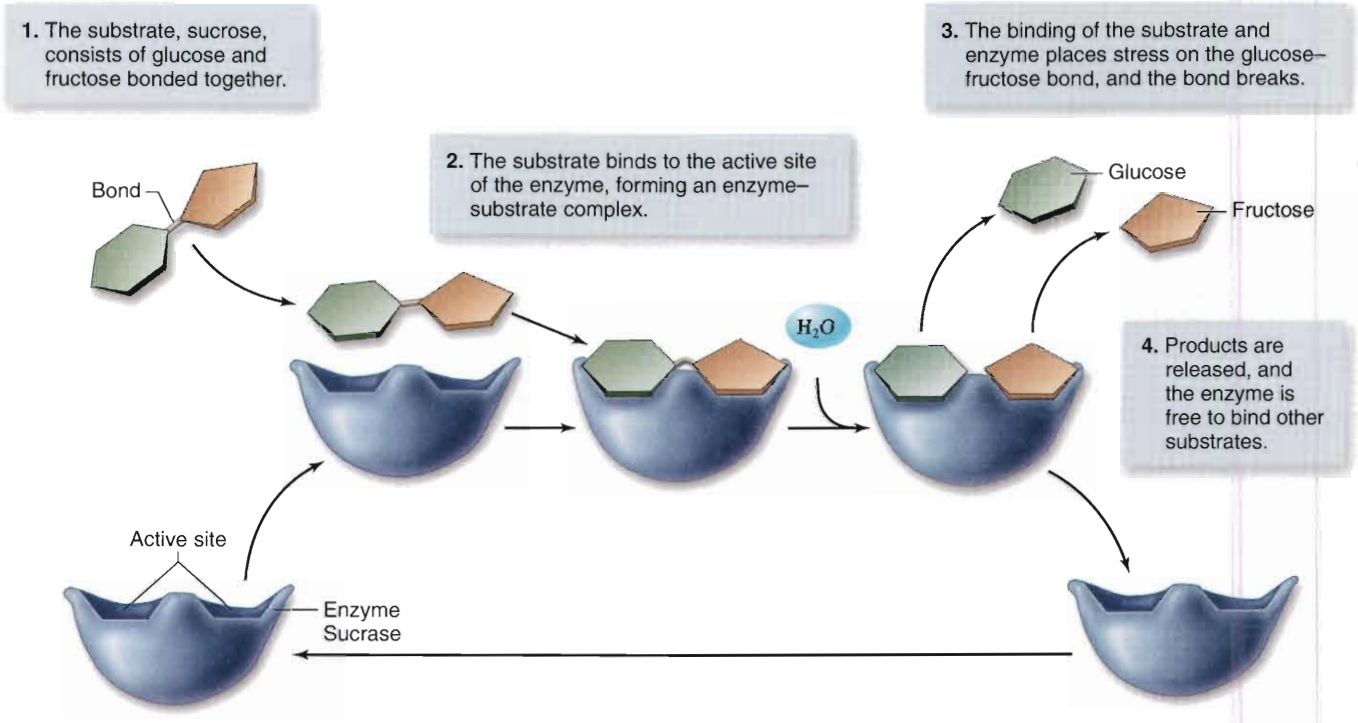


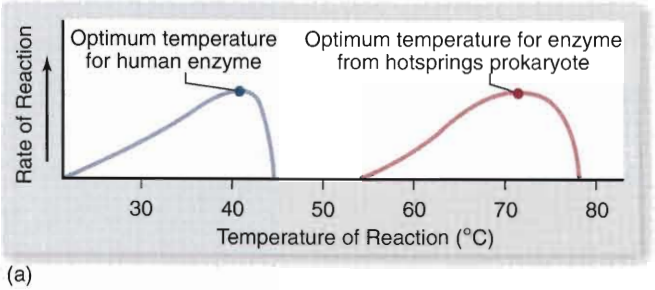
Figure 11.2

The catalytic cycle of an enzyme. Enzymes increase the speed of chemical reactions but are not themselves permanently altered by the process. Here, the enzyme sucrase splits the disaccharide sucrose (steps 1, 2, 3, and 4) into its two parts, the monosaccharides glucose and fructose. After the enzyme releases the glucose and fructose, it can bind another molecule of sucrose and begin the catalytic cycle again.

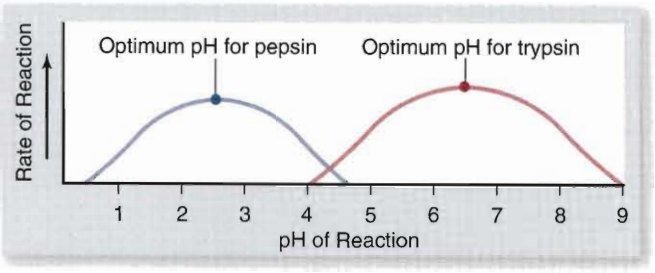
Table 11.1

Experimental Conditions to Test the Effect of Temperature on Catechol Oxidase Activity

Tube	Distilled Water	pH 6 Buffer	Potato Extract (catechol oxidase)	1% Catechol	Temperature
1	2 mL	1 mL			22°C
2	1 mL	1 mL		1 mL	22°C
3	1 mL	1 mL	1 mL		22°C
4		1 mL	1 mL	1 mL	22°C
5		1 mL	1 mL	1 mL	4°C
6		1 mL	1 mL	1 mL	40°C
7		1 mL	1 mL	1 mL	80°C



(a)



(b)

Figure 11.3

Enzymes are sensitive to their environment. The activity of an enzyme is influenced by both (*a*) temperature and (*b*) pH. Most enzymes in humans, such as the protein-degrading enzyme trypsin, work best at temperatures about 40°C and within a pH range of 6 to 8. As you can see, however, pepsin works best at a much lower pH than does trypsin.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

The effects of temperature on enzyme activity can be investigated with **catechol oxidase**, a plant enzyme that oxidizes catechol and converts it to benzoquinone. When fruit is bruised, injured cells release catechol and catechol oxidase, which react to form a brownish product, benzoquinone. Toxic to bacteria, benzoquinone prevents decay in damaged cells. Your source of catechol oxidase will be potato extract.

$$\underset{\text{(substrate)}}{\text{Catechol}} \xrightarrow[\text{(enzyme)}]{\textit{catechol oxidase}} \underset{\text{(product)}}{\text{benzoquinone}}$$

Catechol is toxic. Wash well with soap and water after skin contact.

Procedure 11.1

Observe the effect of temperature on catechol oxidase activity

1. Prepare water-baths at 40°C and 80°C. Locate a refrigerator or ice bath at or below 4°C. Place a test-tube rack in each bath and in the refrigerator.
2. Obtain seven test tubes and number them at the top 1–7.
3. Obtain a tube of potato extract and a tube of 1% catechol from your instructor.
4. Add distilled water, pH buffer, and potato extract to the tubes as listed in table 11.1. Shake or swirl to resuspend the potato extract.

TABLE 11.2

QUALITATIVE AND QUANTITATIVE COLOR CHANGES AS CATECHOL OXIDASE ACTIVITY PRODUCES BROWN BENZOQUINONE

	Qualitative Color Change Results					Quantitative Absorbance Results				
Tube	0 min	5 min	10 min	15 min	20 min	0 min	5 min	10 min	15 min	20 min
1	0	0	0	0	0	0	0	0	0	0
2										
3										
4										
5										
6										
7										

5. Place the tubes in the appropriate bath or refrigerator. Allow each tube to stand undisturbed for 5 min at its respective temperature. Put tubes 1–4 in a test-tube rack at room temperature (approximately 22°C).
6. Add 1% catechol solution to tubes 2 and 4–7 as listed in table 11.1. For each tube immediately record in table 11.2 any color changes for 0 min. Record qualitative color changes on a scale between 0 (no change) and 5 (drastic change).
7. Every 5 min observe and note color changes in the seven tubes over the next 20 min. Always return the tubes to their original temperature locations (e.g., refrigerator, water-bath).
8. If your instructor asks you to further quantify your data, then measure the absorbance of the solution in each tube using a spectrophotometer set to 470 nm with tube 3 as a blank. Refer to Exercise 8 for instructions on how to use the spectrophotometer.
9. Clean your work area and materials. **Catechol must be disposed into waste containers, not down the sink drain.**

Question 1

a. Write a hypothesis and a null hupothesis for the effect of temperature on catechol oxidase activity.

b. What were the enzyme, substrate, and product of the enzymatic reaction?

c. Why was each tube left undisturbed for 5 min in step 5 of procedure 11.1?

d. Explain the results observed for tubes 1–3. What was the purpose of these tubes?

e. Use your results for tubes 4–7 to construct a line graph of *Enzyme Activity* versus *Time* on graph paper provided at the end of the exercise. There will be four curves on the graph.

f. Use your results to argue for or against the statement, "Catechol oxidase functions equally and efficiently at various temperatures."

g. Over what range of temperatures tested was catechol oxidase active? Should other temperatures be tested to more accurately determine the range of activity?

h. At which temperature was catechol oxidase activity greatest? Should more temperatures be tested to determine its optimum?

TABLE 11.3

EXPERIMENTAL CONDITIONS TO TEST THE EFFECT OF pH ON CATALASE ACTIVITY

Tube	Distilled Water	Buffer	Hydrogen Peroxide	HCl	NaOH	pH	Catalase Solution
1	5 mL	1 mL, pH 7					
2	4 mL	1 mL, pH 7					1 mL
3	2 mL	1 mL, pH 7	3 mL				
4	1 mL		3 mL	1 mL			1 mL
5	1 mL	1 mL, pH 5	3 mL				1 mL
6	1 mL	1 mL, pH 7	3 mL				1 mL
7	1 mL	1 mL, pH 9	3 mL				1 mL
8	1 mL		3 mL		1 mL		1 mL
9	1 mL	1 mL, pH 7	3 mL	1 mL			
10	1 mL	1 mL, pH 7	3 mL		1 mL		

i. What temperature apparently denatured catechol oxidase? How do you know?

j. What is the effect of denaturing an enzyme?

k. If an enzyme has a single optimal temperature, then an organism might have difficulty dealing with an environment with wide temperature variation. What adaptive advantage is there in having repetitive enzyme systems (i.e., more than one enzyme to catalyze the same reaction) that we know many organisms have?

l. Do your data support or refute your hypothesis? Explain.

pH AFFECTS THE ACTIVITY OF ENZYMES

Enzymatic activity is sensitive to pH because the surfaces and side groups of enzyme molecules are often charged. Acidic and basic solutions are rich in H^+ and OH^- ions (see Exercise 5), respectively, and they react with the side groups of the enzyme molecules. As the pH is lowered, side groups gain H^+ ions; as the pH is raised, side groups lose H^+ ions. In this way, solutions having an extreme pH can change an enzyme's conformation enough to alter its active site. Extreme pH can denature an enzyme just as drastically as can high temperatures. Many enzymes function optimally in the neutral pH range, while others (such as pepsin, an enzyme in your digestive tract) function optimally as low as pH 1.6.

The effects of pH can be investigated with **catalase**, an enzyme in plants and animals that speeds the breakdown of hydrogen peroxide, toxic to cells. Hydrogen peroxide is broken down by catalase to water and oxygen.

$$2\ H_2O_2 \xrightarrow{catalase} 2\ H_2O + O_2$$

Procedure 11.2

Observe the effects of pH on catalase activity

1. Prepare catalase solution.
   - *a.* Use a mortar and pestle to macerate a marble-size portion of fresh, raw ground meat in 10 mL of distilled water.
   - *b.* Filter the solution through cheesecloth into a test tube and add an equal volume of distilled water.
2. Obtain 10 test tubes and number them at the top 1–10.
3. Obtain stock solutions of distilled water, hydrogen peroxide, buffer pH 5, buffer pH 7, buffer pH 9, 0.1 M HCl, and 0.1 M NaOH.

HCl is a strong caustic acid, and NaOH is a strong caustic base. Follow your instructor's directions for handling, dispensing, and disposing of these chemicals. Rinse immediately with water if you spill any acid or base on your skin.

TABLE 11.4

PRODUCTION OF OXYGEN BY CATALASE ACTIVITY. QUALITATIVE DATA ARE OBSERVATIONS OF INTENSITY OF OXYGEN EFFERVESCENCE RANGING FROM 1–5. QUANTITATIVE DATA ARE MILLILITERS OF OXYGEN PRODUCED.

Tube	Oxygen Production		Explanation
	Qualitative (0–5)	Quantitative (mL O_2)	
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

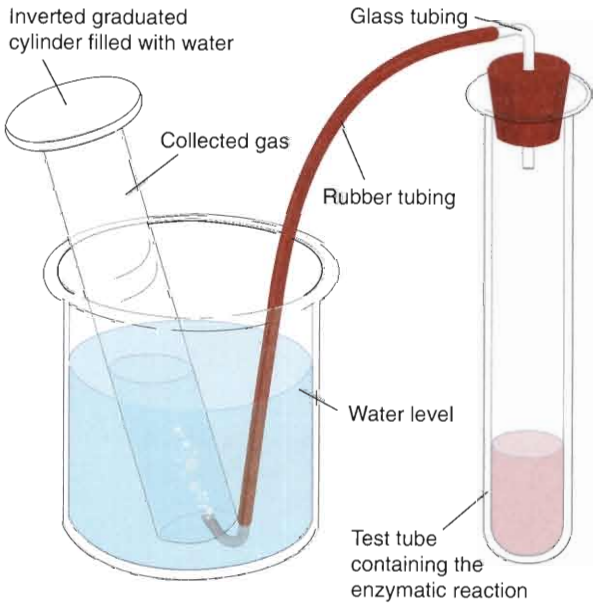


Figure 11.4
A method to capture oxygen released by catalase activity.

4. Add distilled water and hydrogen peroxide to each tube as listed in table 11.3. If you are measuring by drops, then 1 mL equals about 20 medium-sized drops. Wait 2 min before proceeding to step 5.
5. Add 1 mL of HCl to tubes 4 and 9. Verify that the pH is approximately 3 or lower.
6. Add 1 mL of NaOH to tubes 8 and 10. Verify that the pH is approximately 11 or higher.
7. Add 1 mL of the buffer solutions as indicated in table 11.3.
8. Your instructor may ask you to verify that the buffers produce the indicated pH. If so, use pH paper to measure the values for each solution and record them in table 11.3.
9. No catalase is added to tubes 1, 3, 9, or 10.
10. Add catalase to tube 2 according to table 11.3. After adding catalase, swirl the solution gently and immediately record in table 11.4 qualitative changes in the bubbling intensity of oxygen production on a scale of 0 (no bubbling) to 5 (vigorous bubbling).
11. If your instructor asks you to more rigorously quantify your results, then immediately after adding the catalase place a stopper with tubing over each tube to collect and measure the volume of gases produced in a water-filled graduated cylinder inverted in a beaker of water (fig. 11.4). Be sure that the graduated cylinder does not pinch off the rubber tubing. Also be sure the cylinder is vertical when you measure volume. Record these results in table 11.4.
12. Repeat step 10 for each remaining solution.
13. After you have gathered your data for all 10 tubes, record in table 11.4 your explanation for the results of the catalase activity in each of the tubes.
14. Clean your work area and materials. Follow your instructor's directions concerning the disposal of waste solutions containing HCl and NaOH.

Question 2

a. Write a hypothesis and a null hypothesis for the effect of pH on catalase activity.

b. What were the enzyme, substrate, and product of the enzymatic reaction?

c. What was the purpose of completing steps 1–8 for all tubes before adding the catalase in step 10?

d. What was the purpose of tubes 1, 2, 3, 9, and 10?

e. Use your data for tubes 4–8 to construct a line graph of *Enzyme Activity* versus *pH*.

f. Over what pH range was catalase active?

g. What pH levels denatured catalase? Specifically how do solutions of high or low pH change an enzyme's reactivity?

h. At which of the tested pH values did catalase react most rapidly? Should more values be tested to accurately determine its optimum?

i. After experimenting with the effects of pH on enzymes, would you suspect that human blood has a constant pH? Why? What would be the adaptive advantage of this?

j. Do your data support or refute your hypothesis? Explain.

INHIBITORS AFFECT THE ACTIVITY OF ENZYMES

Peroxidase is an enzyme in plants (such as turnips) and some bacteria that converts toxic hydrogen peroxide to H_2O and O_2 in a reaction similar to that of catalase. Peroxidase is a large protein with a reactive iron atom at its active site.

$$2\ H_2O_2 \xrightarrow{peroxidase} 2\ H_2O + O_2$$

Enzymes such as peroxidase can be inhibited by chemicals in various ways. One mechanism is **competitive inhibition.** Competitive inhibitors are molecules structurally similar to the substrate and compete for a position at the active site of an enzyme. This ties up the enzyme, thereby making it unavailable to bind with the substrate. For example, hydroxylamine ($HONH_2$) is structurally similar to hydrogen peroxide (H_2O_2) and binds to the iron atom at the active site of peroxidase. Thus, hydroxylamine competes with hydrogen peroxide for the active site on peroxidase, thereby preventing peroxidase from binding with hydrogen peroxide. This inhibits the reaction. A high enough concentration of enzyme with a constant concentration of inhibitor will reduce the inhibition.

The production of oxygen by peroxidase provides a method to measure the ongoing reaction rate. One method would be to capture liberated bubbles of oxygen and measure their total volume. But in the following procedure you will measure oxygen by combining it with a dye that changes color when it is oxidized. Guaiacol is a convenient dye that turns from colorless to brown as it is oxidized by oxygen. The amount of brown color in the final product is proportional to the amount of oxygen formed by the reaction. You can measure the color change qualitatively by rating the color of a reacting solution on an arbitrary scale from 0 to 5, or quantitatively with a spectrophotometer by measuring the solution's absorbance of 470 nm light. Review Exercise 8 for instructions on using a spectrophotometer.

$$O_2 + \text{guaiacol} \longrightarrow \text{oxidized guaiacol}$$
(colorless) (brown)

Procedure 11.3

Observe the effects of an inhibitor on enzymatic activity

1. Prepare turnip extract.
   - *a.* Thoroughly blend 6 g of the inner portion of a peeled turnip in a blender with 200 mL of cold water.
   - *b.* Filter the turnip slurry through cheesecloth into a beaker.
   - *c.* Pour about 7 mL of the extract into a test tube and determine its absorbance in a spectrophotometer. Refer to Exercise 8 for

TABLE 11.5

EXPERIMENTAL CONDITIONS TO TEST THE INHIBITION OF HYDROXYLAMINE ON PEROXIDASE ACTIVITY

Tube	Distilled Water	Guaiacol (25 mM)	Hydrogen Peroxide (3%)	Turnip Extract	Hydroxylamine (10%)
1	5.9 mL	0.1 mL			
2	5.8 mL		0.2 mL		
3	5.7 mL	0.1 mL	0.2 mL		
4	4.9 mL	0.1 mL		1.0 mL	
5	4.7 mL	0.1 mL	0.2 mL	1.0 mL	
6	4.2 mL	0.1 mL	0.2 mL	1.0 mL	0.5 mL
7	3.7 mL	0.1 mL	0.2 mL	1.5 mL	0.5 mL
8	3.2 mL	0.1 mL	0.2 mL	2.0 mL	0.5 mL
9	2.2 mL	0.1 mL	0.2 mL	3.0 mL	0.5 mL

TABLE 11.6

ABSORBANCE AT 470 NM OF PEROXIDE/PEROXIDASE SOLUTIONS

Tube	0.0 min	0.5 min	1.0 min	1.5 min	2.0 min	2.5 min	3.0 min	3.5 min	4.0 min	4.5 min	5.0 min
1											
2											
3											
4											
5											
6											
7											
8											
9											

instructions on spectrophotometry. The absorbance for the turnip extract should be between 0.1 and 0.2 at 470 nm to give a reasonable concentration of enzyme.

d. Dilute or concentrate the suspension as necessary. Your instructor may provide directions for standardizing this enzyme solution more precisely.

2. Obtain nine test tubes and number them at the top 1–9.
3. Add distilled water to each tube as listed in table 11.5.
4. To tube 1, add 0.1 mL (2 drops) of guaiacol as listed in table 11.5, swirl the contents.
5. Immediately determine the solution's absorbance at 470 nm using a spectrophotometer. Record the absorbance value in table 11.6. Measure the absorbance every 30 sec for 5 min and record the value each time.
6. To tube 2, add 0.2 mL (4 drops) of hydrogen peroxide as listed in table 11.5. Swirl the contents. Repeat step 5 quickly.
7. To tube 3, add 0.1 mL (2 drops) of guaiacol and 0.2 mL of hydrogen peroxide as listed in table 11.5. Swirl the contents. Repeat step 5 quickly.

8. To tube 4, add 0.1 mL (2 drops) of guaiacol and 1.0 mL of turnip extract as listed in table 11.5. Swirl the contents. Repeat step 5 quickly.
9. Complete all of the measurements for steps 4–8 before proceeding to step 10.
10. For each of tubes 5–9, add 0.1 mL (2 drops) of guaiacol and 0.2 mL (4 drops) of hydrogen peroxide as listed in table 11.5.
11. For tube 5, add 1.0 mL of turnip extract and swirl the contents. Repeat step 5 quickly.
12. For tube 6, add 1.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 5 quickly.
13. For tube 7, add 1.5 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 5 quickly.
14. For tube 8, add 2.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 5 quickly.
15. For tube 9, add 3.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 5 quickly.
16. Clean your work area and materials.

Question 3

a. Write a hypothesis and a null hupothesis for the effect of an inhibitor on enzymatic activity.

b. What were the enzyme, substrate, and product of this enzymatic reaction?

c. Explain the results you observed for tubes 1, 2, 3, and 4. What was the purpose of these tubes?

d. Use your data for tubes 5–9 to construct a line graph of *Enzyme Activity (Absorbance)* versus *Time*. There will be five curves on the graph. You will not graph the values for tubes 1–4.

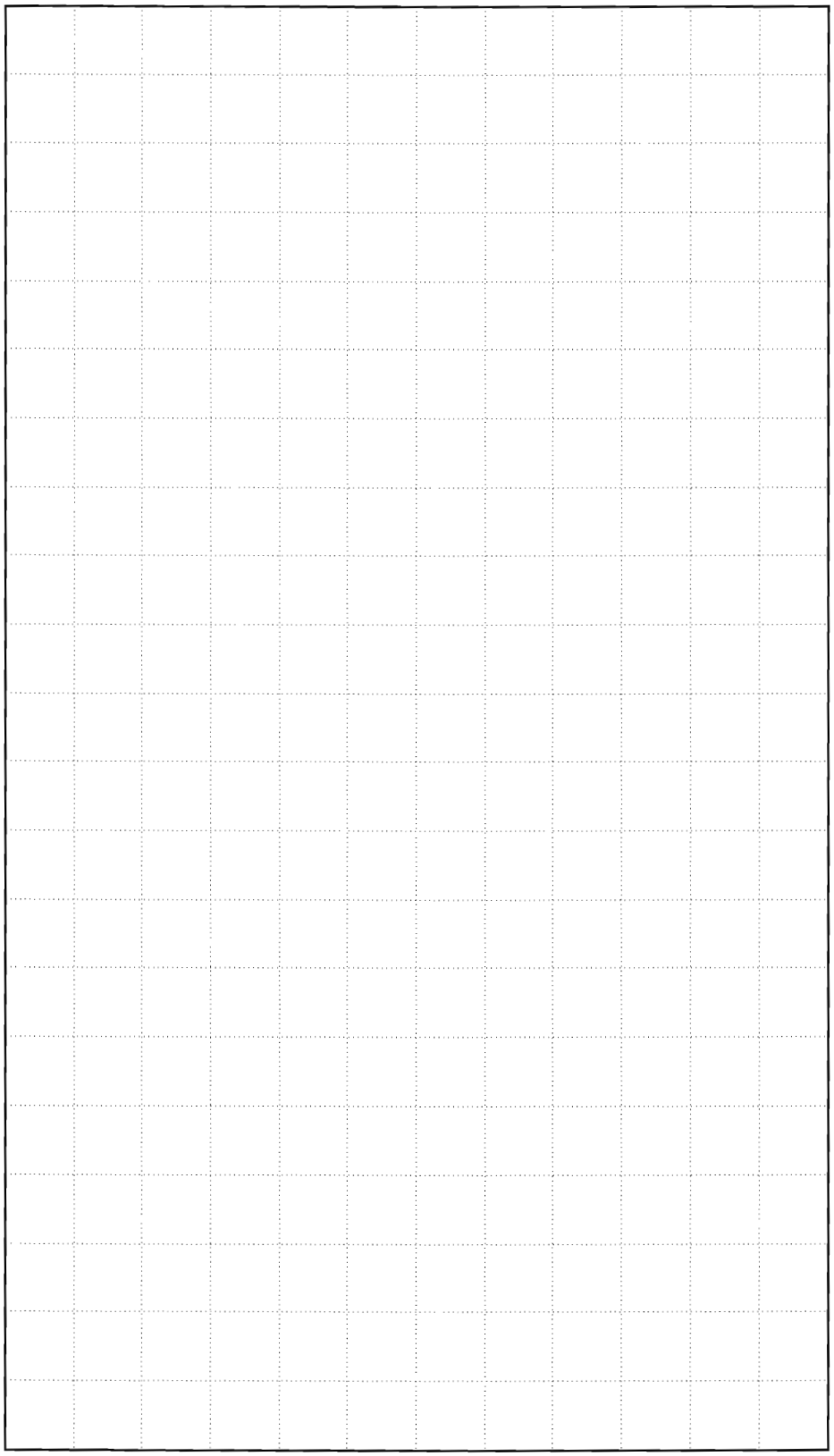
e. For which tubes is peroxidase still active after 5 min?

f. How does hydroxylamine affect peroxidase activity?

g. Was it possible to detect peroxidase activity in the presence of the inhibitor by increasing enzyme concentration? Why or why not?

h. Inhibitors are common in biological systems. Why might some organisms release enzyme inhibitors into their surrounding environment?

i. Do your data support or refute your hypothesis? Explain.



Respiration

Aerobic and Anaerobic Oxidation of Organic Molecules

12

Objectives

By the end of this exercise you should be able to:

1. Demonstrate carbon dioxide production during anaerobic respiration.
2. Understand the effects of inhibitors, intermediate compounds, and cofactors in anaerobic respiration.
3. Determine oxygen consumption during aerobic respiration.
4. Use a pH-indicator to measure the relative production of carbon dioxide by plants and animals.
5. Use a respirometer to determine the metabolic rate of an animal.
6. Demonstrate practical applications of anaerobic respiration such as making wine and kimchee.

All living organisms respire, meaning that they have metabolic pathways that release energy from organic (rarely inorganic) molecules and capture it in ATP. Some need oxygen to do it, some don't, but they all respire because all organisms need usable chemical energy to fuel their life processes. Respiration is the chemistry that provides that energy. Usually the organic carbon molecules are the energy source and CO_2 and H_2O are released as waste. Humans release the waste as they exhale. Respiring yeast don't exhale, but they can "pump up" rising bread by liberating CO_2 as the fungal yeast breaks down sugar (fig. 12.1).

Cellular respiration involves oxidation of organic molecules and a concomitant release of energy. Some of this energy is stored in chemical bonds of **adenosine triphosphate (ATP),** used later as a direct source of energy for cellular metabolism. Organisms use the energy stored in ATP to do work such as transport, synthesize new compounds, reproduce, contract muscles, and remove wastes.

Photosynthesis, the topic of Exercise 13, uses light energy to split H_2O and harvest high-energy electrons. These energetic electrons (and accompanying H^+) are passed to CO_2, thereby reducing CO_2 to energy-storing sugars. Respiration removes electrons from (i.e., oxidizes) glucose, captures some of the energy in ATP, and ultimately passes the electrons to oxygen to form H_2O.

In most cells, respiration begins with the oxidation of glucose to pyruvate via a set of chemical reactions called **glycolysis** (fig. 12.2*a*). During glycolysis, some of the energy released from each glucose molecule is stored in ATP. Glycolysis occurs with or without oxygen. If oxygen is present, most organisms continue respiration by oxidizing pyruvate to CO_2 via chemical reactions of the **Krebs cycle**. Organisms that use oxygen for respiration beyond glycolysis are called **aerobes.**

As aerobes oxidize pyruvate in the Krebs cycle, they store energy in electron carriers such as NAD^+ (nicotinamide adenine dinucleotide). Specifically, aerobes store energy by reducing (adding high-energy electrons to) NAD^+ and FAD^+. These compounds later transfer their high-energy electrons to a series of compounds collectively called the electron transport chain. The **electron transport chain** generates proton gradients from energy stored in reduced

Figure 12.1

Bread dough rises because respiring yeasts break down sugars to obtain their energy for growth and liberate CO_2, thereby forming small bubbles in the dough. The lower loaf has been rising 4 hr longer than the upper loaf.

b. How was the effect of concentration of inhibitor tested in this experiment? How did the concentration of NaF affect anaerobic fermentation in your experiment? Why?

c. Which compounds in the list are intermediates in the respiratory pathway?

d. Why did tube 6 produce CO_2 even though an inhibitor of glycolysis was present?

e. Compare tubes 4 and 5. How was CO_2 production affected by the 10-fold increase in the amount of NaF? For example, was it also changed 10-fold?

f. Did magnesium (a cofactor that activates many enzymes) promote respiration? If not, what are some possible reasons?

g. Smell the contents of the tube containing the most CO_2. What compound do you smell?

h. What is the economic importance of fermentation by yeast?

TABLE 12.1

EXPERIMENTAL TREATMENTS AND CO_2 PRODUCTION DURING ANAEROBIC FERMENTATION

Tube	3 M Na Pyruvate (Activator)	0.1 M $MgSO_4$ (Activator)	0.1 M NaF (Inhibitor)	5.0% Glucose (Activator)	Water	Fill With	CO_2 Produced After 40 min (mm)
1	—	—	—	—	7.5 mL	Yeast suspension	
2	—	—	—	2.5 mL	5.0 mL	Yeast suspension	
3	—	5.0 mL	—	2.5 mL	—	Yeast suspension	
4	—	—	0.5 mL	2.5 mL	4.5 mL	Yeast suspension	
5	—	—	5.0 mL	2.5 mL	—	Yeast suspension	
6	2.5 mL	—	2.5 mL	2.5 mL	—	Yeast suspension	
7	—	—	—	2.5 mL	2.5 mL	Water	

TABLE 12.2

EFFECTS OF FOUR CHEMICAL VARIABLES ON CO_2 PRODUCTION DURING ANAEROBIC FERMENTATION

Variable	Tube # with Variable	Tube # Control	Effect of Variable on Respiration Rate	Mechanism for the Effect
Yeast				
Glucose				
NaF				
Na Pyruvate				
$MgSO_4$				

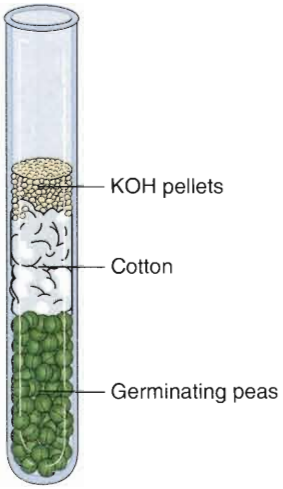


Figure 12.3
Test tube containing germinating peas, cotton, and KOH pellets.

i. What gas is responsible for the holes in baked bread?

If time and facilities are available, repeat procedure 12.1 and incubate the tubes at 4°C (refrigerator), 20°C (incubator), and/or 55°C (incubator). Use your data to explain the effect of temperature on fermentation by yeast.

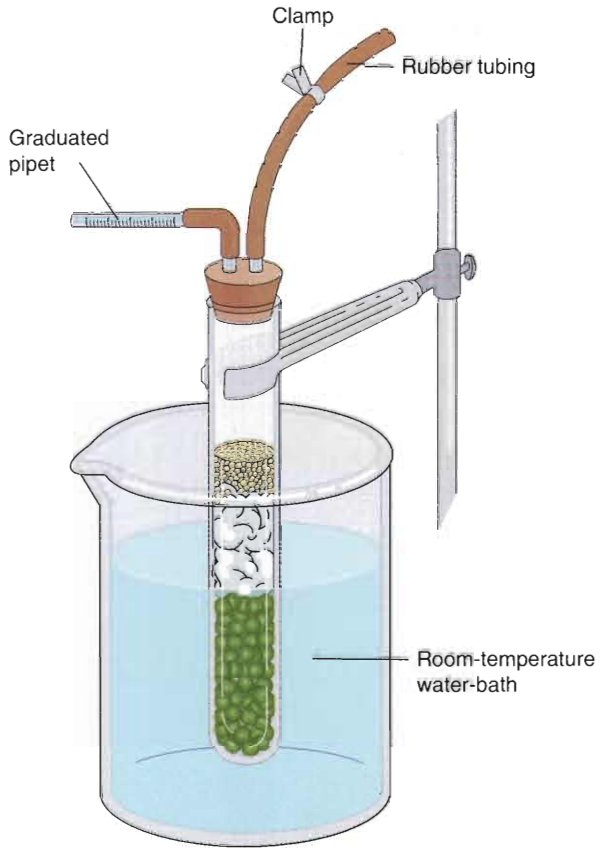


Figure 12.4
Test tube with stopper having capillary tubes attached. The tube will be covered in foil.

OXYGEN CONSUMPTION DURING AEROBIC RESPIRATION

Aerobic respiration uses oxygen as the terminal electron acceptor in the electron transport chain. Because this oxygen is reduced to water, you can measure aerobic respiration by measuring the consumption of oxygen. During respiration, CO_2 is produced while O_2 is consumed. Review the summary equation in the introduction of this exercise. In the following experiment, KOH is used to absorb the CO_2. Therefore, the net change in gas volume is a measure of oxygen consumption.

Procedure 12.2

Determine oxygen consumption during aerobic respiration (may be done as a demonstration)

1. Fill a test tube or flask half-full with germinating peas and another half-full with heat-killed peas. The germinating peas have been soaked in water in the dark for three to four days.
2. Cover the contents of each tube with a loose-fitting plug of cotton.
3. Cover the cotton with approximately 1 cm of loosely packed pellets of potassium hydroxide (KOH) (fig. 12.3).
4. Place a stopper containing a capillary tube or graduated pipet with an attached outlet tube into both tubes containing peas (fig. 12.4). The capillary tube or graduated pipet should be oriented horizontally.
5. Cover the tube with foil to prevent light and photosynthesis.
6. Vertically clamp the tubes to a ring stand so that the bottom of each tube is submerged in a room-temperature water-bath. The water-bath will minimize temperature fluctuations in the tube.
7. Use a Pasteur pipet to inject enough dye into each capillary tube so that approximately 1 cm of dye is drawn into each capillary tube. The capillary tube or graduated pipet should be oriented horizontally.
8. After waiting 1 min for equilibration, attach a pinch clamp to the outlet tube and mark the position of the

TABLE 12.3

OXYGEN CONSUMPTION BY SEEDS AT THREE TEMPERATURES

		mL O_2 Consumed					
		10 min		20 min		30 min	
Treatment	0 min	Alive	Heat-Killed	Alive	Heat-Killed	Alive	Heat-Killed
Room temperature	0	_____	_____	_____	_____	_____	_____
Ice bath	0	_____	_____	_____	_____	_____	_____
Warm water-bath	0	_____	_____	_____	_____	_____	_____

dye with a wax pencil. Write your predicted results and a brief explanation here:

9. Use a wax pencil to mark the position of the dye every 10 min for the next 30 min.
10. After each time interval measure the distance the dye moved from its starting point; record your data in table 12.3.
11. Remove the pinch clamp from the outlet valve and return the dye to the end of the capillary tube by tilting the capillary tube.
12. Repeat steps 1–11 using tubes incubated in an ice bath and warm (35°C) water-bath. You can save time by running all of these treatments simultaneously. Record your results in table 12.3.

Question 4

a. What was the purpose of adding heat-killed peas to a tube?

b. In which direction did the dye move?

c. Why?

d. What does this experiment tell you about the influence of temperature on oxygen consumption during cellular respiration?

PRODUCTION OF CO_2 DURING AEROBIC RESPIRATION

CO_2 produced during cellular respiration can combine with water to form carbonic acid:

$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} \longleftrightarrow \underset{\text{Carbonic acid}}{H_2CO_3}$$

In this procedure (fig. 12.5) you will use phenolphthalein to detect changes in pH resulting from the production of CO_2 (and, therefore, carbonic acid) during cellular respiration. Phenolphthalein is red in basic solutions and colorless in acidic solutions. Thus, you can monitor cellular respiration by measuring acid production as change in pH. **pH** is a measure of the acidic or basic properties of a solution; pH 7 is neutral. Solutions having a pH < 7 are acidic, and solutions having a pH > 7 are basic (see Exercise 5).

In procedure 12.3 you will not directly measure the volume of CO_2 produced by a respiring organism. Instead, you will measure the volume of NaOH used to neutralize the carbonic acid produced by the CO_2, and thereby calculate a relative measure of respiration.

Question 5

The organisms you will study include an animal (snail) and a plant (*Elodea*). Which do you think will respire more? Write your hypothesis here:

Procedure 12.3

Measure relative CO_2 production by aerobic organisms

Experimental Setup

1. Obtain 225 mL of culture solution provided by your instructor. This solution has been dechlorinated and adjusted to be slightly acidic.
2. Place 75 mL of this solution in each of three labeled beakers.

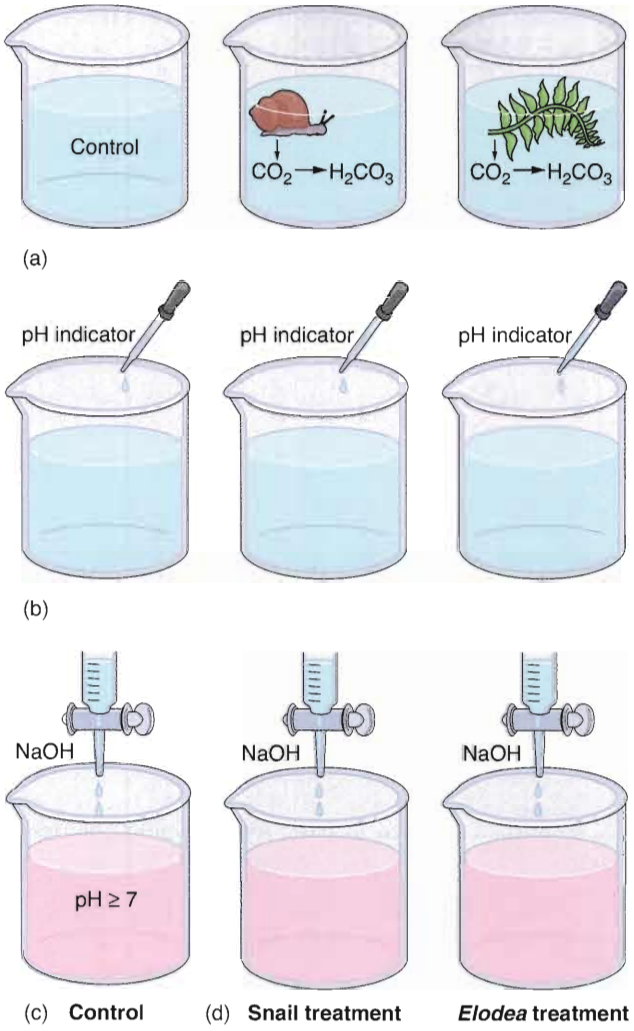


Figure 12.5

The procedure to determine the relative respiration rates of a plant and animal. (*a*) During respiration, organisms release CO_2, which combines with water to form carbonic acid (H_2CO_3). (*b*) The acidic solutions remain colorless after addition of phenolphthalein, a pH indicator. (*c*) Titration of the control with NaOH will make the solution basic and pink when the pH reaches the end point of phenolphthalein. (*d*) The treatment solutions are then titrated to the pink end point matching the control. The volume of NaOH needed to reach the end point indicates the relative amounts of dissolved CO_2 produced during respiration.

3. Obtain the organisms listed in table 12.4 from your instructor and determine the volume of each organism by following steps 4–6.

Determine Volume by Water Displacement

4. Place exactly 25 mL of water in a 50-mL graduated cylinder.
5. Place the organism in the cylinder and note the increase in volume above the original 25 mL. This increase equals the volume of the organism.
6. Record the volumes in table 12.4. Gently place the organism in the appropriate beaker.

Incubate Experimental Treatments

7. Cover each beaker with a plastic film or petri dish top and set them aside on your lab bench. Place the beaker containing the *Elodea* in the dark by covering it with a coffee can or aluminum foil.
8. Allow the organisms to respire for 15 min.
9. Gently remove the organisms from the beakers and return them to their original culture bowls.

Titrate to Gather Your Raw Data

10. Add four drops of phenolphthalein to the contents of each beaker. The solutions should remain clear because the solutions are acidic.
11. Obtain a burette or dropper bottle to dispense NaOH (2.5 mM). Add NaOH drop by drop to the contents of the control beaker. Thoroughly mix the contents of the beaker after adding each drop. Record in table 12.4 the milliliters of NaOH required to reach the end point of phenolphthalein (i.e., make the solution pink in the control beaker).
12. Repeat step 11 for beaker 1; be sure to add NaOH only until the solution is the same shade of pink as the control beaker. Record the number of milliliters of NaOH added to beaker 1 in table 12.4.
13. Repeat step 11 for beaker 2.

TABLE 12.4

DATA FOR MEASURING CO_2 PRODUCTION DURING RESPIRATION

Organisms	Total Volume of Organisms (mL)	Milliliters of NaOH to Reach End Point (mL NaOH)	Relative Respiration Rate of Organisms (mL NaOH)	Respiration Rate per Milliliter of Organism (mL NaOH/mL organisms)
Beaker 1: 4 snails	_____	_____	_____	_____
Beaker 2: *Elodea*	_____	_____	_____	_____
Control beaker	0	_____	0	0

Calculate Your Results

14. For beaker 1, determine the relative respiration rate for organisms by subtracting the milliliters NaOH added to the control beaker from the milliliters NaOH added to beaker 1. Record this value in table 12.4.
15. Repeat step 14 for beaker 2.
16. For beakers 1 and 2, determine the respiration rate per milliliter of organism by dividing the relative respiration rate for organisms by the volume of the organism(s). Record these values in table 12.4.

Question 6

a. In this exercise you measured the relative respiration rates of an animal and a plant. Why were you cautioned about having no algae in the control beaker?

b. Before you gathered your raw data, you formulated a hypothesis about the expected results. After considering your data, do you accept or reject your hypothesis? Why?

c. What is your major conclusion from the results of this procedure?

d. What features of the biology of the organisms that you used most likely contributed to the observed differences in respiration rate?

e. Do you feel justified in drawing conclusions from your work about all plants and animals? Or only about snails and *Elodea?* Why?

f. How would you expand this experiment to further test your conclusions about other plants and other animals?

g. What other organisms might you include in an expanded experiment? Why did you choose these organisms?

DEMONSTRATION: DETERMINATION OF METABOLIC RATE

The rate of O_2 uptake during cellular respiration indicates the metabolic rate of an organism. In procedure 12.4 you will measure O_2 uptake by measuring changes in air pressure as O_2 is removed from the air by a respiring mouse. Changes in air pressure can be attributed primarily to O_2 consumption (rather than CO_2 production or exhalation of water vapor) only if exhaled CO_2 and H_2O are removed from the air. This is accomplished by adding ascarite (which adsorbs CO_2) and drierite (which adsorbs H_2O) to the experimental setup (fig. 12.6). Use procedure 12.4 to estimate the metabolic rate of a mouse.

Procedure 12.4

Estimate the metabolic rate of a mouse

1. Weigh a mouse to the nearest 0.1 g. Record this weight in table 12.5 and place the mouse in the jar of a respirometer (fig. 12.6). Use a fan to circulate air in the jar and allow the mouse to get accustomed to the jar.
2. Attach a 10-mL syringe filled with air to the respirometer.
3. Seal the respirometer jar with a lid. Then close the air escape line with a clamp and record the position of the dye solution in the right column of the curved capillary tube. This tube is called a manometer.
4. Inject 10 mL of air into the respirometer. The level of dye in the right side of the manometer will rise because of the increased presence of air. After injecting the 10 mL of air into the respirometer, record the position of the dye solution in table 12.5.
5. Allow the mouse to respire. The air pressure in the respirometer should decrease as O_2 is consumed, and the dye level in the right column of the manometer should decrease.
6. Record in table 12.5 the elapsed time for the dye level to return to its original position. This is the time for the mouse to consume 10 mL of O_2. Record this time as "A" in the Calculations section of table 12.5.
7. Gently return the mouse to its cage.
8. Calculate the number of liters consumed by the mouse per day by using the following formula:

   Liters of O_2 consumed per day =
   1440 minutes per day ÷ minutes to consume 1 liter of O_2

   Record this as "B" in the Calculations section of table 12.5.
9. Calculate and record in table 12.5 the mouse's metabolic rate in kcal/day, assuming that 4.8 kcal of

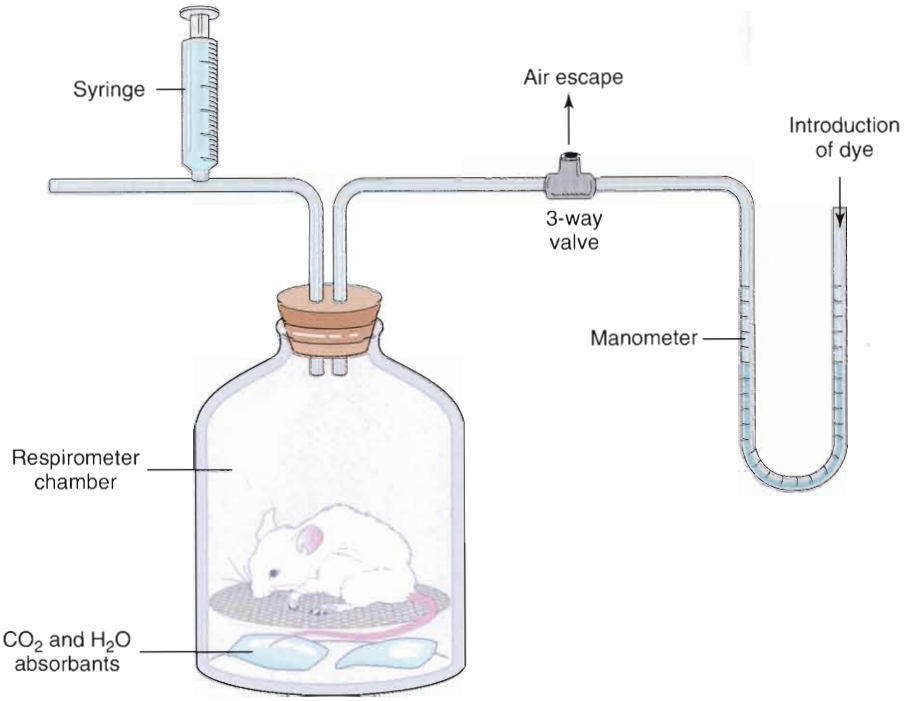


Figure 12.6
Respirometer with mouse.

TABLE 12.5

DATA FOR DETERMINATION OF METABOLIC RATE OF A RESPIRING MOUSE

Observations and Data

Weight of mouse: _____ grams

Initial position of dye solution: _____

Position of dye solution after injection of 10 mL of air: _____

Minutes for dye level to return to initial position: (minutes per 10 mL oxygen): _____ min

Calculations

A	Minutes to consume 1 liter of O_2 = (minutes per 10 mL oxygen) × 100 = _____ min
B	Liters of O_2 consumed per day = 1440 minutes per day ÷ A = _____ liters per day
C	Experimental metabolic rate as kcal per day = B × 4.8 kcal per liter O_2 = _____ kcal per day
D	Predicted metabolic rate = 70 × (weight of mouse)$^{3/4}$ = _____ kcal per day

energy are used for each liter of O_2 consumed. Record this as "C" in the Calculations section of table 12.5.

10. Calculate and record the predicted metabolic rate obtained from the following general equation for metabolic rate of small mammals:

Predicted metabolic rate = 70 × (body weight in kg)$^{3/4}$

Record this rate as "D" in the Calculations section of table 12.5.

11. Compare your experimental value with the predicted value for metabolic rate.

Question 7

a. Is the predicted metabolic rate similar to that which you determined experimentally?

INVESTIGATION

The Effect of Environmental Stimuli on Cellular Respiration

Observations: Respiration, like all biochemical processes, responds to environmental stimuli (e.g., temperature, salinity, acidity, light). However, some organisms tolerate a wider range of conditions than others.

Question: How is the rate of cellular respiration affected by environmental stimuli?

a. Establish a working lab group and obtain Investigation Worksheet 12 from your instructor.
b. The preceding question will give you a general direction for your work, but you'll need to refine it before proceeding. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Review procedures 12.1 and 12.3, which use yeast, snails, and *Elodea* as model organisms to investigate respiration. Outline on Worksheet 12 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

b. What could cause any differences in these values?

c. Determine the metabolic rate of other organisms available in the lab. How do their metabolic rates compare with that of a mouse?

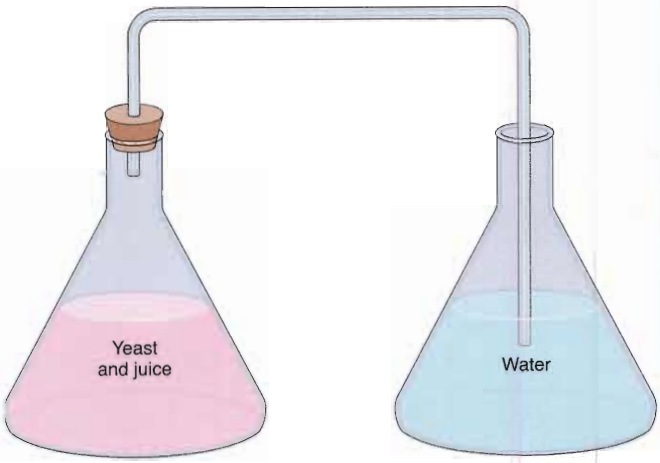


Figure 12.7
Experimental setup for home wine making.

APPLICATIONS OF ANAEROBIC RESPIRATION

Making Wine

In this exercise you've seen how easy it is to demonstrate alcoholic fermentation by yeast. Many biologists as well as nonbiologists use this reaction to make their own wine. If you're game for an introduction to home wine making, try the following procedure.

Procedure 12.5

Making wine

1. Thoroughly clean and sterilize all glassware.
2. Combine a cake of yeast with either bottled grape juice or cranberry juice. Mix the yeast and juice in a ratio of approximately 5 liters of juice to 1 gram of yeast.
3. Add approximately 650 mL of the juice-yeast mix to each of four 1-liter Erlenmeyer flasks (or use 1- to 2-liter recycled plastic pop bottles).
4. Dissolve the following amounts of sucrose in each flask:
   Flask 1: 75 g
   Flask 2: 150 g
   Flask 3: 300 g
   Flask 4: no sucrose
5. For each flask, set up the fermentation apparatus as shown in figure 12.7.
6. Be sure to keep the procedure anaerobic by keeping the end of the exit tube under water in the adjacent

flasks. This will prevent contamination by airborne bacteria and yeast.

7. Incubate the flasks at temperatures between 15°C and 22°C. Although fermentation will continue for a month or so, most fermentation will occur within the first 14 days. Fermentation is complete when bubbling stops.
8. To test your wine, remove the stopper and use a piece of tubing to siphon off the wine solution without disturbing the sediment in the bottom of the flasks. You may then want to filter the solution to remove any remaining yeast cells from the wine.
9. Taste your wine. If your wine has been contaminated by bacteria that produce acetic acid, vinegar may have been formed, so take your first sip cautiously.

Question 8

a. What differences are there in wines produced with different amounts of sugar?

b. What would happen if oxygen were present (i.e., if conditions were not anaerobic)? If you have time, test your hypothesis.

1. How would you modify the experimental setup to introduce oxygen?

2. What results would you predict?

3. Based on your results, was your hypothesis accurate? Explain.

If you're interested in the finer points of wine making, visit your local bookstore or library. There may also be a local society of amateur wine makers in your area who will be glad to give you some pointers on creating "a simply delightful bouquet."

Making Kimchee

Pickling is an ancient way of preserving food. Pickling involves the anaerobic fermentation of sugars to lactic acid; this acid lowers the pH of the medium, thereby creating an environment in which other food-spoiling organisms cannot grow. Common foods preserved with pickling include sauerkraut, yogurt, and dill pickles. The ancient Chinese cabbage product kimchee, still a major part of the Korean diet, is also made with pickling. Here's how to make kimchee.

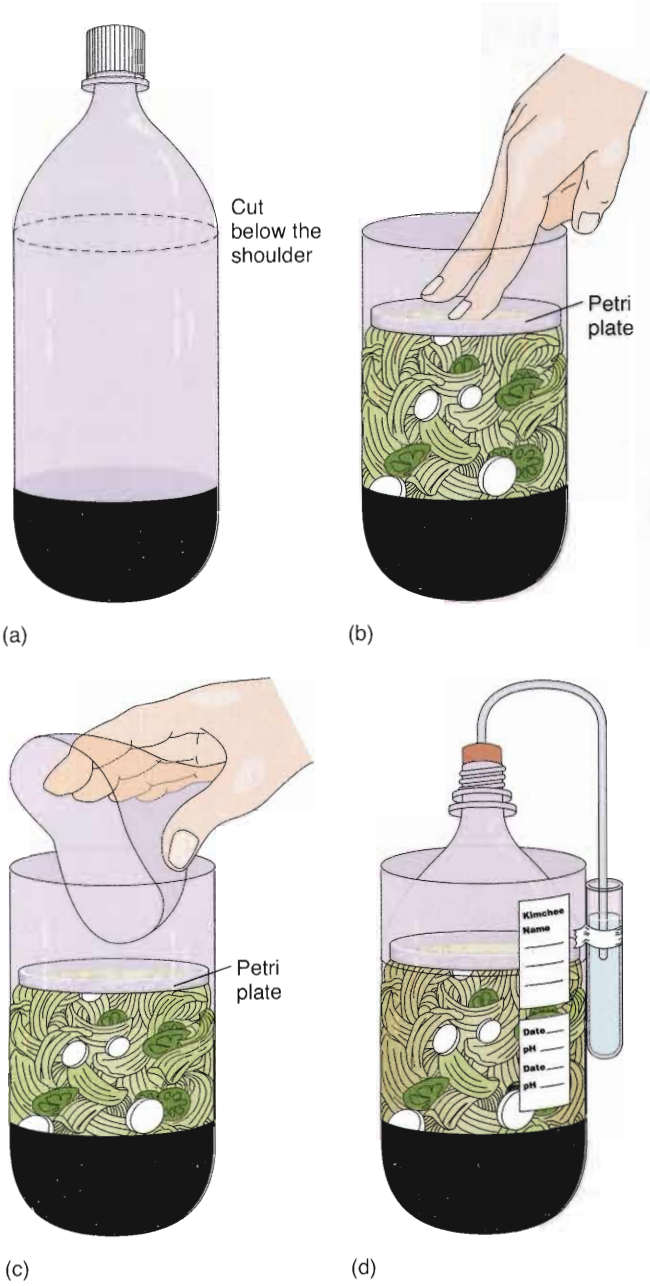


Figure 12.8
Experimental setup for making kimchee. See the text for the recipe and procedure.

Procedure 12.6

Making kimchee

1. Coarsely shred a head of cabbage. Place it in a mixing bowl with salt and allow it to wilt. This will draw some of the liquid out and prevent the finished kimchee from being watery.
2. Cut a 2-liter bottle just below the shoulder, as shown in figure 12.8*a*.

3. Add alternating layers of cabbage, garlic, pepper, and a sprinkling of salt in the bottle, pressing each layer down until the bottle is full. If you're using chilies or pepper, do not touch your eyes or mouth.
4. Place the lid, rim side up, atop the ingredients. Press down (fig. 12.8*b*). Within a few minutes, the salt will draw liquid from the cabbage; that liquid will begin to accumulate in the bottle.
5. For the next hour or so, continue to press the cabbage. You should then be able to fit the bottle top inside the bottle bottom, forming a sliding seal (fig. 12.8*c*). When you press with the sliding seal, cabbage juice will rise above the petri plate and air will bubble out around the edge of the plate.
6. The cabbage will pack half to two-thirds of the bottle's volume (fig. 12.8*d*). Every day, press on the sliding seal to keep the cabbage covered by a layer of juice.

Question 9
What happens when you press on the cabbage? How do you explain this?

7. Use pH-indicator paper to measure and record the pH of the juice each day (see Exercise 5).
8. After 4 to 7 days (depending on the temperature), the pH will have dropped from about 6.5 to about 3.5. Enjoy your kimchee!

Questions for Further Thought and Study

1. What is the difference between respiration and breathing?

2. Does cellular respiration occur simultaneously with photosynthesis in plants? How could you determine the relative rates of each?

3. What role does cellular respiration play in the metabolism of an organism?

4. What modifications of cellular respiration might you expect to find in dormant seeds?

5. In procedure 12.3, why did you subtract the control value from the titrant in beaker 1 and beaker 2?

6. Why is the volume of CO_2 production rather than O_2 uptake an adequate measure of respiration for the study of respiration rate?

DOING BIOLOGY YOURSELF

Repeat the procedure to measure relative CO_2 production by aerobic organisms and include in your design an animal such as a fish. Would you expect greater CO_2 production from a fish or a snail? Why?

DOING BIOLOGY YOURSELF

Repeat procedure 12.1 to measure CO_2 production in yeast and incubate the tubes at 4°C (refrigerator), 20°C (incubator), and/or 50°C (incubator). How does temperature affect the rate of fermentation by yeast?

13 Photosynthesis

Pigment Separation, Starch Production, and CO_2 Uptake

Objectives

By the end of this exercise you should be able to:

1. Relate each part of the summary equation for photosynthesis to the synthesis of sugar.
2. Describe the differences between the light-dependent and light-independent reactions involved in photosynthesis.
3. Separate the photosynthetic pigments using paper chromatography and calculate their R_f numbers.
4. Use a spectroscope to describe the absorption of visible light by chlorophyll.
5. Describe fluorescence and its role in photosynthesis.
6. Describe the process of electron transport in chloroplasts and its role in photosynthesis.
7. Describe the change of pH that occurs as plants take up CO_2 from their environment during photosynthesis.
8. Describe the distribution of starch in leaves resulting from photosynthesis relative to the amount of light they receive and the distribution of pigments.

Figure 13.1
The energy that drives photosynthesis comes from the sun. Less than 1% of all the energy that reaches the earth from the sun is captured by photosynthesis. This 1% fuels virtually all life on earth.

Photosynthesis is the most important series of chemical reactions that occurs on earth (fig. 13.1). Indeed, virtually all life depends on photosynthesis for food and oxygen. **Photosynthesis** is a complex chemical process that converts radiant energy (light) to chemical energy (sugar). The following is a summary equation for photosynthesis:

$$\underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{12\,H_2O} \xrightarrow[\textit{chlorophyll}]{\textit{light}} \underset{\text{Sugar}}{C_6H_{12}O_6} + \underset{\text{Water}}{6\,H_2O} + \underset{\text{Oxygen}}{6\,O_2}$$

Thus, photosynthesis is the light-dependent and chlorophyll-dependent conversion of carbon dioxide and water to sugar, water, and oxygen. Oxygen is released to the environment, and sugar is used to fuel growth or is stored as starch, a polysaccharide. Although water is present on both sides of the summary equation, these are not the same water molecules. The "reactant" water molecules (i.e., those on the left side of the equation) are split to release electrons during the photochemical (i.e., light-dependent) reactions. The "product" water molecules (i.e., those on the right side of the equation) are assembled from hydrogen and oxygen released during the photochemical and biochemical (i.e., light-independent) reactions. The photochemical reactions of photosynthesis are often referred to as the "light reactions." The biochemical reactions are often referred to as the "dark reactions" or the Calvin cycle, in honor of Melvin Calvin, the botanist who described the reactions.

As mentioned, photosynthesis can be divided into two sets of reactions (fig. 13.2). Some characteristics of these reactions are compared here:

Photochemical "Light" Reactions	*Biochemical "Dark" Reactions*
Fast (practically instantaneous)	Slower, but still extremely fast
Light-dependent	Light-independent
Splits water to release oxygen, electrons, and protons	Converts (fixes) carbon dioxide to sugar

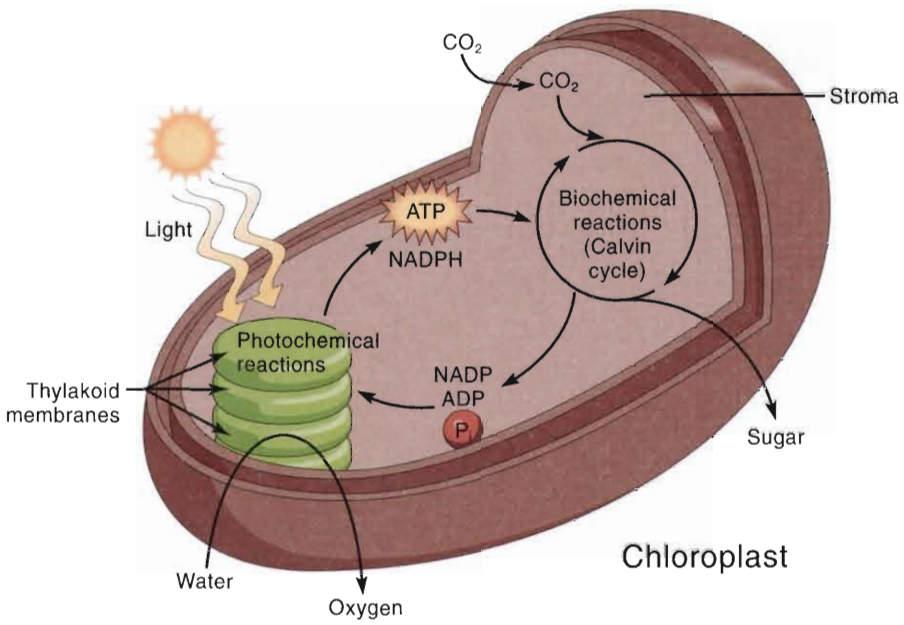


Figure 13.2

Photosynthesis consists of photochemical (the light-dependent "light reactions") and biochemical (the light-independent "dark reactions" including the Calvin cycle) reactions; both occur in chloroplasts. The photochemical (i.e., light) reactions convert light-energy to chemical energy captured in ATP and NADPH. The biochemical (i.e., dark) reactions use the ATP and NADPH produced by the photochemical reactions to reduce CO_2 to sugars. The photochemical reactions occur on thylakoid membranes, whereas the biochemical reactions occur in the stroma.

In today's exercise, you'll investigate some of the major aspects of photosynthesis, beginning with the isolation and identification of photosynthetic pigments.

Before you begin studying photosynthesis, we should remind you that *all* organisms (including plants) carry out respiration in one form or another, but chlorophyll-containing organisms can *also* photosynthesize.

PAPER CHROMATOGRAPHY OF PHOTOSYNTHETIC PIGMENTS

Light must be absorbed before its energy can be used. A substance that absorbs light is a **pigment.** The primary photosynthetic pigments that absorb light for photosynthesis are **chlorophylls *a*** and ***b*.** However, chlorophylls are not the only photosynthetic pigment; **accessory pigments** such as **carotenes** and **xanthophylls** also absorb light and transfer energy to chlorophyll *a*.

Paper chromatography is a technique for separating dissolved compounds such as chlorophyll, carotene, and xanthophyll. When a solution of these pigments is applied to strips of paper, the pigments adsorb onto the fibers of the paper. When the tip of the paper is immersed in a solvent, the solvent is absorbed and moves up through the paper. As the solvent moves through the spot of applied pigments, the pigments dissolve in the moving solvent. However, the pigments do not always keep up with the moving solvent—some pigments move almost as fast as the solvent, whereas others move more slowly. This differential movement of pigments results from each pigment's solubility and characteristic tendency to stick (i.e., be adsorbed) to the cellulose fibers of the paper. A pigment's molecular size, polarity, and solubility determine the strength of this tendency; pigments adsorbed strongly move slowly, whereas those adsorbed weakly move fastest. Thus, each pigment has a characteristic rate of movement, and the pigments can be separated from each other. In procedure 13.1, four bands of color will appear on the strip—a yellow band of xanthophylls, a yellow-orange band of carotenes, a blue-green band of chlorophyll *a*, and a yellow-green band of chlorophyll *b*.

The relationship of the distance moved by a pigment to the distance moved by the solvent front is specific for a given set of conditions. We call this relationship the R_f number and define it as follows:

$$R_f = \frac{\text{Distance moved by pigment}}{\text{Distance from pigment origin to solvent front}}$$

Thus, paper chromatography can be used to identify each pigment by its characteristic R_f. This R_f is constant for a given pigment in a particular solvent-matrix system.

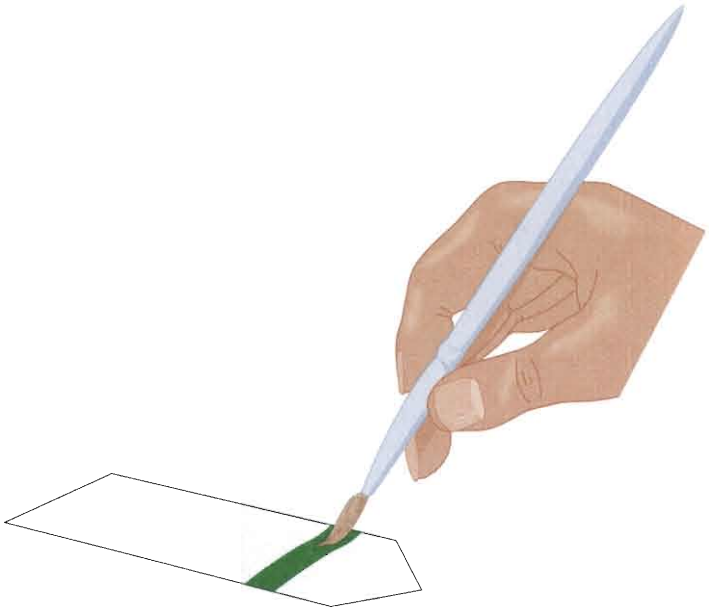


Figure 13.3
Application of pigment extract to a chromatography strip.

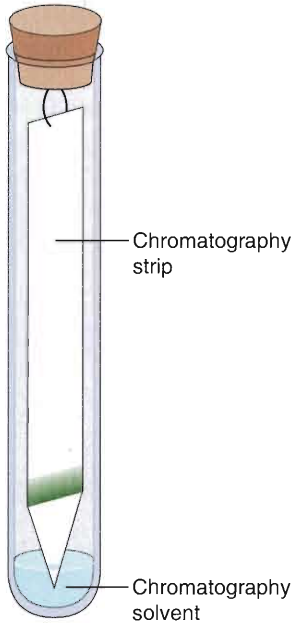


Figure 13.4
Chromatography setup.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 13.1

Separate plant pigments by paper chromatography

1. Observe the contents of the provided container labeled "Plant Extract." You'll use paper chromatography to separate its pigments.

Extinguish all hotplates and flames before you do this experiment. Keep all solvents away from hotplates and flames at all times.

Question 1
What color is the plant extract, and why is it this color?

2. Obtain a strip of chromatography paper from your lab instructor. Handle the paper by its edges so that oil on your fingers does not contaminate the paper.
3. Use a Pasteur pipet or a fine-tipped brush to apply a stripe of plant extract approximately 2 cm from the tip of the paper (fig. 13.3). Blow the stripe dry and repeat this application at least 15 times. For this separation to work well, you must start with an extremely concentrated application of extract on the paper.
4. An alternate procedure is to place a fresh leaf directly on the paper, then press and roll the edge of a coin (quarter) over the leaf to crush the cells and form a stripe of pigment.
5. Place the chromatography strip in a test tube containing 2 mL of chromatography solvent (9 parts petroleum ether : 1 part acetone). Position the chromatography strip so that the tip of the strip (but not the stripe of plant extract) is submerged in the solvent. You can do this by hooking the strip of paper with a pin inserted in the tube's stopper (fig. 13.4).
6. Place the tube in a test-tube rack and watch as the solvent moves up the paper. Keep the tubes capped and undisturbed during solvent movement.
7. Remove the chromatography strip before the solvent front reaches the top of the strip (i.e., after 2–3 min). Mark the position of the solvent front with a pencil and set the strip aside to dry. Observe the bands of color, then draw your results on figure 13.5. Use your textbook or other materials in lab to identify the

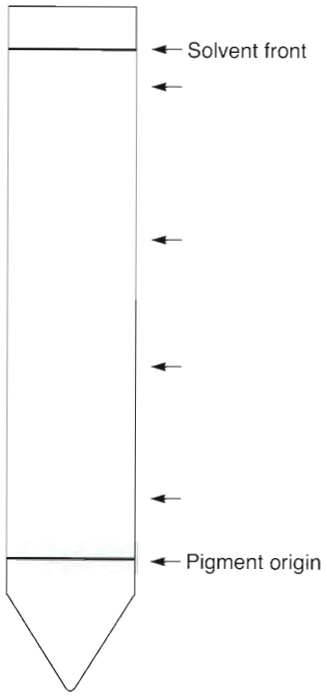


Figure 13.5

Completed chromatogram. On the chromatogram indicate the color of the pigment band to the left of the arrows. To the right of the arrows write the name of the pigment.

different bands of pigments according to their position and color. For example, xanthophylls appear yellow.

8. Use a ruler to measure the distance from the pigment origin to the solvent front and from the origin to each pigment band. Calculate the R_f number for each pigment; record your data in table 13.1.

Question 2

a. What does a small R_f number tell you about the characteristics of the moving molecules?

b. Which are more soluble in the chromatography solvent, xanthophylls or chlorophyll *a*? How do you conclude this?

TABLE 13.1

R_f NUMBERS FOR FOUR PLANT PIGMENTS

Pigment	R_f
Carotene	
Xanthophyll	
Chlorophyll *a*	
Chlorophyll *b*	

c. Would you expect the R_f number of a pigment to change if you altered the composition of the solvent? Why or why not?

d. If yellow xanthophylls were present in the extract, why did the extract appear green?

e. Is it possible to have an R_f number greater than 1? Why or why not?

ABSORPTION OF LIGHT BY CHLOROPHYLL

A **spectroscope** is an instrument that separates white light into its component colors. These colors range from red to violet and appear as a spectrum when separated (fig. 13.6). Observe this spectrum by looking through the spectroscope provided in the lab. Now insert a chlorophyll sample between the light and spectroscope, and observe the resulting spectrum. Light not visible through the extract has been absorbed.

Question 3

What colors are diminished or absent?

Based on this observation, complete the following absorption spectrum for chlorophyll. For each color, estimate the relative absorbance of that color by placing an X above the color name at the appropriate position along the y-axis. Connect the Xs for all colors to complete the absorption spectrum.

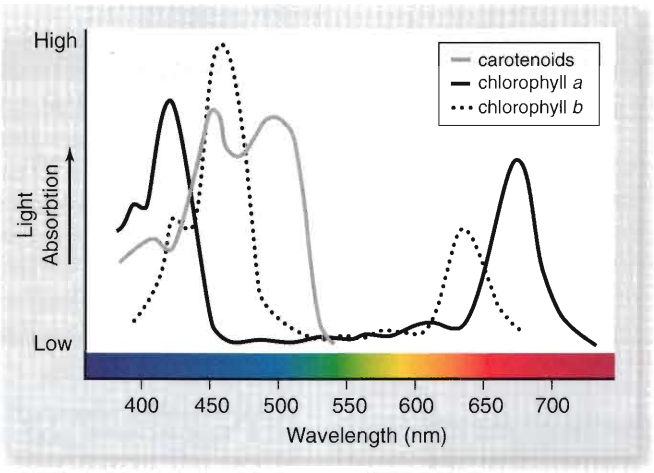
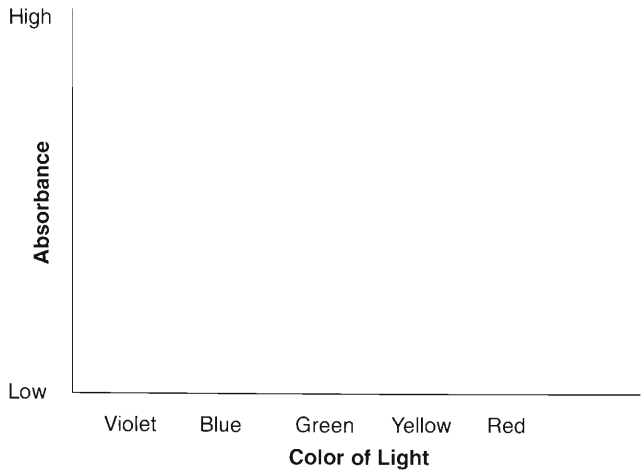


Figure 13.6

The absorption spectrum of chlorophyll and carotenoids. The peaks represent wavelengths of sunlight absorbed by the two common forms of photosynthetic pigment, chlorophylls *a* and *b*, and by the carotenoids. Chlorophylls absorb predominantly violet-blue and red light in two narrow bands of the spectrum, and reflect green light in the middle of the spectrum. Carotenoids absorb mostly blue and green light and reflect orange and yellow light.



Question 4

What color of light would be least effective for plant photosynthesis? Why?

FLUORESCENCE

Light produces reactions only if it is absorbed by a molecule. When sunlight strikes a plant, the chlorophyll absorbs some of the light and reflects some of the light. The green light is reflected and is responsible for the plant's green color. The absorbed light "excites" the chlorophyll by boosting electrons to a higher-energy orbital. During photosynthesis, the energy of these excited electrons from chlorophyll and chlorophyll's central magnesium atom is passed efficiently to another pigment molecule and photosynthesis proceeds. However, to easily observe these energized electrons, we can disrupt the photosynthetic system by blending the cells during the preparation of the plant extract. The chlorophyll electrons in the extract are still energized if you shine light on them, but they are left with nowhere to go. They quickly release their energy by falling back to their original orbitals rather than continuing photosynthesis. As they fall back, they emit a photon of red light. This release of light energy is **fluorescence.** The wavelength of reemitted light is determined by the structure of the molecule reemitting the light.

Procedure 13.2

Observe fluorescence by chlorophyll

Place a glass test tube of chlorophyll extract in front of a bright light. View the extract from the side.

Question 5

What color light does the extract fluoresce?

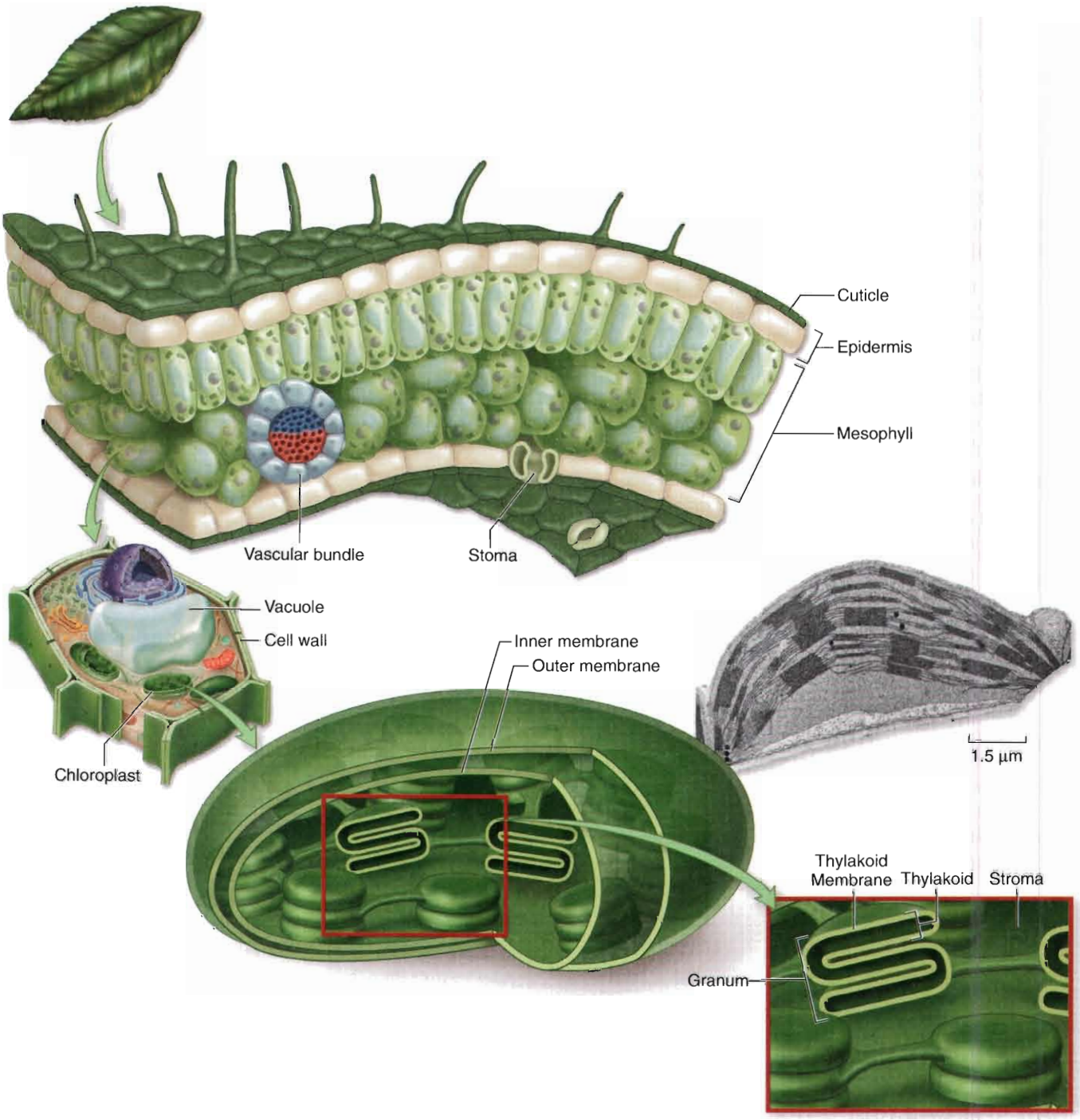


Figure 13.8

The structure of a leaf and chloroplast. Chloroplasts are bounded by a double membrane and contain photosynthetic membranes called thylakoids. Stacked one on top of the other, a column of thylakoids is a granum. The interior of the entire chloroplast is bathed by a semiliquid, the stroma. The openings that enable CO_2 to enter the leaf are stomata (singular, stoma).

Question 8

a. Was starch stored in the leaf? How can you tell?

b. Would you expect leaves to be the primary area for starch storage? Why or why not?

Procedure 13.7

Observe the requirement of light for photosynthesis

1. Obtain a *Geranium* leaf that has been half or completely covered with metal foil or thick paper for three or four days.
2. Repeat the bleaching and staining steps described in procedure 13.6.

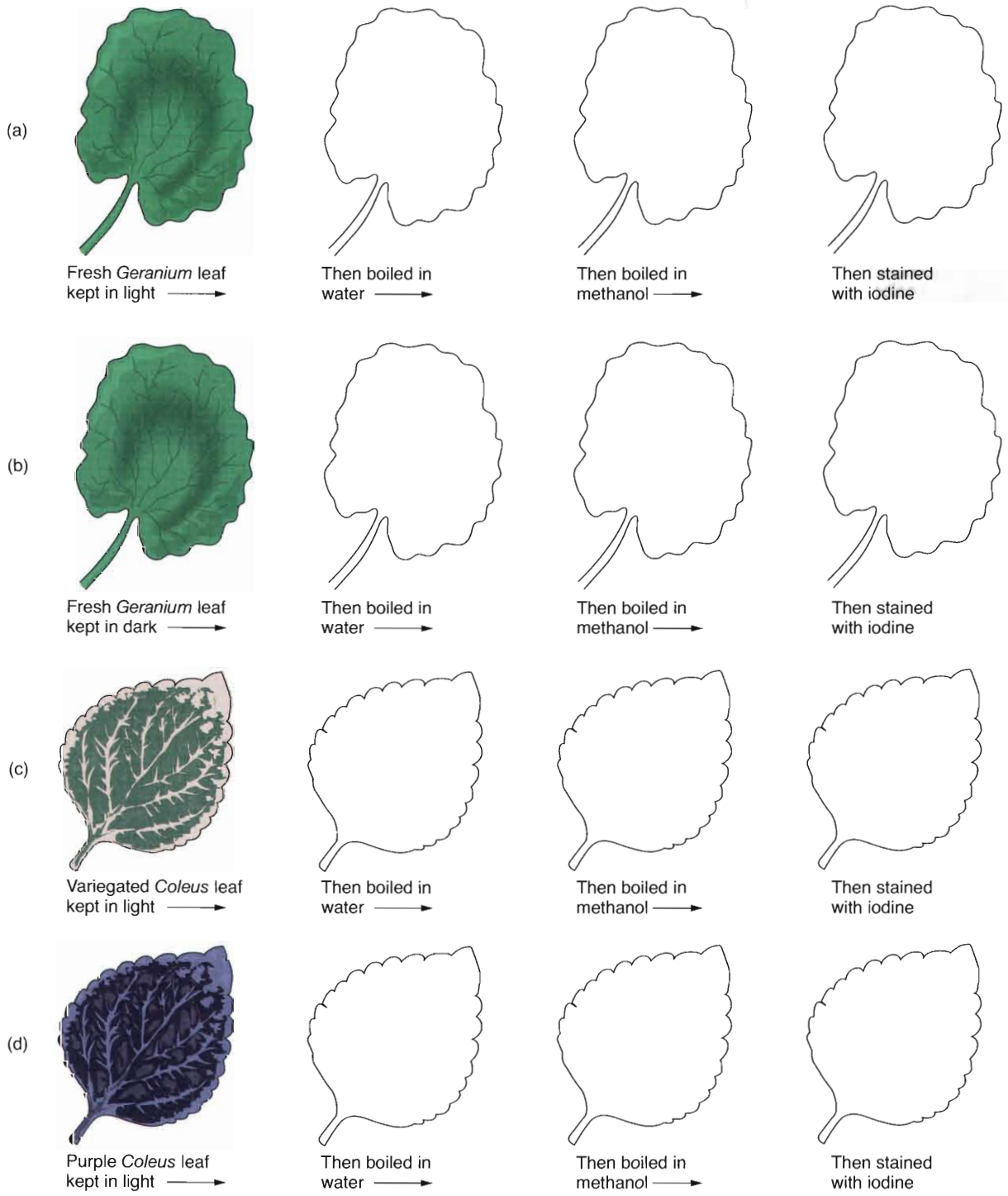


Figure 13.9

The requirement of light and chlorophyll and the production of starch during photosynthesis. Within each diagram, record the color of the leaf following the treatments to indicate (*a*) the production of starch, (*b*) the need for light, and (*c, d*) the need for chlorophyll for photosynthesis. Record your results from the appropriate procedure by writing the resulting color of each treated leaf directly onto the leaf outline.

3. Describe and explain any color change in the leaf.
4. Record in figure 13.9*b* the color of the leaves after each successive treatment.

Question 9

Does a leaf produce starch if it has been deprived of light?

(a)

(b)

Figure 13.10

Coleus plants. (*a*) Leaves of this variegated plant have green, white, purple, and pink areas resulting from combinations of chlorophylls and anthocyanin (red) pigments. (*b*) Leaves of this purple *Coleus* have the same pigment combination throughout the leaf.

Procedure 13.8

Observe the requirement of chlorophyll for photosynthesis

1. Obtain leaves of a variegated *Coleus* plant (fig. 13.10*a*) and a purple-leafed *Coleus* plant (fig. 13.10*b*). Make sketches of their original pigmentation patterns in figure 13.9*c*, *d*. Indicate which areas are green, red, green/red, and white.
2. Extract the pigments and stain for starch according to procedure 13.6. Boiling the leaf in water will remove the water-soluble pigments such as the red cyanins, and boiling the leaf in alcohol will remove chlorophyll. These pigments must be removed for you to see the color changes of the iodine starch test.
3. Record in figure 13.9*c*, *d* the color of the leaves after each successive treatment.

Question 10

a. How does the pattern of starch storage relate to the distribution of chlorophyll?

b. Photosynthesis requires chlorophyll (green), but some of the *Coleus* leaves that you tested were purple. How do you explain your results?

INVESTIGATION

Relative Uptake and Production of CO_2 during Photosynthesis

Observations: Recall that aerobic cellular respiration releases CO_2, which can combine with water to form carbonic acid and lower the pH (see procedure 12.3). *Elodea* growing in light respires and photosynthesizes. *Elodea* in darkness only respires. Design an experiment to measure *Elodea*'s relative uptake and production of CO_2.

Question: What is the relative uptake versus production of CO_2 during photosynthesis and respiration?

a. Establish a working lab group and obtain Investigation Worksheet 13 from your instructor.

b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record it on Worksheet 13.

c. Translate your question into a testable hypothesis and record it.

d. Review procedure 12.3 that provides a method to quantify CO_2 production. Outline on Worksheet 13 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. Why does chlorophyll appear green?

2. Is starch produced when a leaf is kept in the dark? Why or why not?

3. What causes leaves to turn from green to yellow and red in autumn?

4. Of what value to plants is starch?

5. What is the significance of electron transport in the photochemical (i.e., light-dependent) reactions of photosynthesis?

6. Design an experiment to determine if plants respire. Be sure to explain how you would measure respiration and the controls you would include in the experimental design.

DOING BIOLOGY YOURSELF

Recall that respiration produces CO_2, which combines with water to form carbonic acid that lowers the pH of a surrounding solution. Design an experiment to measure the relative dynamics (mass balance) of photosynthesis versus respiration for *Elodea*.

WRITING TO LEARN BIOLOGY

Use a reference to determine the relative penetration of different wavelengths of light through water. Describe how this could affect the existence and distribution of submerged plants.

14 Mitosis
Replication of Eukaryotic Cells

Objectives

By the end of this exercise you should be able to:

1. Describe events associated with the cell cycle.
2. Describe events associated with mitosis.
3. Distinguish the stages of mitosis on prepared slides of mitotic cells.
4. Stain and examine chromosomes in mitotic cells.
5. Estimate the duration of various stages of mitosis from experimental observations.

Cells grow, have specialized functions, and usually replicate during their life. Although cell enlargement is part of organismal growth, cell replication is also required and allows growth without each cell becoming too large. All of these activities are part of a repeating set of events called the **cell cycle.** A major feature of the cell cycle is cellular replication, and a major feature of cellular replication is mitosis. **Mitosis** is the replication and division of the nucleus of a eukaryotic cell in preparation for cytokinesis. During mitosis, replicated chromosomes within the cell are separated into two identical sets—each set is then surrounded by a nuclear membrane. Each of the two new nuclei has a full set of chromosomes containing a copy of all of the genetic information for the organism. Prokaryotic cells lack nuclei and do not undergo mitosis. Instead, they replicate their chromosome during a process called binary fission (described in Exercise 24).

Mitosis is usually associated with **cytokinesis,** the division of the cell and cytoplasm into halves that each contain a nucleus. In some tissues, cytokinesis is delayed or does not occur at all, and the cells are multinucleate. Mitosis and cytokinesis are important because they provide a mechanism for orderly growth of living organisms.

Question 1
Consider the surface-to-volume ratios of large versus small cells. Is it adaptive for cells of a growing organism to remain small? Explain your answer.

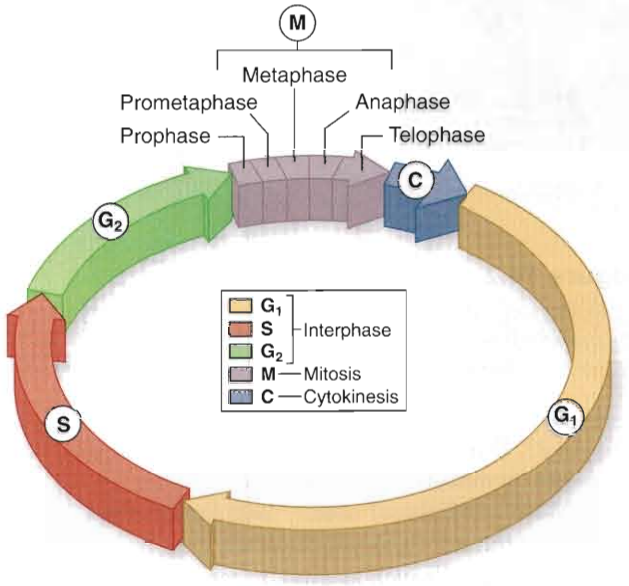


Figure 14.1
The cell cycle. The cell cycle is depicted as a circle. The first gap phase, G_1, involves growth and preparation for DNA synthesis. During S phase, a copy of the genome is synthesized. The second phase, G_2, prepares the cell for mitosis. During mitosis, replicated chromosomes are partitioned. Cytokinesis divides the cell into two cells with identical genomes.

THE CELL CYCLE

This exercise emphasizes events associated with mitosis, but mitosis is only part of the cell cycle (fig. 14.1). The remainder of the cycle is called the **interphase** and is subdivided further into **cytokinesis (C), gap 1 (G_1), synthesis (S),** and **gap 2 (G_2)** phases.

The cell cycle begins with the formation of a new cell and ends with replication of that cell. The G_1 phase of the cell cycle occurs after mitosis and cytokinesis, and is when the majority of cellular activity for the functions of the cell occurs. Many cell-specific proteins and other molecules are produced for the metabolism of the cell during G_1. During the S phase, the DNA composing the chromosomes is duplicated. At the end of the S phase each chromosome consists of an identical pair of chromosomal DNA strands, called sister **chromatids,** attached at a **centromere**

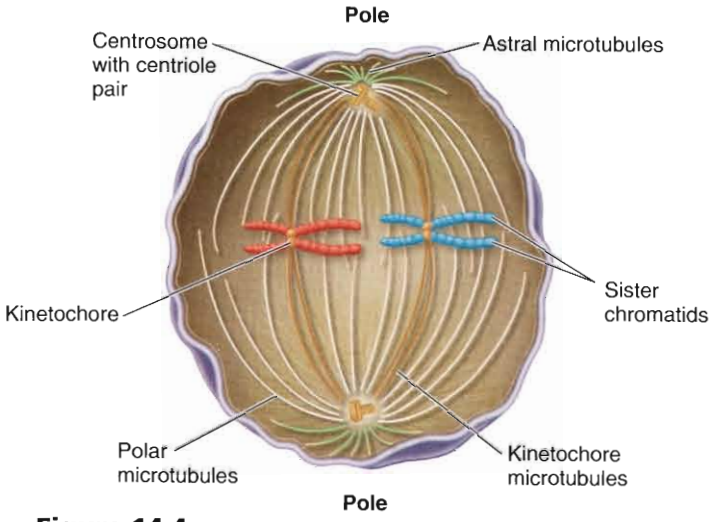


Figure 14.4

The structure of the mitotic spindle. The mitotic spindle is formed by the centrosomes from three types of microtubules. The astral microtubules emanate away from the region between the poles. The polar microtubules project into the region between the two poles. The kinetochore microtubules are attached to the kinetochores of sister chromatids.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 14.1

Describe the specific events of mitosis

Before your lab meeting, review in your textbook the events associated with each stage of mitosis in addition to the preparatory stage, interphase. List these events in table 14.1. Some events and structures occur only in plant cells and some occur only in animal cells. Mark these events in your list with an asterisk. This list can serve as an excellent study guide, so be as complete as possible. One event for each stage is provided in figure 14.4.

Question 3

a. If a nucleus has eight chromosomes during interphase, how many chromosomes does it have during metaphase?

b. How many does it have after mitosis is complete?

Understanding the movements of chromosomes is crucial to understanding mitosis. You can simulate these movements easily with chromosome models made of pipe cleaners or popsicle sticks. This is a simple procedure but a valuable one. It will be especially helpful when you are comparing the events of mitosis to the events of meiosis, which you will simulate in the next exercise.

Procedure 14.2

Simulate chromosomal replication and movement during mitosis

1. Examine the materials to be used as chromosome models provided by your instructor.
2. Identify the differences in chromosomes represented by various colors, lengths, or shapes of materials. Also identify materials representing centromeres.
3. Place a sheet of notebook paper on your lab table to use in representing the boundaries of the mitotic cell.
4. Assemble the chromosomes needed to represent nuclear material in a cell of a diploid organism with a total of six chromosomes. Place the chromosomes in the cell.
5. Arrange the chromosomes to depict the position and status of chromosomes during interphase G_1. (During G_1 the chromosomes are usually not condensed, as the chromosome models imply, but the models are an adequate representation.)
6. Depict the status of chromosomes after completing interphase S. Use additional "nuclear material" if needed.
7. Move the chromosome models appropriately to depict prophase.
8. Move the chromosome models appropriately to depict metaphase.
9. Move the chromosome models appropriately to depict anaphase.
10. Move the chromosome models appropriately to depict telophase.
11. Draw the results of cytokinesis and the re-formation of nuclear membranes.
12. Chromosomal events occur as a continuous process of movements rather than in distinct steps. Therefore, repeat steps 4–11 as a continuous process and ask your instructor to verify your simulation.

MITOSIS IN ANIMAL CELLS

The most distinctive features of cellular replication in animal cells are the formation of asters with centrioles at their center (discussed earlier) and cytokinesis. Cytokinesis includes formation of a **cleavage furrow** that begins on the periphery of the cell, pinches inward, and eventually divides the cytoplasm into two cells (fig. 14.5). Cells of a whitefish

TABLE 14.1

EVENTS OF MITOSIS AND INTERPHASE

Interphase	Although interphase is not part of nuclear replication, understanding its events is essential to understanding mitosis.
Prophase	
Prometaphase	
Metaphase	
Anaphase	
Telophase	

blastula provide good examples of the stages of mitosis and cytokinesis. Whitefish are commonly cultured fish whose eggs and early developmental stages undergo rapid cell divisions (as do all embryonic cells). A blastula is an early embryonic stage of a vertebrate and consists of a sphere of 25–100 cells with a high frequency of different mitotic stages. Exercise 50 (Embryology) details the formation of a blastula during embryonic development.

Procedure 14.3

Observe and describe mitosis in animal cells

1. Obtain a prepared slide of a cross section through the blastula of a whitefish.
2. Examine the cells first on low (10×) then high (40× or 100×) magnification. Some of the cells contain condensed and stained chromosomes.
3. Refer to figure 14.4 for a summary of the stages of mitosis. Identify examples of each stage on your prepared slide (fig. 14.6). Verify these stages with your lab partner or teaching assistant.
4. Also identify cells that you believe are between stages.
5. Examine the whitefish cells for signs of cytokinesis.
6. Prepared cross sections of cells show only two dimensions, but mitosis is a three-dimensional process. In the following space draw two cells in metaphase: one in which the cross section is parallel

to the axis of the spindle apparatus and one in which the cross section is perpendicular to the spindle apparatus.

Question 4

a. Why would we choose an embryonic mass of cells for procedure 14.3 in which to study the stages of mitosis?

b. Which stage of mitosis most often is associated with the beginning of cytokinesis?

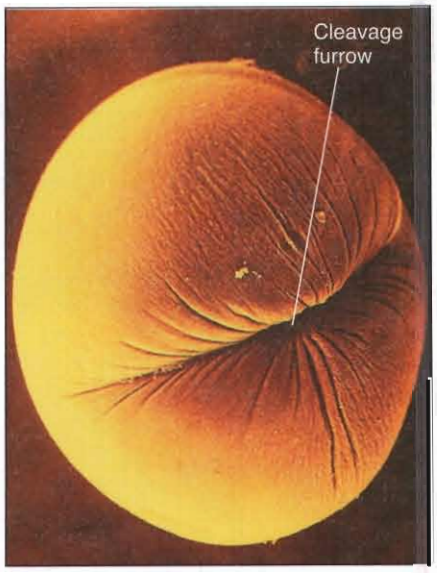


Figure 14.5
Cytokinesis in an animal cell. Cytokinesis, the physical division of the cell's cytoplasm, usually occurs after nuclear division is complete. A cleavage furrow is forming around this dividing sea urchin egg.

MITOSIS IN PLANT CELLS

Our model to study cellular replication in plants is the root tip of *Allium* (onion). Root tips of plants contain **meristems,** localized areas of rapid cell division due to active growth at the root tips. In plant cells, cytokinesis includes formation of a partition called a **cell plate** perpendicular to the axis of the spindle apparatus. The cell plate forms in the middle of

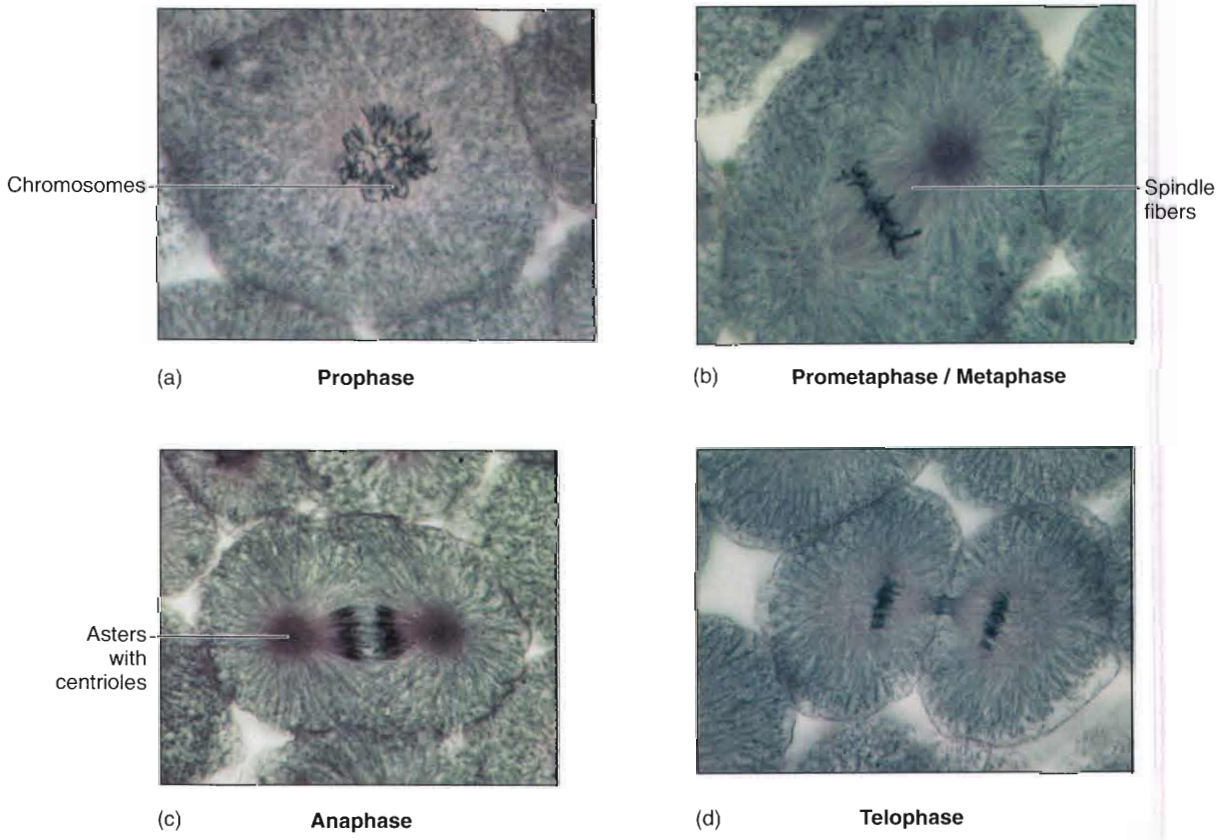
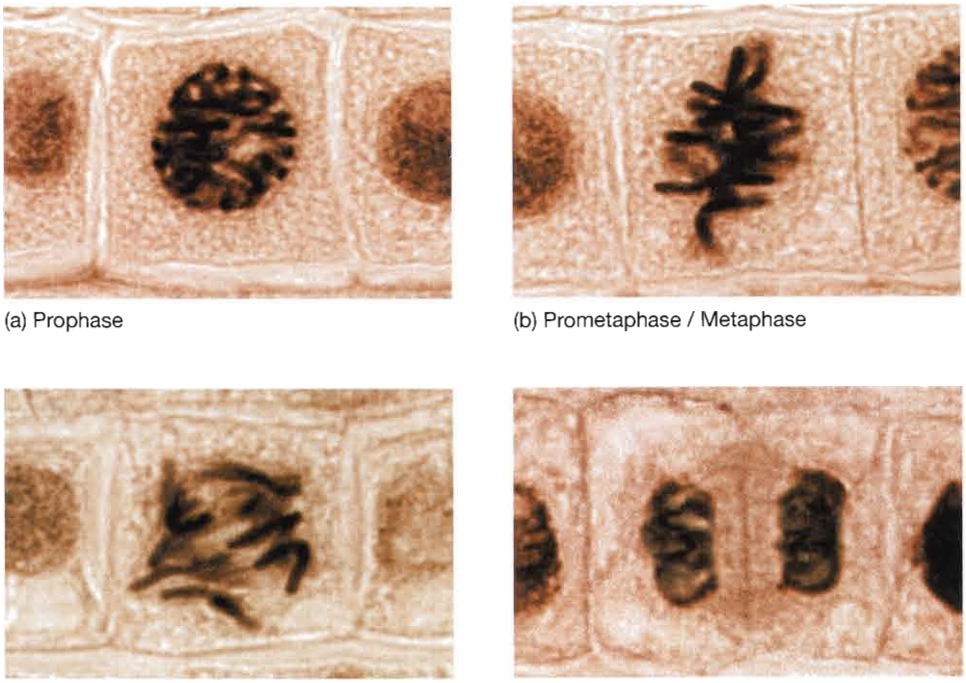


Figure 14.6
Stages of mitosis in the cells of a whitefish embryonic blastula (400×). Prometaphase and metaphase may not always be distinguishable by light microscopy.



(a) Prophase (b) Prometaphase / Metaphase

(c) Anaphase (d) Telophase

Figure 14.7
Stages of mitosis in a plant cell (1000×). The dark structures are chromosomes.

the cell and grows out to the periphery. It will separate the two new cells.

Interestingly, the formation of the spindle apparatus and other microtubule systems in plant and fungal cells is organized by centrosomes, as in animal cells. But plant and fungal cells have no centrioles within the centrosomes. Thus, the function and necessity of centrioles remain somewhat of a mystery.

Procedure 14.4

Observe and diagram mitosis in plant cells

1. Examine a prepared slide of a longitudinal section through an onion root tip.
2. Search for examples of all stages of mitosis (fig. 14.7). Notice that most cells are in some part of interphase. Prometaphase may be difficult to distinguish from metaphase.
3. Search for signs of cell plate formation.
4. In figure 14.8, diagram a plant cell with a diploid number of three pairs of chromosomes in each of the stages of mitosis. **Diploid** refers to a nucleus with two of each type of chromosome. Be sure to label the cell wall and cell plate.
5. Prepared cross sections of cells show only two dimensions, but mitosis is a three-dimensional process. In the following space draw two cells in metaphase: one in which the plane of section is parallel to the axis of the spindle apparatus and one in which the cross section is perpendicular to the spindle apparatus.

Question 5

a. What region of a root has the most mitotic activity?

b. How does cytokinesis differ in plant versus animal cells?

c. Why is pinching of the cytoplasm inadequate for cytokinesis in plant cells?

d. What is a cell plate, and in what stage of mitosis does it form?

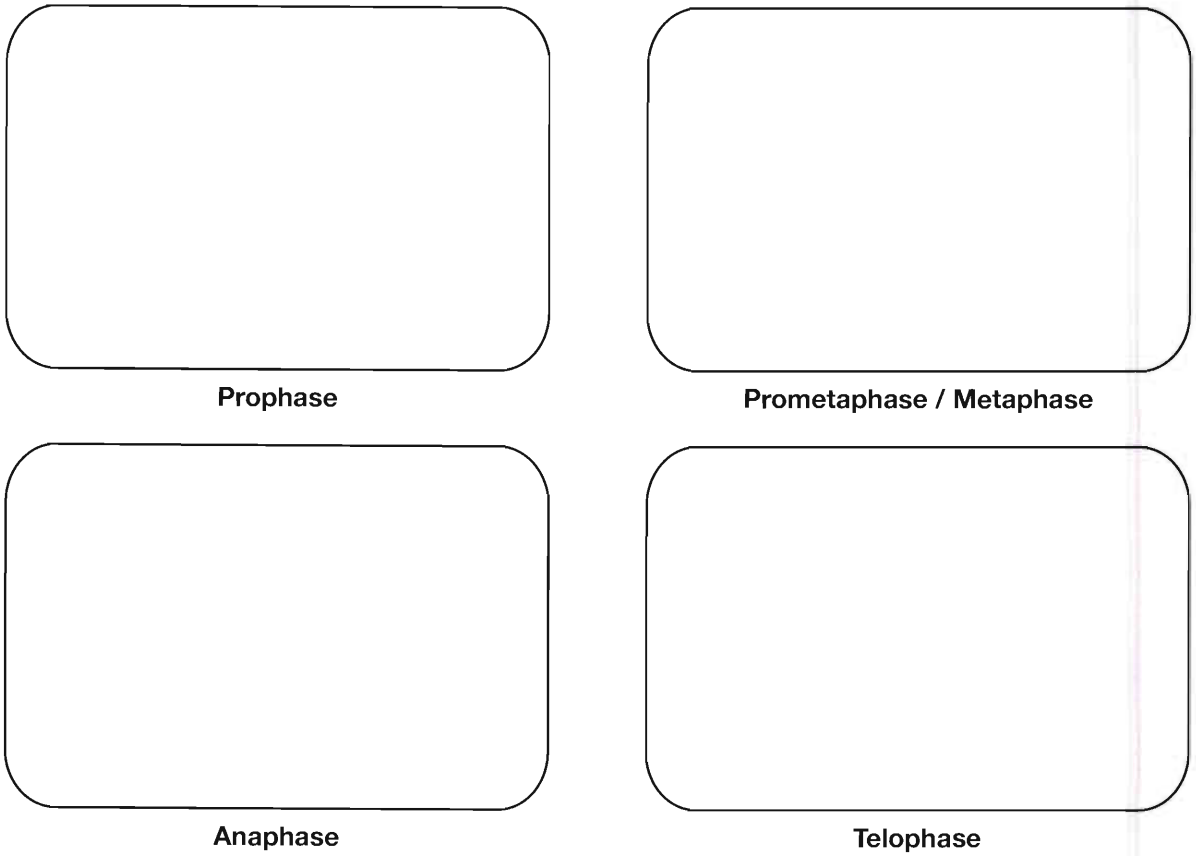


Figure 14.8

Diagram of the stages of mitosis in a plant cell with six chromosomes. The outlines represent the cell walls of each of four cells.

e. Locate a plant cell in late telophase. What is the volume of the two new cells relative to a mature cell?

d. What were some of the assumptions you made for calculations in procedure 14.5?

Question 6

a. Why are the combined data from all the class members more meaningful than your results alone?

b. How accurate were your predictions for the length of each stage of mitosis?

c. What sources of error can you list for this technique to determine the time elapsed during each stage of mitosis?

PREPARING AND STAINING CHROMOSOMES

Your instructor has prepared some living onion root tips for you to process further and use to observe the stages of mitosis.

Procedure 14.5

Stain chromosomes

1. Obtain an onion root tip and place it in a small vial with Schiff's reagent for 30 min. Handle Schiff's reagent carefully because it is a colorless liquid that becomes bright red after reaction.

   Keep the vial in the dark and at room temperature until the root tip becomes purple. Your instructor may have already stained some root tips for you.

INVESTIGATION

The Time Elapsed during the Various Stages of Mitosis

Observations: The cell cycle of actively dividing cells of root tips of *Allium* is approximately 24 h long. The phases of mitosis usually occupy only a small portion of that time.

Question: How long does mitosis take?

a. Establish a working lab group and obtain Investigation Worksheet 14 from your instructor.
b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record it on Worksheet 14.
c. Translate your question into a testable hypothesis and record it.
d. Each prepared slide of a root tip of *Allium* reveals a snapshot in time of all stages of mitosis.
e. The relative abundance of cells in a phase of mitosis is directly proportional to the length of time for that phase.
f. Outline on Worksheet 14 your experimental design and supplies needed to test your hypothesis. Table 14.2 offers insight to a reasonable design. Ask your instructor to review your proposed investigation.
g. Conduct your procedures, record your data, answer your question, and make relevant comments.
h. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

TABLE 14.2

DURATION OF EACH STAGE OF MITOSIS IN AN ONION ROOT CELL BASED ON A 24-HOUR CELL CYCLE

Stage of Mitosis	Predicted Duration (hours)	Number of Cells in Each Stage			Total Number of Cells in Each Stage	Class Total Number for Each Stage	Calculated Duration (hours)
Interphase							
Prophase							
Prometaphase/ Metaphase							
Anaphase							
Telophase							
Totals							

2. Place the root tip in a drop of 45% acetic acid on a slide and cut away all except the terminal 1 mm of the tip.

Acetic acid is corrosive. Do not spill it.

3. Crush the root tip with a blunt probe and cover the tissue with a coverslip.
4. Smash the tissue by pressing on the coverslip with the eraser of your pencil. Your instructor will demonstrate this procedure.
5. Scan your preparation at low magnification to locate stained chromosomes. Then switch to high magnification and locate formations of chromosomes that indicate each of the stages of mitosis.
6. Add a drop of acetic acid to the edge of the coverslip to avoid desiccation.
7. Locate as many stages of mitosis as you can. Be sure to look at preparations done by other students.

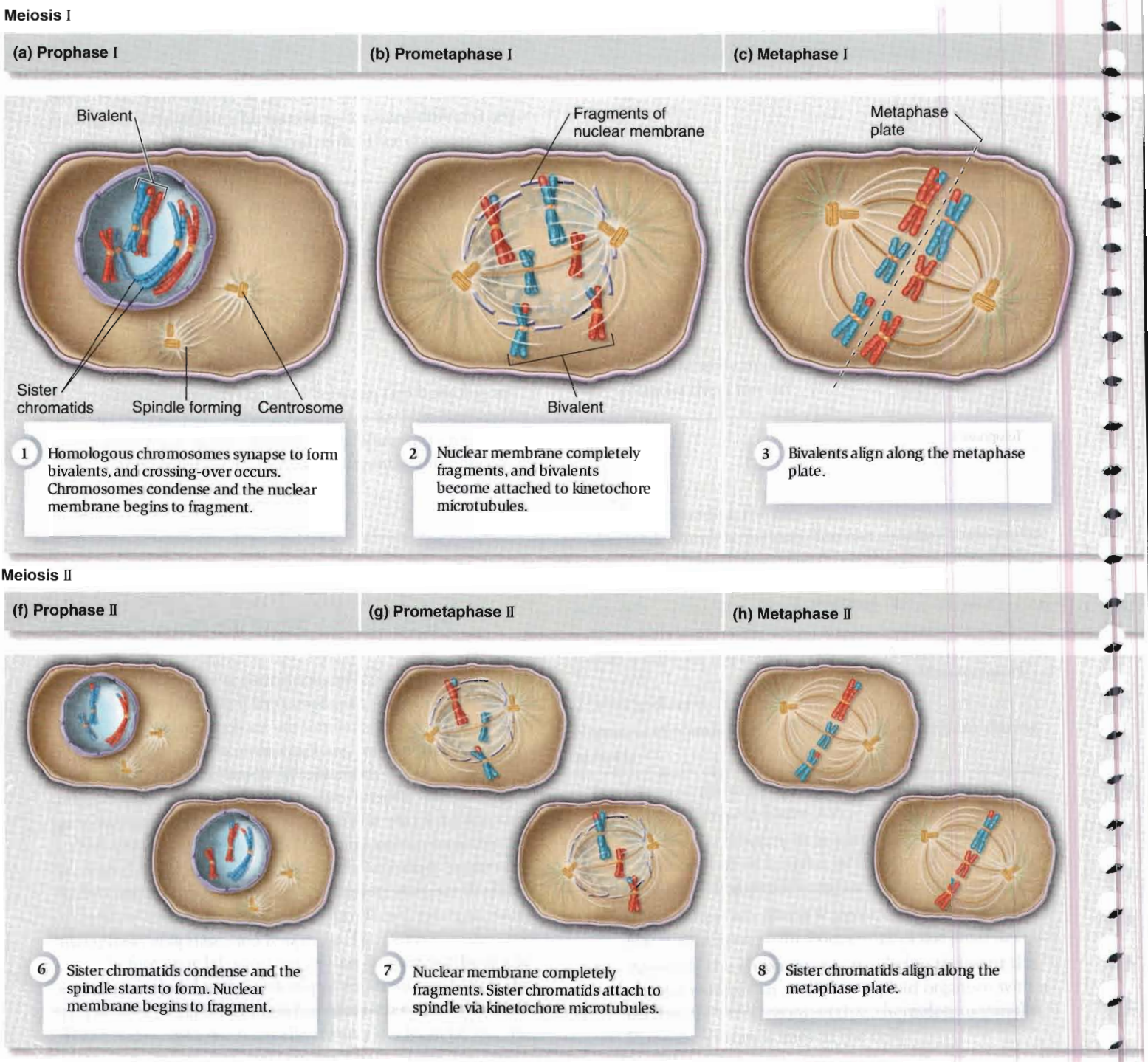
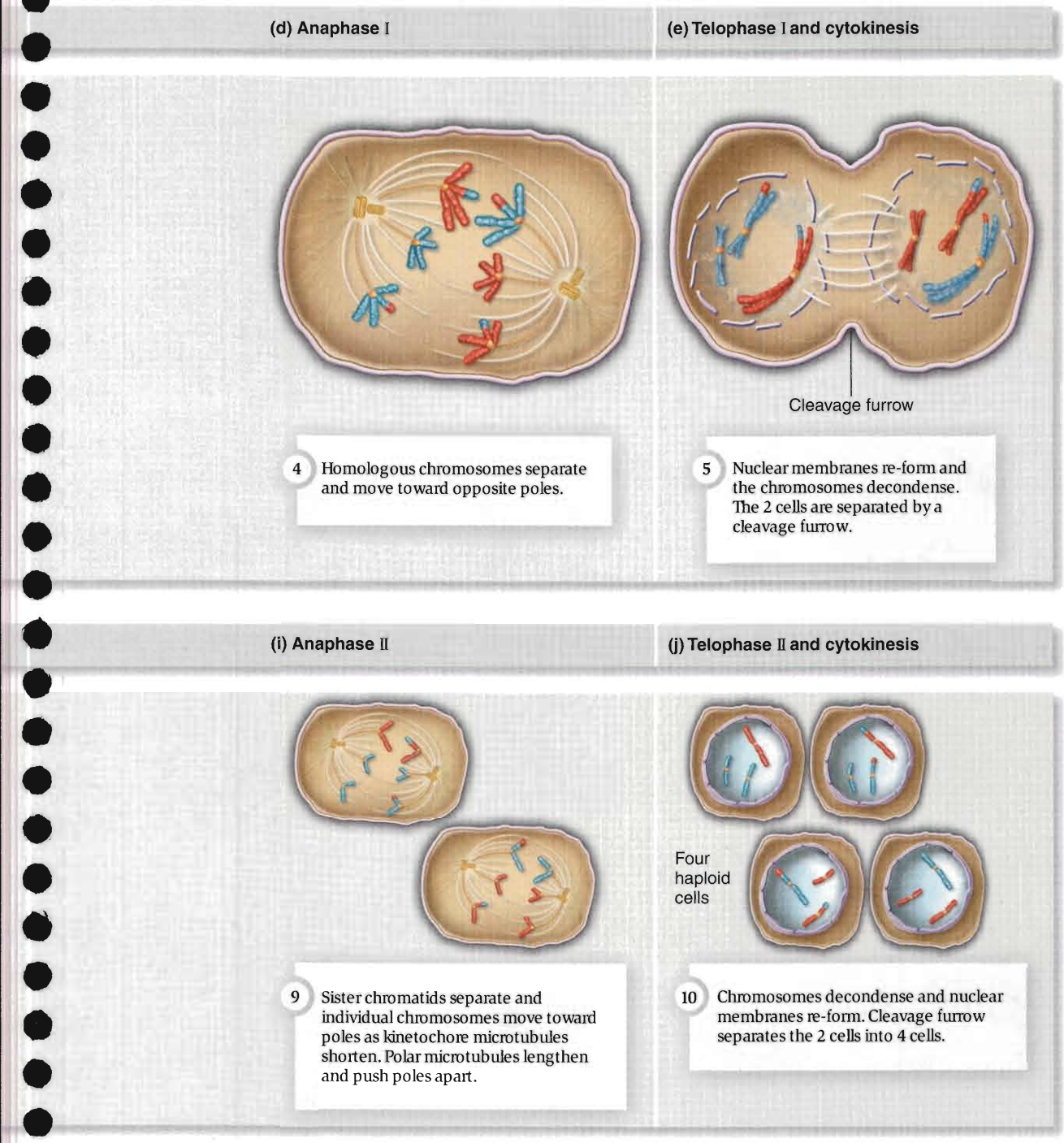


Figure 15.2
Stages of meiosis.

GAMETOGENESIS

Meiosis occurs in all sexually reproducing eukaryotes and produces haploid nuclei. However, organisms vary in the timing and structures associated with producing functional gametes. **Gametes** are reproductive cells with haploid nuclei resulting from meiosis, and the formation of gametes is called **gametogenesis.** Meiosis is the primary element of gametogenesis in animals, but the cells must mature and usually change their morphology before a newly formed cell from meiosis becomes a functional gamete.

In this exercise you will examine mammalian gametogenesis. Male gametes of mammals are different from female gametes. Gametogenesis is divided into **spermatogenesis,** the formation of sperm cells, and **oogenesis,** the formation of egg cells (fig. 15.3).



Mammalian Spermatogenesis

Spermatogenesis occurs in male testes, made of tightly coiled tubes called **seminiferous tubules** (fig. 15.4). Examine a prepared slide of a cross section through the seminiferous tubules of a monkey, rat, or grasshopper. Packed against the inner walls of the tubules are diploid cells called **spermatogonia,** which constantly replicate mitotically during the life of males. Some of the daughter cells move inward toward the lumen of the tubule and begin meiosis. These cells are called **primary spermatocytes.** Meiosis I of a primary spermatocyte produces two **secondary spermatocytes,** each with a haploid set of double-stranded chromosomes.

Meiosis II separates the strands of each chromosome and produces two haploid cells called **spermatids.** Spermatids mature and differentiate into **sperm** cells as they move along the length of the tubule. Review these basic

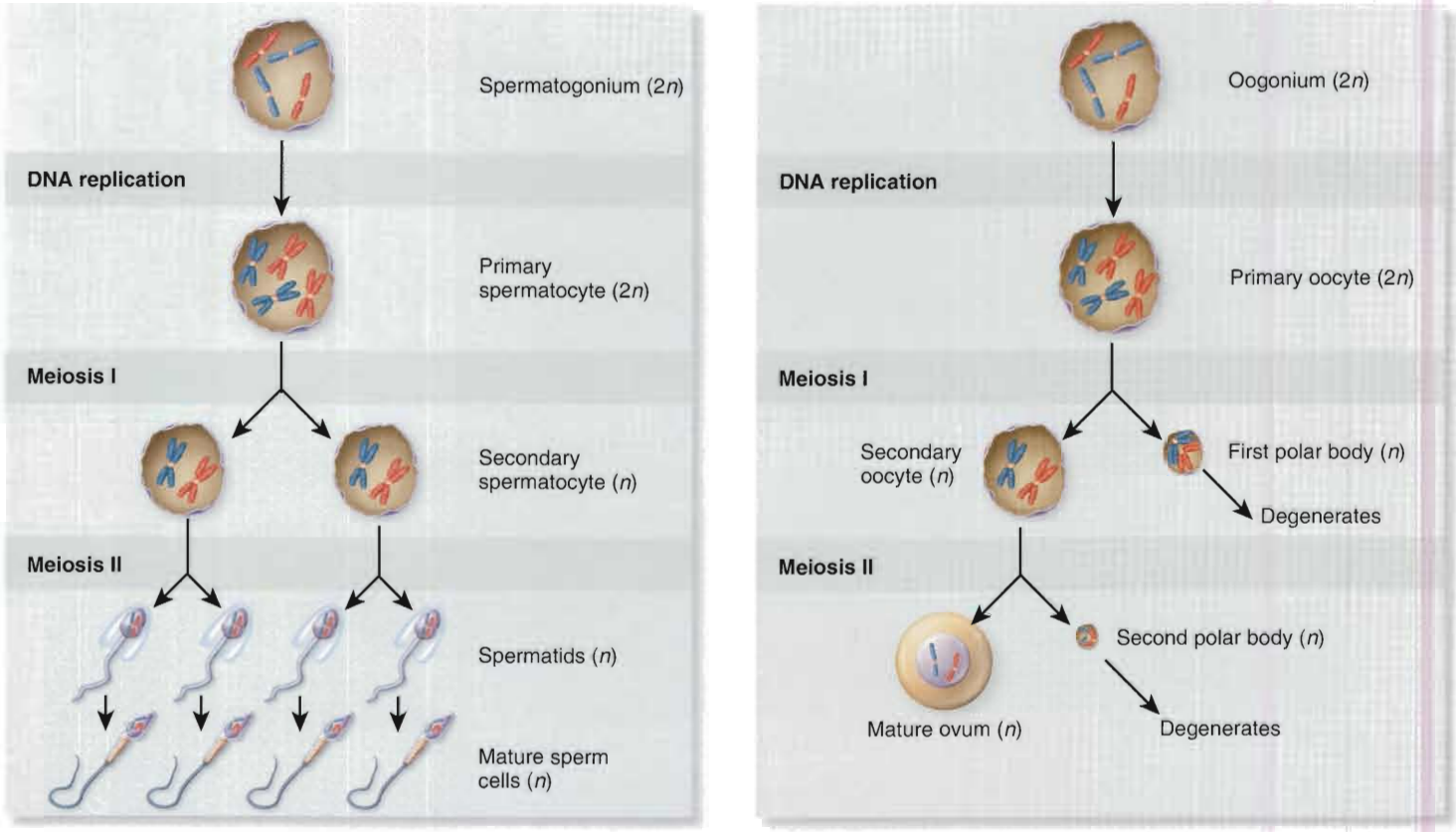


Figure 15.3

Gametogenesis in (*a*) males and (*b*) females. Both male and female germ cells are diploid (2*n*) cells that undergo two meiotic divisions to produce mature haploid (*n*) gametes.

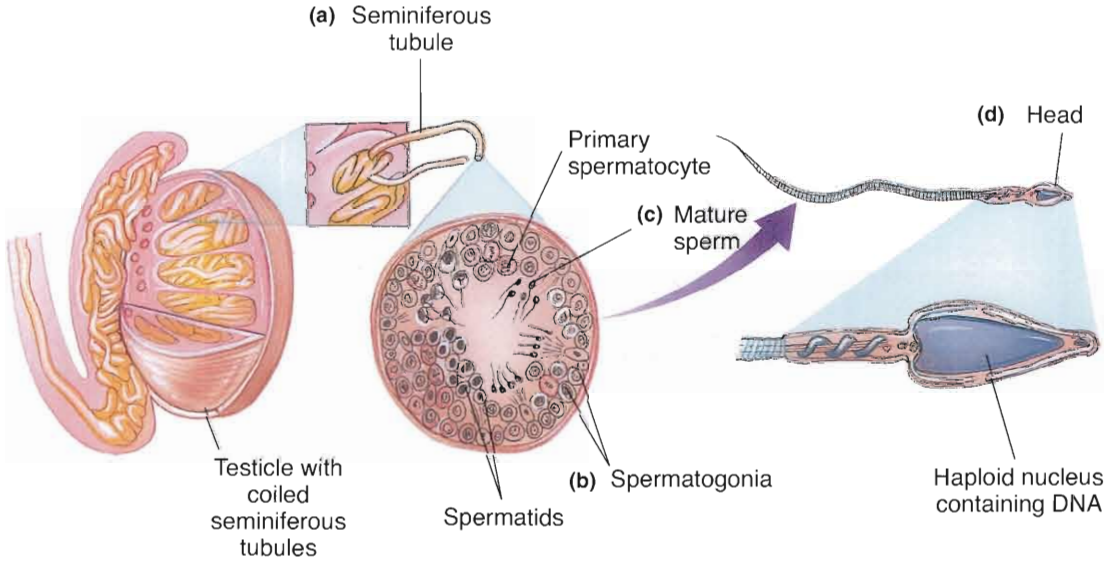


Figure 15.4

The interior of the testis, the site of spermatogenesis. Within the seminiferous tubules of the testis (*a*), cells called spermatogonia (*b*) pass through the spermatocyte and spermatid stages to develop into sperm. Each sperm (*c*) possesses a long tail coupled to a head (*d*), which contains a haploid nucleus.

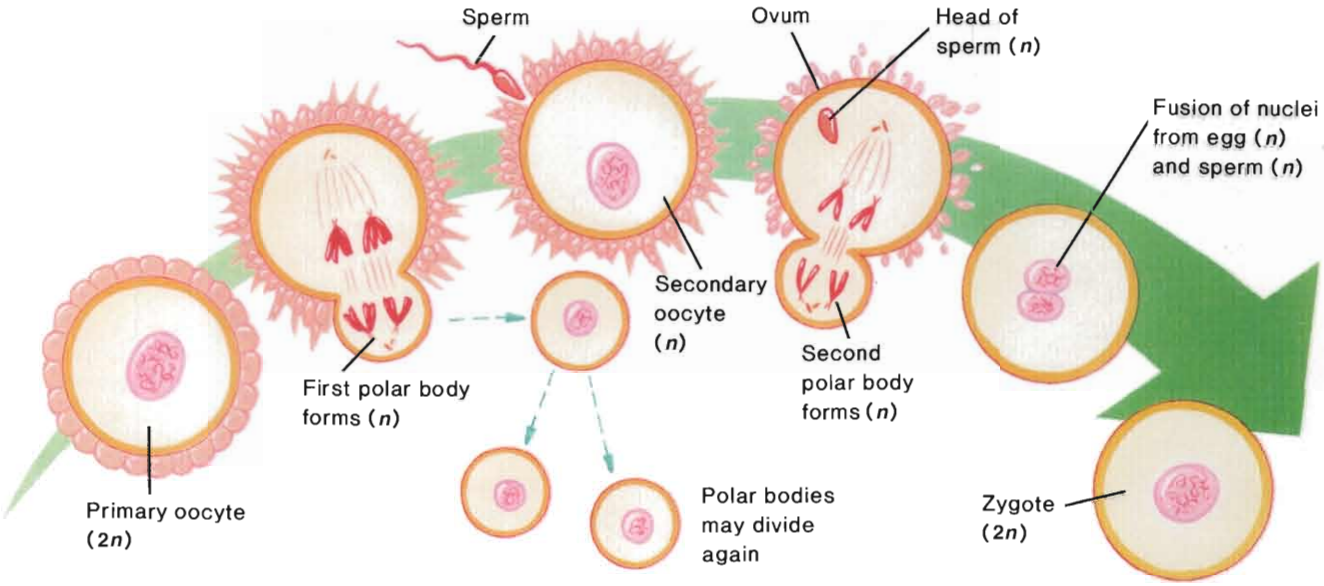


Figure 15.5

Oogenesis. A primary oocyte is diploid ($2n$). After its first meiotic division, one product is eliminated as a polar body. The other product, the secondary oocyte, is released during ovulation. Sperm penetration stimulates the second meiotic division, and a second polar body and a haploid ovum are produced. Fusion of the haploid (n) ovum nucleus with a haploid (n) sperm nucleus produces a diploid ($2n$) zygote that subsequently forms an embryo.

stages of spermatogenesis in figure 15.3. Then examine some prepared slides of sperm cells from vertebrates such as guinea pig, rat, and human.

Question 4

a. During gametogenesis a sperm cell undergoes considerable structural change. What are the basics of sperm structure and how does it relate to function?

b. What is the advantage of producing sperm in a system of tubes rather than in solid tissue?

c. What is each strand of a double-stranded chromosome called?

Mammalian Oogenesis

Oogenesis occurs in ovaries of females (fig. 15.5). Cells of the ovary that produce female gametes are called **oocytes.** However, oocytes are not produced continually by the ovary, as spermatocytes are produced by the testes. During early fetal development, oogonia are produced in the ovaries. These oogonia replicate mitotically to produce as many as two million **primary oocytes.** In humans, ovaries of a newborn female contain all of the primary oocytes that she will ever have (i.e., oogonia produce no more primary oocytes). At birth the primary oocytes in a female have begun meiosis I but are arrested in prophase I. They are surrounded by supportive **follicular cells,** and together they are called **follicles.** At puberty, circulating hormones stimulate growth of one or two of these dormant follicles (and their primary oocytes) each month. The oocyte enlarges and the number of follicular cells increases. Just before **ovulation** (release of the oocyte from the ovary) the oocyte completes meiosis I, which produces a **secondary oocyte** and a **polar body.** This mature follicle is called a **Graafian follicle** and contains a secondary oocyte (fig. 15.6). Each secondary oocyte contains a haploid set of double-stranded chromosomes (two chromatids), but cytoplasmic cleavage is unequal. The secondary oocyte retains most of the cytoplasm and the polar body usually disintegrates.

Examine a prepared slide of a mammalian ovary cross section. In the following space sketch a Graafian follicle and two or three less mature stages.

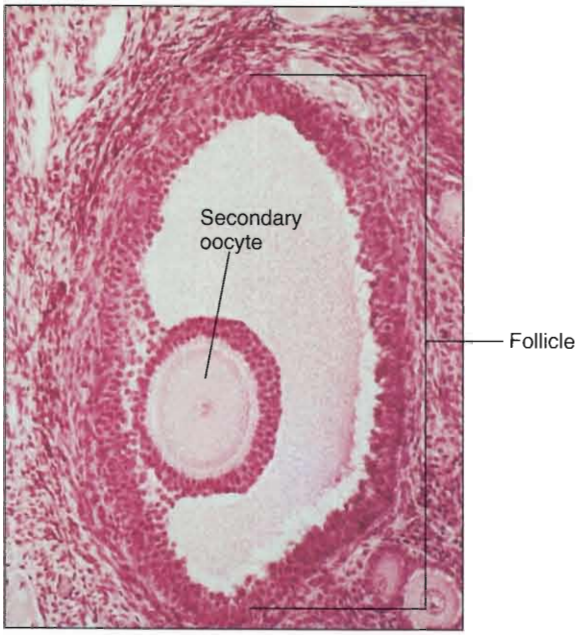


Figure 15.6
A mature secondary oocyte in an ovarian follicle of a cat. This secondary oocyte awaits ovulation.

Question 5
How would retaining extra cytoplasm enhance survival of a developing oocyte?

Meiosis II proceeds but is not completed until after a sperm cell penetrates the egg. Completion of meiosis II produces another polar body and a haploid egg cell ready for **fertilization** (fusion of nuclei). Review these basic stages of oogenesis in figure 15.3. Then examine a cross section of a cat ovary.

Question 6

a. What are the relative sizes of oocytes in a dormant follicle, a growing follicle, and a Graafian follicle?

b. Are polar bodies visible in your prepared slide of a cat ovary? Why or why not?

After ovulation the remaining follicle cells form the **corpus luteum** on the surface of the ovary. The corpus luteum produces hormones that prepare the uterus for the potential arrival of a fertilized egg.

Plant Gametogenesis

The formation of gametes in plants is somewhat different because their sexual life cycle includes an alternation of generations between haploid and diploid forms. However, meiosis is still the critical process by which plants reduce the number of chromosomes by half to prepare for gamete production.

In flowering plants, meiosis occurs in the anthers and ovary of the flowers. In the anther the spores resulting from meiosis produce a stage of the life cycle (pollen) that will eventually produce male gametes. In the ovary the spores resulting from meiosis produce a stage of the life cycle (ovule) that will eventually produce female gametes. You'll learn more about these events in Exercise 31. In this procedure you will observe prepared slides showing stages of the beginning, middle, and end of meiosis I and II in a representative plant.

Procedure 15.2

Diagram and observe stages of meiosis

1. In figure 15.7, diagram a plant cell with three pairs of chromosomes in each of the stages of meiosis. Be sure to label the cell wall and cell plate.
2. Examine the following prepared slides of stages of meiosis in a *Lilium* anther (see figs. 31.10, 31.11).
   - *a.* *Lilium* anther—early prophase I
   - *b.* *Lilium* anther—late prophase I
   - *c.* *Lilium* anther—first meiotic division
   - *d.* *Lilium* anther—second meiotic division
   - *e.* *Lilium* anther—pollen tetrads. Each of these cells will produce a pollen grain.
3. Examine the following prepared slides of stages of meiosis in a *Lilium* ovary.
   - *a.* *Lilium* ovary—"mother cell," prophase I
   - *b.* *Lilium* ovary—binucleate stage, end of meiosis I
   - *c.* *Lilium* ovary—four nucleate stage, end of meiosis II

MITOSIS VERSUS MEIOSIS

Mitosis and meiosis are both forms of cellular replication but they play different roles in the life cycle of animals and plants. Mitosis may occur in either haploid or diploid cells and is necessary for cell production and growth. Meiosis occurs in diploid cells. Its role is to produce cells with a reduced number of chromosomes and shuffle the genetic material so an organism can reproduce sexually. To compare mitosis and meiosis, review table 15.2 and complete the column with the contrasting features of meiosis.

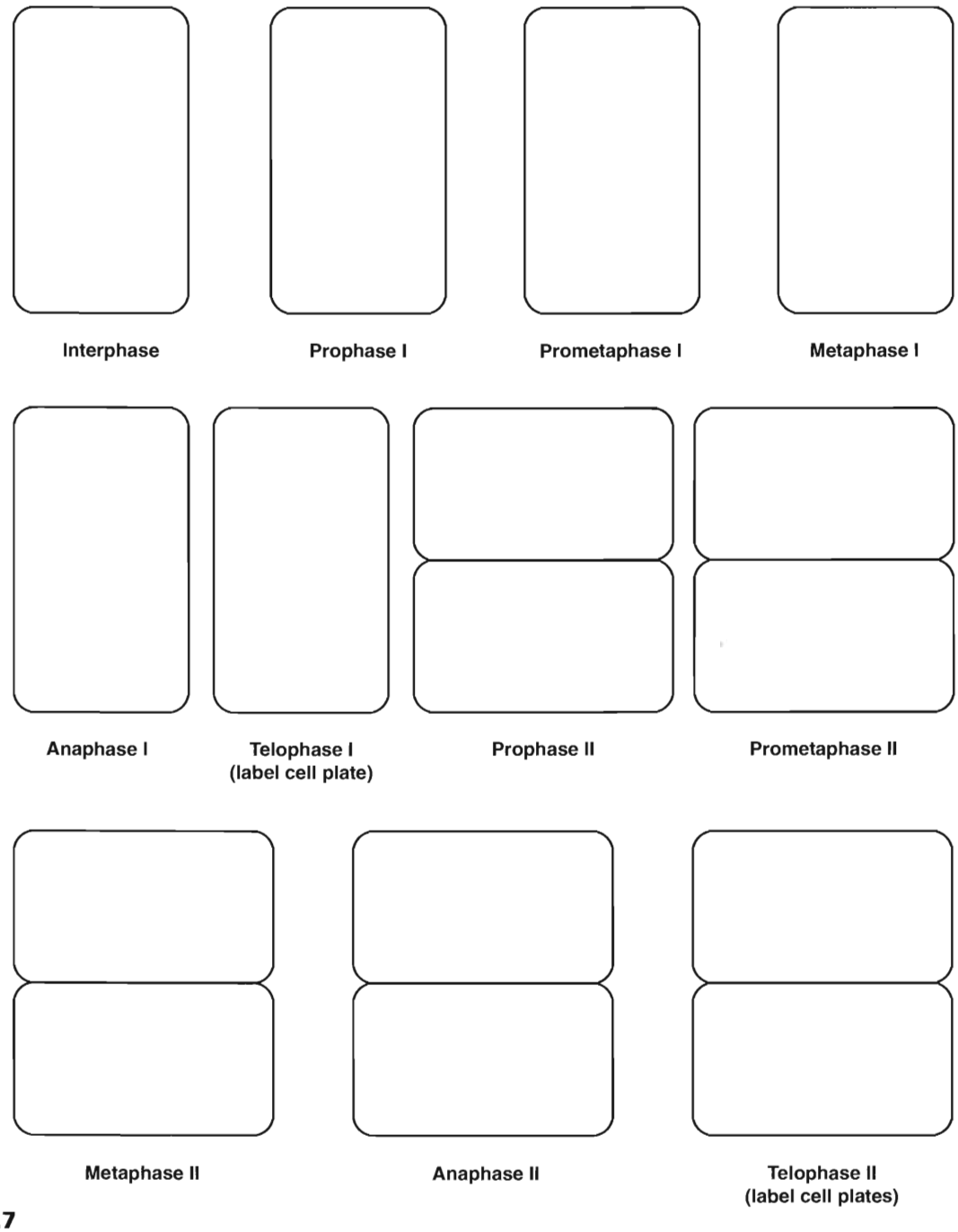


Figure 15.7
Stages of meiosis in plants.

INVESTIGATION

Variation in the Morphology of Vertebrate Sperm Cells

Observation: The morphology of sperm cells directly relates to their function. Sperm of vertebrates such as guinea pigs, rats, and humans vary in size and shape.

Question: How does sperm cell morphology vary among species of vertebrates?

a. Establish a working lab group and obtain Investigation Worksheet 15 from your instructor.

b. Discuss with your group the measurements and observations you might make to reveal variation in sperm morphology. Pose a well-defined question relevant to the preceding observation and question. Record it on Worksheet 15.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 15 the procedures and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

TABLE 15.2

A COMPARISON OF THE MAJOR FEATURES OF MITOSIS AND MEIOSIS

	Mitosis	Meiosis
Purpose of process		
Number of cells produced		
Number of nuclear divisions		
Haploidy or diploidy of resulting cells		
Genetically identical cells (yes or no)		
Pairing of homologues (yes or no)		
Occurrence of crossing over (yes or no)		

Questions for Further Thought and Study

1. How would you diagram the chromosomal arrangement for transitional stages such as late prophase/early metaphase I? Or late anaphase/early telophase I?

2. Would evolution occur without the events of meiosis and sexual reproduction? Why or why not?

3. What are the general characteristics of sexual reproduction in humans and other vertebrates that are associated with continuous production of many sperm cells but intermittent, finite production of egg cells?

4. Which process is most accurately referred to as nuclear division: meiosis or mitosis?

5. What special event does interkinesis lack compared to premeiotic interphase?

6. How are mammalian sperm cells produced and incubated at a lower temperature than body temperature?

7. How old is an ovulated oocyte of a 35-year-old woman? What consequences does this have?

WRITING TO LEARN BIOLOGY

Wouldn't it be easier for a cell simply to divide the chromosomes once rather than duplicating them and then dividing them twice during meiosis? Why do you suppose this isn't done?

Genetics
The Principles of Mendel

17

Objectives

By the end of this exercise you should be able to:

1. Describe simple genetic dominance, incomplete dominance, and lethal inheritance.
2. Describe possible genotypes for some of your personal traits inherited as dominant and recessive genes.
3. Explain the importance of Mendel's Law of Segregation and Law of Independent Assortment.
4. Distinguish between an organism's phenotype and genotype.

Published papers are the primary means of communicating scientific discoveries. One of the most famous of these papers, entitled "Experiments in Plant Hybridization," was written in 1866 by Gregor Mendel, an Austrian monk. Although this paper later became the basis for genetics and inheritance, it went largely unnoticed until it was rediscovered independently by several European scientists in 1900. The experiments and conclusions in Mendel's paper now form the foundation of **Mendelian genetics,** the topic of today's exercise.

Mendel's greatest contribution was to replace the blending theory of inheritance, which stated that all traits blend with each other, with the **particulate theory.** Mendel's particulate theory states that (1) inherited characters are determined by particular factors (now called genes), (2) these factors occur in pairs (i.e., genes occur on maternal and paternal homologous chromosomes), and (3) when gametes form, these genes segregate so that only one of the homologous pair is contained in a particular gamete. Recall from Exercise 15 (Meiosis) that each gamete has an equal chance of possessing either member of a pair of homologous chromosomes. This part of the particulate theory is collectively known as Mendel's First Law, or the **Law of Segregation.** Mendel's Second Law, or the **Law of Independent Assortment,** states that genes on nonhomologous or different chromosomes will be distributed randomly into gametes (fig 17.1).

Before you start this exercise, briefly review in your textbook some principles and terms pertinent to today's exercise. A gene is a unit of heredity on a chromosome. A **gene** has alternate states called **alleles,** contributed to an organism by its parents. Alleles for a particular gene occur in pairs. Alleles that mask expression of other alleles but are themselves expressed are **dominant;** these alleles are usually designated by a capital letter (for example, *P*). Alleles whose expression is masked by dominant alleles are **recessive,** and they are designated by a lowercase letter (for example, *p*). The **genotype** of an organism includes all the alleles present in the cell, whether they are dominant or recessive. The physical appearance of the trait is the **phenotype.** Thus, if purple flowers (*P*) are dominant to white flowers (*p*), a plant with purple flowers can have a genotype *PP* or *Pp*. A plant with white flowers can only have a genotype *pp*. When the paired alleles are identical (*PP* or *pp*), the genotype is **homozygous. Heterozygous** refers to a pair of different (*Pp*) alleles. With this minimal review, you're prepared to apply this information to solve some genetics problems.

SIMPLE DOMINANCE

Assume that purple flowers are dominant to white flowers. If a homozygous purple-flowered plant is crossed (mated) with a homozygous white-flowered plant, what will be the phenotype (physical appearance) and genotype of the offspring?

Parents:	*PP* (homozygous dominant = purple flowers) × *pp* (homozygous recessive = white flowers)
Gametes:	*P* from the purple-flowered parent *p* from the white-flowered parent
Offspring:	genotype = *Pp* phenotype = purple flowers

This first generation of offspring is called the **first filial** or **F_1 generation** (fig. 17.2).

Each of the F_1 offspring can produce two possible gametes, *P* and *p*. Mendel noted that the gametes from each of

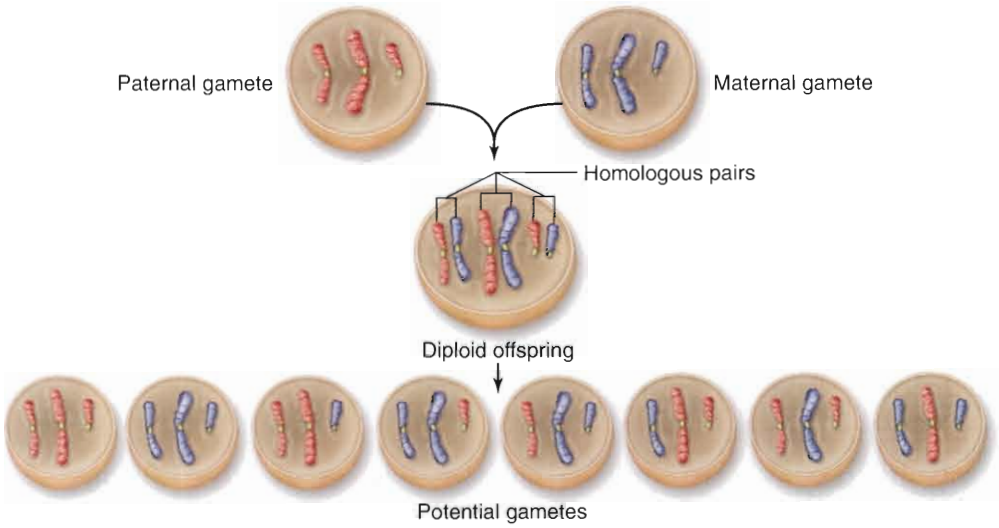
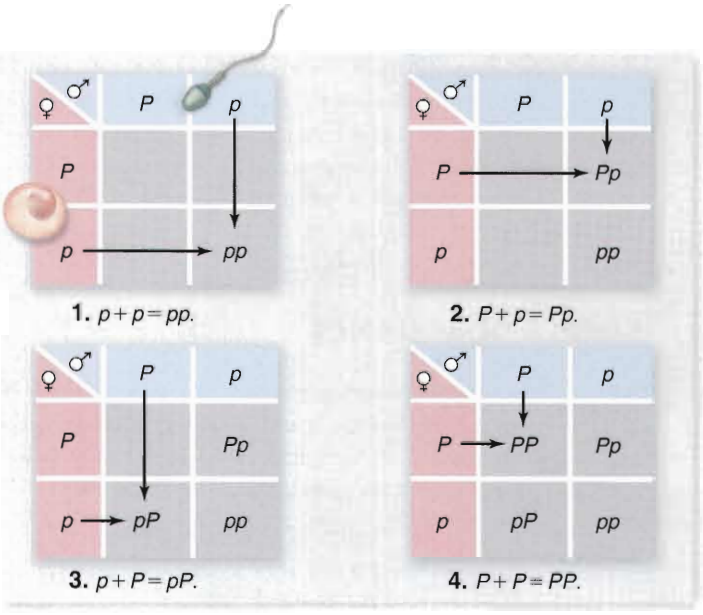


Figure 17.1

Independent assortment increases genetic variability. Independent assortment contributes new gene combinations to the next generation because the orientation of chromosomes on the metaphase plate is random. For example, in cells with three chromosome pairs, eight different gametes can result, each with different combinations of parental chromosomes.



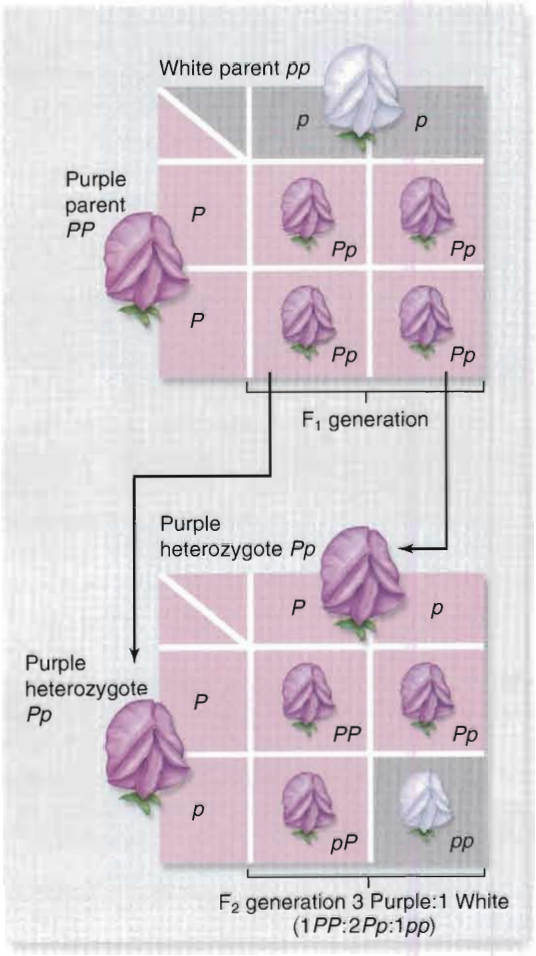


Figure 17.2

In Mendel's cross of purple by white flowers, the original parents each only make one type of gamete. The resulting F_1 generation are all *Pp* heterozygotes with purple flowers. These F_1 then each make two types of gametes that can be combined to produce three kinds of F_2 offspring: *PP* homozygotes (purple flowers); *Pp* heterozygotes (also purple flowers); and *pp* homozygotes (white flowers). The ratio of dominant to recessive phenotypes is 3:1. The ratio of genotypes is 1:2:1 (1 *PP*: 2 *Pp*: 1 *pp*).

TABLE 17.1

RESULTS OF COIN-FLIPPING EXPERIMENT SIMULATING RANDOM MATING OF HETEROZYGOUS (*Pp*) INDIVIDUALS

Response	Number
Heads-heads = *PP* = purple flowers	
Heads-tails = *Pp* = purple flowers	
Tails-tails = *pp* = white flowers	

the parents combine with each other randomly. Thus, you can simulate the random mating of gametes from the F_1 generation by flipping two coins simultaneously. Assume that heads designates the purple-flower allele (*P*), and tails designates the white-flower allele (*p*). Flipping one coin will determine the type of gamete from one parent and flipping the other will determine the gamete from the other parent. To demonstrate this technique, flip two coins simultaneously 64 times and record the occurrence of each of the three possible combinations in table 17.1.

Question 1
What is the ratio of purple-flowered (*PP* or *Pp*) to white-flowered (*pp*) offspring?

Keep these results in mind and return to the original problem: What are the genotypes and phenotypes of the offspring of the F_1 generation?

Parents:	*Pp* × *Pp*
Gametes:	(*P* or *p*) × (*P* or *p*)
Offspring:	*PP Pp pP* *pp*
	3 purple 1 white

Thus, the theoretical genotypic ratio for the offspring of the F_1 generation is 1 *PP* : 2 *Pp* : 1 *pp*, and the phenotypic ratio is 3 purple : 1 white.

Question 2

a. How do these ratios compare with your data derived from coin flipping?

b. Would you have expected a closer similarity if you had flipped the coins 64,000 times instead of 64 times? Why or why not?

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 17.1

Determine genotypic and phenotypic ratios for albinism

Albinos are homozygous recessive for the pair of alleles that produce pigments of skin, hair, and eyes. Suppose a woman having normal colored skin and an albino mother marries an albino man. Record the genotypic and phenotypic ratios of their children.

Genotype of children's mother _____
Genotype of children's father _____
Possible gametes of mother _____
Possible gametes of father _____
Possible offspring _____
Genotypic ratio of children _____
Phenotypic ratio of children _____

Procedure 17.2

Determine color and height ratios for corn plants

Color of grains (karyopses) and height of *Zea mays* (corn) plants are often determined by a single gene.

1. Examine (a) the ears of corn having red and yellow grains and (b) the tray of tall and dwarf plants on demonstration.
2. Record your observations here and determine the probable genotypes of the parents of each cross.
   Probable genotypes of parents:

Color of Corn Grains

Number of red grains _____
Number of white grains _____
Ratio of red : white grains _____
Probable genotypes of parents _____ × _____

Height of Plants

Number of tall plants _____
Number of dwarf plants _____
Ratio of tall : dwarf plants _____
Probable genotypes of parents _____ × _____

3. The preceding crosses involved only one trait and thus are termed **monohybrid crosses.** Let's now examine a cross involving two traits; that is, a **dihybrid cross.** Your instructor will review with you the basis for working genetics problems involving dihybrid crosses.

In corn, red (*R*) seed color is dominant to white (*r*) seed color, and smoothness (*S*) is dominant to wrinkled (*s*) seed. Observe the cobs of corn derived from a cross between parents having genotypes *RRSS* and *rrss*.

Question 3

a. What is the expected genotype for the F_1 generation?

b. Will all F_1 offspring (seeds) have the same genotype?

Question 4

a. What are the predicted genotypes for the F_2 (i.e., second) generation?

b. In what ratio will they occur?

4. To test your prediction in Question 4 count the number of kernels for five rows for each of the following phenotypes:

Red, smooth _____

Red, wrinkled _____

White, smooth _____

White, wrinkled _____

Question 5

a. What are the genotypes of the F_1 generation?

b. How did your data compare with those that you predicted?

INCOMPLETE DOMINANCE

Some traits such as flower color are controlled by incomplete dominance. In this type of inheritance, the heterozygous genotype results in an intermediate characteristic. For example, if a plant with red flowers (*RR*) is crossed with a plant having white flowers (*rr*), all of the offspring in the first filial (F_1) generation will have pink flowers (*Rr*).

Parents:	*RR* (red) × *rr* (white)
Gametes:	*R* × *r*
Offspring:	*Rr* (pink)

Question 6

What are the expected ratios of red, pink, and white flowers in a cross involving two pink-flowered parents?

LETHAL INHERITANCE

Lethal inheritance involves inheriting a gene that kills the offspring. Observe the tray of green and albino seedlings of corn. The albino plants cannot photosynthesize and therefore die as soon as their food reserves are exhausted.

Question 7

a. What is the ratio of green to albino seedlings?

b. Based on this ratio, what might you expect were the genotypes of the parents?

Question 8

Why is it impossible to cross a green and an albino plant?

OTHER SOURCES OF GENETIC DIVERSITY

Genetic diversity can also result from multiple alleles, gene interactions (epistasis), continuous variation, pleiotropy, environmental effects, linkage, and sex linkage. Although time limitations prohibit exercises about these topics, be sure to review them in your textbook.

BLOOD TYPE

Blood type of humans provides an excellent example of **codominance,** another type of Mendelian inheritance. In codominance, both alleles contribute to the phenotype of a heterozygote. For example, all individuals have one of four blood types: A, B, AB, and O (fig. 17.3). These blood groups are determined by the presence of compounds called

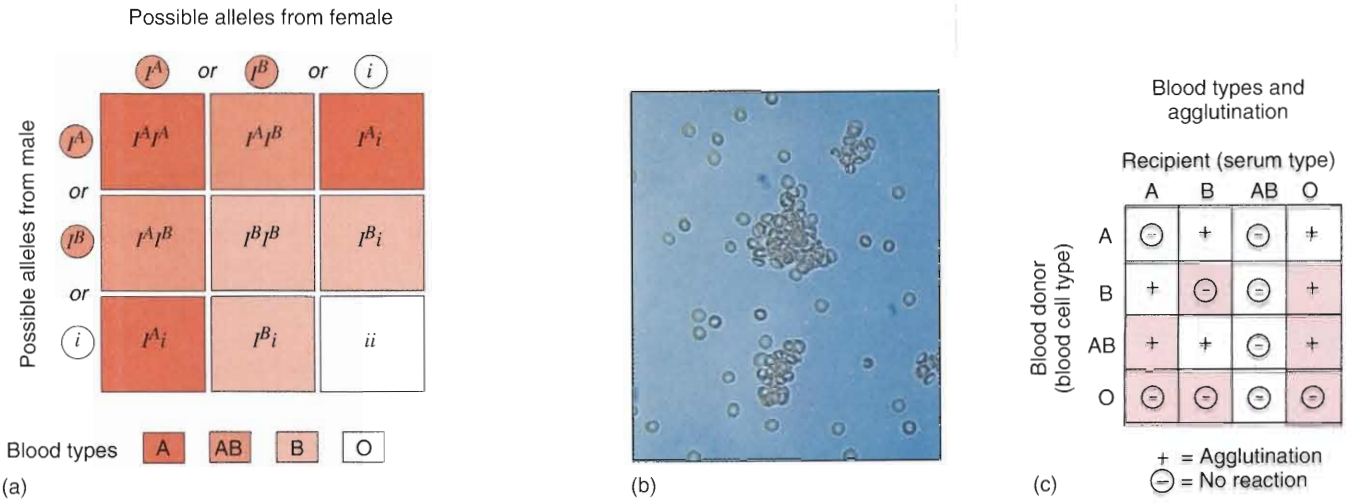


Figure 17.3

ABO blood groups. (*a*) Multiple alleles control the ABO blood groups. Different combinations of the three *I* gene alleles result in four different blood type phenotypes: type A (either I^AI^A homozygotes or I^Ai heterozygotes), type B (either I^BI^B homozygotes or I^Bi heterozygotes), type AB (I^AI^B heterozygotes), and type O (*ii* homozygotes). (*b*) The blood agglutination reaction. Agglutination occurs when blood cells stick and clump together. (*c*) Agglutination will occur when donor blood cells are incompatible with recipient serum, as designated by a +.

TABLE 17.2

CHARACTERISTICS OF INDIVIDUALS WITH THE FOUR MAJOR BLOOD TYPES

Blood Type	Antigen on Red Blood Cell	Antibody	Genotype
A	A	anti-B	I^AI^A or I^Ai
B	B	anti-A	I^BI^B or I^Bi
AB	A and B	none	I^AI^B
O	O (none)	anti-A and anti-B	*ii*

antigens on the surfaces of their red blood cells. If antigen A or B is present, no antibodies against this antigen are produced. Thus, if a person has antigen-A on his or her blood cells, then the person has type A blood and possesses blood **antibodies** (proteins) that agglutinate type B blood cells. Similarly, a person having antigen-B on his or her blood cells has type B blood and has antibodies that agglutinate type A blood cells. If a person has antigen-A and antigen-B on his or her blood cells, then the person has type AB blood and lacks A and B antibodies. If a person has no A or B antigens on his or her blood cells, the blood type is O and the person possesses antibodies against both A and B antigens (table 17.2). This system is rather unusual in that individuals have antibodies against the blood antigens that they do not possess.

Blood typing is often important for establishing the possible identity of an individual in forensic work and paternity suits. For example, assume that a woman with type O blood has a child having type O blood. The suspected father has type AB blood.

Could the suspected father with type AB blood be the child's father? The answer is no, because the cross would have the following results:

Parents: *ii* (type O) × I^AI^B (type AB)
Gametes: *i* and *i*, I^A and I^B
Offspring: I^Ai or I^Bi

Half of the offspring from the mother and the suspected father would have type A blood (genotype = I^Ai), and the other half would have type B blood (genotype = I^Bi). Thus, the suspected father could not have fathered a child with blood type AB or with type O blood with this mother.

ABO blood typing can be used to eliminate a person as a potential parent, but not to prove paternity. To appreciate this, suppose there is a mix-up of children in the maternity ward of a hospital after the genotypes of the children are determined from the parents' blood types. The following unidentified children have these blood types:

Child 1: type A (genotype I^AI^A or I^Ai)

Child 2: type B (genotype I^BI^B or I^Bi)

Child 3: type AB (genotype I^AI^B)

Child 4: type O (genotype *ii*)

Question 9

Which child or children could belong to a couple having AB and O blood types?

Blood typing is also important for determining the safety of blood transfusions. Your body automatically produces antibodies for antigens you do not carry (fig. 17.3). For example, people with type A blood have antibodies against B antigens, and people with type B blood produce antibodies against A antigens. If someone having type B blood received blood from someone having type A blood, the recipient's antibodies would react with and agglutinate the red blood cells received from the donor (fig. 17.3*b*). As a result, the recipient would die.

Question 10

a. Can a person with type O blood safely donate blood to a person having type A blood? Why or why not?

b. Which blood type would be a universal donor?

c. Which blood type would be a universal recipient?

Procedure 17.3

Determine blood type for ABO system

1. You will be provided with various samples of synthetic blood. This material is designed to simulate the blood type characteristics of human blood, and it is safe. Also obtain two bottles of antisera.
2. Obtain a clean slide and label the ends A and B. Near one end of the slide place a drop of antiserum A (containing antibodies against antigen-A), and near the other end of the slide place a drop of antiserum B (containing antibodies against antigen-B).
3. Place drops of blood near (but not touching) the two drops of antisera.
4. Mix one of the drops of blood with antiserum A and one with antiserum B. Use a different toothpick to mix each antiserum.
5. Dispose of all used materials properly.
6. Observe any agglutination of blood cells in either of the two antisera.

Agglutination of blood mixed with an antiserum is indicated by a grainy appearance. Agglutination indicates the presence of the respective antigen on red blood cells (fig. 17.3). Determine and record the blood type from your sample based on the presence of antigens.

Question 11

a. What antigens are present on the artificial red blood cells that you tested?

b. What is the blood type of your sample?

You are probably familiar with another characteristic of blood called **Rh factor.** Although more than two alleles determine Rh, we'll use "positive" and "negative" for simplicity and convenience.

Procedure 17.4

Determine Rh

1. Place a drop of anti-Rh serum on a clean slide.
2. Using the procedure just described, mix a drop of blood from the synthetic blood sample provided with the antiserum.
3. Label the slide with your initials and place it on the warming plate in the lab.
4. The blood sample will agglutinate within a few minutes if it is Rh-positive. The absence of agglutination indicates the blood is Rh-negative.
5. Dispose of all materials properly.

Rh Incompatibility

You've probably heard of the incompatibility (agglutination) problems that Rh-negative women may have with their Rh-positive babies (the Rh-positive trait is inherited from the child's father). This problem usually occurs with the second and subsequent children, because women with the Rh blood system must be sensitized to the antigen before antibody production begins. This sensitization usually occurs during birth of the first child.

If you are a woman having Rh-negative blood, you should be concerned but not alarmed. Rh incompatibility is handled routinely by injections of anti-Rh antibodies.These antibodies destroy Rh-positive red cells and thus eliminate the Rh-associated risk of subsequent childbirth.

TABLE 17.3

PHENOTYPES AND GENOTYPES OF HUMAN TRAITS

Characteristic	Your Phenotype	Your Genotype*	Phenotypes of Class	
			Dominant	Recessive
Widow's peak				
Bent little finger				
Albinism				
Pigmented iris				
Attached earlobes				
Hitchhiker's thumb				
Interlacing fingers				
PTC tasting				
Middigital hair				
Dimpled chin				
Six fingers				

*Homozygous dominant, heterozygous, or homozygous recessive

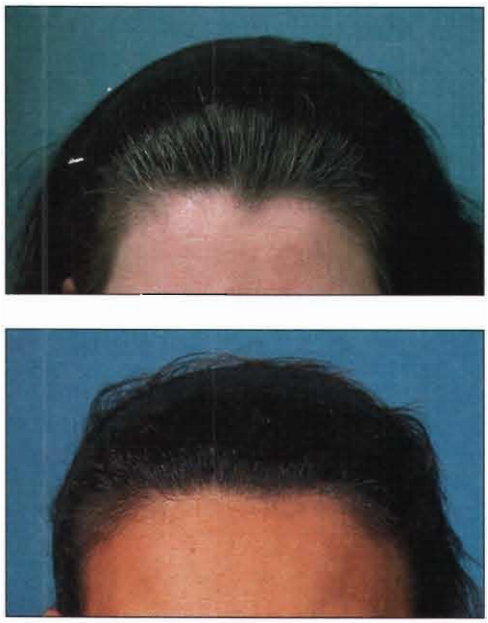


Figure 17.4

Widow's peak hairline *(top)*. People lacking a widow's peak have a relatively straight hairline *(bottom)*.

OTHER HUMAN TRAITS

The following traits are determined by a single gene. List your phenotype for each trait in table 17.3 and, if possible, list your genotype. If you have the recessive trait for gene G, for example, your genotype is homozygous recessive (*gg*). If you have the dominant trait, your genotype could be GG or *Gg*, in which case you should enter G in table 17.3. If you have the dominant trait and one of your parents shows the recessive trait, you must be heterozygous (*Gg*) for that trait. Give your results to your instructor so that she or he can provide you with the phenotypic results for your class.

Widow's peak—The *W* allele for widow's peak (i.e., a pointed hairline) is dominant to the *w* allele for a straight hairline (fig. 17.4).

Bent little finger—Lay your hands flat on the table and relax them. If the last joint of your little finger bends toward the fourth finger, you have the dominant allele *B* (fig. 17.5).

Albinism—The *A* allele is dominant and leads to production of melanin, a pigment. Individuals with an *aa* genotype lack pigment in their skin, hair, and iris.

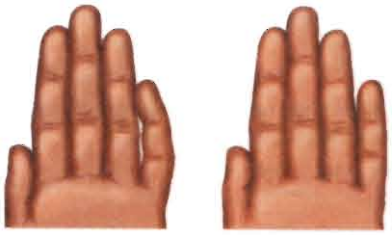


Figure 17.5
Bent little finger.

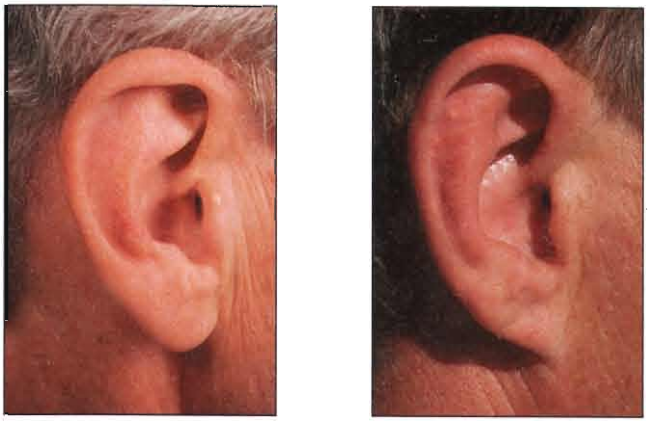


Figure 17.6
(*a*) Unattached earlobe. (*b*) Attached earlobe.

Pigmented iris—If you are homozygous for the recessive allele *p*, you do not produce pigment in the front layer of your iris, and your eyes are either blue or gray (i.e., your eyes are the color of the back layer of the iris). The *P* allele produces pigment in the front layer of the iris (green, hazel, brown, or black), which masks the blue or gray color of the back layer of the iris.

Attached earlobes—The A allele for free earlobes is dominant to the recessive *a* allele for attached earlobes (fig. 17.6).

Hitchhiker's thumb—Bend your thumb backward as far as possible. If you can bend the last joint of the thumb back at an angle of 60° or more, you are showing the recessive allele *h* (fig. 17.7).

Interlacing fingers—Casually fold your hands together so that your fingers interlace. The C allele for crossing the left thumb over the right thumb when you interlace your fingers is dominant over the *c* allele for crossing your right thumb over your left.

PTC tasting—Obtain a piece of paper impregnated with phenylthiocarbamide (PTC). Taste the paper by chewing on it for a few seconds. If you detect a bitter taste, you have the dominant allele *T*.

Middigital hair—The allele M for hair on the middle segment of your fingers is dominant to the *m* allele for no middigital hair. If hair is present on the middigit of any finger you have the dominant allele.

Dimpled chin—A dimpled chin is caused by a dominant allele M. People who have a dimpled chin are either homozygous dominant (MM) or heterozygous (*Mm*) for this trait. Homozygous recessive (*mm*) individuals do not have a dimpled chin.

Six fingers—In humans, the occurrence of six fingers results from a dominant allele S. People who have six fingers are either homozygous dominant (*SS*) or heterozygous (*Ss*). People who have only five fingers are homozygous recessive (*ss*) for this trait.

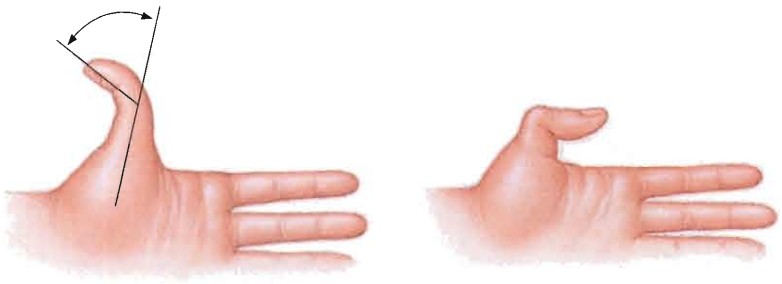


Figure 17.7
Hitchhiker's thumb.

Several diseases are inherited as single-gene traits. These include

Cystic fibrosis, a disease characterized by chronic bronchial obstruction and growth reduction. This disease is inherited as a recessive trait; people who are heterozygous or homozygous dominant do not have this disease.

Galactosemia, an inability to metabolize galactose, a sugar in human milk. Inherited as an autosomal recessive trait. Approximately five cases occur per million births. Prenatal diagnosis can be performed on cells obtained through amniocentesis or chorionic villi sampling. This disease is inherited as a recessive trait; people who are heterozygous or homozygous dominant do not have this disease.

Phenylketonuria (PKU), an inability to metabolize the amino acid phenylalanine. Approximately 100 cases occur per million births. If untreated, this disease produces mental retardation. This disease is inherited as a recessive trait; people who are heterozygous or homozygous dominant do not have this disease.

Juvenile retinoblastoma, a cancer of the retina. The allele is located on chromosome 13. This disease is inherited as a recessive trait; people who are heterozygous or homozygous dominant do not have this disease.

Huntington's disease, a mental disorder involving uncontrollable, involuntary muscle movements. The disease occurs relatively late in life, so many affected individuals bear children before they realize that they are carriers. Approximately 100 cases occur per million births. Unlike most other genetic diseases, Huntington's disease is inherited as a dominant trait; people who are homozygous recessive (*hh*) do not have the disease, and people who are heterozygous (*Hh*) or homozygous dominant (*HH*) have the disease.

Question 12
What conclusion about your genotype is evident if one of your siblings, but neither parent, shows the recessive trait?

ANALYZING PEDIGREES

Many human traits display both dominant and recessive inheritance (table 17.4). Researchers cannot control crossses in humans the way Mendel did with pea plants, so to analyze human inheritance, geneticists study crosses that have been performed already—in other words, family histories. The methodology used is a **pedigree**, a consistent graphical presentation of matings and offspring over multiple generations for a particular trait. Information in a pedigree allows genetecists to deduce the mode of inheritance of the trait.

If you understand the simple patterns of inheritance presented in this lab, you can trace a trait in a pedigree (i.e., family tree) to determine if it is inherited in a dominant or recessive pattern of inheritance.

Question 13
a. What features would characterize pedigrees of dominant traits?

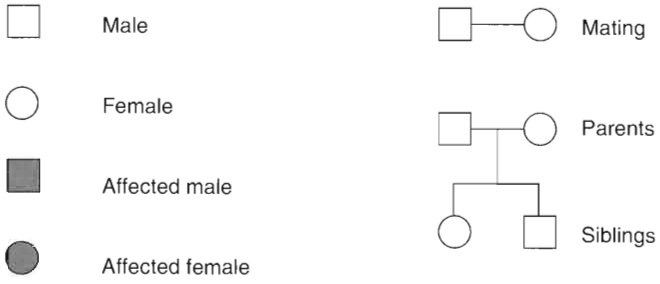
TABLE 17.4

SOME DOMINANT AND RECESSIVE TRAITS IN HUMANS

Recessive Traits	Phenotypes	Dominant Traits	Phenotypes
Albinism	Lack of melanin pigmentation	Middigital hair	Presence of hair on middle segment of fingers
Alkaptonuria	Inability to metabolize homogentisic acid	Brachydactyly	Short fingers
Red-green color blindness	Inability to distinguish red or green wavelengths of light	Huntington disease	Degeneration of nervous system, starting in middle age
Cystic fibrosis	Abnormal gland secretion, leading to liver degeneration and lung failure	Phenylthiocarbamide (PTC) sensitivity	Ability to taste PTC as bitter
Duchenne muscular dystrophy	Wasting away of muscles during childhood	Camptodactyly	Inability to straighten the little finger
Hemophilia	Inability of blood to clot properly, some clots form but the process is delayed	Hypercholesterolemia (the most common human Mendelian disorder)	Elevated levels of blood cholesterol and risk of heart attack
Sickle cell anemia	Defective hemoglobin that causes red blood cells to curve and stick together	Polydactyly	Extra fingers and toes

b. What features would characterize pedigrees of recessive traits?

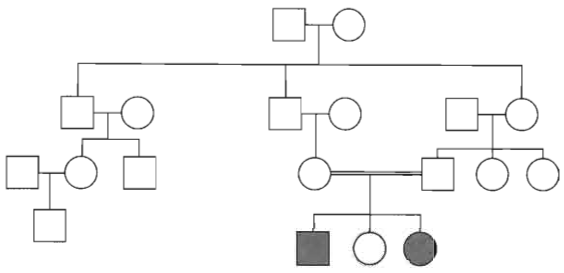
Biologists use the following symbols in pedigrees:



Procedure 17.5

Analyze a pedigree of inheritance of cystic fibrosis

1. Among Caucasians, about 1 of every 2500 newborn infants is born with cystic fibrosis. In these individuals, a defective membrane protein results in the production of unusually thick and dry mucus that lines organs such as the tubes in the respiratory system. People having cystic fibrosis often have recurrent and serious infections, and most die in their 20s or 30s.
2. Determine whether the allele for cystic fibrosis is inherited as a dominant or recessive allele.



Question 14

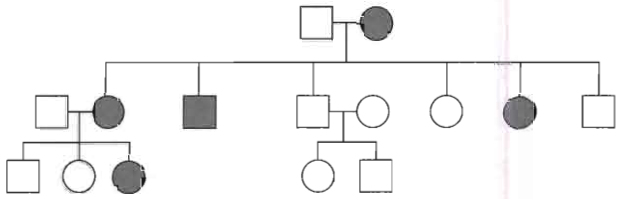
a. What is the inheritance pattern for the cystic fibrosis allele? What is your reasoning for this conclusion?

b. Can you determine the genotypes of any individuals in the pedigree? If so, which ones? Explain your reasoning.

Procedure 17.6

Analyze a pedigree of inheritance of Huntington's disease

1. Huntington's disease is a severe disorder of the nervous system that usually causes death.
2. Determine whether the allele for Huntington's disease is inherited as a dominant or recessive allele.



Question 15

a. What is the inheritance pattern for the Huntington's disease allele? What is your reasoning for this conclusion?

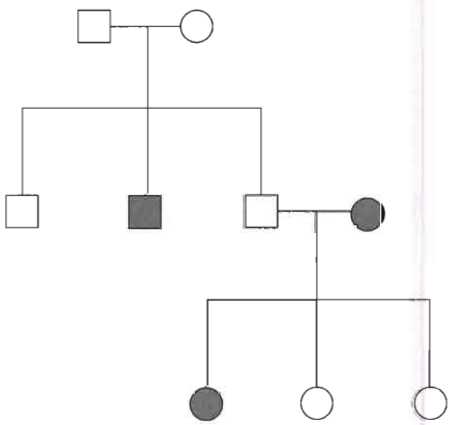
b. Can you determine the genotypes of any individuals in the pedigree? If so, which ones? Explain your reasoning.

c. Examine fig. 17.8. Shana's mother has Huntington's disease, and Shana has a 50-50 chance of developing Huntington's disease. Explain the genetic basis for Shana's chances of inheriting Huntington's disease.

Procedure 17.7

Analyze a pedigree of inheritance of phenylketonuria

1. Phenylketonuria, or PKU, results from an inability to metabolize the amino acid phenylalanine. If untreated, PKU leads to mental retardation.
2. Determine whether the allele for phenylketonuria is inherited as a dominant or recessive allele.



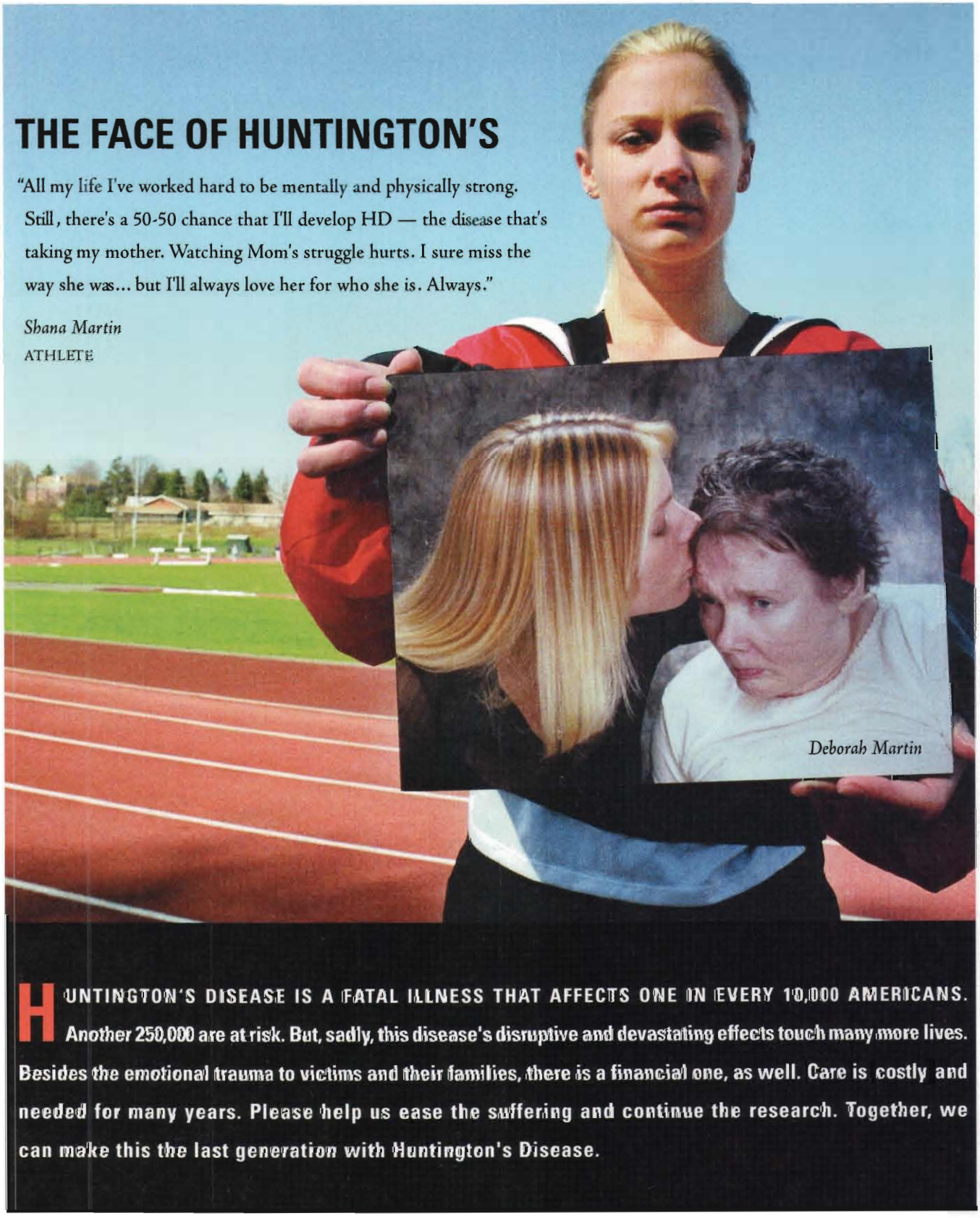


Figure 17.8

Huntington's disease is a degenerative disease inherited as a dominant trait. What is the genetic basis for Shana's statement that she has a 50-50 chance of getting Huntington's disease?

Question 16

a. What is the inheritance pattern for the phenylketonuria allele? What is your reasoning for this conclusion?

b. Can you determine the genotypes of any individuals in the pedigree? If so, which ones? Explain your reasoning.

TRANSPOSONS

For much of this century, geneticists thought that genes do not move in cells. However, in 1947 Barbara McClintock proposed that genes could move within and between chromosomes. McClintock based her conclusion on a series of experiments involving genetic crosses in corn. Specifically, McClintock showed that there is a fragment of DNA that can move to and be inserted at the locus for the production of pigments in corn kernels. Because this insertion renders the cell unable to make the purple pigment, the resulting kernel is yellow or white. However, subsequent removal of the DNA fragment results in the cell resuming production of the purple pigment; therefore, the resulting kernel is purple. Thus, Indian corn often has kernels with varying pigmentation, depending on when the DNA fragment was inserted or removed.

A similar phenomenon occurs with the production of other pigments in corn kernels. The translocation to and from the locus for production of these pigments several times during kernel development produces the red-orange swirls characteristic of many kernels of Indian corn.

The fragments of DNA that McClintock studied are now called **transposons.** Transposons are a useful tool for genetic engineering because they provide a way of inserting foreign DNA into a host cell's chromosome. For her work McClintock received the Nobel Prize in 1983.

Procedure 17.8

Observe corn kernels to understand the effects of transposons

1. Work in a group of two to four. Obtain an ear of Indian corn for your group.
2. Look for examples of kernels with (a) purple or white spots, (b) red-orange swirls, and (c) other unusual color patterns. Sketch the pigmentation patterns in 2–3 kernels.

3. Use the information presented in this exercise and in your textbook to determine how transposons could produce such unusual patterns of pigmentation.

INVESTIGATION

The Frequency of Homozygous Recessive Traits in Humans

Observation: In humans, traits such as widow's peak, attached earlobes, and a dimpled chin are homozygous recessive traits. In many people, these traits are easily observed.

Question: How common are homozygous recessive traits such as widow's peak, attached earlobes, and a dimpled chin among your classmates?

a. Establish a working lab group and obtain Investigation Worksheet 17 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 17 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Testing for Understanding: Solving Genetics Problems

1. When reclusive billionaire Howard Hughes died in 1976, a variety of people claimed that they were entitled to Hughes' estate because they were his children. Hughes had type AB blood. One man who claimed that Hughes was his father had type O blood, and the man's mother had type A blood. If you were the judge in the case, what would you rule? Explain your answer.
2. Suppose that flower color is inherited by simple dominance and that purple flowers are dominant to white flowers. If a homozygous recessive individual is crossed with a homozygous dominant individual, what is the probability of obtaining a purple-flowered offspring?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
3. Bob is heterozygous for phenylketonuria, and Loretta is homozygous recessive for phenylketonuria. What is the probability that their first child will have phenylketonuria?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
4. Suppose that (1) Randy is heterozygous for the allele that causes Huntington's disease, (2) Susan is homozygous recessive for the allele that causes Huntington's disease, and (3) Randy and Susan decide to have a child. What is the probability that their child will get Huntington's disease?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
5. In question 4, what is the probability that their first daughter will get Huntington's disease?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
6. Suppose that someone having type AB blood has a child with someone having type O blood. What is the probability that their child will have type A blood?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
7. Suppose that you cross a red-flowered carnation with a white-flowered carnation. All of the offspring have pink flowers. What can you conclude?
   a. Flower color in carnations is inherited by incomplete dominance, and the red-flowered carnation is homozygous dominant for the trait.
   b. At least one of the parents is heterozygous for flower color.
   c. Flower color is inherited by simple dominance.
   d. Half of the offspring are heterozygous and half are homozygous for flower color.
   e. None of the above statements are true.
8. Suppose that a trait is inherited by simple dominance. If two heterozygotes are mated, what is the probability of having a homozygous recessive offspring?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
9. Tay-Sachs disease is characterized by the inability to produce an enzyme needed to metabolize lipids in brain cells. If this enzyme is not present, lipids accumulate in the brain and gradually destroy its ability to function (homozygous recessive children usually die by the age of four or five). Suppose that you are a carrier for Tay-Sachs disease and that your partner is not. What is the probability that you and your partner will have a child with Tay-Sachs disease?
   a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
10. A normally pigmented man marries a normally pigmented woman. Their second child is an albino.
    a. What is the genotye of the man? _____
    b. What is the genotype of the woman? _____
    c. What is the genotype of the albino child? _____
    d. What is the probability that their next child will be an albino? _____
11. Darrell and Matilda each have type O blood. If they start a family, the probability that they will have a child having type A blood is _____.
    a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
12. Suppose that a person having type B blood is married to someone having type A blood. Is it possible for this couple to have a child having type O blood? Explain your answer.
13. Wanda, who has type O blood, gives birth to a baby having type O blood. The woman then claims that the child's father is Randy, who has type A blood.
    a. Could Randy be the father?
    b. Can this information alone prove that Randy is the father? Explain your answer.
14. Suppose that two people having free earlobes marry and start a family. Their first child has free earlobes and their second has attached earlobes.
    a. What are the genotypes of the parents? _____
    b. What is the genotype of their first child? _____
    c. What is the genotype of their second child? _____
15. Suppose that a trait is inherited by simple dominance. If two heterozygotes are mated, what is the probability of having an offspring that has the same phenotype as the parents?
    a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%
16. Suppose that a trait is inherited by incomplete dominance. If two heterozygotes are mated, what is the probability of having an offspring that has the same phenotype as the parents?
    a. 100% **b.** 75% **c.** 50% **d.** 25% **e.** 0%

Answers to Genetics Problems

1. The man could not be Hughes' son. Hughes had type AB blood. Regardless of the blood type of the mother, a child of Hughes could not have type O blood.
2. a
3. c
4. c
5. c
6. c
7. a
8. d
9. e

10*a*. *Aa*
10*b*. *Aa*
10*c*. *aa*
10*d*. 25%

11. e
12. Yes, but only if the person having type B blood has a BO genotype, and if the person having type A blood has an AO genotype.

13*a*. Yes, if he has an AO genotype.
13*b*. No, information about blood type cannot prove that anyone is the parent of a child; it can only eliminate people who are not parents of the child.

14*a*. *Ee*
14*b*. *EE* or *Ee*
14*c*. *ee*

15. b
16. c

Questions for Further Thought and Study

1. What determines how often a phenotype occurs in a population?

2. Are dominant characteristics always more frequent in a population than recessive characteristics? Why or why not?

3. Is it possible to determine the genotype of an individual having a dominant phenotype? How?

4. Why is hybrid seed so expensive to produce?

5. What blood types are not expected for children to have if their parents have AB blood? O blood?

WRITING TO LEARN BIOLOGY

Organisms heterozygous for a recessive trait are often called carriers of that trait. What does this mean?

18 Evolution

Natural Selection and Morphological Change in Green Algae

Objectives

By the end of this exercise you should be able to:

1. Give a working definition of evolution, fitness, selection pressure, and natural selection.
2. Determine the genotypic and phenotypic frequency of a population while properly using the terms allele, dominant, recessive, homozygous, and heterozygous.
3. Explain the Hardy-Weinberg Principle and use it to demonstrate negative selection pressures on a population.
4. Describe the significance of the Volvocine line, particularly in the areas of cellular specialization and colonial complexity.
5. Describe examples of how a mutation affecting the plane of cellular division could result in the evolution of morphologically different body plans.

Figure 18.1
Mutations produce new alleles and genetic combinations that may be adaptive. One branch of this peppermint peach tree has grown from a meristem that had a mutation for red petals. If this mutation is adaptive, by attracting different pollinators for example, it will probably persist in the gene pool of subsequent generations.

The theory of **evolution** broadly describes genetic change in populations. The existence of genetic change (and therefore evolution) is universally accepted by biologists. We know that many mechanisms can change the genetic makeup of populations, but the relative importance of each mechanism remains to be fully described. Events such as **mutations** (changes in the genetic message of a cell, fig. 18.1) and catastrophes (e.g., meteor showers, ice ages) are all responsible to some degree for genetic change. However, Charles Darwin formulated a theory that explains a major force behind genetic change (fig. 18.2).

Darwin postulated that organisms that survive and reproduce successfully have genetic traits aiding survival and reproduction. These traits enhance an organism's **fitness,** its tendency to produce more offspring than competing individuals, and therefore contribute more genes to the next generation. Darwin noticed that fit individuals (that is, ones that reproduce the most) produce more offspring because their traits are better adapted for survival and reproduction than the traits of their competitors. He further reasoned that if the traits of the more fit individuals are transmitted to the next generation more often, then more of these traits will be found in the next generation. After many generations, the frequency of these traits will increase in the population, and the nature of the population will gradually change. Darwin called this overall process **natural selection** and proposed it as a major force that guides genetic change and the formation of new species. Review in your textbook the theories of evolution and the mechanism of natural selection.

Showing the effects of natural selection in living populations is usually time-consuming and tedious. Therefore, in this exercise you will simulate reproducing populations with nonliving, colored beads representing organisms and their gametes. With this artificial population you can quickly follow genetic change over many generations.

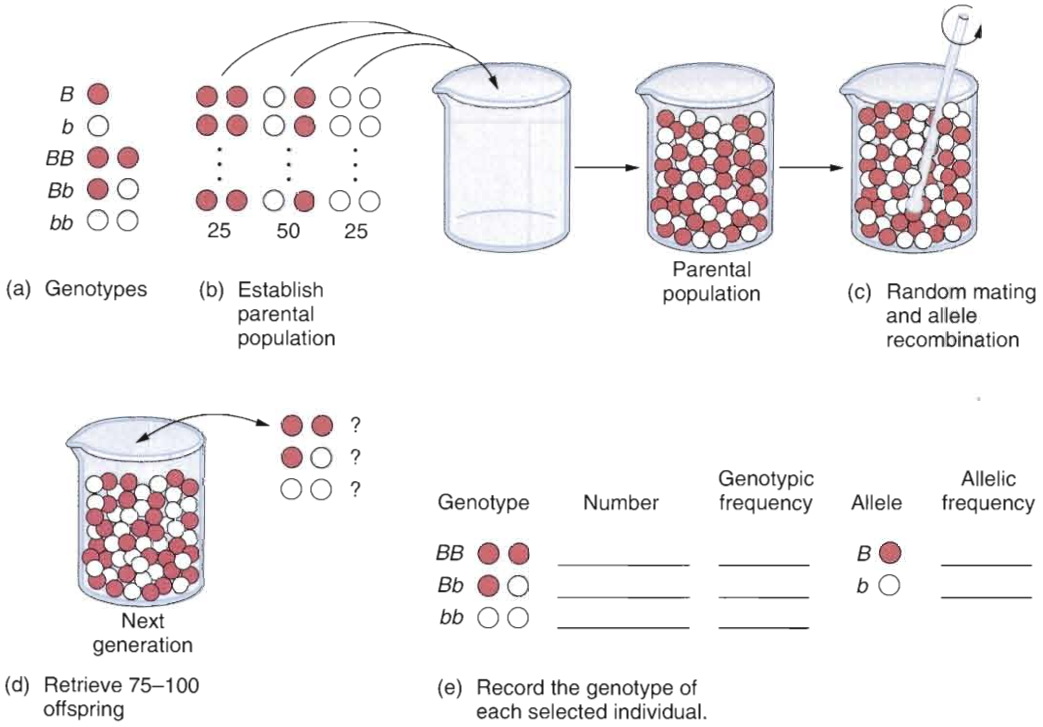


Figure 18.3

Verification of the Hardy-Weinberg Principle. See procedure 18.2 for an explanation of each step.

Procedure 18.2

Verify the Hardy-Weinberg Principle

1. Examine figure 18.3 for an overview of the steps of this procedure.
2. Establish the parental population from procedure 18.1 (fig. 18.3*a*, 18.3*b*).
3. Simulate the random mating of individuals by mixing the population (fig. 18.3*c*).
4. Reach into the parental container (without looking) and randomly select two gametes. Determine their genotype (fig. 18.3*d*). This pair of gametes with colored or white alleles represents an individual offspring.
5. Record the occurrence of the genotype in figure 18.3*e* as a mark under the heading "Number" or temporarily on a second sheet of paper and return the beads to the container.
6. Repeat steps 4 and 5 (100 times) to simulate the production of 100 offspring.
7. Calculate the frequency of each genotype and allele, and record the frequencies in figure 18.3*e*. Beside each of these new-generation frequencies write (in parentheses) the original frequency of that specific genotype or allele from table 18.1.

Question 3

a. The Hardy-Weinberg Principle predicts that genotypic frequencies of offspring will be the same as those of the parental generation. Were they the same in your simulation?

b. If the frequencies were different, then one of the assumptions of the Hardy-Weinberg Principle was probably violated. Which one?

EFFECT OF A SELECTION PRESSURE

Selection is the differential reproduction of phenotypes (fig. 18.4); that is, some phenotypes (and their associated genotypes) are passed to the next generation more often than others. In positive selection, genotypes representing adaptive traits in an environment increase in frequency because their bearers are more likely to survive and reproduce. In negative selection, genotypes representing nonadaptive traits in an environment decrease in frequency because their bearers are less likely to survive and reproduce.

Figure 18.4

Reproduction. These snakes hatching from eggs in a Costa Rican rain forest may or may not survive to reproduce. On average, snakes with characteristics best adapted to their environment will survive and reproduce more than those with less adaptive characteristics. As a result, the frequencies of adaptive traits (and their alleles) in the population will increase from generation to generation.

Selection pressures are factors such as temperature and predation that affect organisms and result in selective reproduction of phenotypes. Some pressures may elicit 100% negative selection against a characteristic and eliminate any successful reproduction of individuals having that characteristic. For example, mice with white fur may be easy prey for a fox if they live on a black lava field. This dark environment is a negative selection pressure against white fur. If survival and reproduction of mice with white fur were eliminated (i.e., if there is 100% negative selection), would the frequency of white mice in the population decrease with subsequent generations? To test this, use the following procedure to randomly mate members of the original parental population to produce 100 offspring (fig. 18.5).

Procedure 18.3

Simulate 100% negative selection pressure

1. Establish the same parental population that you used to test the Hardy-Weinberg prediction.
2. Simulate the production of an offspring from this population by randomly withdrawing two gametes to represent an individual offspring.
3. If the offspring is *BB* or *Bb*, place it in a container for the accumulation of the "Next Generation." Record the occurrence of this genotype on a separate sheet of paper.
4. If the offspring is *bb*, place this individual in a container for those that "Cannot Reproduce." Individuals in this container should not be used to produce subsequent generations. Record the occurrence of this genotype on a sheet of paper.
5. Repeat steps 2–4 until the parental population is depleted, thus completing the first generation.
6. Calculate the frequencies of each of the three genotypes recorded on the separate sheet and record these frequencies for the first generation in table 18.2. Individuals in the next generation will serve as the parental population for each subsequent generation.
7. Repeat steps 2–5 to produce a second, third, fourth, and fifth generation. After the production of each generation, record your results in table 18.2.
8. Graph your data from table 18.2 using the graph paper at the end of this exercise. *Generation* is the independent variable on the *x*-axis and *Genotype* is the dependent variable on the *y*-axis. Graph three curves, one for each genotype.

Because some members (i.e., the *bb* that you removed) of each generation cannot reproduce, the number of offspring from each successive generation of your population will decrease. However, the frequency of each genotype, not the number of offspring, is the most important value.

Question 4

a. Did the frequency of white individuals decrease with successive generations? Explain your answer.

b. Was the decrease of white individuals from the first to second generation the same as the decrease from the second to the third generation? From the third to the fourth generation? Why or why not?

c. How many generations would be necessary to eliminate the allele for white fur?

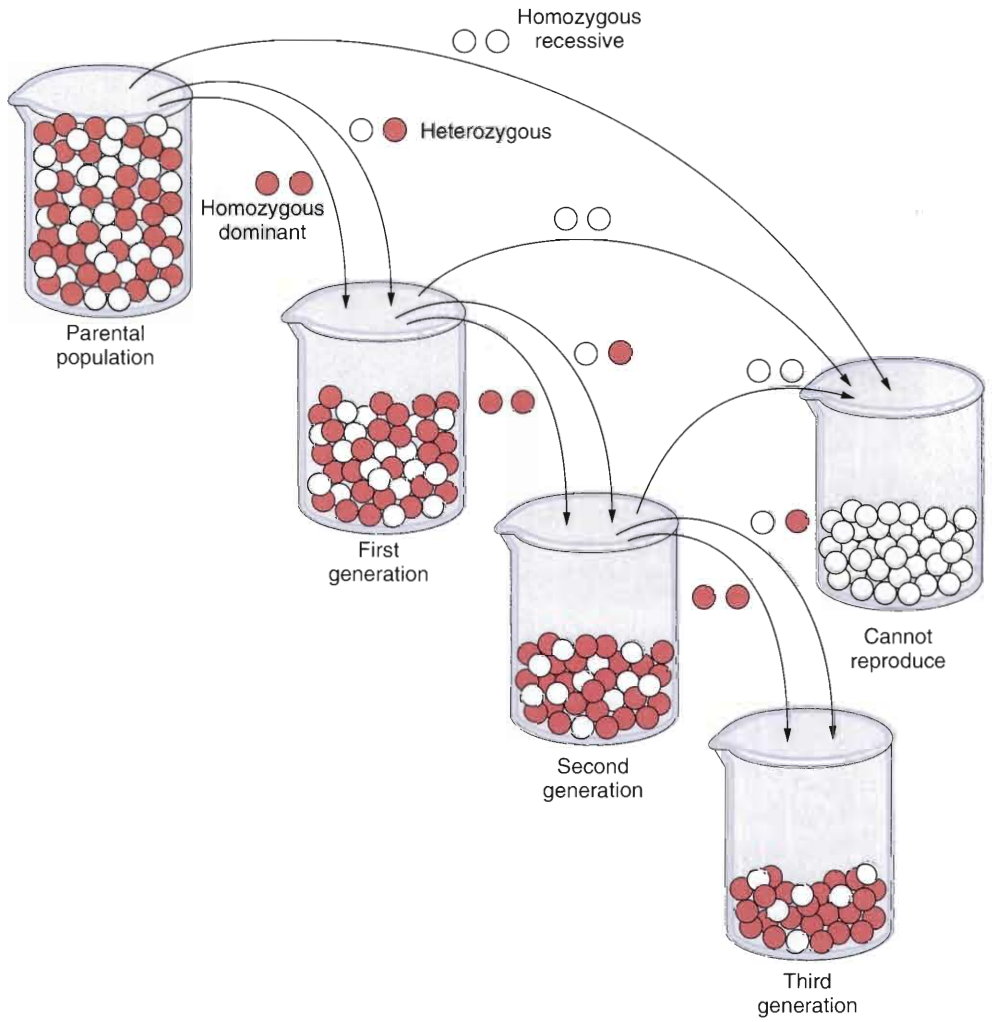


Figure 18.5

Demonstrating the effect of 100% selection pressure on genotypic and phenotypic frequencies across three generations. Selection is against the homozygous recessive genotype. Random mating within the parental population is simulated by mixing the gametes (beads), and the parental population is sampled by removing two alleles (i.e., one individual) and placing them in the next generation. Homozygous recessive individuals are removed (selected against) from the population. The genotypic and phenotypic frequencies are recorded after the production of each generation. The production of each generation depletes the beads in the previous generation in this simulation.

TABLE 18.2

GENOTYPIC FREQUENCIES FOR 100% NEGATIVE SELECTION

	Generation				
Genotype	First	Second	Third	Fourth	Fifth
BB ● ●					
Bb ● ○					
bb ○ ○					
Total	1.0	1.0	1.0	1.0	1.0

Most naturally occurring selective pressures do not eliminate reproduction by the affected individuals. Instead, their reproductive capacity is reduced by a small proportion. To show this, use procedure 18.4 to eliminate only 20% of the *bb* offspring from the reproducing population.

TABLE 18.3

GENOTYPIC FREQUENCIES FOR 20% NEGATIVE SELECTION

Genotype	Generation				
	First	Second	Third	Fourth	Fifth
BB ● ●					
Bb ● ○					
bb ○ ○					
Total*	1.0	1.0	1.0	1.0	1.0

*Note: The total of frequencies for each generation must equal 1.0.

INVESTIGATION

The Effect of Selection against Heterozygotes

Observations: Natural selection can change allelic frequencies in populations. Negative selection pressure (i.e., an environment that reduces reproduction by a particular phenotype) against a homozygous genotype can reduce allelic frequencies in only a few generations. The results of selection against heterozygotes may differ.

Question: How would selection against heterozygous individuals over many generations affect allelic frequencies in a population?

a. Establish a working lab group and obtain Investigation Worksheet 18 from your instructor.
b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record it on Worksheet 18.
c. Translate your question into a testable hypothesis and record it.
d. Review procedures 18.3 and 18.4. Outline on Worksheet 18 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Procedure 18.4

Simulate 20% negative selection pressure

1. Establish the same parental population that you used to test the Hardy-Weinberg prediction.
2. Simulate the production of an offspring from this population by randomly withdrawing two gametes to represent an individual offspring.
3. If the offspring is *BB* or *Bb*, place it in a container for production of the "Next Generation." Record the occurrence of this genotype on a separate sheet of paper.
4. If the offspring is *bb*, place every fifth individual (20%) in a separate container for those that "Cannot Reproduce." Individuals in this container should not be used to produce subsequent generations. Place the other 80% of the homozygous recessives in the container for production of the "Next Generation." Record the occurrence of this genotype on a sheet of paper.
5. Repeat steps 2–4 until the parental population is depleted, thus completing the first generation.
6. Calculate the frequencies of each of the three genotypes recorded on the separate sheet and record these frequencies for the first generation in table 18.3.
7. Repeat steps 2–5 to produce a second, third, fourth, and fifth generation. Individuals in the "Next Generation" will serve as the parental population for each subsequent generation. After the production of each generation, record your results in table 18.3.
8. Graph your data from table 18.3 using the graph paper at the end of this exercise. *Generation* is the independent variable on the *x*-axis and *Genotype* is the dependent variable on the *y*-axis. Graph three curves, one for each genotype.

Because some members of each generation cannot reproduce, the number of offspring from each generation of your population will decrease. However, the frequency of each genotype, not the number of offspring, is the most important value.

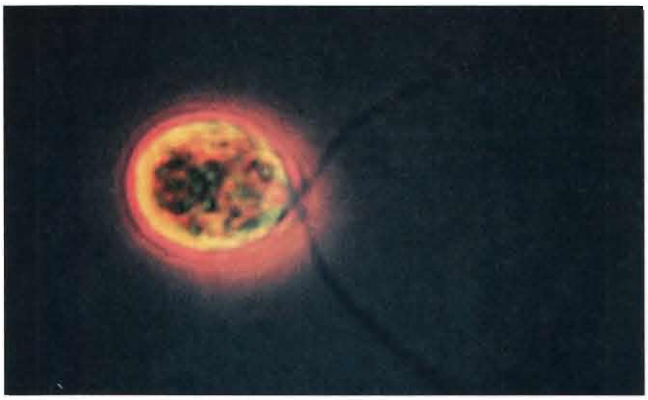


Figure 18.6
Chlamydomonas, a unicellular green alga (1700×). *Chlamydomonas* has two flagella.

Question 5

a. Did the frequency of white individuals decrease with successive generations?

b. Consult your graphs and compare the rate of selection for procedures in 18.3 and 18.4. Was the rate of decrease for 20% negative selection similar to the rate for 100% negative selection? If not, how did the rates differ?

c. How many generations would be necessary to eliminate the allele for white fur?

AN EXAMPLE OF EVOLUTION: THE VOLVOCINE LINE

The evolution of most species is too slow to witness in the lab, but we can examine modern species to learn about changes that likely occurred over evolutionary time. Researchers might ask which characteristics are conserved throughout an evolutionary lineage of species and which ones evolve rapidly and consistently. Are more complex species always more successful?

The **Volvocine line** of algae is a group of modern species that reflects an easily recognized sequence of changes as their common ancestors evolved. In this case the changes were in colony complexity.

Studies of morphology and molecular genetics indicate that an ancient species similar to today's flagellated *Chlamydomonas* (fig. 18.6) was probably the original and most ancient common ancestor to the Volvocine line. The probable sequence of events was that an ancestor of unicellular *Chlamydomonas* evolved a novel colonial morphology that was successful and gave rise to *Gonium* (figs. 18.7 and 18.8). In turn that ancestor evolved greater colonial complexity to give rise to today's *Pandorina* (fig. 18.9) and then *Eudorina* (fig. 18.10). That colonial ancestor later gave rise to *Volvox* (fig. 18.11), the most complex alga of the Volvocine line. These five genera are modern representatives of a lineage of species that evolved along a path of colonial complexity.

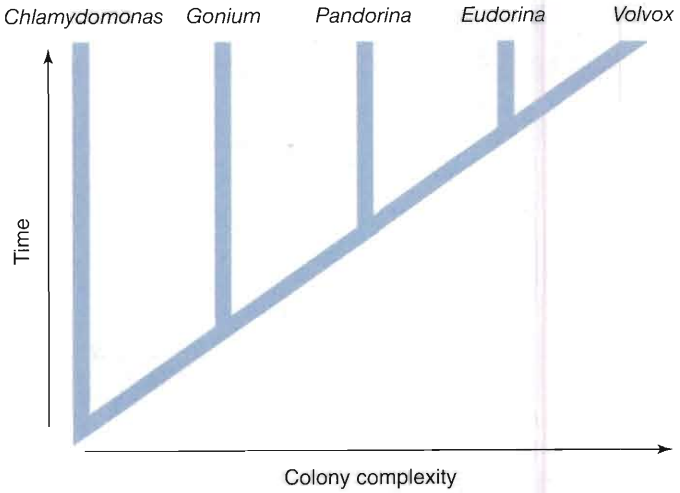


Figure 18.7
A cladogram representing the simplified phylogeny (family tree) of the Volvocine line. Proposed common ancestors are represented by the branching points called nodes.

Figure 18.8
Gonium, a colonial green alga composed of 16 cells (400×).

Procedure 18.5

Examine members of the Volvocine line of algae

1. Follow steps 2–6 to sequentially examine each of the organisms with your microscope. When preparing each of the colonial specimens, try both a standard microscope slide and a deep-well or depression slide. Determine which works best for colonies of cells.
2. *Chlamydomonas* is among the most primitive and widespread of the green algae. It is a unicellular biflagellate alga (fig. 18.6). All species of the Volvocine line consist of cells similar to *Chlamydomonas*, but the cells are in different configurations.

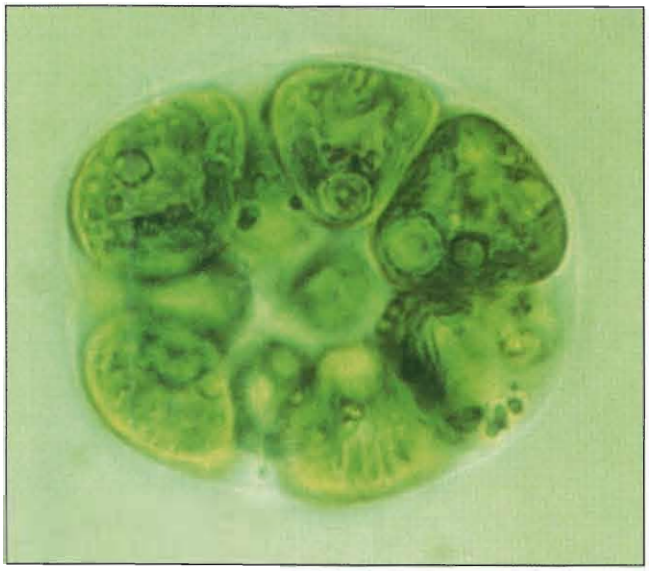


Figure 18.9
Pandorina, a colony of 16 or 32 flagellated green algal cells (150×).

3. *Gonium* is the simplest colonial member of the Volvocine line (fig. 18.8). A *Gonium* colony consists of 4, 8, 16, or 32 *Chlamydomonas*-like cells held together in the shape of a disk by a gelatinous matrix. Each cell in the *Gonium* colony can divide to produce cells that produce new colonies. Like *Chlamydomonas*, *Gonium* is isogamous.

Question 6
Why do colonies of *Gonium* consist of only 4, 8, 16, or 32 cells? Why are there no 23-celled colonies?

4. *Pandorina* consists of 16 or 32 *Chlamydomonas*-like cells held together by a gelatinous matrix (fig. 18.9). Examine how *Pandorina* moves. Flagella on *Pandorina* move the ellipsoidal alga through the water like a ball. After attaining its maximum size, each cell of the colony divides to form a new colony. The parent matrix then breaks open like Pandora's box (hence the name *Pandorina*) and releases the newly formed colonies. *Pandorina* is isogamous.

Question 7
What is the significance of a specialization at one end of the colony?

Figure 18.10
Eudorina, a colony of 32 flagellated green algal cells.

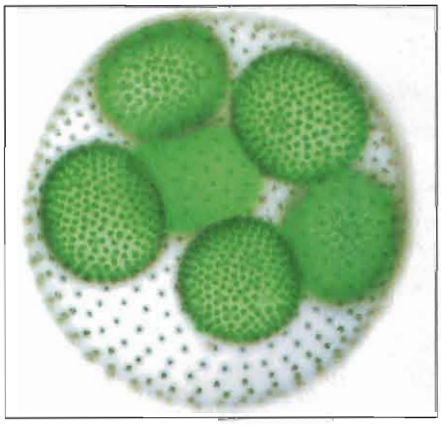


Figure 18.11
Volvox, a common green alga (100×). Colonies of *Volvox* often consist of hundreds of cells. Daughter colonies are visible within the larger parent colony.

5. *Eudorina* is a spherical colony composed of 32, 64, or 128 cells (fig. 18.10). Cells in a colony of *Eudorina* differ in size; smaller cells are located at the anterior part of the colony. The anterior surface is determined by the direction of movement.

Question 8
What is the significance of these structural and functional specializations of *Eudorina*?

6. *Volvox* is the largest and most spectacular organism of the Volvocine line. *Volvox* is a spherical colony made of thousands of vegetative cells and a few

reproductive cells (fig. 18.11). Flagella spin the colony on its axis. In some species of *Volvox* and *Gonium*, cytoplasmic strands form a conspicuous network among the cells.

Question 9

a. Does the *Volvox* colony spin clockwise or counterclockwise?

b. What is the significance of the cytoplasmic network in *Volvox*?

To organize your information and observations complete table 18.4.

TABLE 18.4

EVOLUTIONARY SPECIALIZATION OF MEMBERS OF THE VOLVOCINE LINE

Characteristic	*Chlamydomonas*	*Gonium*	*Pandorina*	*Eudorina*	*Volvox*
Number of cells					
Colony size					
Structural and functional specializations of cells					
Reproductive specialization (isogamy versus oogamy)					

Questions for Further Thought and Study

1. How would selection against heterozygous individuals over many generations affect the frequencies of homozygous individuals? Would the results of such selection depend on the initial frequencies of p and q? Could you test this experimentally? How?

2. How are genetic characteristics associated with nonreproductive activities such as feeding affected by natural selection?

3. Although Charles Darwin wasn't the first person to suggest that populations evolve, he was the first to describe a credible mechanism for the process. That mechanism is natural selection. What is natural selection, and how can it drive evolution?

4. Do you suspect that evolutionary change always leads to greater complexity? Why or why not?

5. Is natural selection the only means of evolution? Explain.

6. What change in a population would you expect to see if a selection pressure was against the trait of the dominant allele?

7. The application of an evolutionary approach to understanding disease is widespread and productive. What is the benefit of applying Darwinian principles to medical practice?

DOING BIOLOGY YOURSELF

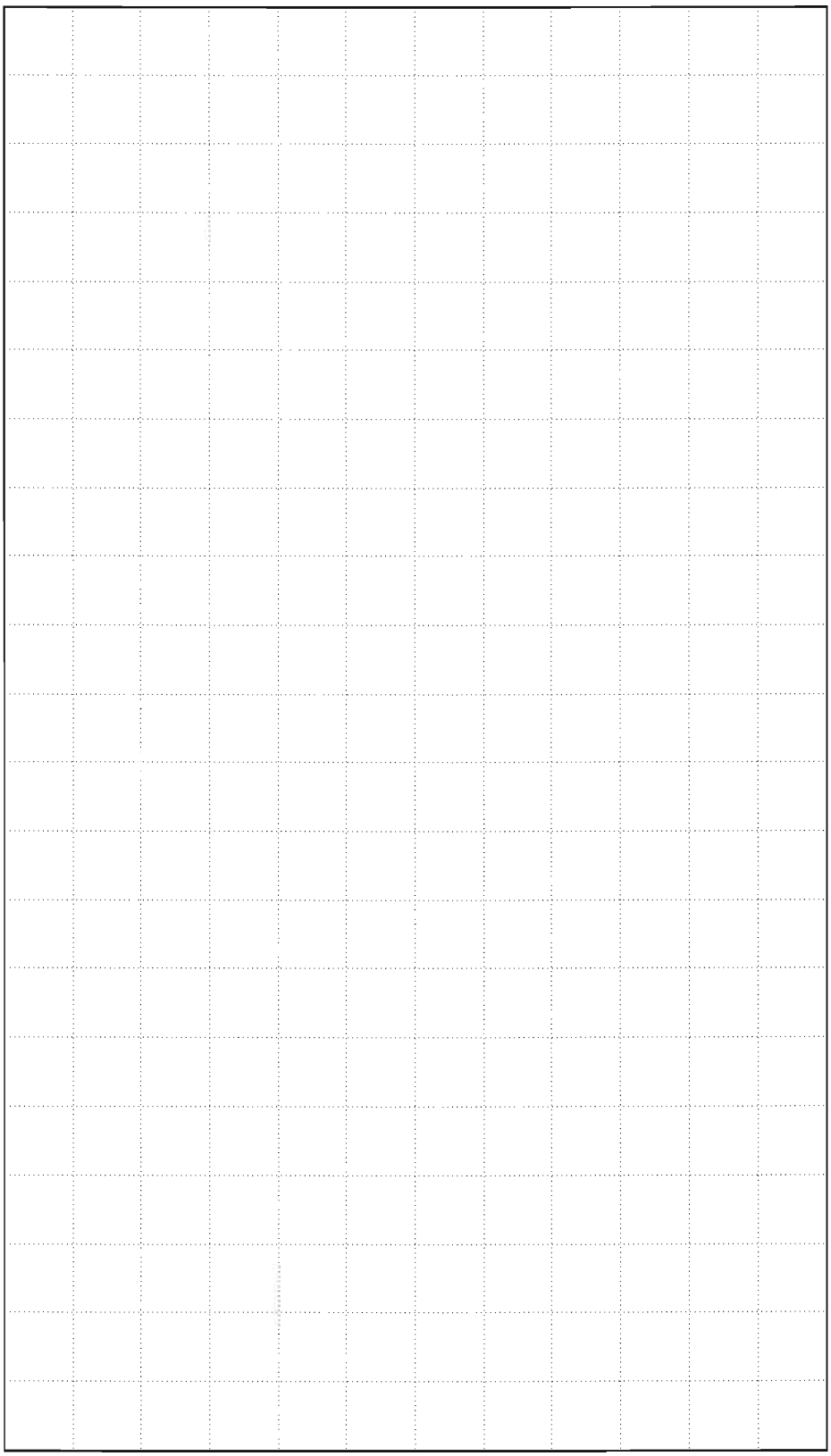
Design an experiment to determine the phylogenetic relationships among members of the Volvocine line of algae. What information about their DNA sequences would be useful?

WRITING TO LEARN BIOLOGY

Summarize the most recent books and publications that review the benefits of applying Darwinian principles to medical practice.

WRITING TO LEARN BIOLOGY

The Hardy-Weinberg equilibrium assumes that pollination and subsequent fertilization must be random. Is that true for most wildflower populations? What characteristics of these plants influence pollination patterns?



28

Survey of the Plant Kingdom

Liverworts, Mosses, and Hornworts of Phyla Hepaticophyta, Bryophyta, and Anthocerophyta

Objectives

By the end of this exercise you should be able to:

1. Describe the life histories and related reproductive structures of bryophytes.
2. Describe the distinguishing features of liverworts, mosses, and hornworts.
3. Describe some of the key adaptations that allow liverworts, mosses, and hornworts to live on land.

The plant kingdom comprises a remarkably diverse group of multicellular organisms. With few exceptions, plants are autotrophic, contain chlorophyll *a*, and have cell walls containing cellulose. Life cycles of all members of the plant kingdom are variations on alternation of generations. Be sure to review in your textbook this generalized life cycle and be familiar with the major stages presented in figure 28.1.

The major groups of plants that you will examine in this and the next three laboratory exercises are bryophytes (mosses, liverworts, and hornworts), ferns and fern allies, gymnosperms, and angiosperms. These groups of plants are distinguished by morphology, life cycle, and the presence or absence of vascular tissues. Pay special attention to variations in each of these characteristics as you survey the plant kingdom in upcoming weeks.

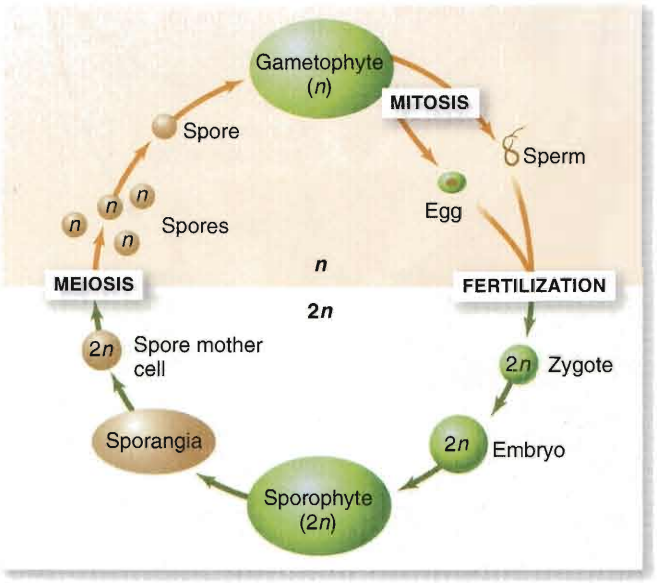


Figure 28.1

In a generalized plant life cycle, gametophytes, haploid (n), alternate with sporophytes, diploid (2n). Antheridia (male) and archegonia (female) are the sex organs produced by the gametophyte; they produce sperm and eggs, respectively. The sperm and egg fuse during fertilization to produce the first diploid cell of the sporophyte generation, the zygote. Meiosis occurs within sporangia, which are the spore-producing organs of the sporophyte. The resulting spores are haploid and are the first cells of the gametophyte generation.

Bryophytes include liverworts, mosses, and hornworts and are the most primitive group of terrestrial plants. Bryophytes are green, have rootlike structures called **rhizoids,** and may have stem and leaflike parts. Bryophytes do not generally possess specialized vascular tissues, which transport materials between roots and shoots. This lack of developed vascular tissues in bryophytes typically limits their distribution to moist habitats, because their rhizoids neither penetrate the soil very far nor absorb many nutrients. Also, the lack of vascular tissues necessitates that their photosynthetic and nonphotosynthetic tissues be close together. Because vascular tissues, along with supporting tissues, are often absent, bryophytes are relatively small and inconspicuous. Despite their diminutive size, however, bryophytes occur throughout the world in habitats ranging from the tropics to Antarctica.

There are approximately 24,000 species of bryophytes, more than any other group of plants except the flowering plants. Bryophytes fix CO_2, degrade rocks to soil, stabilize soil, and reduce erosion. Humans have used bryophytes in a number of ways, including as a fuel, in the production of Scotch whiskey, and as packing materials.

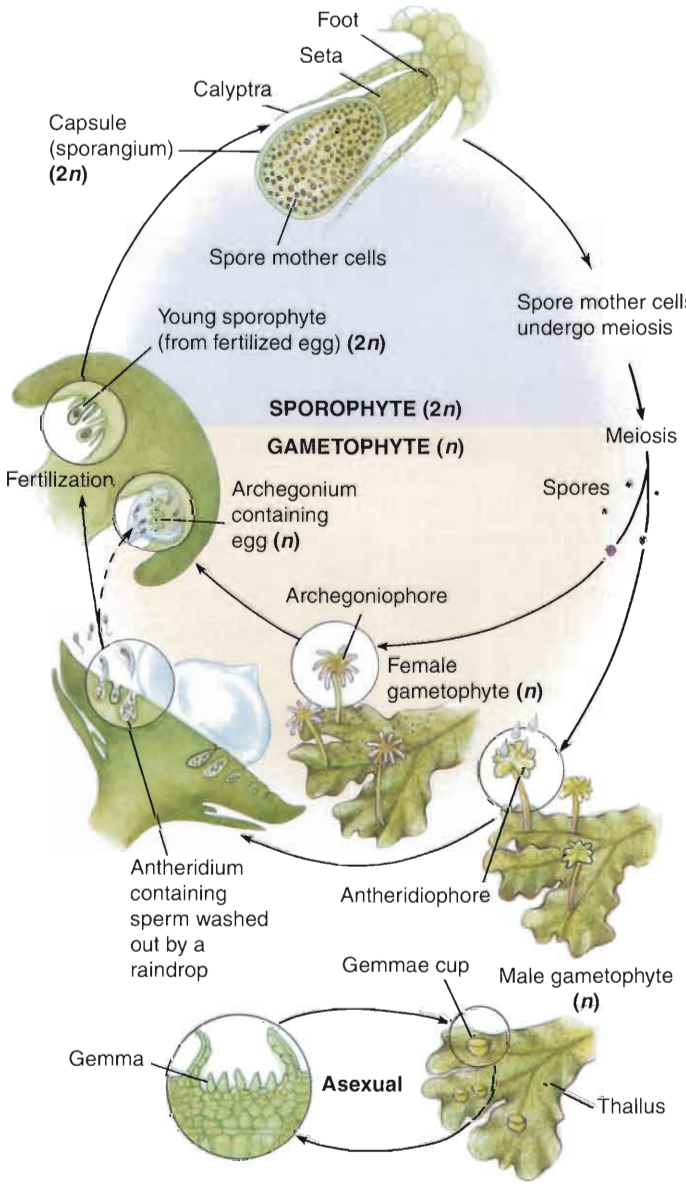


Figure 28.2

Life cycle of *Marchantia*, a liverwort. During sexual reproduction, spores produced in the capsule germinate to form independent male and female gametophytes. The archegoniophore produces archegonia, each of which contains an egg; the antheridiophore produces antheridia, each of which produces many sperm. After fertilization, the sporophyte develops within the archegonium and produces a capsule with spores. *Marchantia* reproduces asexually by fragmentation and gemmae.

The plant body of bryophytes is called a **thallus** (pl., *thalli*). Liverwort thalli are flattened dorsoventrally (from back and front plane, rather than from side to side plane) and are bilaterally symmetrical (i.e., have two equal halves). For comparison, moss thalli are erect and radially symmetrical (circular). Hornwort thalli are similar to those of liverworts.

The life cycle of bryophytes is characterized by a distinct alternation of generations that includes both gametophyte and sporophyte phases, *but the gametophyte is the dominant phase* (fig. 28.2). Bryophytes have multicellular sex organs in which gamete-producing cells are enclosed in a jacket of sterile cells. **Antheridia** are male sex organs that produce swimming, biflagellate sperm. Bryophytes require free water for sexual reproduction because their sperm must swim to eggs. These sperm fertilize eggs produced in **archegonia**, the female sex organs. The fertilized egg is called a **zygote**. This zygote divides and matures in the **archegonium** to produce the **sporophyte**, which remains attached to and nutritionally dependent on the **gametophyte**. The mature sporophyte produces haploid spores (via meiosis), each of which can develop into a gametophyte.

PHYLUM HEPATICOPHYTA: LIVERWORTS

Liverworts are the earliest land plants. Although many liverworts are "leafy," we will restrict our observations to a thallus-type liverwort, *Marchantia*. The gametophytic thallus of this liverwort grows as a large, flat, photosynthetic structure on the surface of the ground (fig. 28.3).

Liverwort Gametophyte

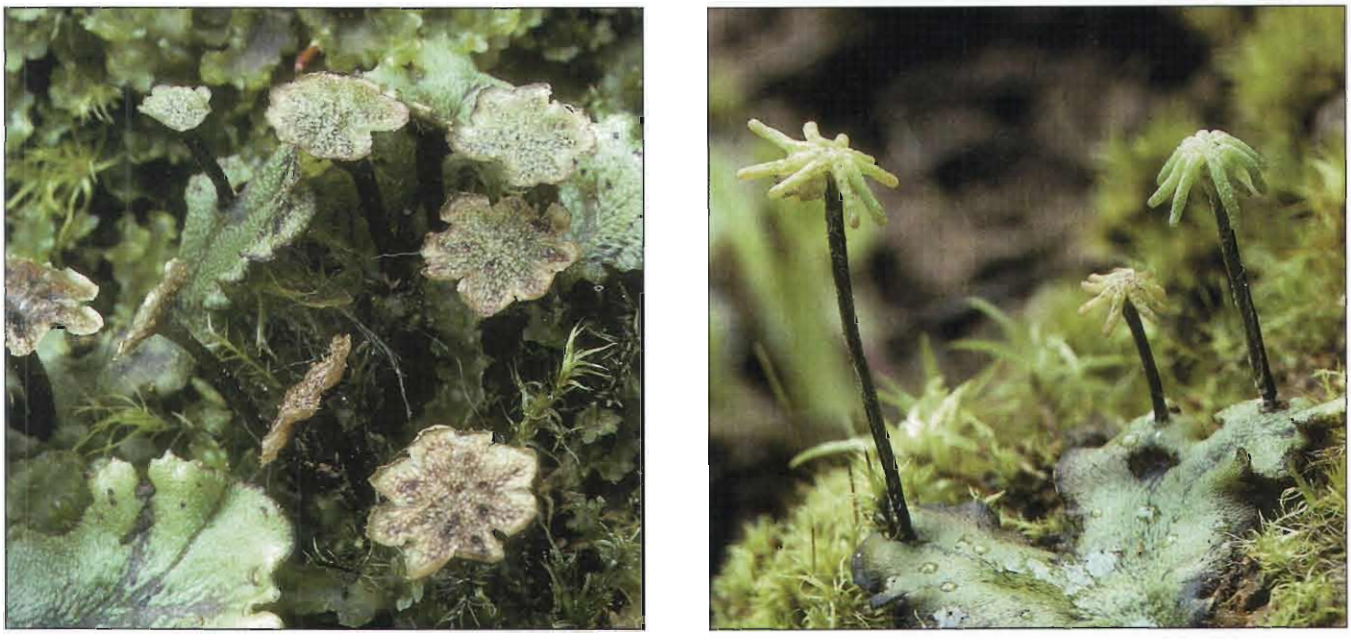
Procedure 28.1

Examine the thallus of *Marchantia*

1. Observe some living *Marchantia* and note the Y-shaped (dichotomous) growth. Rhizoids extend downward from the lower (ventral) surface of the thallus.

Question 1
What are the functions of rhizoids?

2. View the upper (dorsal) surface of the thallus with a dissecting microscope and note the pores in the center of the diamond-shaped areas. Obtain a prepared slide of a thallus of *Marchantia* and compare what you see with figure 28.4. The pores in the dorsal surface of the thallus overlie air chambers containing **chlorenchyma** (chloroplast-containing) cells.



(a) (b)

Figure 28.3
Marchantia. The flat, leafy thallus of this liverwort grows close to the ground. (*a*) A thallus bearing upright male reproductive structures called antheridiophores. (*b*) A thallus bearing upright female reproductive structures called archegoniophores.

Question 2
What is the function of these pores?

Asexual Reproduction in Liverworts

Liverworts can reproduce asexually via fragmentation. In this process, the older, central portions of the thallus die, leaving the growing tips isolated to form individual plants.

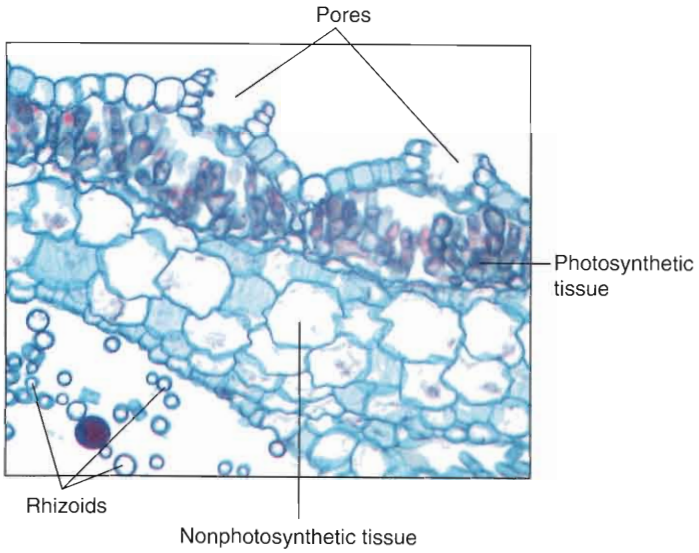


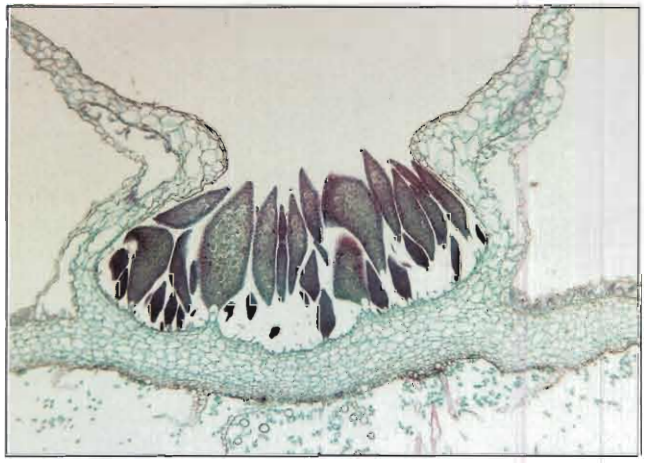
Figure 28.4
Section of *Marchantia* gametophyte showing pores, rhizoids, photosynthetic tissue, and nonphotosynthetic tissue.

Structures called **gemmae cups** occur on the dorsal surface of some thalli near the midrib (fig. 28.5). Gemmae cups represent another means of asexual reproduction by liverworts. Inside the gemmae cups are lens-shaped outgrowths called **gemmae** (sing., *gemma*), which are splashed out of the cup by falling drops of rain. If a gemma lands in an adequate environment, it can produce a new gametophyte plant. Examine a prepared slide of gemmae cups. Also examine available live or preserved material. In the following space, diagram and label what you see, and compare it to figure 28.5.

Sexual Reproduction in Liverworts

Many species of *Marchantia* are **dioecious,** meaning that they have separate male and female plants. Gametes from each plant are produced in specialized sex organs borne on upright stalks (fig. 28.3). **Archegoniophores** are specialized stalks on female plants that bear archegonia. Each flask-shaped archegonium consists of a **neck** and a **venter**, which contains the **egg** (fig. 28.6*a*). **Antheridiophores** are specialized stalks on male plants that bear antheridia (fig. 28.3).

(a)



(b)

Figure 28.5
(*a*) Gemmae cups ("splash cups") containing gemmae on the gametophytes of a liverwort. Gemmae are splashed out of the cups by raindrops and can then grow into new gametophytes, each identical to the parent plant that produced it by mitosis. (*b*) Longitudinal section of a gemmae cup (10×).

Sperm form in antheridia (fig. 28.6*b*). Flagellated sperm are released and washed from the antheridia during wet conditions and eventually fertilize the egg located in the venter. The zygote remains in the venter and grows into a sporophyte plant.

Procedure 28.2

Examine archegonia and antheridia of liverworts

1. Examine living or prepared liverworts having mature archegoniophores that bear archegonia. Archegonia at various stages of development are located on the ventral surface.
2. Locate an egg in an archegonium. Notice a pattern of evolution in plants as well as in animals—eggs are larger and fewer in number, while sperm cells are smaller but greater in number.
3. Examine living or preserved liverworts with mature antheridiophores bearing antheridia.
4. Examine a prepared slide of cross sections of an antheridiophore. Antheridia are located just below the upper surface of the disk in a chamber that leads to the surface of the disk through a pore.

Question 3

How do the positions of the archegonium and antheridium relate to their reproductive function?

Liverwort Sporophyte

Procedure 28.3

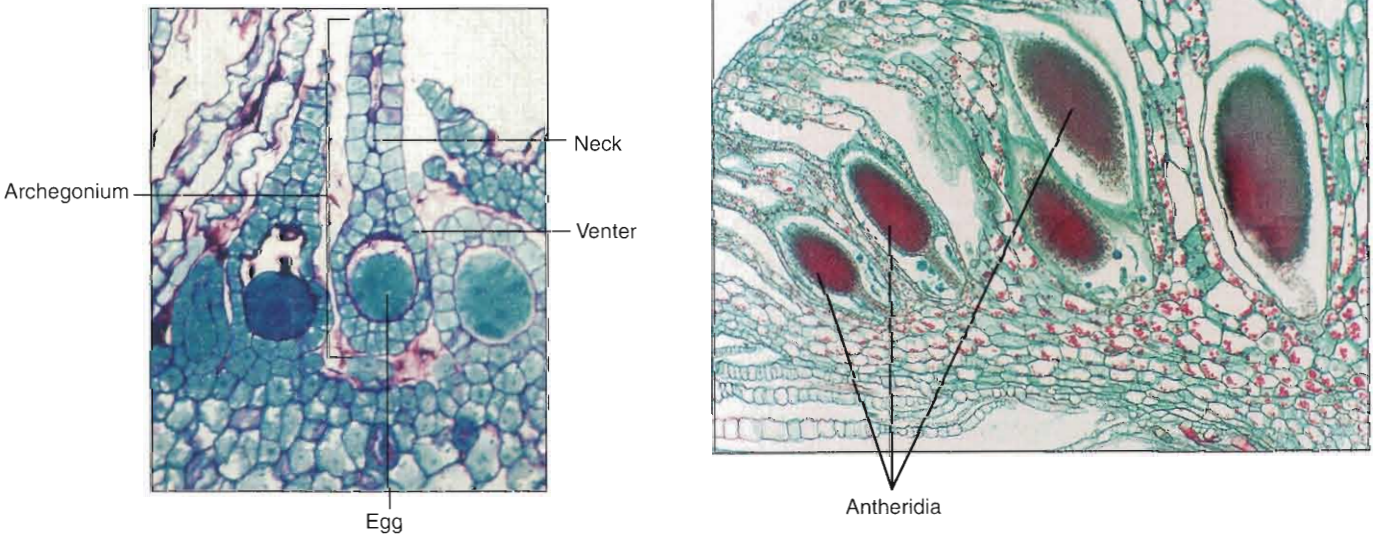
Examine sporophytes of liverworts

1. Examine a prepared slide of a sporophyte of *Marchantia*. The nonphotosynthetic sporophyte is connected to the gametophyte by a structure called the **foot**. **Spores** are produced by meiosis in a **capsule** located on a **seta** (stalk) that extends downward from the foot (fig 28.2).
2. Locate elongate cells called **elaters** among the spores. Elaters help disperse spores by twisting. In humid conditions the elaters coil, but when it is dry the elaters expand, pushing the spores apart and rupturing the spore case to release the spores.
3. Understand that gamete release, fertilization, spore release, and germination are most efficient in individually specific environmental conditions.

Question 4

a. What is the function of the foot?

b. Are the spores haploid or diploid?



(a) (b)

Figure 28.6

(*a*) Section through an archegonium of *Marchantia*. A single egg differentiates within the archegonium. (*b*) Section through an antheridiophore, showing individual antheridia (100×).

c. What is the functional significance of the response of elaters to moisture?

PHYLUM BRYOPHYTA: MOSSES

Mosses are often more visible than liverworts because of their greater numbers, more widespread distribution, and because gametophyte plants of mosses are leafy and usually stand upright. Mosses also withstand desiccation better than do liverworts. Consequently, mosses often grow in a greater diversity of habitats than do liverworts. The moss gametophyte is radially symmetrical and is the most conspicuous phase of the moss life cycle (fig. 28.7).

Moss Gametophyte

Procedure 28.4

Examine mosses

1. Observe the living moss on display called *Polytrichum* (fig. 28.8). The "leafy" green portions of the moss are the gametophytes and are often only one cell thick (except at the midrib).
2. Make a wet mount of a single leaflet and examine it with low magnification.

Question 5

a. How many cells thick is the leaflet?

b. Is there a midrib vein?

c. Are stomata or pores visible on the leaf surface?

d. How does the symmetry of a moss gametophyte compare with that of a liverwort gametophyte?

Moss gametophytes have specialized cells that aid in the absorption and retention of water. Mats of moss act, in effect, like sponges. The following procedure demonstrates the water-absorbing potential of mosses.

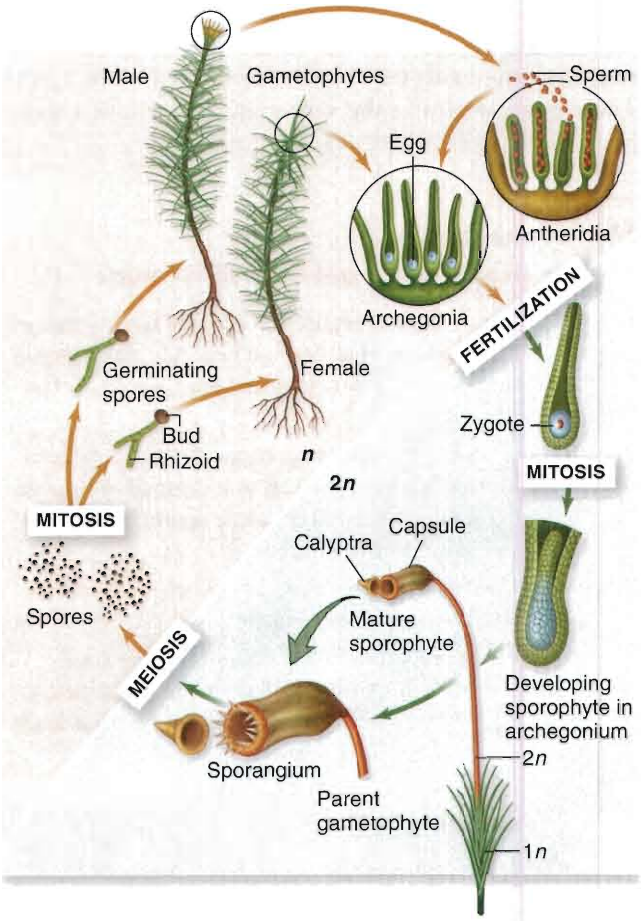


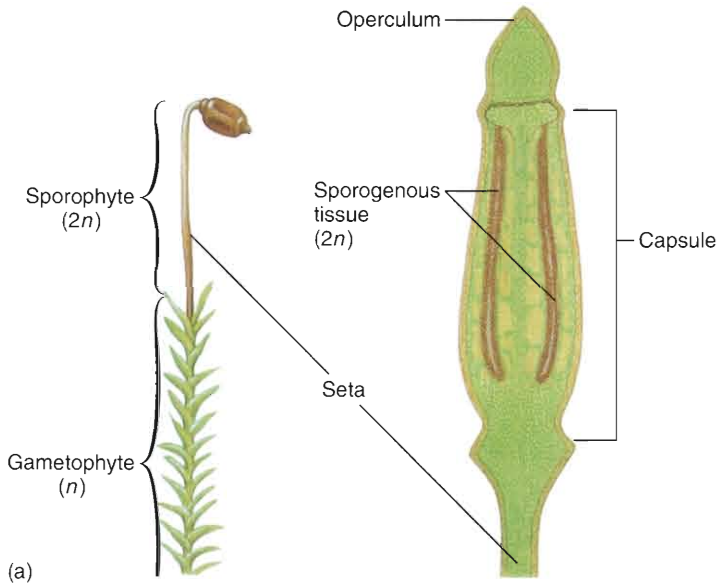
Figure 28.7

Moss life cycle. Haploid (*n*) sperm are released from antheridia on the male gametophytes. The sperm then swim through water to the archegonia and down their necks to fertilize the eggs. The resulting diploid (2*n*) zygote develops into a diploid sporophyte. The sporophyte grows out of the archegonium and differentiates into a slender seta with a swollen capsule at its apex. The capsule is covered with a cap, or calyptra, formed from the archegonium. The sporophyte grows on the gametophyte and eventually produces spores by meiosis. The spores are shed from the capsule after the cap drops off. The spores germinate, giving rise to gametophytes. The gametophytes initially grow along the ground. Ultimately, buds produce leafy gametophytes.

Procedure 28.5

Water absorption by moss

1. Weigh 3 g of *Sphagnum*, a peat moss, and 3 g of paper towel.
2. Add the moss and towel to separate beakers each containing 100 mL of water.
3. After several minutes, remove the materials from the beaker.




(b)

Figure 28.8

The structure of a moss. (*a*) Diagram of the parts of a mature mass sporophyte. (*b*) A hair-cup moss, *Polytrichum*. The leaves belong to the gametophyte. Each of the yellowish-brown stalks, with the capsule at its summit, is a sporophyte. Although moss sporophytes may be green and perform a limited amount of photosynthesis when they are immature, they are soon completely dependent, in a nutritional sense, on the gametophyte.

4. Measure the amount of water left in each beaker by pouring the water into a 100-mL graduated cylinder. Remember that 1 mL of water weighs 1 g.
5. Record your data.

Question 6

a. How many times its own weight did the moss absorb?

b. How does this compare with the paper towel?

c. Why is *Sphagnum* often used to ship items that must be kept moist?

Asexual Reproduction in Mosses

Unlike liverworts, mosses lack structures such as gemmae for asexual reproduction. Mosses reproduce asexually by fragmentation.

Sexual Reproduction in Mosses

Most mosses, like liverworts, are dioecious (i.e., male and female reproductive structures are on separate individuals). Archegonia or antheridia are borne either on tips of the erect gametophyte stalks or as lateral branches on the stalks. The apex of stalks of the female plant (the plant that bears archegonia) appears as a cluster of leaves, with the archegonia buried inside.

Procedure 28.6

Examine archegonia and antheridia of mosses

1. Examine living or preserved mosses having mature archegonia.
2. Examine a prepared slide of moss archegonia (fig. 28.9). Note the canal that leads through the neck and terminates in the venter of the archegonium. When the archegonium matures, cells lining the neck disintegrate and form a canal leading to the egg. Sperm, following a chemical attractant released by the archegonium, swim through this canal to reach the egg.

Question 7

Where is the egg located in the archegonium?

3. Examine living or preserved mosses having mature antheridia. The male plant (i.e., the plant that bears antheridia) has a platelike structure on the tip with the "leaves" expanding outward to form a rosette.

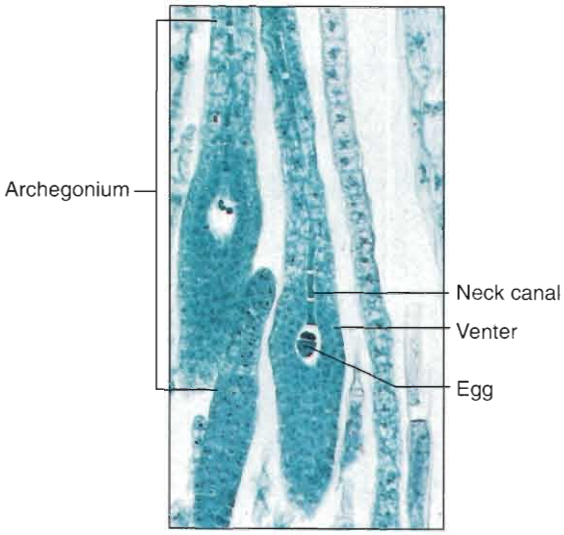


Figure 28.9
A longitudinal section through the tip of a female gametophyte of a moss.

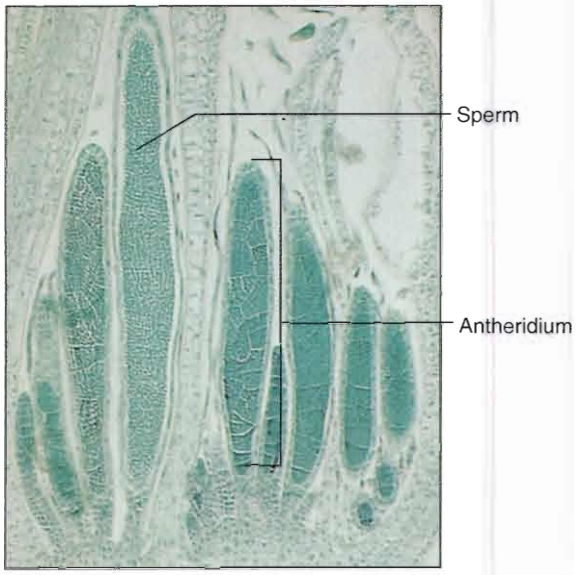


Figure 28.10
A longitudinal section through the tip of a male gametophyte of a moss.

This structure is sometimes called a "moss flower" because of its appearance or a "splash cup" because of its function (i.e., the dispersal of sperm by falling raindrops).

4. Examine a prepared slide of moss antheridia, which appear as elongate, saclike structures (fig. 28.10).
5. Locate the outer sterile jacket and the inner mass of cells destined to become sperm.

Question 8
Are sperm haploid or diploid?

Moss Sporophyte

Moss sporophytes consist of **capsules** located atop stalks, or **seta,** that extend upward from the moss gametophyte (fig. 28.8*a*). A sporophyte is attached to the gametophyte by a structure called a **foot.**

Question 9
Is the sporophyte more prominent in mosses or liverworts?

The capsule atop the seta is covered by the **calyptra**, the upper portion of archegonium that covers the apex of the capsule. The calyptra falls off when the capsule matures. Inside the capsule are numerous haploid spores formed by meiosis.

Question 10
What is the adaptive significance of the seta of the sporophyte growing well above the mat of the gametophytes?

If enough living moss is available in the lab, remove the calyptra from a sporophyte capsule. On the tip of the capsule is a caplike structure called the **operculum**. Remove the operculum and notice the hairlike teeth lining the opening of the capsule. These teeth help control the release of spores from the capsule. In wet weather these teeth bend inward and prevent release of the spores. In dry weather they bend outward, facilitating distribution of spores by the wind.

Crush the capsule in water on a microscope slide and note the large number of spores released.

Question 11
a. What process produces spores?

b. Is the capsule haploid or diploid?

Moss spores germinate and form a photosynthetic **protonema**, which resembles a branching, filamentous alga. Leafy moss plants arise from "buds" located along the protonema.

Question 12
Can you think of any evolutionary implications of the similarity between a moss protonema and a filamentous green alga?

PHYLUM ANTHOCEROPHYTA: HORNWORTS

The hornworts are the smallest group of bryophytes; there are only about 100 species in six genera. Hornworts have several features that distinguish them from most other bryophytes. The sporophyte is shaped like a long, tapered horn that protrudes from a flattened thallus. Also, archegonia are not discrete organs. Rather, they are embedded in the thallus and are in contact with surrounding vegetative cells.

The most familiar hornwort is *Anthoceros*, a temperate genus (fig. 28.11). Examine some living and preserved *Anthoceros*. Locate the gametophyte thallus and the rhizoids extending from its lower surface. Also locate the hornlike sporophytes extending from the upper surface. Spores are produced in the horn of the sporophyte. If prepared slides are available, locate spores in various stages of development within the sporophyte.

Figure 28.11
Anthoceros, a hornwort.

INVESTIGATION

The Roles of Bryophytes in the Environment

Observation: Although they are usually overlooked, bryophytes often grow in places that other plants cannot grow and are important parts of many ecosystems. Find a place where bryophytes are growing on campus. Consider what these plants are doing in that ecosystem and why they are not in other ecosystems on campus.

Question: What conditions are best suited for the growth of bryophytes?

a. Establish a working lab group and obtain Investigation Worksheet 28 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 28 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

DOING BIOLOGY YOURSELF

Liverworts and mosses are surprisingly diverse. Make a simple collection of living liverworts and mosses from two or three sites. Bring the collection to class and compare yours with those of other students. Use reference books in your lab or library to identify the plants that you've collected.

WRITING TO LEARN BIOLOGY

Because water is required for the swimming sperm to reach the archegonium, would you say that this means that bryophytes are not truly land plants? Why or why not?

Questions for Further Thought and Study

1. Compare and contrast the complexity of bryophytes and algae regarding their morphology, habitat, asexual reproduction, and sexual reproduction.

2. What event begins the sporophyte phase of the life cycle? Where does this event occur in liverworts and mosses?

3. What event begins the gametophyte phase of the life cycle? Where does it occur in liverworts and mosses?

4. What features distinguish a moss from a liverwort?

5. Diagram the life cycle of a liverwort, indicating which stages are sporophytic and which are gametophytic.

6. Diagram the life cycle of a moss, indicating which stages are sporophytic and which are gametophytic. Also indicate where meiosis and syngamy occur.

7. How did liverworts obtain their name?

8. What ecological roles do liverworts, mosses, and hornworts play in the environment?

9. Is the sporophyte of mosses ever independent of the gametophyte? Explain.

10. Why do you think that bryophytes are sometimes referred to as the amphibians of the plant kingdom?

11. What limits the height of mosses?

Survey of the Plant Kingdom

Seedless Vascular Plants of Phyla Pterophyta and Lycophyta

29

Objectives

By the end of this exercise you should be able to:

1. Discuss similarities and differences between ferns and other plants you have studied in the lab.
2. Describe the life cycles of ferns and their allies.
3. Describe the distinguishing features of true ferns, club mosses, whisk ferns, and horsetails.

Seedless vascular plants include two phyla of nonflowering plants having a vascular system of fluid-conducting xylem and phloem: phylum Pterophyta (true ferns, whisk ferns, and horsetails) and phylum Lycophyta (club mosses) (table 29.1). The life cycle of seedless vascular plants includes both gametophyte and sporophyte phases, but in contrast to bryophytes (Exercise 28), in this group the sporophyte is the dominant phase (fig. 29.1).

All ferns and fern allies possess **sporophylls** (*sporo* = spore forming, *-phyll* = leaf). Sporophylls are leaflike structures of the sporophyte generation that bear spores. They may be

TABLE 29.1

THE TWO PHYLA OF SEEDLESS VASCULAR PLANTS

Phylum	Examples		Key Characteristics	Approximate Number of Living Species
Pterophyta	Ferns		Primarily homosporous (a few heterosporous) vascular plants. Sperm motile. External water necessary for fertilization. Leaves are megaphylls that uncoil as they mature. Sporophytes and virtually all gametophytes photosynthetic. About 365 genera.	11,000
	Horsetails		Homosporous vascular plants. Sperm motile. External water necessary for fertilization. Stems ribbed, jointed, either photosynthetic or nonphotosynthetic. Leaves scalelike, in whorls, nonphotosynthetic at maturity. One genus.	15
	Whisk ferns		Homosporous vascular plants. Sperm motile. External water necessary for fertilization. No differentiation between root and shoot. No leaves; one of the two genera has scalelike enations and the other leaflike appendages.	6
Lycophyta	Club mosses		Homosporous or heterosporous vascular plants. Sperm motile. External water necessary for fertilization. Leaves are microphylls. About 12–13 genera.	1150

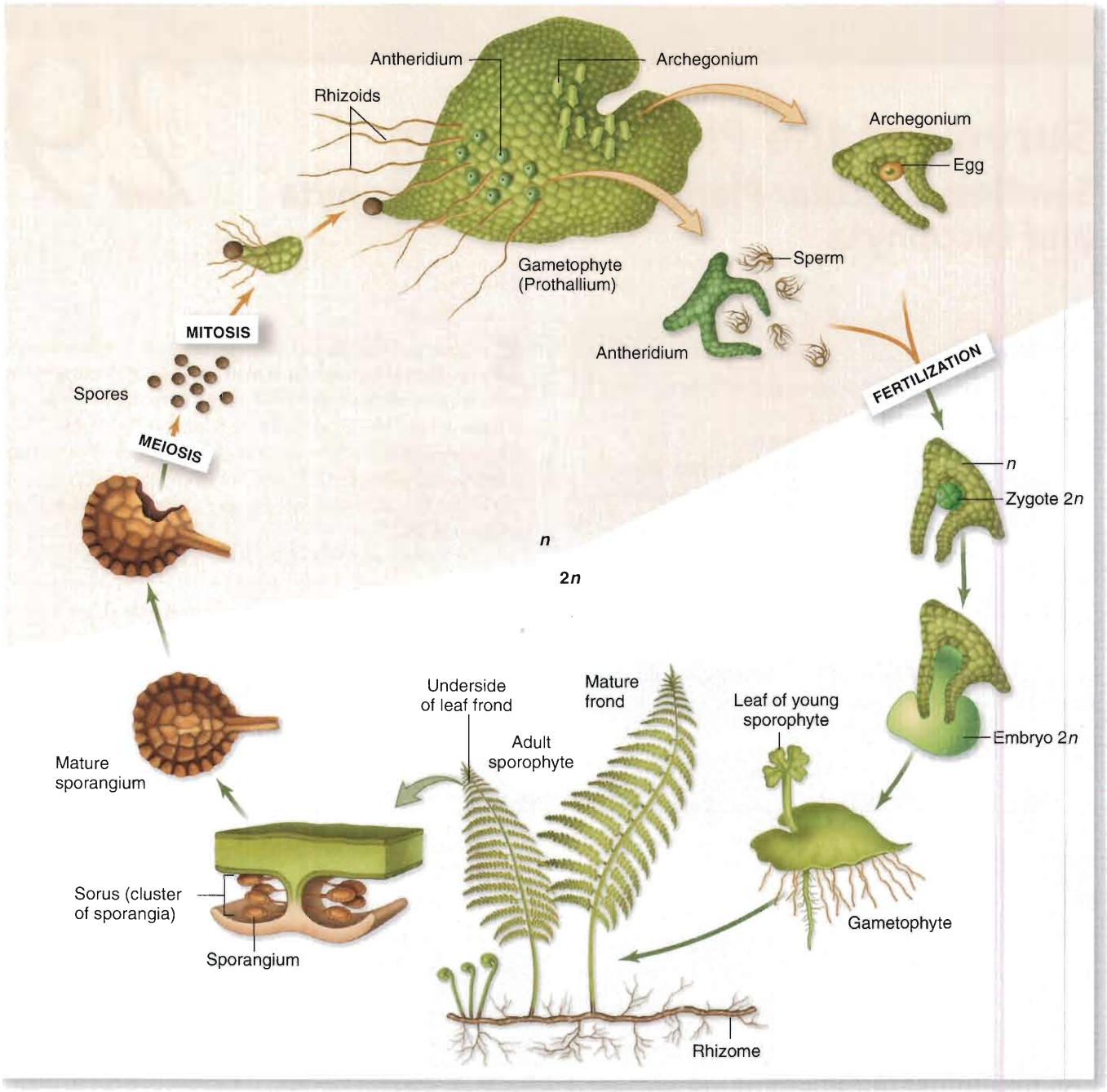


Figure 29.1

Fern life cycle. The haploid gametophyte (i.e., the prothallium) grows in moist places. Rhizoids are anchoring structures that project from the lower surface of a prothallium. Eggs and sperm develop in archegonia and antheridia, respectively, on gametophytes' lower surface. Sperm released from antheridia swim to archegonia and fertilize the single egg. The zygote—the first cell of the diploid sporophyte generation—starts to grow within the archegonium and eventually becomes much larger than the gametophyte. Most ferns have horizontal stems, called rhizomes, that grow below the ground. On the sporophyte's leaves (fronds) are clusters of sporangia within which meiosis occurs and spores are formed. When released, the spores germinate and become new gametophytes called prothallia.

large **megaphylls** (*mega* = large, *-phyll* = leaf) with several to many veins (as in the megaphylls of true ferns) or they may be smaller **microphylls** (*micro* = small, *-phyll* = leaf) with one vein (as in whisk ferns, scouring rush, and club mosses). **Sporangia**, which form on sporophylls, are where spores are produced by meiosis (review fig. 28.1 and compare to fig. 29.1). Sporangia occur somewhere on all plants. In ferns, the sporangia are on the backs of leaves; this is why the leaves are called sporophylls. Like bryophytes, ferns require water for fertilization.

PHYLUM PTEROPHYTA
FERNS

True ferns (phylum Pterophyta) inhabit almost all types of environments and possess characteristics of the more advanced seed plants as well as the less advanced bryophytes. Ferns have well-developed vascular tissue. Unlike bryophytes, ferns have an independent sporophyte (fig. 29.1) and stomata, pores that open and close on leaves and, in doing so, regulate gas exchange.

The diversity of ferns is striking; they range from majestic tree ferns to bizarre staghorn ferns. Tree ferns reach heights of up to 16 m. Along with other plants, these ferns once formed forests that were transformed into coal deposits. Today, humans use ferns as decorations and in rice cultivation. Review the fern life cycle shown in figure 29.1.

Question 1

a. Which parts of the life cycle are haploid?

b. Which are diploid?

Fern sporophytes grow indefinitely via underground stems called **rhizomes** (fig. 29.1). Examine the fern rhizomes on display. Also examine the different ferns available in the lab and note the different shapes of the leaflike fronds. Identify the **stalk**, **blade**, and **pinnae**.

Question 2

a. How many veins are present in each frond?

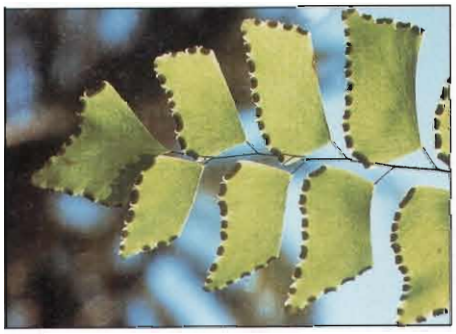
b. What tissues comprise a vein of vascular tissue?

c. What is the function of the stalk? The blade? The pinnae?

Groups of sporangia called **sori** (singular = **sorus**) form on the underside of fern fronds (fig. 29.2). Sporangia may be

(a)

(b)



(c)

Figure 29.2

Fern sporangia. Most ferns have sporangia aggregated into clusters, called sori, on the undersides of their leaves. (*a*) In some ferns, such as the marginal woodfern (*Dryopteris marginalis*), each sorus is covered by a flap of leaf tissue called an indusium (also see fig. 29.3). (*b*) Other ferns bear uncovered sori, as shown here in *Alsophila sinuata*. (*c*) In still other ferns, as in the giant maidenhair fern (*Adiantum trapeziforme*), sori are enfolded by the edge of the leaf itself.

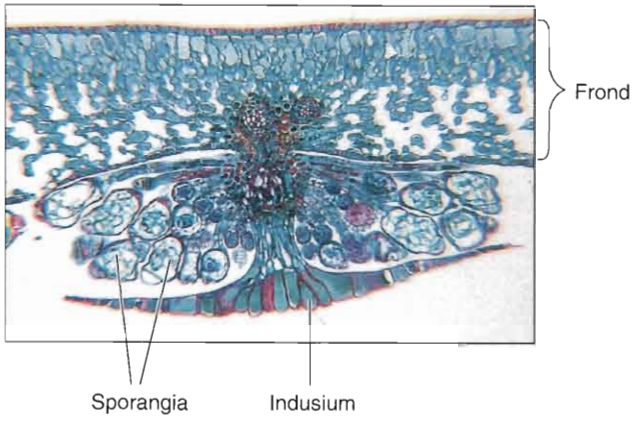


Figure 29.3
Fern sorus.

protected by a shield-shaped **indusium** (fig. 29.3), a specialized outgrowth of a frond. Meiosis in the sporangium produces haploid spores, the first stage of the gametophyte.

Procedure 29.1

Examine sori

1. Scrape a sorus into a drop of water on a microscope slide and use the low power on your microscope to observe the sorus.

Question 3
Are any spores in the sporangium?

Be careful when handling acetone; it is a strong solvent.

2. Place a few drops of acetone on the sporangium while you observe it with a dissecting microscope.
3. Watch the sporangium for a few minutes, adding acetone as needed.
4. Describe what you see.

Question 4

a. Did the application of acetone cause the spores of the fern to disperse?

b. How is the mechanism for spore dispersal in ferns similar to that of bryophytes?

5. Examine prepared slides of fern sori, referring to figure 29.3. Diagram and label each structure that you see, listing its function.

Fern Reproduction

Fern spores germinate and form a threadlike **protonema**. Subsequent cellular divisions produce an independent, heart-shaped **prothallium** ("valentine plant").

Question 5

a. Is the prothallium haploid or diploid?

b. Is the prothallium sporophyte or gametophyte?

Rhizoids and male and female reproductive structures occur on the underside of the prothallium. However, a prothallium rarely fertilizes itself because the antheridia and archegonia mature at different times. Globe-shaped antheridia form first, followed by archegonia. Archegonia are vase-shaped and are located near the cleft of the heart-shaped prothallium. After producing sperm, antheridia drop off, leaving sperm to swim to the archegonia of the other prothallia.

Procedure 29.2

Observe archegonia and antheridia

1. Observe archegonia and antheridia (see fig. 29.1) on prepared slides.
2. Observe archegonia and antheridia on a living prothallium.

Question 6

a. What is the adaptive significance of having these structures on the lower surface of the prothallium rather than on the upper surface?

b. What is the adaptive significance of having sperm and egg produced at different times?

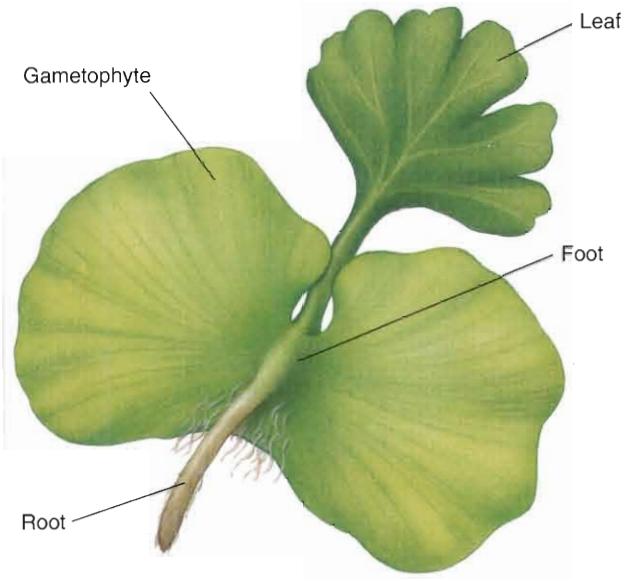


Figure 29.4
Young fern sporophyte growing out of its gametophyte parent. Shortly after this stage, the gametophyte dies and begins to decompose.

The zygote develops in the archegonium and is nutritionally dependent on the gametophyte for a short time (fig. 29.4). Soon thereafter, the sporophyte becomes leaflike and crushes the prothallium. Fronds of the growing sporophyte break through the soil in a coiled position called a **fiddlehead** (fig. 29.5). The fiddlehead then unrolls to display the frond, a single leaf. Fiddleheads are considered a culinary delicacy in some parts of the world.

Most terrestrial ferns are **homosporous;** they produce one kind of spore that develops into a single kind of gametophyte that produces both antheridia and archegonia (see fig. 29.1). Conversely, aquatic ferns such as *Salvinia* and *Azolla* are **heterosporous**, meaning that they produce two types of spores: **megaspores** and **microspores**. You will learn more about heterospory in the next exercise. A megaspore forms a gametophyte with only archegonia, and a microspore forms a gametophyte with only antheridia. Examine the living *Salvinia* and *Azolla* in the lab (fig. 29.6).

Question 7
How do *Salvinia* and *Azolla* differ from other ferns you examined earlier?

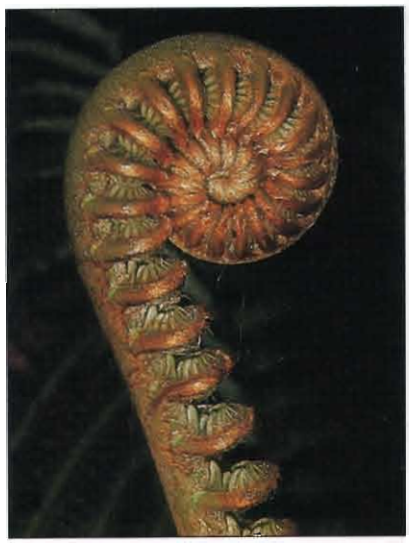


Figure 29.5
A fiddlehead of a tropical tree fern.

(a)

(b)

Figure 29.6
(*a*) *Salvinia* and (*b*) *Azolla* are ferns that grow in aquatic habitats.

WHISK FERNS

Whisk ferns (phylum Pterophyta) include only two extant representatives: *Psilotum* (fig. 29.7) and *Tmesipteris*. *Psilotum* has a widespread distribution, whereas *Tmesipteris* is restricted to the South Pacific. *Psilotum* lacks leaves and roots and is homosporous.

Procedure 29.3

Examine *Psilotum*

1. Examine a prepared slide of a *Psilotum* sporangium (fig. 29.8).
2. Study living *Psilotum* plants in the lab.

Figure 29.7

Whisk ferns (*Psilotum* sp.) are so called because their branching pattern gives the impression of a whisk broom. The stems bear lobed sporangia (fig. 29.8).

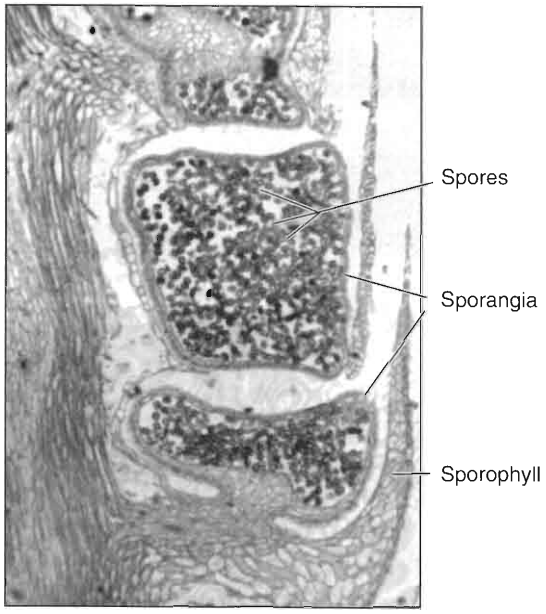


Figure 29.8

Psilotum sporangium.

Question 8

a. How would you describe the branching pattern of *Psilotum*?

b. Are any roots present?

c. Are any leaves present?

d. Where are the sporangia?

e. Where does photosynthesis occur in *Psilotum*?

HORSETAILS

Equisetum (also called scouring rush) is the only extant genus of horsetails (phylum Pterophyta) (fig. 29.9). *Equisetum* is an example of a plant whose vegetative structure identifies the plant better than does its reproductive struc-

Figure 29.9

Scouring rush, *Equisetum telmateia*, of the phylum Pterophyta. This species forms two types of erect stems, a green, photosynthetic type and a brownish type terminating in spore-producing cones. The spores produced by meiosis in the cones give rise to a single kind of tiny, green, nutritionally independent gametophyte.

ture: *Equisetum* is distinguished by its jointed and ribbed stem. Examine the living *Equisetum* plants.

Question 9

a. Where are the leaves?

b. What part of the plant is photosynthetic?

c. Which part of the life cycle of *Equisetum* is dominant, the sporophyte or gametophyte?

Feel the ribbed stem of an *Equisetum* plant. Its rough texture results from siliceous deposits in its epidermal cells. During frontier times, *Equisetum* was used to clean pots and pans, sand wooden floors, and scour plowshares, thus accounting for its common name of "scouring rush."

Strobili of *Equisetum* occur at the tips of reproductive stems. Within a strobilus, sporangia form atop umbrellalike modified branches called **sporangiophores**. Elaters in sporangia of *Equisetum* help disperse spores. Examine prepared slides of *Equisetum* strobili (fig. 29.10). Diagram, label, and state the function of each major structure composing the strobilus.

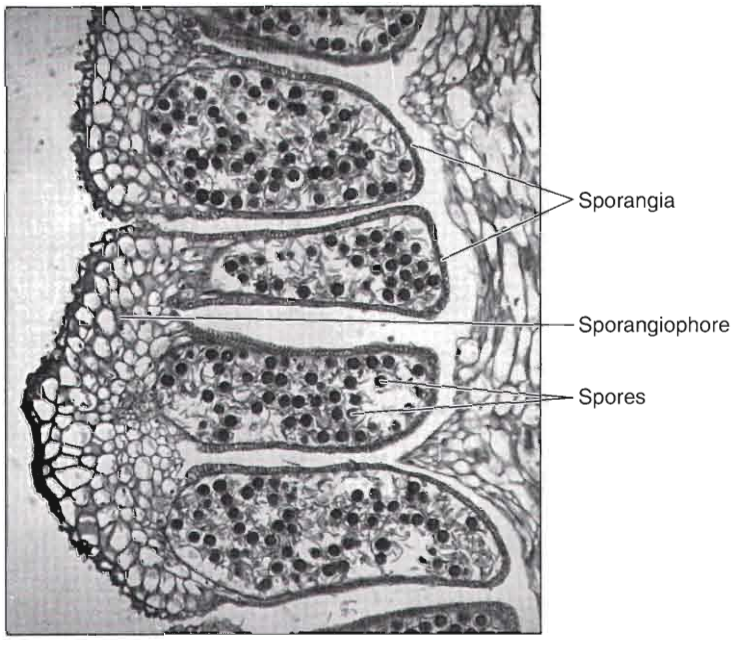
Question 10

How do elaters aid in the dispersal of spores?

PHYLUM LYCOPHYTA
CLUB MOSSES

During the Devonian and Carboniferous periods (300–400 million years ago), club mosses, whisk ferns, and scouring rush were among the dominant plants on earth. Indeed, most of our coal deposits consist largely of these plants. However, the "giant" species of these phyla are now extinct, and modern representatives are relatively small compared to their giant ancestors.

(a)



(b)

Figure 29.10

Equisetum. (*a*) Strobili occur at the tips of reproductive stems. (*b*) Cross section of a strobilus showing spores within sporangia at the tips of sporangiophores.

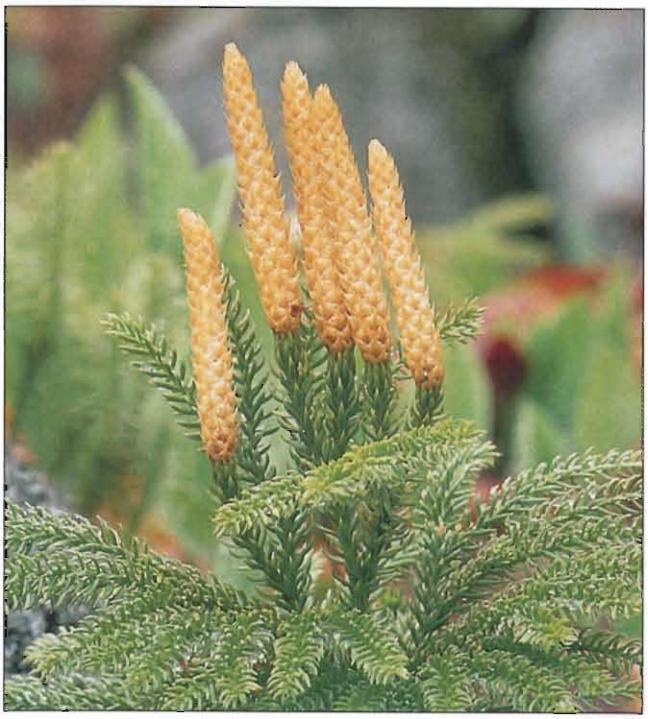


Figure 29.11

Strobili, or cones, are aggregations of closely packed sporangium-bearing branches or leaves. Shown here are strobili of *Lycopodium obscurum*, a club moss.

Club mosses (phylum Lycophyta) possess true roots, stems, and leaves. Most asexual reproduction by club mosses occurs via rhizomes. If one is available, locate the rhizome on a *Lycopodium*, a club moss. Study the aboveground portion of the *Lycopodium* plant (fig. 29.11). *Lycopodium* is evergreen, as are some ferns, most gymnosperms, and some angiosperms.

Question 11

a. How could a rhizome be involved in asexual reproduction?

b. How is a rhizome different from a rhizoid?

c. Does the rhizome have leaves?

d. What is the shape and size of the leaves?

e. What is the significance of the form of the leaves?

f. Is a midvein visible?

g. What does the term "evergreen" mean?

h. Is being evergreen a good characteristic for classifying plants? Why or why not?

Sporangia of *Lycopodium* occur on small modified leaves called **sporophylls** clustered in **strobili** (cones) that form at the tips of branches. Species with these cones probably shared common ancestry with the familiar cone-bearing gymnosperms such as pine (*Pinus*) (see Exercise 30).

Procedure 29.4

Examine club mosses

1. Examine strobili on a living *Lycopodium* plant. Also examine prepared slides of strobili of *Lycopodium* (fig. 29.12).
2. Diagram, label, and state the function of each major feature of the strobilus.

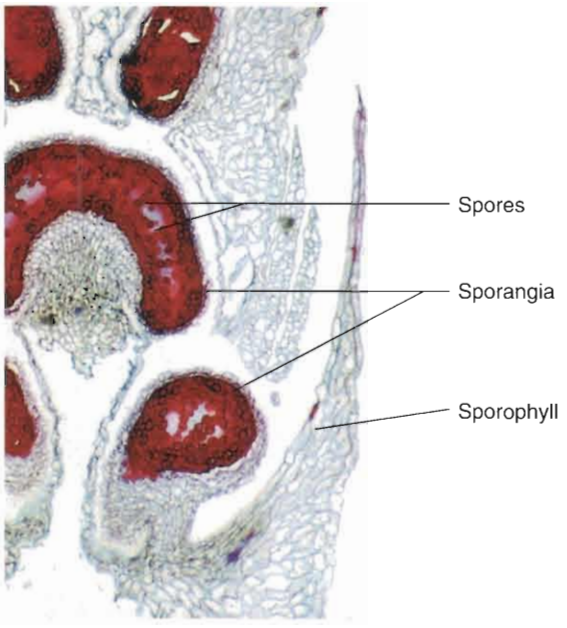


Figure 29.12
Sporophyll and sporangium of *Lycopodium*, a club moss.

3. Examine prepared slides of spores of *Lycopodium* as well as those available on plants growing in the lab.

Question 12

a. How many sporangia occur on each sporophyll?

b. Can you see why spores of *Lycopodium* are sometimes called "vegetable sulfur"?

c. Why are the spores a good dry lubricant?

d. Which is the dominant part of the *Lycopodium* life cycle, the sporophyte or gametophyte?

4. Examine living *Selaginella* plants (fig. 29.13). Many species of *Selaginella* produce two types of strobili, typically red and yellow. If strobili are present, examine spores derived from these cones.
5. Examine hydrated and dehydrated resurrection plants (*Selaginella lepidophylla*).

Figure 29.13
Selaginella, a club moss.

Question 13

a. Are spores of *Selaginella* similar in size?

b. What is this condition called?

c. What is the functional significance of the difference in the appearance of dehydrated and rehydrated *Selaginella*?

d. Can you see why these plants are sometimes referred to as "resurrection plants"?

e. How does the formation of strobili in *Equisetum* compare with that in *Lycopodium* and *Selaginella*?

Procedure 29.5

Examine *Isoetes*

1. Examine living *Isoetes* (quillwort) plants (fig. 29.14).
2. Compare and contrast the following features of *Isoetes* with *Lycopodium* and *Selaginella*:
   - Shape of aerial portion of plant
   - Branching patterns
   - Shape, size, and arrangement of leaves

At the branching points along the stem of *Isoetes*, you'll see an unusual runnerlike organ. These proplike axes are called **rhizophores** and have structural features that are intermediate between stems and roots.

To review the structures and characteristics of seedless vascular plants, complete table 29.2 on the next page.

Figure 29.14
Quillworts (*Isoetes*) are so-named because of their narrow, quill-like leaves. Most quillworts live in aquatic environments.

INVESTIGATION

The "Resurrection" of a "Resurrection Plant"

Observation: The "resurrection" of a "resurrection plant" (*Selaginella*) involves the rapid absorption of water. This, in turn, triggers other changes in the plant that "revive" the plant.

Question: What changes accompany the "resurrection" of a "resurrection plant"?

a. Establish a working lab group and obtain Investigation Worksheet 29 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 29 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

TABLE 29.2

SUMMARY OF STRUCTURES COMMON TO SEEDLESS VASCULAR PLANTS

Plant Structure	Sporophyte or Gametophyte	Function
Prothallium		
Pinna		
Spore		
Frond		
Annulus		
Sporangium		
Antheridium		
Archegonium		
Microspore		
Megaspore		
Microphyll		
Megaphyll		

Questions for Further Thought and Study

1. Compare ferns and bryophytes. What structures and features do ferns possess that bryophytes do not that may have contributed to their success in a broader range of environments?

2. What are the advantages of vascular tissues in land plants?

3. What are the distinguishing features of club mosses, whisk ferns, and horsetails? How are these plants different from ferns?

WRITING TO LEARN BIOLOGY

What problems did plants face as they moved onto land? What adaptations of mosses, liverworts, ferns, and other seedless plants are relevant to the transition?

30

Survey of the Plant Kingdom

Gymnosperms of Phyla Cycadophyta, Ginkgophyta, Coniferophyta, and Gnetophyta

Objectives

By the end of this exercise you should be able to:

1. Describe the distinguishing features of the gymnosperms.
2. Understand the life cycle of pine, a representative gymnosperm.
3. Understand the evolutionary significance of pollen and seeds.
4. Identify the parts and understand the function of a cone.
5. Identify the parts and understand the function of a seed.

Gymnosperms are plants with exposed seeds borne on scalelike structures called **cones** (strobili). Like ferns, gymnosperms have a well-developed alternation of generations. Unlike most ferns, however, gymnosperms are **heterosporous**; that is, they produce two types of spores (fig. 30.1). **Microspores** occur in male cones and form male gametophytes. **Megaspores** occur in female cones and form female gametophytes. Gametophytes of gymnosperms are microscopic and completely dependent on the large, free-living sporophyte.

In gymnosperms, pollination is the transfer of pollen from male cones (where the pollen is produced) to female cones, which house the eggs. In these plants, pollen is carried from male cones to female cones by wind.

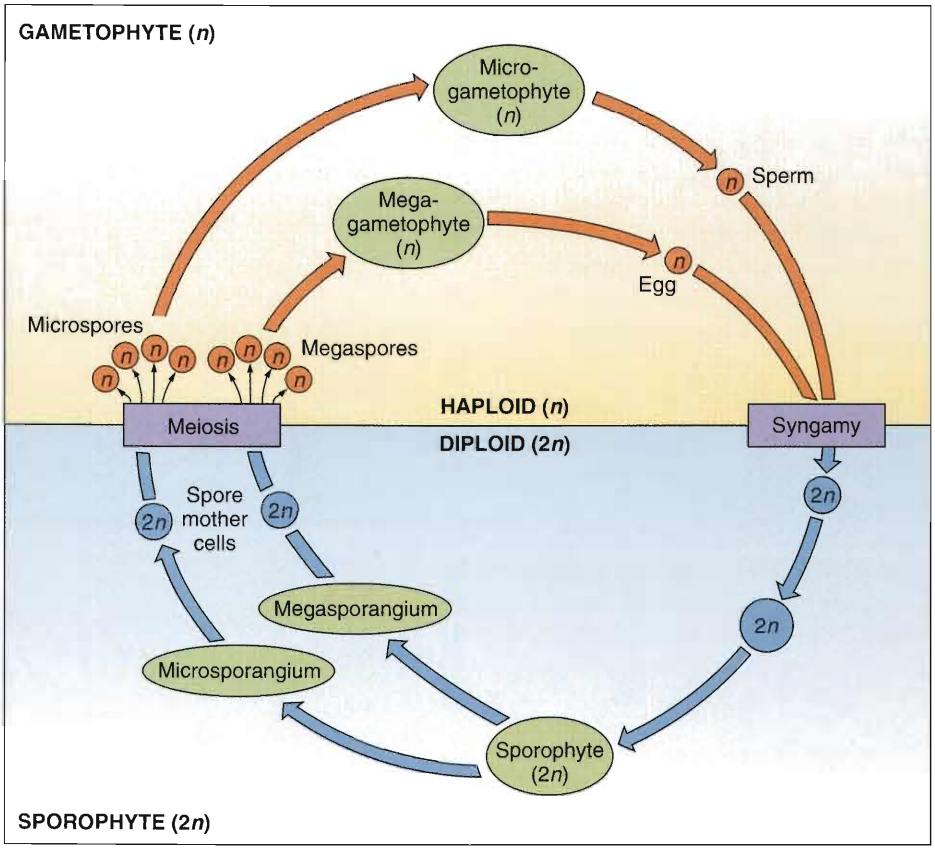


Figure 30.1
Diagram of the life cycle of a heterosporous vascular plant.

TABLE 30.1

THE FIVE PHYLA OF EXTANT GYMNOSPERMS

Phylum	Examples		Key Characteristics	Approximate Number of Living Species
Cycadophyta	Cycads		Heterosporous vascular seed plants. Sperm flagellated and motile but confined within a pollen tube that grows to the vicinity of the egg. Palmlike plants with pinnate leaves. Secondary growth slow compared with that of the conifers. Ten genera.	206
Ginkgophyta	*Ginkgo*		Heterosporous vascular seed plants. Sperm flagellated and motile but conducted to the vicinity of the egg by a pollen tube. Deciduous tree with fan-shaped leaves that have evenly forking veins. Seeds resemble a small plum with fleshy, ill-scented outer covering.	1
Coniferophyta	Conifers (including pines, spruces, firs, yews, redwoods, and others)		Heterosporous seed plants. Sperm not motile; conducted to egg by a pollen tube. Leaves mostly needlelike or scalelike. Vascular. Trees, shrubs. About 50 genera.	601
Gnetophyta	Gnetophytes		Heterosporous vascular seed plants. Sperm not motile; conducted to egg by a pollen tube. The only gymnosperms with vessels. Trees, shrubs, vines. Three very diverse genera (*Ephedra, Gnetum, Welwitschia*).	71

Gymnosperms were the first plants to evolve that did not need free water to transform sperm to egg, and were therefore able to colonize terrestrial habitats.

Gymnosperms include four phyla: Cycadophyta, Ginkgophyta, Coniferophyta, and Gnetophyta (table 30.1). The last of these, the Gnetophyta, is discussed only briefly here because the phylum consists of a few rare genera.

PHYLUM CYCADOPHYTA

The Cycadophyta (cycads) once flourished, but today the phylum consists of only about 10 genera and 200 species. Cycads resemble palms because they have unbranched trunks and large, closely packed leaves that are evergreen and tough (fig. 30.2). Sperm of cycads are flagellated. Examine a branch of a cycad such as *Zamia* bearing developing seeds. The seeds are fleshy and exposed to the environment.

Question 1

a. Based on your knowledge of reproduction in bryophytes (Exercise 28) and ferns (Exercise 29), is the presence of flagellated sperm in cycads surprising? Why or why not?

b. Is the possession of flagellated sperm a primitive or advanced characteristic in the plant kingdom? Why?

PHYLUM GINKGOPHYTA

The Ginkgophyta consists of one species, *Ginkgo biloba* (Maidenhair tree), a large dioecious tree that does not bear cones. *Ginkgo* are hardy plants in urban environments and tolerate insects, fungi, and pollutants. Males are usually planted because females produce fleshy, smelly, and messy fruit that superficially resembles cherries. Leaves of *Ginkgo* have a unique shape (fig. 30.3). *Ginkgo* has not been found in the wild and would probably be extinct but for its cultivation in ancient Chinese gardens.

Question 2

What does dioecious mean?

(a)

(b)

Figure 30.2

Cycads. (*a*) A male cycad with a strobilus. (*b*) A female cycad with strobili. *Zamia pumila* is the only species of cycad native to the United States. The starchy roots and stems (mostly underground) were used by Native Americans for food.

Figure 30.3

Maidenhair tree, *Ginkgo biloba*, the only living representative of the phylum Ginkgophyta, a group of plants abundant 200 million years ago. Among living seed plants, only the cycads and *Ginkgo* have swimming sperm. This photograph shows *Ginkgo* leaves and fleshy seeds.

PHYLUM CONIFEROPHYTA

The Coniferophyta are a large group of cone-bearing plants that includes the 5000-year-old bristlecone pines, the earth's oldest living individual organisms. The cones they bear are reproductive structures of the sporophyte generation that consist of several scalelike sporophylls arranged about a central axis (fig. 30.4). Sporophylls, also present in ferns and their allies, are modified leaves specialized for reproduction.

(a)

(b)

Figure 30.4

Pine cones. (*a*) First-year ovulate (seed) cones open for pollination. (*b*) Second-year ovulate pine cones at time of fertilization.

Figure 30.5
Pollen-bearing cones of *Pinus*. These cones are usually found on the lower branches of pine trees.

Sporophylls bear spores. In conifers, sporophylls of male cones are called **microsporophylls**. On the surface of each microsporophyll is a layer of cells called a **microsporangium** that produces spores. Male cones live only a few weeks; after these cones release their pollen, they fall off the tree. Sporophylls of female cones are **megasporophylls**; each bears two spore-producing **megasporangia** on its upper surface. Microsporangia and megasporangia are patches of cells near the central axis of the sporophylls composing the cones on the respective sporophylls. Male cones are small and similar in all conifers (fig. 30.5). However, female cones are variable; they may be small (1–3 cm in *Podocarpus*) and fleshy (a *Juniperus* "berry") or large and woody (6–40 cm in *Pinus*).

In this exercise you'll study pine (*Pinus*), a representative and widely distributed conifer. Pine has considerable economic value because it is used to produce lumber, wood pulp, pine tar, resin, and turpentine. Other examples of conifers include spruce, cedar, and fir.

The Pine Sporophyte

The life cycle of *Pinus* is typical of conifers (fig. 30.6). You are already familiar with the sporophyte of pine—it is the tree.

Procedure 30.1

Examine pine twigs and leaves

1. Examine pine twigs having leaves (needles) and a terminal bud. Notice that the leaves are borne on short branches only a few millimeters long. The length and number of leaves distinguish many of the species of *Pinus*.
2. Examine a prepared slide of a cross section of a pine leaf; locate the structures labeled in figure 30.7.

Question 3

a. Where on the branch was the terminal bud last year?

b. How can you tell?

c. How are needles or leaves arranged?

d. How many leaves are in a bundle?

e. How are pine leaves different from those of broad-leaved trees such as maples and oaks?

f. Why are pines called evergreens?

g. What other plants have you studied in the lab that are evergreen?

h. What is the function of each of the structures labeled in figure 30.7?

i. How do the structural features of pine leaves adapt the tree for life in dry environments?

Male cones usually form on the lower branches of pine trees, and female cones usually form on the upper branches. Examine pine branches with staminate (male) and ovulate (female) cones. Examine a prepared slide of a young staminate cone and note the pine pollen in various stages of development. Each scale (microsporophyll) of the male cone

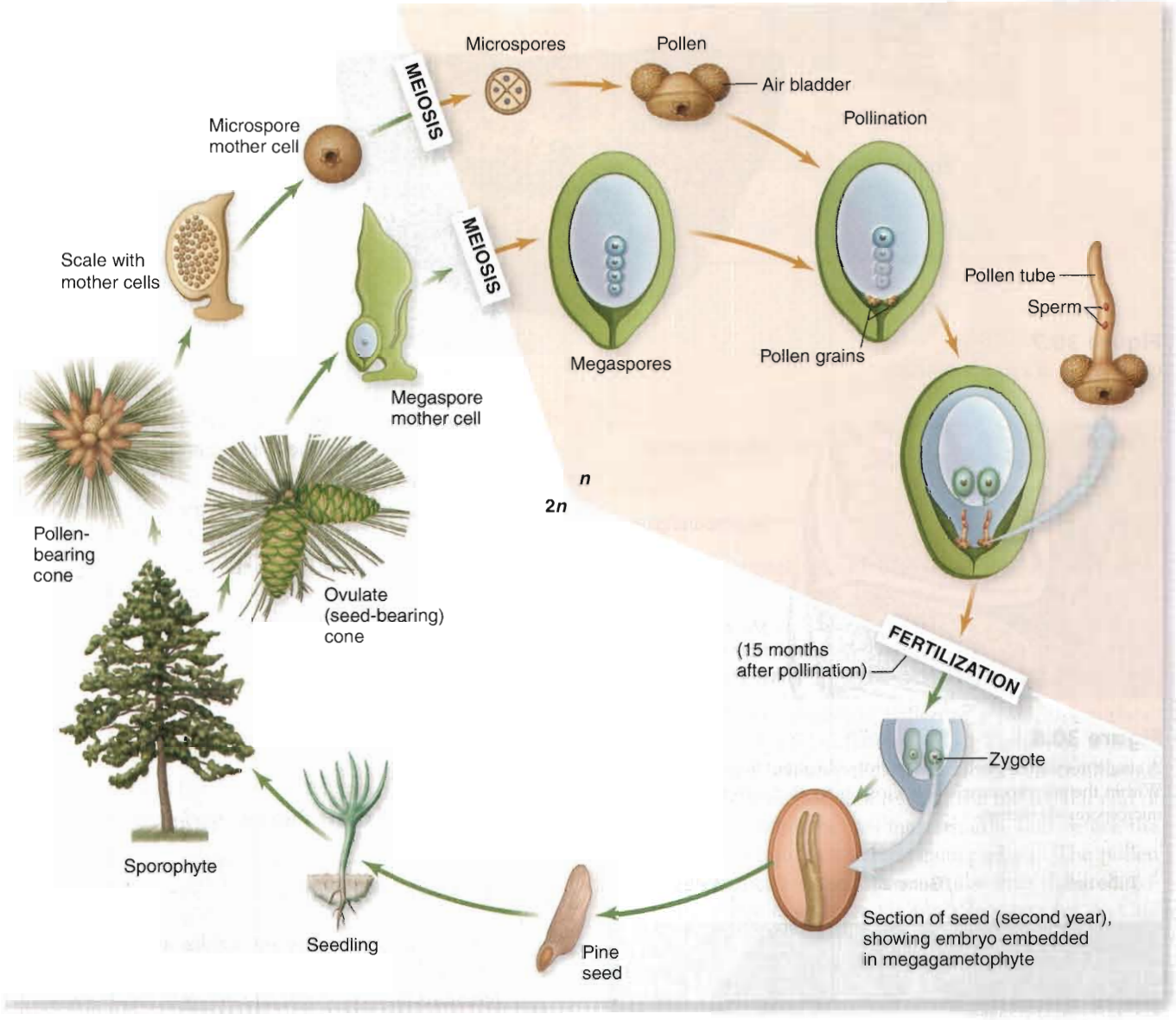


Figure 30.6

Pine life cycle. In seed plants, the gametophyte generation is greatly reduced. A germinating pollen grain is the mature microgametophyte of a pine. Pine microsporangia are borne in pairs on the scales of the delicate pollen-bearing cones. Megagametophytes, in contrast, develop within the ovule. The familiar seed-bearing cones of pines are much heavier than the pollen-bearing cones. Two ovules, and ultimately two seeds, are borne on the upper surface of each scale of a cone. In the spring, when the seed-bearing cones are small and young, their scales are slightly separated. Drops of sticky fluid, to which the airborne pollen grains adhere, form between these scales. Pollination occurs more than a year before the ovule produces a mature female gametophyte. These pollen grains germinate, and slender pollen tubes grow slowly toward the egg. When a pollen tube grows to the vicinity of the megagametophyte, sperm are released, fertilizing the egg and producing a zygote there. The development of the zygote into an embryo occurs within the ovule, which matures into a seed. Seeds mature up to 6 months after fertilization. Eventually, the seed falls from the cone and germinates, the embryo resuming growth and becoming a new pine tree.

bears a microsporangium, which in turn produces diploid **microspore mother cells** (fig. 30.8). Microspore mother cells undergo meiosis to produce **microspores** that develop via mitotic divisions into microgametophytes called pollen grains (fig. 30.9). Each **pollen grain** consists of four nuclei and a pair of bladderlike wings. The gametophytes of gymnosperms are reduced to only a few cells.

Procedure 30.2

Examine staminate pine cones and pollen

1. Prepare a wet mount of some pine pollen; notice their characteristic shape.
2. Remove a scale from a mature staminate cone and tease open the microsporangium.

seed plants in today's environment, because a seed permits a small but multicellular sporophyte to remain dormant until conditions are favorable for continued growth. While dormant, the young sporophyte is protected by a seed coat and surrounded by a food supply.

Refer to the pine life cycle shown in figure 30.6 to answer Question 6. If plant material and time are available, examine other gymnosperms in the lab.

Question 6

a. Where are spores located?

b. What is the male gametophyte?

c. What is the female gametophyte?

d. What is an ovule?

e. What is an integument?

f. What is the function of the winglike extensions of a pine seed?

g. How are other gymnosperms similar to pine?

h. How are they different?

i. What evolutionary advantages might arise from not needing free water for fertilization?

PHYLUM GNETOPHYTA

The gnetophytes (71 species in three genera) include some of the most distinctive (if not bizarre) of all seed plants (fig. 30.11). They have many similarities with angiosperms, such as flowerlike compound strobili, vessels in the secondary xylem, loss of archegonia, and double fertilization. The slow-growing *Welwitschia* plants are extraordinary in appearance and live only in the deserts of southwestern Africa. Most of their moisture is derived from fog that rolls in from the ocean at night. *Welwitschia* stems are short and broad (1 m). Mature, 100-year-old plants have only two leaves that are wide and straplike.

(a)

(b)

(c)

Figure 30.11

Gnetophytes. (*a*) Leaves and immature seeds of *Gnetum*. *Gnetum* grows as a shrub or woody vine in tropical or subtropical forests. (*b*) *Welwitschia* plants in the Namib Desert of southwest Africa. (*c*) *Ephedra*, the only living genus of Gnetophyta found in the United States, is a common dietary supplement.

INVESTIGATION

Release of Pollen from Pinecones

Observation: Male pinecones produce and release large amounts of pollen. In some people this pollen contributes to hay fever and other sinus-related problems.

Question: How much pollen is released by a male pinecone?

a. Establish a working lab group and obtain Investigation Worksheet 30 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 30 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What is the difference between pollination and fertilization?

2. What does the term *gymnosperm* mean? Why is this term an appropriate description?

3. How is alternation of generations different in ferns and pines?

4. How are the environmental agents for uniting sperm and egg different in pines and bryophytes?

5. Compare and contrast alternation of generations in mosses, ferns, and pines.

6. What is the evolutionary significance of pollen and seeds?

WRITING TO LEARN BIOLOGY

Describe the ecological advantages that the production of seeds has over the reproductive process in ferns.

Survey of the Plant Kingdom
Angiosperms

31

Objectives

By the end of this exercise you should be able to:

1. Relate the life cycle of angiosperms to the other phyla of the plant kingdom.
2. Discuss the events associated with development of microspores, megaspores, gametophytes, gametes, seeds, and fruit.
3. List and discuss the reasons why angiosperms are considered to be the most advanced land plants.

Flowering plants (phylum Anthophyta—also referred to as angiosperms) are the most abundant, diverse, and widespread of all land plants. They owe their success to several factors, including their structural diversity, efficient vascular systems, and mutualisms with fungi and insects (fig. 31.1).

PHYLUM ANTHOPHYTA

Angiosperms range in size from 1 mm (*Wolffia*) to over 100 m tall (*Eucalyptus*). As in gymnosperms, the sporophyte of angiosperms is large and heterosporous. The microgametophyte (pollen) and megagametophyte (embryo sac) are completely dependent on the sporophyte. Angiosperms are important to humans because our world economy is overwhelmingly based on them. We eat and use **vegetative structures** (roots, stems, leaves) as well as **reproductive structures** (flowers, fruits, seeds) of angiosperms.

Botanists commonly divide angiosperms into monocots and dicots (fig. 31.2). The "typical" features of these groups of plants are described in table 31.1.

Having studied gymnosperms, you probably concluded that vegetative adaptations of angiosperms and gymnosperms are similar. This is true. However, in today's exercise you will study two unique adaptations of angiosperm reproduction: flowers and fruit.

Figure 31.1
This bee is pollinating a desert poppy in eastern Arizona. The evolution of flowers and the success of flowering plants correlate with the development of mutualisms with insects and other pollinators.

Figure 31.2
In monocots such as this lily (*Lilium longiflorum*), flower parts usually occur in multiples of three. A lily has six stamens, three petals, three petal-like sepals, and a three-chambered ovary.

TABLE 31.1

CHARACTERISTICS OF THE TWO CLASSES OF ANGIOSPERMS

Monocotyledons	Dicotyledons
1. One cotyledon per embryo	1. Two cotyledons per embryo
2. Flower parts in sets of three	2. Flower parts in sets of four or five
3. Parallel venation in leaves	3. Reticulate (i.e., netted) venation in leaves
4. Multiple rings of vascular bundles in stem	4. One ring of vascular bundles or cylinder of vascular tissue in stem
5. Lack a true vascular cambium (lateral meristem)	5. Have a true vascular cambium (lateral meristem)
6. Fibrous root system	6. Tap root system

STRUCTURE AND FUNCTION OF FLOWERS

Luckily for florists, angiosperms have a seemingly infinite variety of flowers, ranging from the microscopic flowers of *Lemna* (duckweed) to the rare, monstrous blossoms of *Rafflesia*, which measure up to 1 m across. In today's exercise, we'll consider only the "typical" flower depicted in figure 31.3.

Examine the flower model(s) available in the lab and the living flowers on display and identify the following parts:

- **Peduncle**—flower stalk.
- **Receptacle**—the part of the flower stalk that bears the floral organs; located at the base of the flower; usually not large or noticeable.
- **Sepals**—the lowermost or outermost whorls of structures, which are usually leaflike and protect the developing flower; the sepals collectively constitute the **calyx**.
- **Petals**—whorls of structures located inside and usually above the sepals; may be large and pigmented (in insect-pollinated flowers) or inconspicuous (in wind-pollinated plants); the petals collectively constitute the corolla.

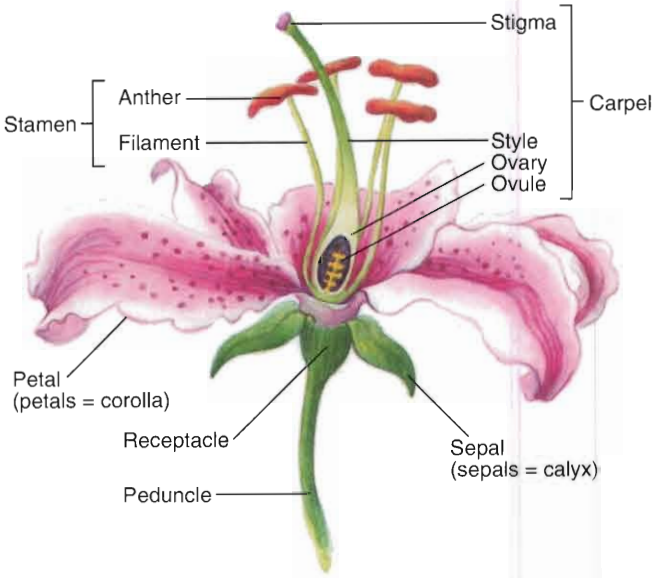


Figure 31.3
The parts of a flower. This is a generalized flower with four primary parts: sepals, petals, stamens, and carpels. The carpels and stamens are the fertile parts of a flower. The roles of carpels and stamens in the life cycle of angiosperms are shown in figure 31.4.

- **Androecium**—the male portion of the plant that rises above and inside the petals; consists of stamens, each of which consists of a **filament** atop which is located an **anther**; inside the anthers are **pollen grains**, which are the microgametophytes and contain the male gametes.
- **Gynoecium**—the female portion of the plant that rises above and inside the androecium; consists of one or

more **carpels**, each made up of an **ovary**, **style**, and **stigma**; the ovary contains **ovules** that contain the megagametophyte; the megagametophyte is called the embryo sac and contains female gametes. During pollination, pollen grains are transferred to the stigma, where they germinate and grow a tube through the style to the ovary.

The sepals and petals are usually the most conspicuous parts of a flower, and a variety of flower types are described by their characteristics. In **regular** flowers such as tulips, the members of the different whorls of the flower consist of similarly shaped parts that radiate from the center of the flower and are equidistant from each other. The flowers are **radially symmetrical**. In other flowers such as snapdragons, one or more parts of at least one whorl are different from other parts of the same whorl. These flowers are generally bilaterally symmetrical and are said to be **irregular**.

Procedure 31.1

Examine flowers and their parts

1. Obtain a flower provided in the laboratory.
2. Remove the parts of the flower, beginning at the lower, outside rosettes of sepals and petals.
3. Locate the petals and sepals and determine their arrangement and point of attachment.

Question 1
How would you describe this flower?

4. Remove the petals and sepals.
5. Locate and remove a stamen and place it on a slide. Examine the stamen with low magnification.
6. Add a drop of water to the preparation and open the anther (if necessary) to disperse pollen. Cover with a coverslip.
7. Use high magnification to locate the generative and tube nucleus of a pollen grain. The **generative nucleus** is usually small, spindle-shaped, and off center (you cannot see it easily). The **tube nucleus** is larger and centered. You'll learn more about pollen nuclei later in this exercise.
8. To enhance visibility of the nuclei, stain them with acetocarmine. Add one or two drops to the preparation and gently heat it. Do not overheat. Re-examine the preparation.
9. Locate the gynoecium of the flower, and make longitudinal and transverse sections. The gynoecium consists of one or more fused carpels, each with an interior cavity called a locule containing ovules.

Question 2

a. How many carpels (locules) are apparent?

b. How many ovules are developing in each locule?

10. Examine the other types of flowers available in the lab. Repeat procedure 31.1 to guide your examination. Preserved specimens of *Lilium* (lily) and *Ranunculus* (buttercup) may also be provided for you to study and dissect.

Alternation of Generations in Flowering Plants

The life cycle of flowering plants involves the alternation of a multicellular haploid stage with a multicellular diploid stage as is typical for all plants (see fig. 30.1, fig. 31.4). The diploid **sporophyte** produces haploid spores by meiosis. Each haploid spore develops into the **gametophyte** by mitosis and cellular differentiation. In angiosperms the sporophyte is the large, mature organism with flowers that you easily recognize. The gametophyte within the flower is reduced to a pollen grain (that contains a sperm nucleus) or an embryo sac (that produces an egg) within an ovule. Be sure to review your textbook's description of alternation of generations.

Production of spores in the sporophyte by meiosis is part of a larger process called **sporogenesis**. Flowering plants produce two types of spores: **microspores** and **megaspores**. Production of gametes by the gametophytes is **gametogenesis**.

Microsporogenesis, Production of Pollen, and Microgametogenesis

Microsporogenesis is the production of microspores within **microsporangia** of a flower's anthers via meiosis of **microspore mother cells** (microsporocytes) (fig. 31.5). These microspores grow and mature into microgametophytes, also known as pollen grains (fig. 31.6). The haploid nuclei in a mature pollen grain include a **tube nucleus** (or vegetative nucleus) and a **generative nucleus**. The pollen grain will germinate when it lands on a flower stigma, and the tube nucleus will control the growth of the pollen tube. The generative nucleus will replicate to produce two **sperm nuclei**.

Pollen of some plants cause allergies in many people. However, studies of pollen are important to science beyond the treatment of allergies. For example, geologists examine pollen brought up in sediment cores during oil drilling. Dark brown to black pollen indicate temperatures too high for oil deposits and indicate that a well will likely produce natural

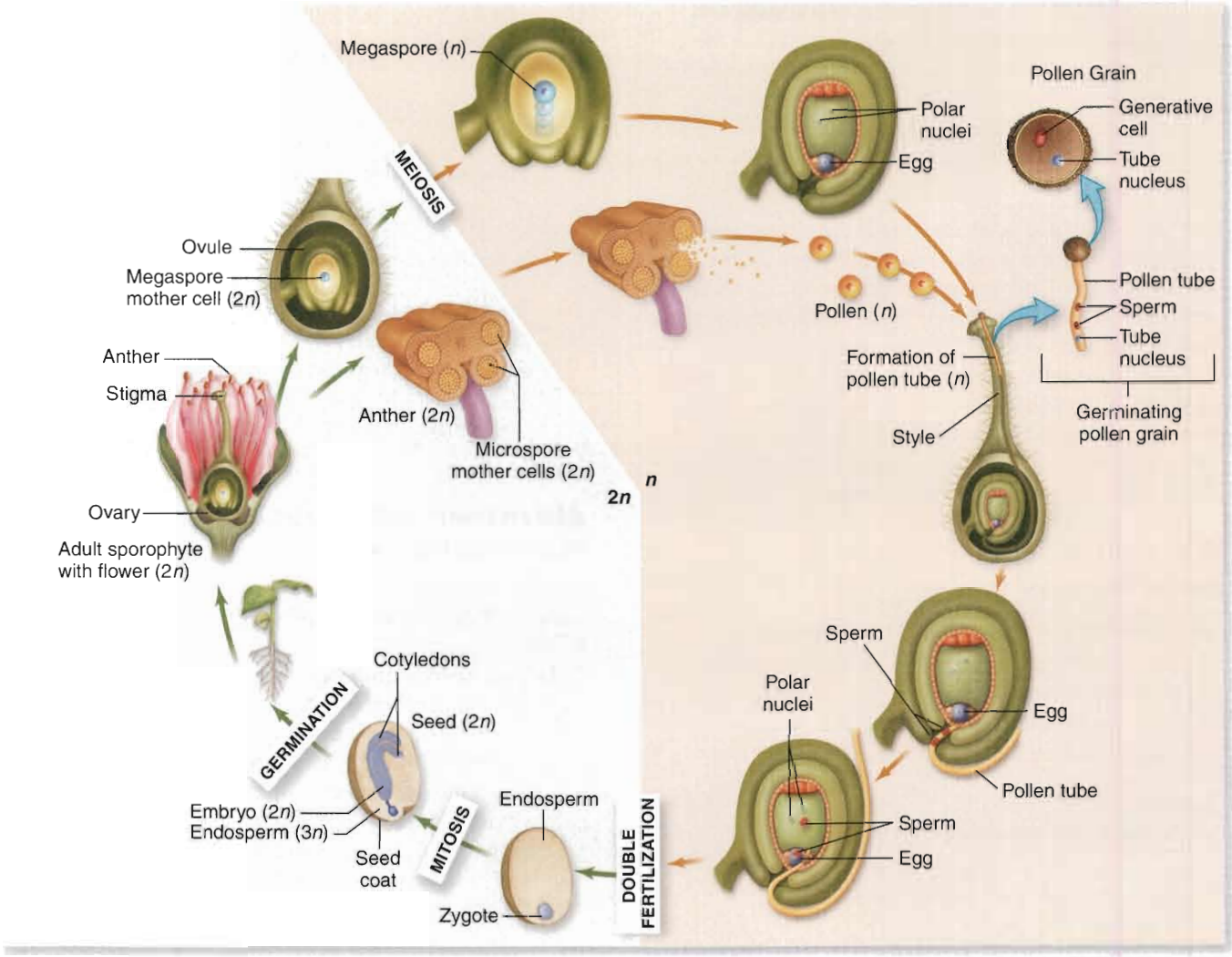


Figure 31.4

Angiosperm life cycle. Eggs form within the embryo sac inside the ovules, which, in turn, are enclosed in the carpels. The pollen grains, meanwhile, form within the sporangia of the anthers and are shed. Fertilization is a double process. A sperm and an egg come together, producing a zygote; at the same time, another sperm fuses with the polar nuclei to produce the endosperm. The endosperm is the tissue, unique to angiosperms, that nourishes the embryo and young plant.

gas. Orange pollen indicate the less intense heat associated with high-quality oil production. In addition, examination of fossil pollen tells us about the diversity of ancient flora and climatic change through the ages, and helps us locate ancient seas and their shorelines where pollen is known to accumulate.

Procedure 31.2

Examine stages of microsporogenesis and microgametogenesis in *Lilium*

1. Examine fresh or preserved specimens of dehiscent (split-open) and predehiscent anthers (fig. 31.7).

Question 3

a. What differences can you detect between the structures of dehiscent and predehiscent anthers?

b. Which stage is the most mature?

2. Examine a prepared cross section of a young anther. Note the immature sporangia tissue that will form microsporocytes.
3. Examine a prepared cross section of a lily anther showing microsporocytes in early prophase I (fig. 31.8*a*).
4. Examine a prepared slide showing stages of meiosis II (fig. 31.8*b*).
5. Examine a prepared slide showing pollen tetrads of microspores produced by meiosis (fig. 31.8*c*).

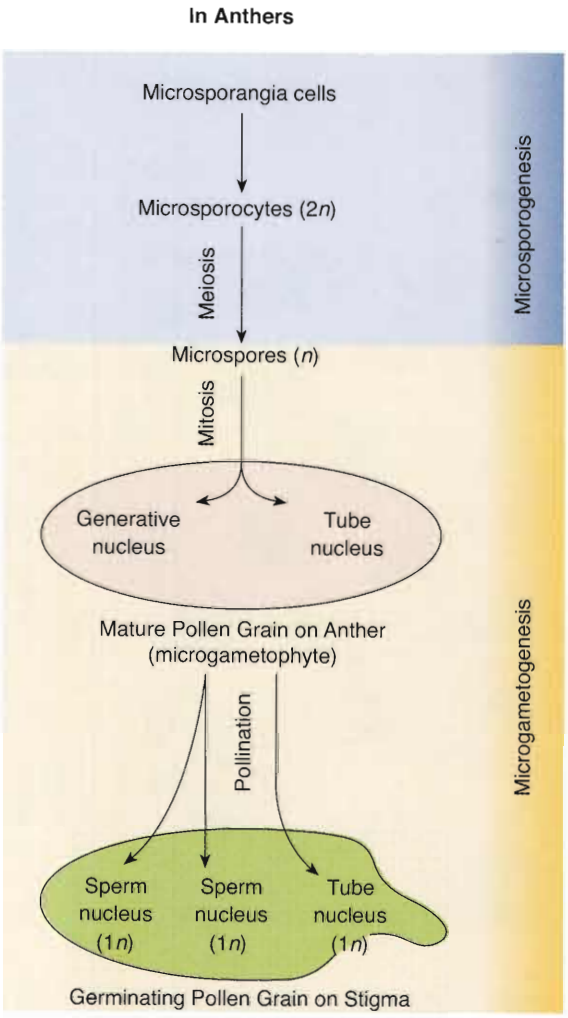


Figure 31.5
Microsporogenesis and microgametogenesis in the anthers of flowers. Mature pollen grains (see fig. 31.6) are microgametophytes. Micrographs of microsporogenesis and microgametogenesis are shown in figure 31.8.

6. Examine a prepared slide showing mature pollen with two or more nuclei (fig. 31.8*d*).
7. Examine living or prepared pollen from various plants if available. Note any differences among pollen grains and differences between pollen of monocots and dicots.

Procedure 31.3

Observe germination of pollen grains

NOTE

Start this experiment at the beginning of the lab period so that the pollen grains will germinate before the lab is over.

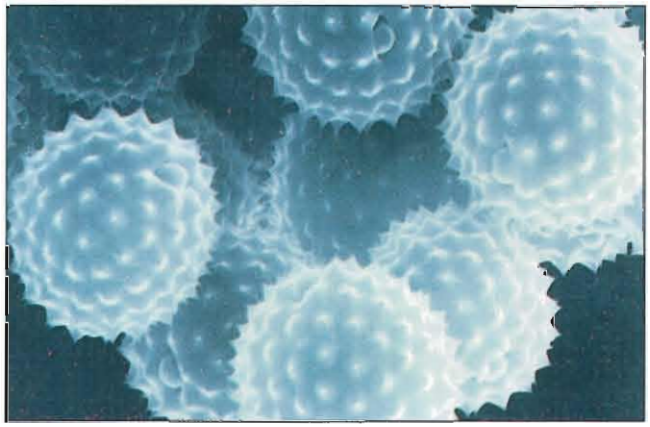


Figure 31.6
Scanning electron micrograph of pollen grains. Pollen grains of ragweed are spherical and elaborately sculptured. Their outer wall contains proteins that regulate germination of the pollen grain when it lands on a stigma of a ragweed flower. These same proteins also cause allergies in many people.

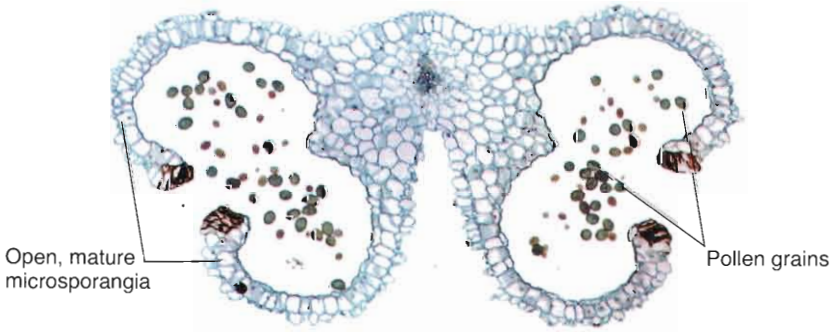


Figure 31.7
Lilium anther (40×). Mature pollen grains are the product of microsporogenesis and microgametogenesis within the microsporangia of flower anthers. A higher-magnification view of developing pollen grains is shown in figure 31.8.

1. At the beginning of the lab period, place some pollen in a drop of 1% sucrose within a ring of petroleum jelly on a microscope slide. Alternatively, a preparation of pollen germinating on 5% sugar and agar may be available for observation.
2. Apply a coverslip and examine the pollen intermittently with your microscope throughout the lab period. Incubate the slide in a warm place.
3. Ring the coverslip with petroleum jelly to prevent the preparation from drying.

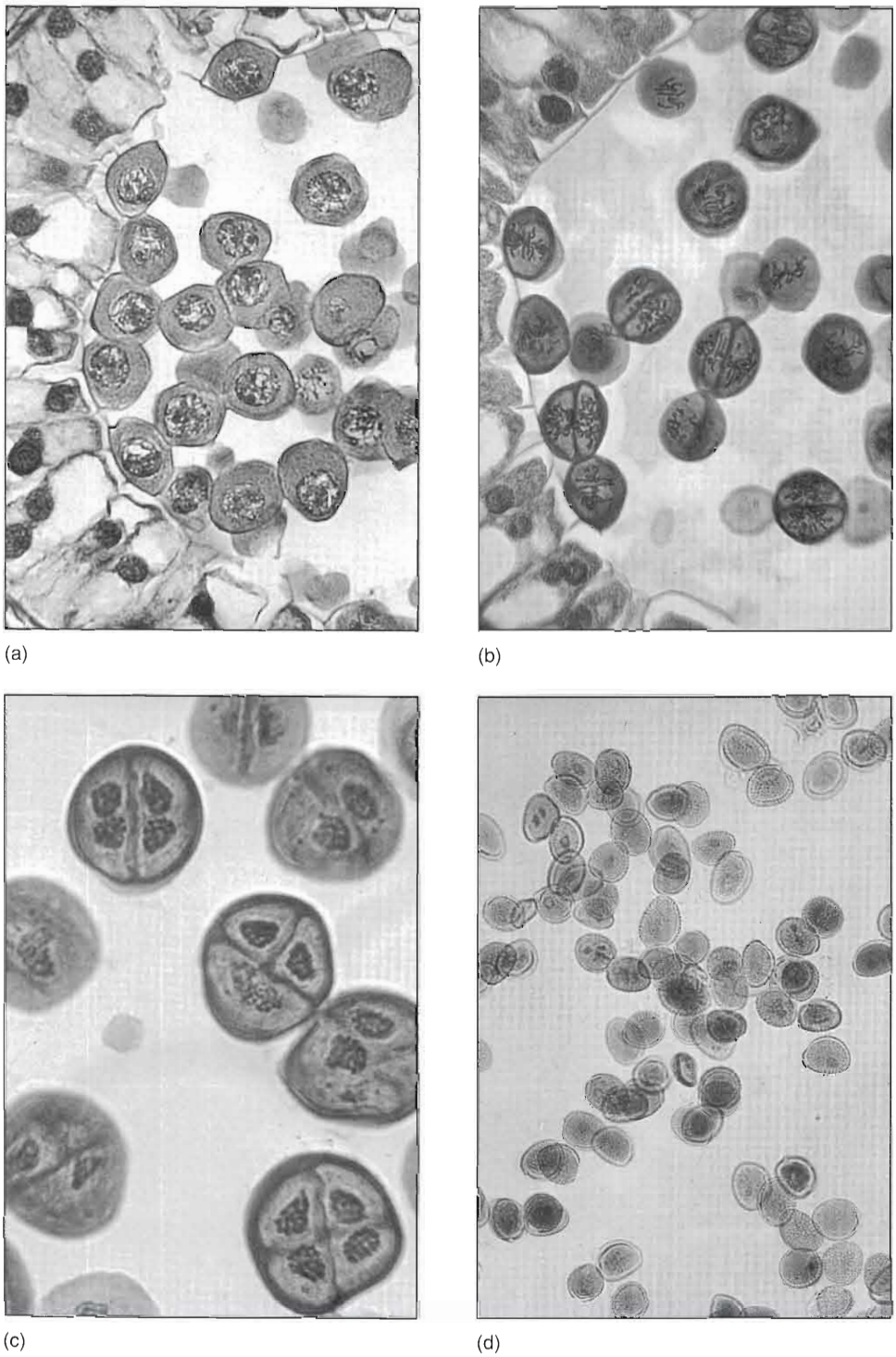


Figure 31.8

Stages of microsporogenesis and microgametogenesis in *Lilium*. (*a*) Cells in early prophase I. (*b*) Cells in second meiotic division. (*c*) Tetrads of microspores from meiosis. (*d*) Mature pollen. For an overview of these stages, see figure 31.5.

Question 4

a. How many pollen grains germinated?

b. Can you see vegetative and generative nuclei in the pollen tubes?

Megasporogenesis, Production of Ovules, and Megagametogenesis

Megasporogenesis is the production of megaspores (fig. 31.9); it occurs in the sporangia of the flower ovary by meiosis of **megaspore mother cells** (megasporocytes). These megaspores undergo **megagametogenesis**; that is, they develop into **megagametophytes**. The megagametophyte and its surrounding tissues are called an **ovule**. Ovules usually have two coverings called **integuments**. The entire haploid structure is called the **embryo sac** and consists of only six to ten nuclei, one of which is an egg. A seven- or eight-celled embryo sac is most common (fig. 31.10).

Procedure 31.4

Examine stages of megasporogenesis and megagametogenesis in *Lilium*

1. Examine a cross section of a *Lilium* ovary and locate the six megasporangia. Within each of these megasporangia a megasporocyte will form and develop. The stages for development of a megasporocyte are shown in figures 31.9, 31.10, and 31.11.
2. Examine a prepared slide of a cross section of a *Lilium* ovary showing a diploid megasporocyte within the sporangium before meiosis (fig. 31.11*a*).
3. Examine a prepared slide showing the four-nucleate embryo sac after meiosis (fig. 31.11*b*). Meiosis produced four haploid megaspores in the embryo sac. In most angiosperms, three of the four nuclei degenerate and the single remaining nucleus passes through two mitotic divisions before the next stage. *Lilium* is atypical because all four products of meiosis

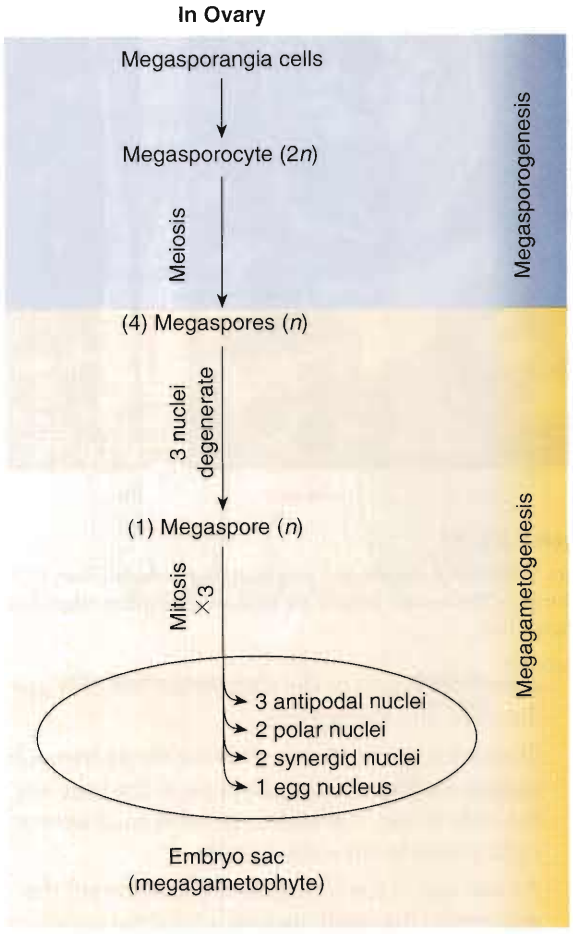


Figure 31.9

Megasporogenesis and megagametogenesis in the ovaries of flowers. The embryo sac is the mature megagametophyte. The locations of these processes are shown in figures 31.10 and 31.11.

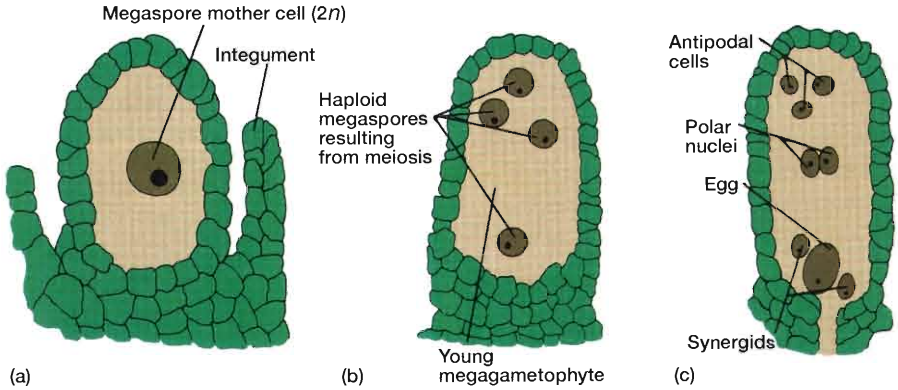


Figure 31.10

Megasporogenesis and megagametogenesis in *Lilium*. (*a*) The megaspore mother cell (megasporocyte) is diploid and undergoes meiosis. (*b*) Four haploid megaspores result from meiosis. (*c*) The mature megagametophyte contains eight nuclei, one of which is the egg.

attachment of the cotyledons and the stem apical meristem; it has not elongated in the mature embryo.

Procedure 31.5

Examine development of a *Capsella* embryo

1. Obtain and examine prepared slides showing the various stages of embryo development.
2. The cross section most likely passes through an entire fruit of *Capsella* and shows a number of sectioned, developing seeds each in a slightly different stage. Some seeds were sectioned off center and the stage may not be obvious. Nevertheless, each slide should include an example of at least one stage.
3. Locate among all the sectioned seeds examples of the globular, heart, torpedo, and mature embryos. These stages are continuous, so each slide may have multiple stages and intermediates between stages.
4. You may need to examine several slides. Find examples of as many stages as you can.
5. Compare your observations with figure 31.13.

Question 5

a. Why is the endosperm being digested?

b. Is *Capsella* a monocot or a dicot? How can you tell?

SEED STRUCTURE

Do not eat any seeds or fruits used in this laboratory.

Procedure 31.6

Observe parts of a bean seed

1. Obtain some beans that have been soaked in water for 24 h.
2. Peel off the seed coat and separate the two cotyledons. Between the cotyledons you'll see the young root and shoot.
3. Examine the opened seed and compare its structure with that shown in figure 31.14. Look for these features:
   - **Micropyle**—a small opening on the surface of the seed through which the pollen tube grew.
   - **Hilum**—an adjacent, elliptical area at which the ovule was attached to the ovary.
   - **Cotyledon**—food for the embryo.
   - **Embryo with young root and shoot**—develops into the new sporophyte.

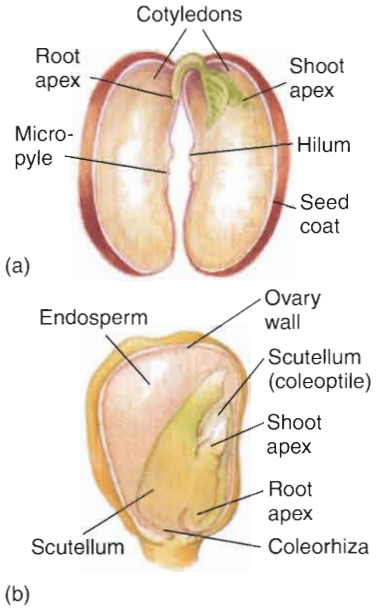


Figure 31.14

Seed structure of a garden bean (dicot) and corn (monocot). (*a*) The two cotyledons in each seed of garden bean (*Phaseolus vulgaris*) absorb the endosperm before germination. (*b*) Corn (*Zea mays*) has seeds in kernels (grains); the single cotyledon is an endosperm-absorbing structure called a scutellum.

4. Add a drop of iodine to the cut surface and observe the staining pattern. Indicate this pattern on figure 31.14.
5. Repeat steps 2–4 with soaked peas.

Question 6

How are seeds of peas and beans similar? How are they different?

Procedure 31.7

Examine a corn grain with embryo

1. Examine a prepared slide of a corn grain (fig. 31.15). Identify the following features:
   - **Endosperm**
   - **Scutellum** (cotyledon; this structure helps absorb the endosperm)
   - **Coleoptile** (sheath enclosing shoot apical meristem and leaf primordia of grass embryos)
   - **Root**
   - **Root cap**
   - **Coleorhiza** (sheath enclosing embryonic root of grass embryo)
   - **Shoot apical meristem**
2. Use a razor blade to longitudinally split a water-soaked corn grain.

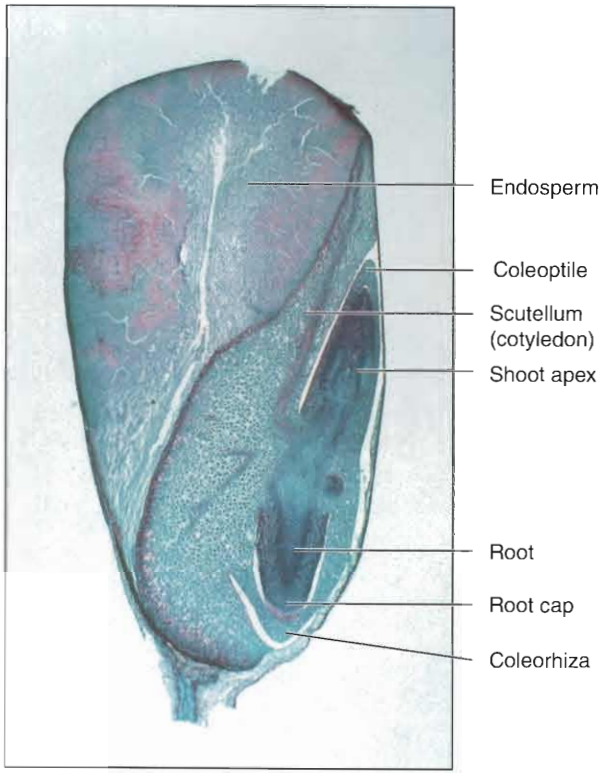


Figure 31.15
The structure of a corn grain, longitudinal section. The embryo is nourished by the starchy endosperm.

3. Add a drop of iodine to the cut surface and observe the staining pattern. Recall from Exercise 6 that iodine stains starch black. Indicate this pattern on figure 31.15.

Question 7

a. What does this staining pattern tell you about the content of the endosperm and embryo?

b. Do mature seeds of monocots or dicots store most of their food in cotyledons? How can you tell?

FRUIT

A **fruit** is a mature, ripened ovary plus any associated tissues. Therefore, a fruit contains seeds. Are you surprised that tomatoes and okra are fruit? A mature fruit is often larger than the ovary at the time of pollination and fertilization, which indicates that a great deal of development occurs while the seeds are maturing. Most fruits are either dry or fleshy. Dry fruits crack or split at maturity and release their seeds. Sometimes the dry wall surrounds the seed until it germinates. The fruit wall is usually tough and hard and is sometimes referred to as stony. The seeds of fleshy fruits remain in the tissue until germination.

A typical fruit has an outer wall called a **pericarp** composed of an **exocarp**, **mesocarp**, and **endocarp**. Within the pericarp are seeds, various partitions, and placental tissues. The fruit often includes the receptacle of the flower.

Procedure 31.8

Observe diversity of fruits

1. Use the following descriptions to study and classify fruits available in the lab. This list is not complete but describes a few common types of fruit.

Procedure 31.9

Observe the structure of a bean, sunflower, corn grain, apple, and tomato

1. Examine the pod of a string bean. This single carpel has two seams that can open to release seeds. Remove the seeds; then locate and remove the seed coat. Split the seed and locate the embryo.

Question 8

a. Does the pod appear to be a single carpel with one cavity containing seeds?

b. Is the micropyle near the attachment of the seed to the pod?

2. Crack the outer coat of a sunflower fruit and remove the seed. Locate the embryo and determine whether sunflower is a monocot or dicot.

Question 9

a. Before today would you have referred to the uncracked sunflower achene as a fruit? Why or why not?

b. Is sunflower a monocot or dicot?

3. Examine an ear of corn from which the husks have been removed without disturbing the "silk." The strands of silk are styles of the gynoecium.

Dichotomous Key to Major Types of Fruit

I. Fleshy fruits
- A. Simple fruits (i.e., from a single ovary)
  1. Flesh mostly of ovary tissue
     - a. Endocarp hard and stony; ovary superior and single-seeded (cherry, olive, coconut): **drupe**
     - b. Endocarp fleshy or slimy; ovary usually many-seeded (tomato, grape, green pepper): **berry**
  2. Flesh mostly of receptacle tissue (apple, pear, quince): **pome**
- B. Complex fruits (i.e., from more than one ovary)
  1. Fruit from many carpels on a single flower (strawberry, raspberry, blackberry): **aggregate fruit**
  2. Fruit from carpels of many flowers fused together (pineapple, mulberry): **multiple fruit**

II. Dry fruits
- A. Fruits that split open at maturity (usually more than one seed)
  1. Split occurs along two seams in the ovary. Seeds borne on one of the halves of the split ovary (pea and bean pods, peanuts): **legume**
  2. Seeds released through pores or multiple seams (poppies, irises, lilies): **capsule**
- B. Fruits that do not split open at maturity (usually one seed)
  1. Pericarp hard and thick, with a cup at its base (acorn, chestnut, hickory): **nut**
  2. Pericarp thin and winged (maple, ash, elm): **samara**
  3. Pericarp thin and not winged
     (sunflower, buttercup): **achene**
     (cereal grains): **caryopsis**

Question 10

a. If a corn grain is actually a fruit, where is the pericarp?

b. Does a corn grain have a cotyledon as well as an endosperm?

4. Examine an apple, an example of a pome. Section the apple longitudinally and transversely. Compare its structure with figure 31.16. Most of the outer flesh of the apple is derived from tissue other than the ovary. Locate the outer limit of the pericarp and the limit of the endocarp.

Question 11

a. How many carpels are fused to form an apple?

b. How might the fleshy pericarp aid in seed dispersal?

5. Examine a tomato and section it transversely. Compare its structure with that shown in figure 31.16. The jellylike material is the placenta giving rise to the ovules.

Question 12

How many carpels are fused to form a tomato?

INVESTIGATION

Grocery Store Botany

Observation: The produce sections of grocery stores and markets contain a vast array of plant organs, each having a characteristic shape, taste, and texture. These plants have been the subject of intensive artificial selection over many generations, and today we enjoy the many varied products of that selection.

Question: What plant organ (e.g., modified root, stem, leaf, flower) are you purchasing when you buy items for a salad? Assume that your salad includes beets, radishes, sweet potatoes, celery, carrots, broccoli, asparagus, eggplant, lettuce, and onion.

a. Establish a working lab group and obtain Investigation Worksheet 31 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 31 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

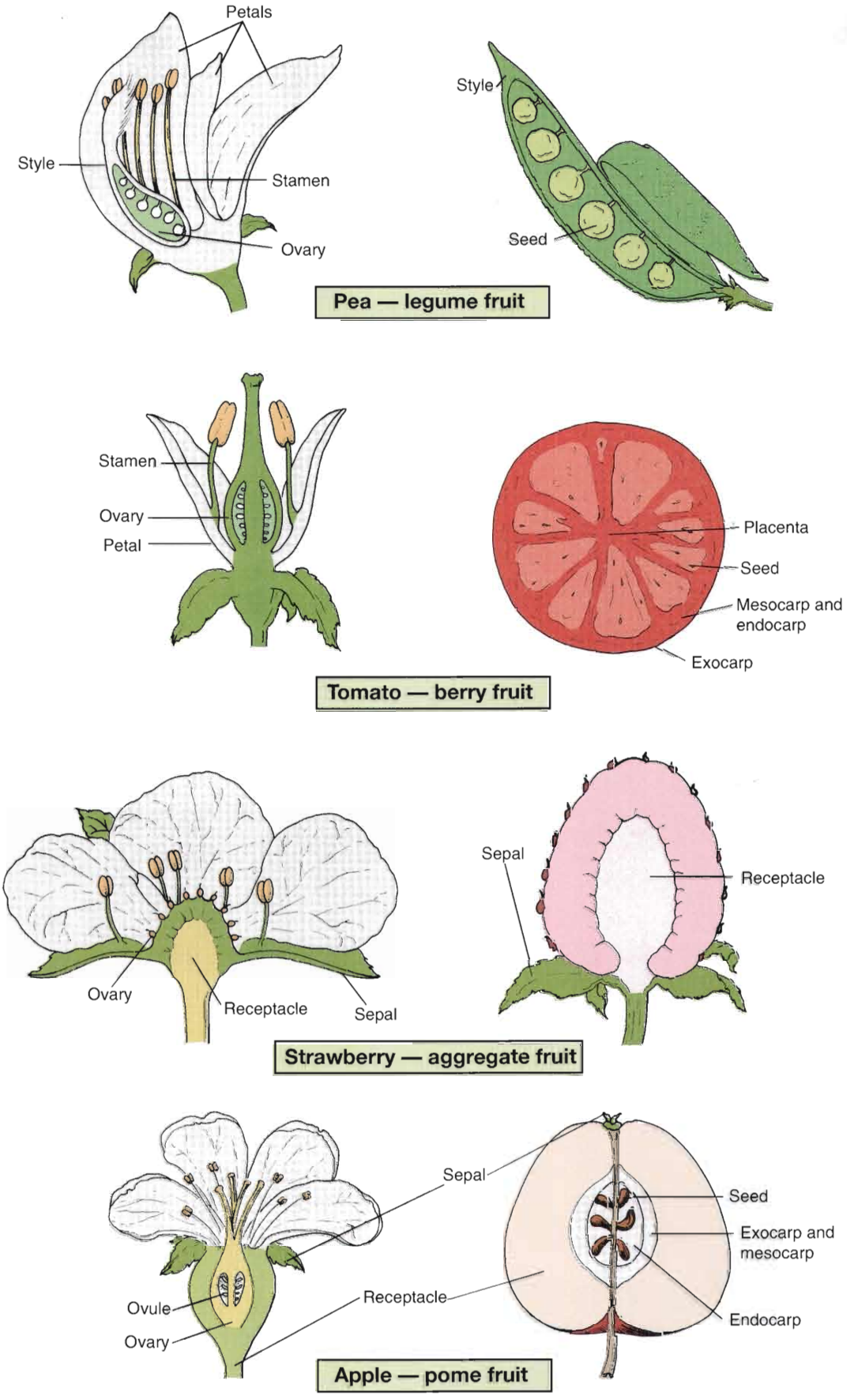


Figure 31.16
Diagrams of fruit and their originating flowers.

Questions for Further Thought and Study

1. What is meant by "double fertilization"?

2. What features of seeds and fruits have enabled angiosperms to become so widespread?

3. What are the similarities and differences between cones and flowers?

4. What is the difference between a fruit and a vegetable?

5. Diagram the life cycle of an angiosperm. Which parts are haploid? Which are diploid?

6. How can you distinguish a monocot from a dicot?

7. How are insects such as bees important for the reproduction of angiosperms?

8. What advantages do flowers give angiosperms over gymnosperms?

9. Draw three fruits (including one dry fruit) that you observed in lab. Describe a probable method for dispersal of each.

WRITING TO LEARN BIOLOGY

What are the functions of a flower? Describe how a flower of your choice is adapted to each function.

Plant Anatomy
Vegetative Structure of Vascular Plants

32

Objectives

By the end of this exercise you should be able to:

1. Describe the functions of roots, stems, and leaves.
2. Distinguish between primary and secondary growth.
3. Describe the functional significance of the internal and external structure of roots, stems, and leaves.
4. Explain what causes growth rings in wood.

The structure of plants varies greatly among species; compare, for example, an oak tree with a cactus. However, these structural differences are typically quantitative rather than qualitative; that is, the differences among roots, stems, and leaves result not from unique tissues but rather from different arrangements and proportions of the same tissues. These differences among plants represent different ways of achieving the same "goals": survival and reproduction. This exercise will concentrate on the structure of roots, stems, and leaves of vascular plants.

ROOTS

During seed germination, a **radicle** or young **primary root** emerges from the seed and grows down. The primary root soon produces numerous **secondary roots** and forms a root system that absorbs water and minerals, anchors the plant, and stores food. Root systems have different morphologies. For example, a **taproot system** has a large main root and smaller secondary roots branching from it (e.g., carrot). In a **fibrous root system**, the primary and secondary roots are similar in size (e.g., roots of many grasses) (fig. 32.1). Examine the displays of taproot and fibrous root systems available in the lab.

Primary growth of roots and all primary tissues is formed by **apical meristems**. A meristem is a localized area of cellular division. Apical meristems occur at the tips of roots and stems. Primary growth (i.e., growth in length) produces herbaceous (nonwoody) tissue. **Secondary growth** refers to growth in girth resulting from nonapical meristems, some of which are discussed later in this exercise.

Question 1

a. How do taproot systems and fibrous root systems help plants survive and reproduce?

b. Would one type of root system provide more adaptive advantages in a particular environment such as a rain forest? A desert?

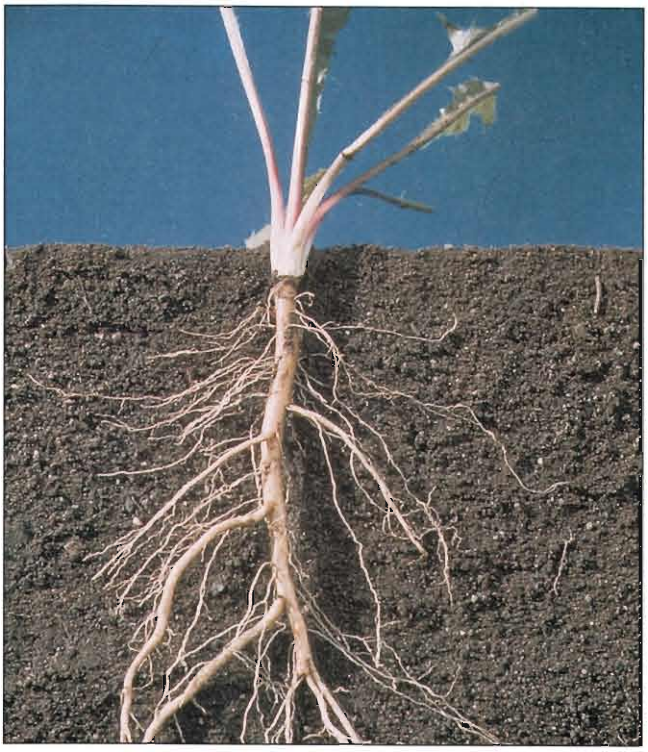
The Root Apex

Examine the root tips of two-day-old seedlings of radish (*Raphanus*) and corn (*Zea*) with your dissecting microscope. Refer to figure 32.2 and identify the **root cap**, **root apical meristem**, **zone of elongation**, and **zone of maturation**.

The cone of loosely arranged cells at the root cap perceives gravity and protects the root apical meristem. The root cap protects the root by secreting mucilage and sloughing cells as the root grows through the soil (fig. 32.3). The root apical meristem is behind the root cap and produces all of the new cells for primary growth. These cells elongate in the zone of elongation, 1–4 mm behind the root tip. This elongation produces primary growth.

Question 2

a. In the following space, sketch the root tips that you examined. In which area of a root tip are cells largest? In which area are they smallest?



(a)

(b)

Figure 32.1

Two common types of root systems of vascular plants. (*a*) The taproot system of dandelion (*Taraxacum*) consists of a prominent taproot and smaller lateral roots. (*b*) The fibrous root system of a grass consists of many similarly sized roots. Fibrous root systems form extensive networks in the soil and successfully minimize soil erosion.

b. Aside from their size, do all cells in the root tip appear similar? Why is this significant?

Re-examine the two-day-old radish seedling with your dissecting microscope. Note the **root hairs** in the zone of maturation. Root hairs are outgrowths of epidermal cells and are short-lived. Root hairs increase the surface area of the root.

Question 3

a. Why do you think root hairs occur only in the zone of maturation?

b. What is the function of root hairs?

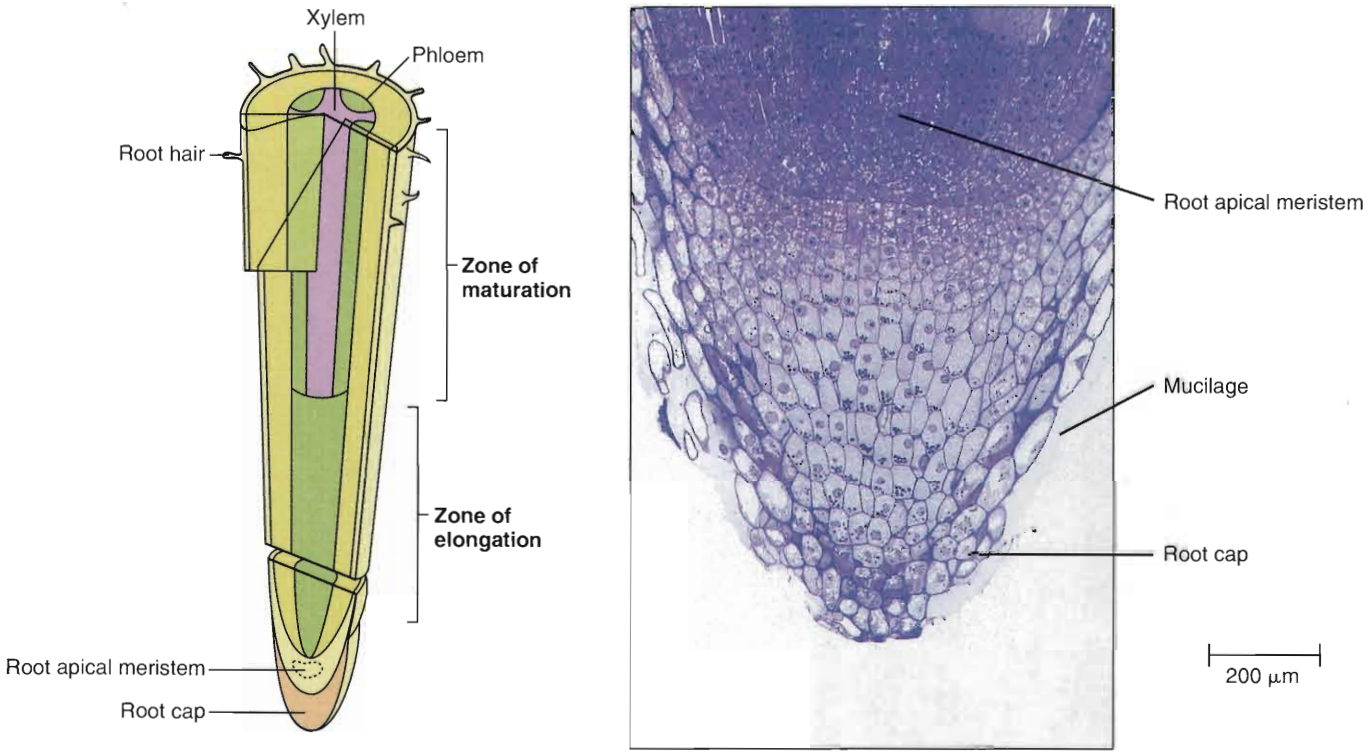
Primary Tissues of the Root

The root apical meristem produces cells that differentiate into primary tissues of the root. The outer layer of cells is the **epidermis**. Just inside the epidermis is the **cortex**, whose cells contain numerous **amyloplasts**, starch-containing plastids. The inner layer of the cortex is the **endodermis**, which regulates water flow to the vascular tissue in the center of the root. Immediately inside the endodermis is the **pericycle**, which can become meristematic and produce **secondary roots** (figs 32.4 and 32.5).

Procedure 32.1

Examine primary tissues of the root

1. Examine a prepared slide of a cross section of a buttercup (*Ranunculus*) root (figs. 32.4 and 32.5). Sketch what you see. Label and state the function of each tissue that is present.



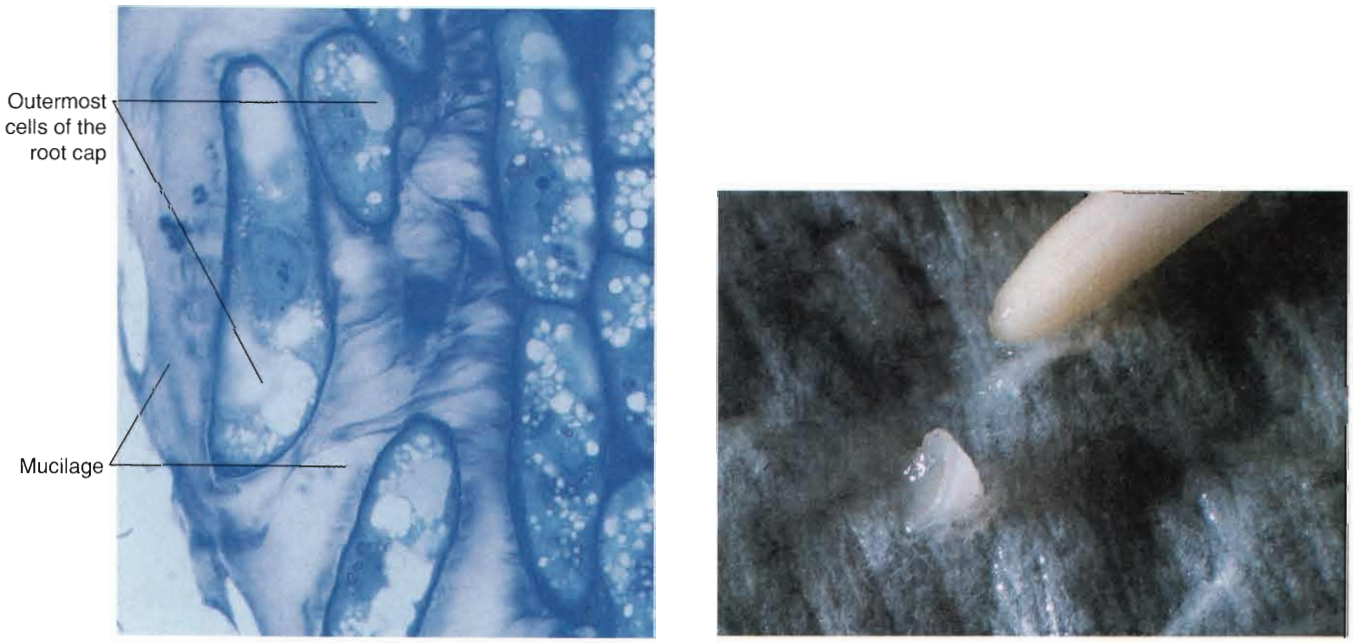


Figure 32.2

(*a*) Root tip showing root cap, root apical meristem, zone of elongation, and zone of maturation. (*b*) The root apical meristem is covered by a thimble-shaped root cap that protects the meristem as the root grows through the soil. (*c*) Mucilage produced by the root cap lubricates the root as it grows through the soil (see fig. 32.3). (*d*) In plants such as corn, the root cap can be removed from the rest of the root.

2. Examine a prepared slide labeled "lateral root origin." Locate the epidermis, cortex, pericycle, and newly formed secondary root. Sketch the lateral root and label its parts.

Figure 32.3

Tips of roots secrete large amounts of mucilage, a lubricant that helps the root force its way through the soil. The mucilage is secreted primarily by the root cap. Movement of the root through soil is also aided by the sloughing of root-cap cells. These sloughed cells are visible in the drop of mucilage on the tip of this root.

3. If time permits, also examine a prepared slide of a cross section of a corn (*Zea*) root.

Question 4

a. Based on the presence of amyloplasts, what do you suppose is the primary function of the cortex?

b. Do secondary roots arise inside the primary root or on its surface?

c. How does the structure of a monocot root differ from that of a dicot?

In the center of the buttercup root is the **vascular** (fluid-conducting) **cylinder** composed of **xylem** and **phloem** (fig. 32.5). Xylem transports water and minerals; phloem transports most organic compounds in the plant, including carbohydrates. Water-conducting cells in the xylem of angiosperms are called **tracheids** and **vessel elements** and are dead and hollow at maturity. Tracheids are long, spindle-shaped cells with thin areas called **pits** where the cell walls of adjacent cells overlap (fig. 32.6). Water moves through these pits from one cell to the next. Vessels are stacks of cylindrical cells with thin or completely open end-walls. Water moves through vessel elements in straight, open tubes. These tubes are usually stained red in slide preparations of buttercup roots.

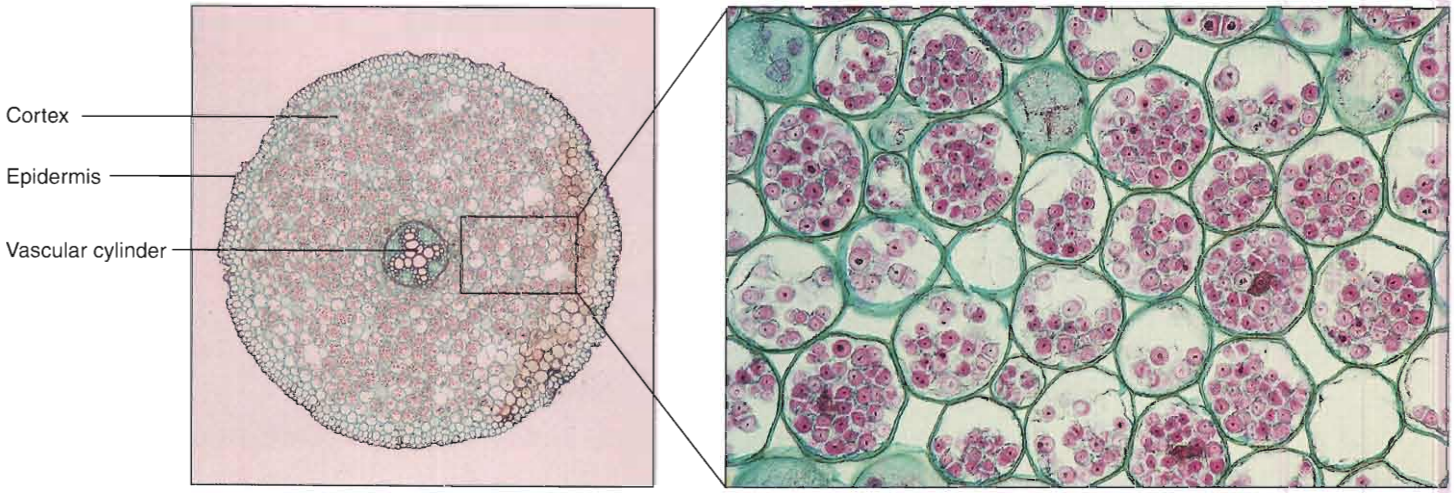


Figure 32.4

Transverse sections of a root of a buttercup (*Ranunculus*). (*a*) Overall view of mature root. The vascular cylinder includes tissues specialized for long-distance transport of water and solutes, whereas the epidermis forms a protective outer layer of the root (16×). (*b*) Detail of cortex (250×). Each parenchyma cell in the cortex contains many amyloplasts, which store starch.

Conducting cells in phloem are called **sieve cells** and **sieve tube members** and are alive at maturity. Phloem cells are small, thin-walled, and arranged in bundles that alternate with the poles of xylem. Sieve tube members are usually stained green.

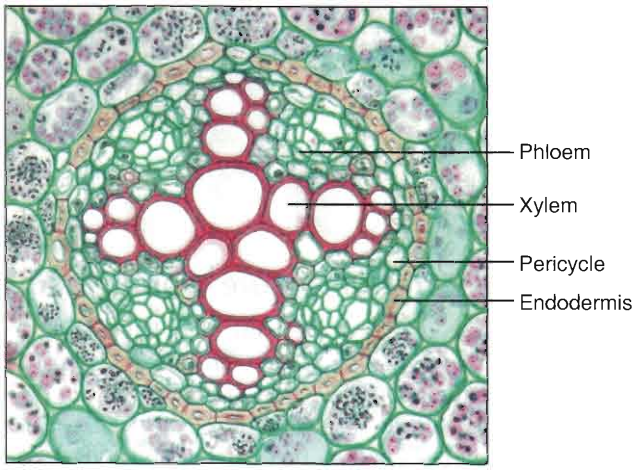


Figure 32.5
A cross section through the center of a root of a buttercup (*Ranunculus*), a dicot (125×). The phloem and xylem are vascular tissues of the vascular cylinder shown in figure 32.4*a*.

Procedure 32.2

Examine carrot root

1. Prepare two thin cross sections of a carrot root.
2. Stain one slice with iodine (a stain for starch) and examine it with your microscope.
3. Stain the other section of carrot root with phloroglucinol. Phloroglucinol stains **lignin**, a molecule that strengthens xylary cell walls.

Phloroglucinol contains hydrochloric acid. Do not spill any on yourself or your belongings!

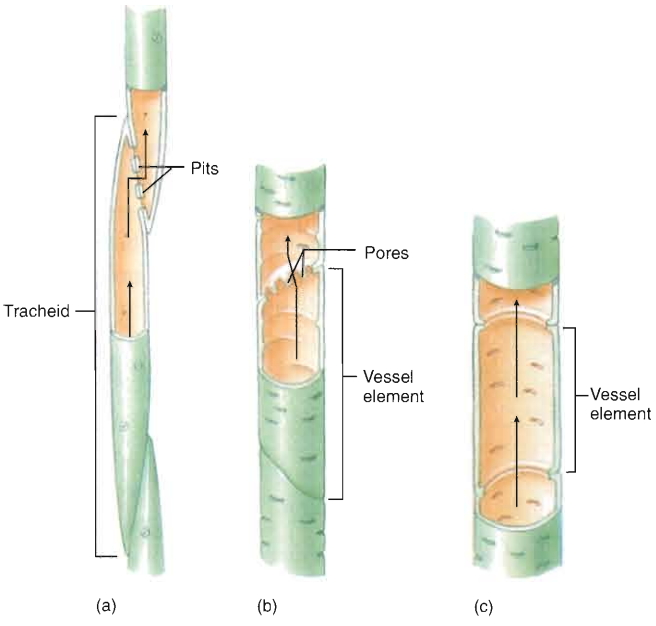
Question 5

a. Where is starch located in a carrot root?

b. What can you conclude from this observation?

STEMS

Stems are often conspicuous organs of plants and function for support and transport of water and solutes. Some stems (e.g., cacti) also photosynthesize and store food.



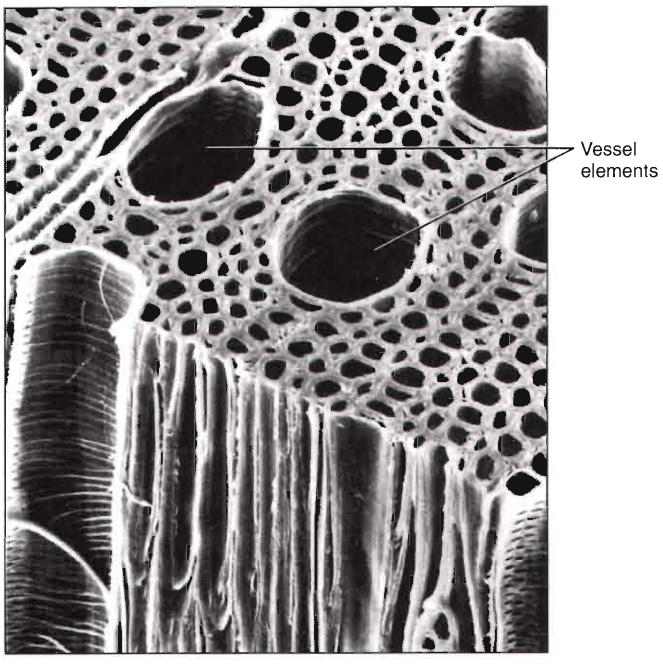


Figure 32.6
Comparison of vessel elements and tracheids. (*a*) In tracheids, water passes from cell to cell through pits. (*b*, *c*) In vessel elements, water moves through pores, which may be simple or interrupted by bars. (*d*) The large openings shown in this scanning electron micrograph of the wood of a red maple (*Acer rubrum*) are vessel elements (350×).

Question 7

a. What is the significance of a coating of cutin on the epidermis?

b. How does the arrangement of xylem and phloem in stems differ from that in roots?

The darkly stained, thick-walled cells just outside the phloem in figure 32.10 are **sclerenchyma fibers**, which function in support. Sclerenchyma fibers from some plants are used to make linen, rope, and burlap.

The ring of vascular bundles in sunflower stems is typical of **dicots**, flowering plants with two **cotyledons** (seed leaves). Examine a prepared slide of a cross section of a corn stem (fig. 32.11). Corn is a **monocot** (a flowering plant with only one cotyledon). In the following space sketch a cross section of a sunflower stem and a corn stem. Note the distribution of the vascular bundles.

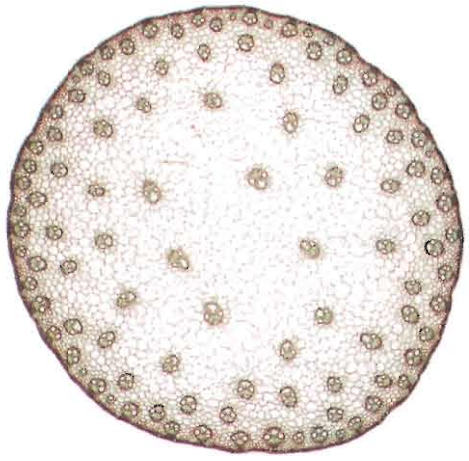


Figure 32.11

Cross section of a stem of corn (*Zea mays*), a monocot (5×). Unlike in dicots such as sunflower (fig. 32.9), bundles of vascular tissue in monocots occur throughout the ground tissue. The stem is surrounded by an epidermis.

Question 8

How does the arrangement of vascular bundles differ in stems of monocots as compared to dicots?

Secondary Growth of Stems

Between the xylem and phloem and each vascular bundle in dicot stems is a meristematic tissue called **vascular cambium**. The vascular cambium is a secondary meristem that produces secondary growth (i.e., growth in girth). The vascular cambium is cylindrical and produces secondary xylem to its inside and secondary phloem to its outside.

Question 9

How is secondary growth different from primary growth?

Procedure 32.4

Examine secondary growth in woody stems

1. Examine cross sections of 1-, 2-, and 3-year-old basswood (*Tilia*) stems. The vascular cambium is a narrow band of cells between the xylem and phloem (fig. 32.12).
2. Compare the structures of the three stems. The secondary xylem of older stems consists of concentric annual rings made of alternating layers of large and small cells. The large cells are formed in the spring, and the smaller cells are produced in summer.
3. In the following space draw 1-, 2-, and 3-year-old stem cross sections.

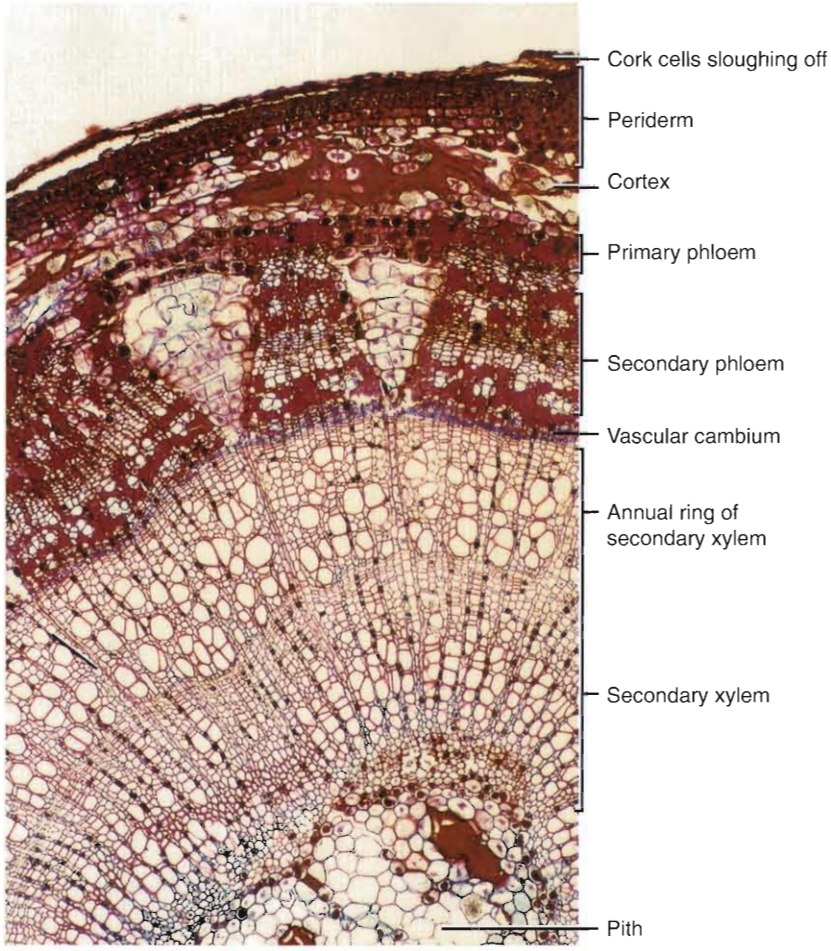


Figure 32.12

A cross section of a portion of a young linden (*Tilia*) stem showing secondary growth (400×). The vascular cambium produces secondary xylem (wood) to the inside and secondary phloem to the outside. Note the annual rings in the secondary xylem. A close-up of a growth ring from pine is shown in figure 32.13.

Question 10

a. How do you account for this seasonal production of different-sized cells?

b. What is the common name for secondary xylem?

c. What is "grain" in wood?

d. Aside from conducting water and minerals, what is another important function of secondary xylem?

4. Examine a prepared slide of secondary xylem of pine (*Pinus*) (fig. 32.13). In cone-bearing plants such as pine, the conducting cells of xylem are all tracheids. The absence of vessel elements gives wood of these plants a relatively uniform appearance.
5. Now examine a prepared slide of secondary xylem of oak (*Quercus*), a flowering plant (fig. 32.14). Sketch what you see.

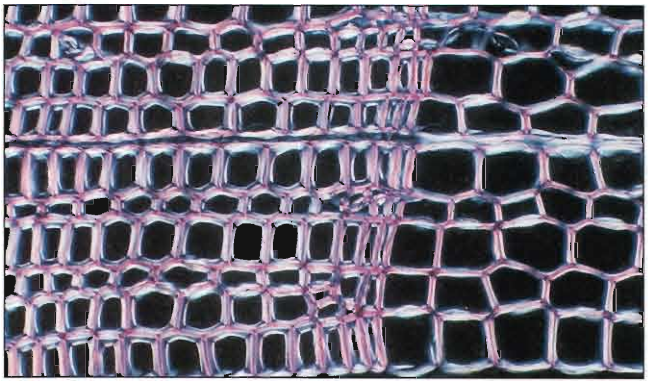


Figure 32.13

The wood of gymnosperms (such as this pine) consists almost exclusively of tracheids. These water-conducting cells are relatively small and help to support the plant. Although water moves slower through tracheids than through vessel elements, tracheids are less likely to be disabled by air bubbles that form in response to freezing and wind-induced bending of branches. The larger cells on the right side of this photo form during the wet days of spring and are called spring wood; the smaller cells at the left form during the drier days of summer and are called summer wood. The change in density between spring and summer wood produces a growth ring, which appears as "grain" in wood.

Question 11

a. What are the large cells in oak wood?

b. What is their function?

c. Which type of wood do you think transports more water per unit area, pine or oak? Why?

Bark

Bark includes all tissues outside of the vascular cambium, including the secondary phloem (fig. 32.12). Secondary phloem consists of pyramidal masses of thick- and thin-walled cells. The thin-walled cells are the conducting cells.

The increase in stem circumference resulting from activity of the vascular cambium eventually ruptures the epidermis. The ruptured epidermis is replaced by a tissue called the **periderm** that, like the epidermis, functions to minimize water loss. Periderm consists of cork cells produced by another secondary meristem called the **cork cambium**.

Locate the cork cambium in cross sections of 1-, 2-, and 3-year-old basswood stems. The cork cambium is a band of thin-walled cells located beneath the epidermis. The cork cambium produces cork cells to the outside and cork parenchyma to the inside. Cork cells stain red because of the presence of suberin, a water-impermeable lipid.

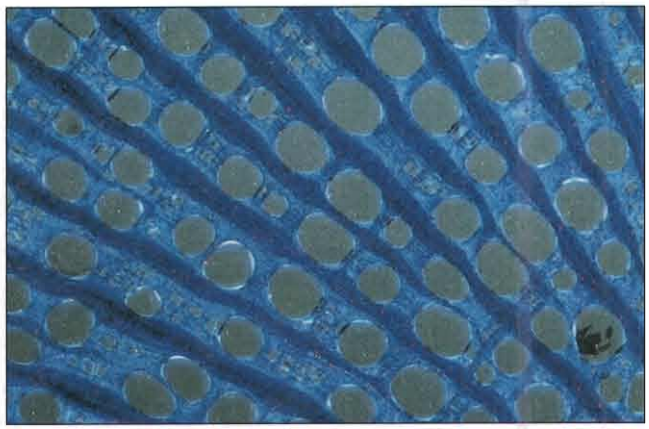


Figure 32.14

Unlike the xylem of gymnosperms, which contains only tracheids, the xylem of angiosperms also contains vessel elements. These vessel elements are much wider than tracheids and appear in this micrograph as large circles. Vessel elements are an adaptation for increased rates of water flow in angiosperms (see fig. 32.6c).

Question 12

Is the amount of cork similar in 1-, 2-, and 3-year-old stems? If not, how does it differ? Why is this important?

As the stem diameter continues to increase, the original periderm ruptures and new periderms form in the underlying tissues. Tissues outside the new periderm die and form encrusting layers of bark.

Gas exchange through peridermal tissues occurs through structures called **lenticels** (fig. 32.15). Examine a prepared slide of a lenticel and locate lenticels on a mature woody stem.

Question 13

How does a lenticel differ from the remainder of the periderm?

LEAVES

With few exceptions, most photosynthesis occurs in leaves, although some may occur in green stems. Leaves typically consist of a **blade** and a **petiole**. The petiole attaches the leaf blade to the stem. **Simple leaves** have one blade connected to the petiole, whereas **compound leaves** have several **leaflets** sharing one petiole (fig. 32.16). **Palmate** leaflets of

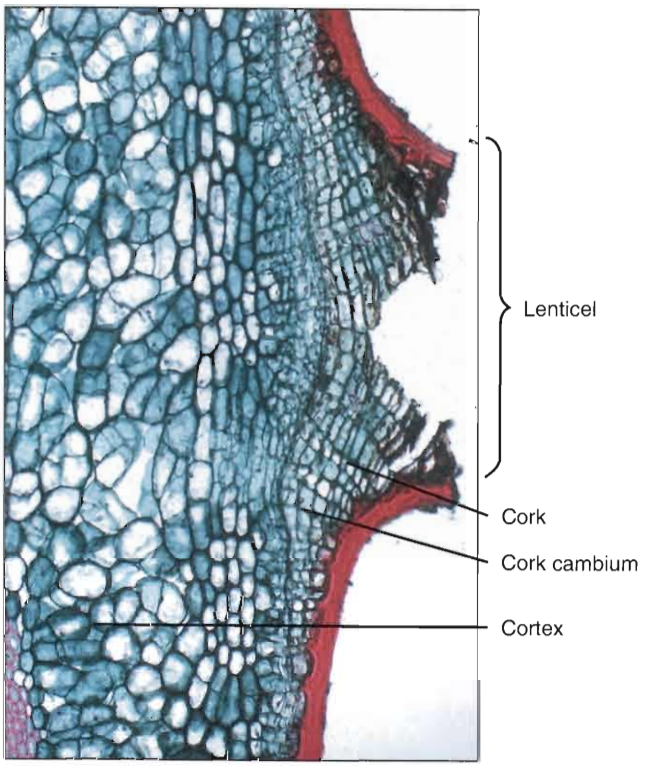


Figure 32.15
A lenticel. Cross section of part of a young stem of elderberry (*Sambucus*). Gas exchange across the cork layer of the stem occurs through lenticels.

a compound leaf arise from a central area, as your fingers arise from your palm. **Pinnate** leaflets arise in rows along a central midline. Examine the simple and compound leaves on display, and sketch a representative of each type of leaf.

Leaves are also classified according to their **venation** (i.e., arrangement of veins) (fig. 32.17). **Parallel veins** extend the entire length of the leaf with little or no cross-linking. **Pinnately veined** leaves have one major vein (i.e., a midrib) from which other veins branch. **Palmately veined** leaves have several veins each having branches. Veins of vascular tissue in leaves are continuous with vascular bundles in stems.

Examine the leaves on display in the lab and determine their venation. Sketch a few of these leaves to show their venation. List the names of common leaves on demonstration and indicate whether each is simple, pinnately compound, or palmately compound. Also indicate whether venation in each leaf is parallel, pinnate, or palmate.

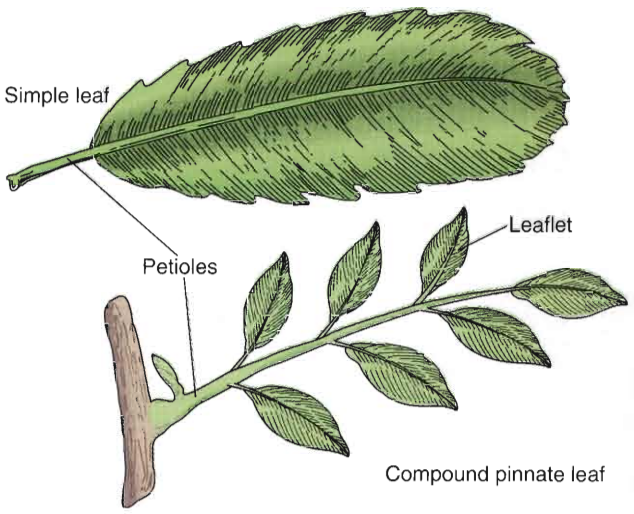


Figure 32.16
Simple and compound leaves.

What Good Is Bark?

You've doubtless seen chips of bark used as garden mulch to spread around plants. Because bark cells (secondary phloem and periderm) were once alive, they contain many nutrients released into the soil as the bark decays. However, sawdust (wood) has few nutrients in it and may result in nitrogen deficiency due to rapid decomposition. Consequently, sawdust is a poor mulch for plants.

Tree Girdling

You've probably heard of "tree girdling," the stripping of a ring of bark from a branch. In the wild, porcupines also girdle trees. Girdling removes secondary phloem from a tree, thereby leaving no pathway for photosynthate to move from the leaves to the roots. As a result, the shoot accumulates sugars and grows rapidly. The following spring it is impossible to send sugars from the roots to the girdled branch to renew growth. Thus, the branch or tree dies the year after it is girdled.

The arrangement of leaves on a stem is called **phyllotaxis** and characterizes individual plant species (fig. 32.18). **Opposite phyllotaxis** refers to two leaves per node located on opposite sides of the stem. **Alternate phyllotaxis** refers to one leaf per node, with leaves appearing first on one side of the stem and then on another. **Whorled phyllotaxis** refers to more than two leaves per node. Examine the plants on display in the lab to determine their phyllotaxis.

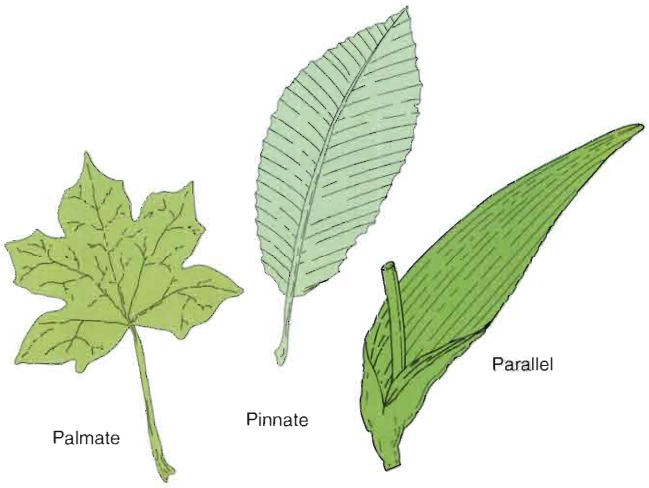


Figure 32.17
Palmate, pinnate, and parallel venation of leaves.

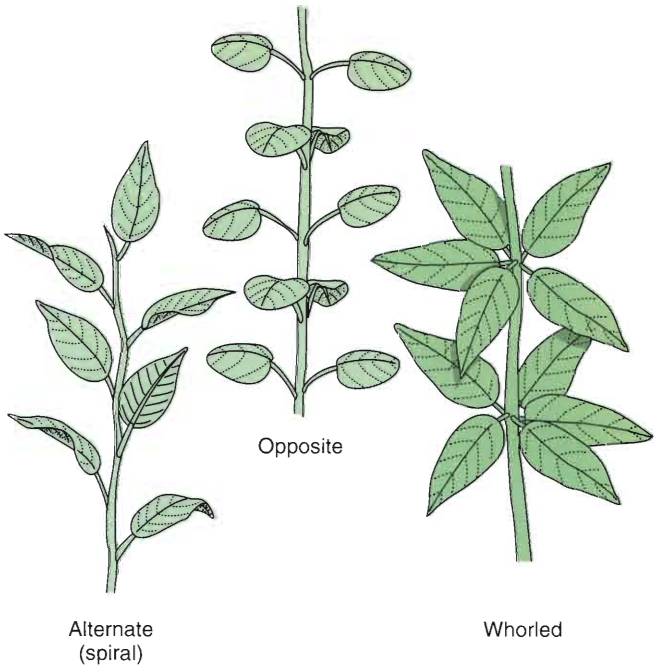


Figure 32.18
Patterns of leaf arrangement.

Internal Anatomy of a Leaf

Examine a cross section of a leaf of *Ligustrum* (privet) (figs. 32.19 and 13.8). The leaf is only 10–15 cells thick—pretty thin for a solar collector! The epidermis contains pores called **stomata**, each surrounded by two guard cells (you will study stomata again in the next exercise). Just below the upper epidermis are closely packed cells called **palisade mesophyll** cells; these cells contain about 50 chloroplasts per cell. Below the palisade layers are **spongy mesophyll** cells with numerous intercellular spaces. Examine and sketch a cross section of a corn leaf.

Question 14

a. What is the function of stomata?

b. Do epidermal cells of leaves have a cuticle? Why is this important?

c. What is the significance of chloroplasts being concentrated near the upper surface of the leaf?

d. What are the functions of air spaces near the lower surface of the leaf?

e. How is the internal anatomy of a corn leaf different from that of *Ligustrum*?

f. How is it similar?

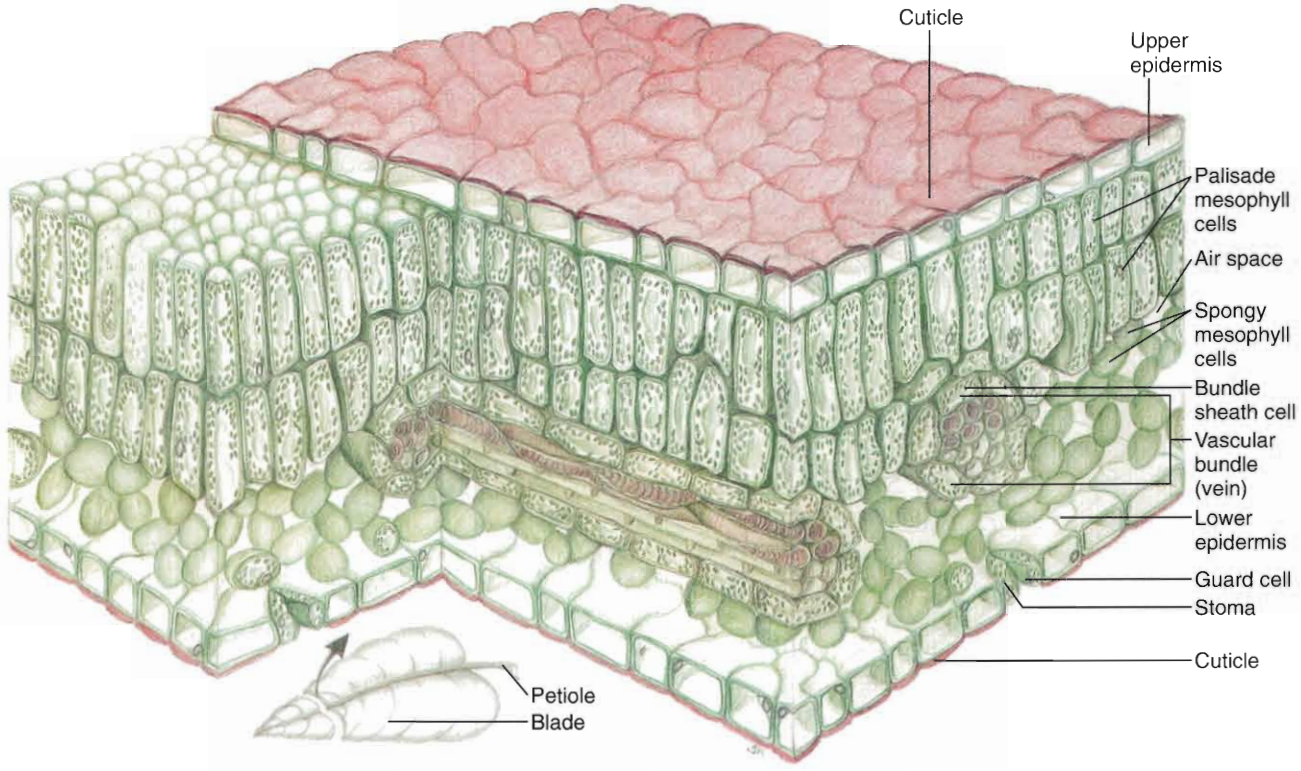


Figure 32.19

Ligustrum leaf, cross section. Most photosynthesis occurs in the densely packed palisade mesophyll cells, just beneath the upper epidermis of the leaf. Gas exchange occurs through stomata, usually most abundant on the lower side of the leaf. Water loss is minimized by the waxy cuticle that covers the leaf.

g. Based on the arrangement of vascular tissue, how could you distinguish the upper versus lower surfaces of a leaf?

h. If time permits, also examine prepared slides of leaves of corn (*Zea mays*, a monocot) and pine (*Pinus*, a gymnosperm). What differences are there in the structures of these leaves? How do these structural differences correlate with functional differences?

INVESTIGATION

How Plants Sense and Respond to Light and Gravity

Observation: The ability of plant roots to grow downward and shoots to grow upward is adaptive because it increases the plants' chances of encountering water (by the roots) and light (by the shoot).

Question: When the roots of a corn (*Zea mays*) seedling grow downward, is this a negative response to light or a positive response to gravity?

a. Establish a working lab group and obtain Investigation Worksheet 32 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 32 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What is the function of xylem? Phloem? Vascular cambium? Epidermis?

2. What are the functions of stomata and lenticels? In what ways do these structures differ?

3. How is the internal anatomy of a stem different from that of a root?

4. How is primary growth different from secondary growth?

5. How is a leaf structurally adapted for its function?

6. Why does a stem typically contain more sclerenchyma and collenchyma than does a leaf?

7. An old friend tells you that 30 years ago she nailed a sign into a tree trunk at a height of 1 meter. She now says the sign is 25 meters up in the tree. Should you believe her? Why or why not?

8. Compare and contrast (*a*) monocot and dicot roots, and (*b*) monocot and dicot stems.

DOING BIOLOGY YOURSELF

Choose a couple of defined environments nearby, such as a vacant field or riverside. Survey the variety of leaf morphologies of the dominant plants. What can you conclude about the predominance of monocots versus dicots in each environment?

DOING BIOLOGY YOURSELF

Roots grow downward. Design and conduct an experiment using *Zea mays* (corn) seedlings to demonstrate whether this is a negative response to light or a positive response to gravity.

WRITING TO LEARN BIOLOGY

Why is leaf abscission especially important for temperate plants?

WRITING TO LEARN BIOLOGY

Would you expect to find annual rings in wood of a tropical dicot tree? Why or why not?

Survey of the Animal Kingdom
Phyla Porifera and Cnidaria

36

Objectives

By the end of this exercise you should be able to:

1. Describe how structures specific to poriferans and cnidarians help them survive and reproduce in their environment.
2. List the fundamental characteristics of members of phylum Porifera and phylum Cnidaria.
3. Recognize members of the three major classes of cnidarians.
4. Describe the body forms of cnidarians and describe reproduction of those species alternating between polyps and medusae.
5. Compare the feeding methods of sponges and jellyfish.
6. Examine the morphology of representative sponges and of the major classes of cnidarians.
7. Discuss characteristics likely to promote long-term evolutionary success of sponges and cnidarians and relate the discussion to objective 1.

To most people, ancient animals such as sponges and jellyfish appear simple and unsophisticated. Their bodies and body cavities are simple and they lack the complex behavior and sensory capabilities of most higher animals. But don't let that simplicity fool you. What sponges and sea jellies (sometimes called jellyfish) lack in complexity is more than compensated for by their extraordinarily elegant design. After all, their overall body plans (table 36.1) have persisted in changing environments for many millions of years; their "simple" morphology accomplishes the primary functions of food getting, reproduction, and adaptive response to their environment as does the morphology of other more familiar animals. Like all animals, sponges of **phylum Porifera** and cnidarians of **phylum Cnidaria** are eukaryotic, **multicellular**, and **ingestive-feeding heterotrophs**. Heterotrophs derive their energy from organic molecules made by other organisms.

(a)

(b)

Figure 36.1

Sponges (phylum Porifera) such as (*a*) the yellow tube sponge, *Verongia*, and (*b*) *Axiomella* have a variety of colors. A seemingly inactive sponge may filter 1000 times its own volume of water per day through walls filled with small canals and chambers. There are up to 18,000 chambers per square millimeter of sponge. Flagellated cells line the chambers to circulate water and filter extremely small particles of food. Eighty percent of the organic matter captured by sponges is too small to be seen with a microscope. Use of this small-size fraction of dissolved matter has made sponges successful for hundreds of millions of years.

PHYLUM PORIFERA

Sponges are the simplest of the major animal phyla and comprise 5000 species (fig. 36.1). Most sponges live in the ocean, but a few encrust rocks and wood in freshwater. Sponges lack tissues and organs and are typically **asymmetrical** assemblages of cells. Bodies of asymmetrical organisms have no symmetry or pattern such as left and right halves or anterior and posterior regions. Sponge cells are so loosely assembled that if a sponge is forced through a

TABLE 36.1

PHYLA PORIFERA AND CNIDARIA

Phylum	Typical Examples		Key Characteristics	Approximate Number of Named Species
Porifera (sponges)	Barrel sponges, boring sponges, basket sponges, vase sponges		Asymmetrical bodies without distinct tissues or organs; saclike body consists of two layers breached by many pores; internal cavity lines with food-filtering cells called choanocytes; most marine (150 species live in freshwater)	5150
Cnidaria (cnidarians)	Jellyfish, hydra, corals, sea anemones		Soft, gelatinous, radially symmetrical bodies whose digestive cavity has a single opening; possess tentacles armed with stinging cells called cnidocytes that shoot sharp harpoons called nematocysts; almost entirely marine	10,000

fine mesh, the disassociated cells will survive. Even more remarkably, the disassociated cells of some species can reassemble as a functioning organism.

Grantia

Examine a preserved specimen of *Grantia*, one of the simplest sponges (fig. 36.2). At first glance, sponges such as *Grantia* appear plantlike because they are **sessile** (i.e., attached to the substrate). Some sponges even appear green because symbiotic algae live in their bodies. However, sponges are **filter-feeding** heterotrophs and have no photosynthetic pigments. Notice that *Grantia* is a tubular, open-ended chamber surrounded by a thin, porous, folded wall of cells. Phylum Porifera gets its name from the many pores in the chamber walls.

Question 1

Do any features of *Grantia* clearly distinguish this organism as an animal? If so, which ones?

Figure 36.2

Grantia is a common tubular sponge with a folded body wall filled with pores for filtering water.

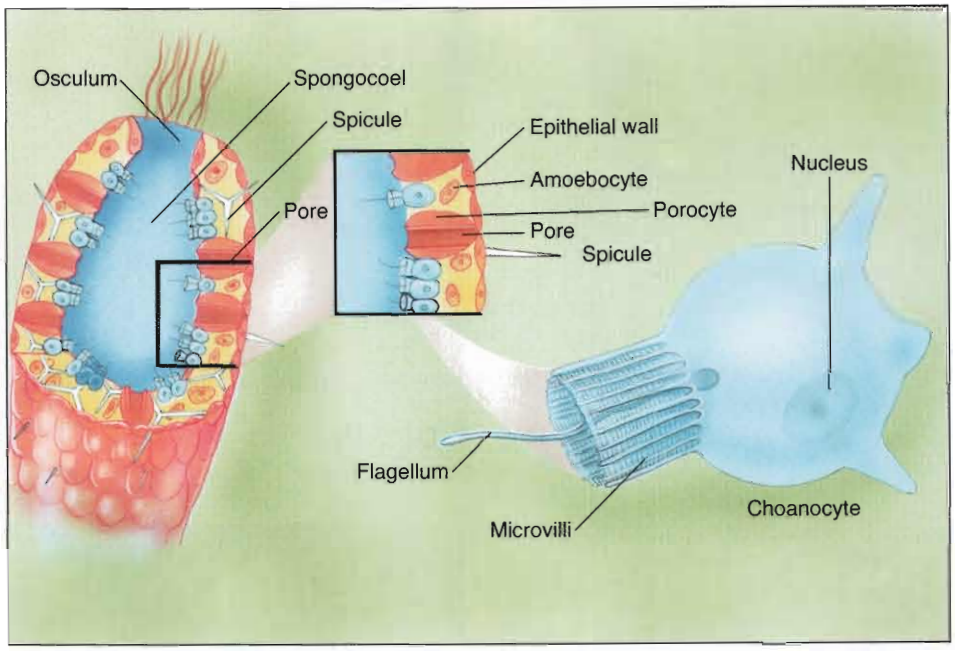


Figure 36.3

Morphology of a simple sponge. An epithelial layer forms the outer wall, reinforced by spicules. Pores are formed by porocytes that extend through the unfolded body wall of this simple sponge. Choanocytes are cells with a flagellum surrounded by a collar of microvilli that traps food particles. Food is moved from the microvilli toward the base of the cell, where the food is incorporated into a food vacuole. The food vacuole is passed to amoeboid cells, where digestion occurs.

Structure of Sponges

Sponge walls filter seawater and remove food particles. In the simplest sponges this wall is lined on the outside by an **epithelial layer** of flat cells (fig. 36.3). Inside the sponge is the **spongocoel**, a central cavity lined by flagellated cells called **choanocytes** (sometimes called collar cells). Their moving flagella draw water through pores within **porocytes** into the spongocoel and across the collars of the choanocytes to trap food particles (fig. 36.3). Choanocytes produce a constant flow of water into a sponge. Eventually the filtered water exits through a large hole in the end of the sponge called the **osculum**.

Question 2

Consider objective 1 listed at the beginning of this exercise. Are choanocytes significant to a fundamental process for sponges? What is the process and how are choanocytes significant?

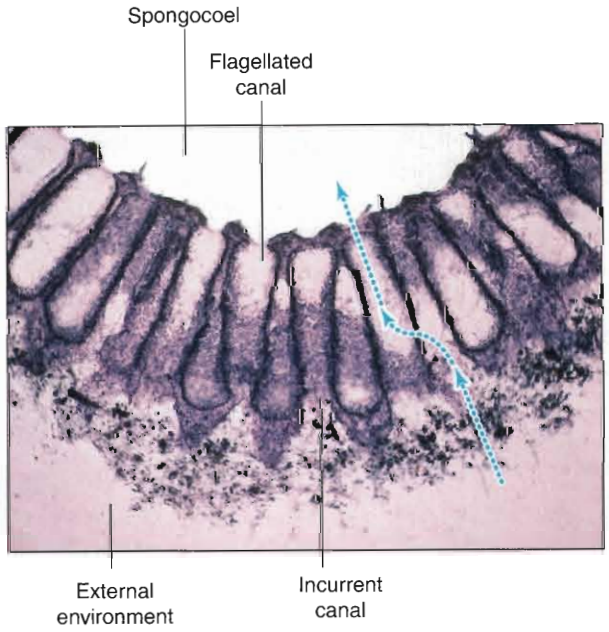


Figure 36.4

Cross section through the folded body wall of *Grantia*. Arrows show the path of water flow. The beating flagella of choanocytes move the water containing food particles from the external environment into an incurrent canal, through the folded wall lined with choanocytes, into the flagellated canal, into the spongocoel, and out the osculum.

Grantia has a folded wall. Examine a prepared slide of a cross section through *Grantia* (fig. 36.4). These folds form **incurrent canals** opening to the outside and **flagellated canals** opening to the central spongocoel. The flagellated canals are lined with choanocytes that move their flagella to draw water into the incurrent canals, through porocytes, through the canals, and on to the spongocoel. A specialized collar of microvilli surrounding the flagellum of a choanocyte traps food particles engulfed by the cell body.

Digestion is **intracellular**, meaning that it occurs inside cells. Some sponges are more complicated than *Grantia* and have highly folded walls or a complicated series of small chambers lined with choanocytes. A spongocoel may be

difficult to distinguish in these complicated sponges (see fig. 36.1*b*).

The wall of a sponge also contains **amoebocytes**, crystalline skeletal structures called **spicules** (fig. 36.5), and a gelatinous matrix called **mesenchyme**. As their name implies, amoebocytes are creeping, mobile cells with a variety of functions including digestion and a rather amazing ability to differentiate into other cell types as needed. They also secrete the skeleton of calcareous spicules (containing calcium), siliceous spicules (containing silicon), or proteinaceous **spongin** fibers.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 36.1

Examine spicules

1. Examine a prepared slide of cleaned and isolated spicules.
2. Also locate spicules in a cross section of *Grantia*.
3. If enough preserved sponge is available, make a wet mount of spicules in a depression slide by gently crushing a small piece of sponge on a slide with a coverslip.
4. If bleach is available, add one or two drops to dissolve the protein and expose the spicules.
5. Examine the preparation under low, then high, magnification.

Question 3

How do spicules help sponges survive in their environment (see objective 1)?

Examine the fused lattice of spicules of *Euplectella* (fig. 36.6). Spicules of different sponges have many shapes and may be fused in an ornate lattice. The beautiful lattice of the sponge *Euplectella* is unusual because it often houses several species of shrimp. Interestingly, when a male and female shrimp enter the spongocoel they may grow too large to escape. A dried specimen of *Euplectella* containing remnants of permanent residents was formerly used in Japan as a wedding present symbolizing the permanent bond of marriage.

Figure 36.5

This photomicrograph shows a variety of sponge spicules (150×).

Figure 36.6

The fused glass (silicon) spicules of *Euplectella* form a beautiful, intricate lattice. The living tissue has been removed from this dried specimen.

Spongia

Examine some large, dried sponges on display. *Spongia* has a more complex arrangement of chambers than does *Grantia*.

Examine a prepared slide of spongin fibers. Fibers of spongin (made of protein) compose the skeleton of common bath sponges such as *Spongia*. Spongin fibers are flexible and

are not like crystalline spicules. In the past, natural sponges were gathered easily in the Caribbean and Mediterranean, dried, and sold. Today, natural sponges are rare and have been replaced by synthetic products.

Question 4

a. Do sponges appear to have any organs or organ systems?

b. What is the advantage of a folded or convoluted wall in sponges?

c. What function other than support might spicules serve?

d. How many prongs do spicules of *Grantia* have?

e. What characteristics of *Spongia* make them useful as a household sponge?

f. Consider objective 1 listed at the beginning of this exercise. Are spicules significant to a fundamental process for sponges? In what way?

Sponge Reproduction

Sponges reproduce asexually and sexually. Asexual reproduction includes budding and the release of stress-resistant aggregates of amoebocytes called **gemmules**. In favorable conditions, amoebocytes in a gemmule can grow into a mature organism. During sexual reproduction, choanocytes and amoebocytes differentiate into gametes. Eggs remain in the mesenchyme, but sperm are released into the water and are captured by choanocytes or amoebocytes of other sponges. The captured sperm are transported to eggs and fertilization occurs. After a brief development, the embryo is expelled from the sponge. Most species of sponges are hermaphroditic (i.e., have male and female reproductive organs) but produce eggs and sperm at different times.

PHYLUM CNIDARIA (COELENTERATA)

Phylum Cnidaria (also called coelenterates) includes **class Hydrozoa** (hydras), **class Scyphozoa** (sea jellies), and **class Anthozoa** (anemones and corals). Cnidarians are almost all marine carnivores. Their bodies are **radially symmetrical** and more complex than sponges (fig. 36.7). Radial symmetry describes a body plan with repetitive body areas arranged in a circle around a central point such as the pieces of a pie. Sensory organs are exposed to the environment in all directions around the perimeter. This is evolutionarily adaptive for a slow or nonmotile organism. The body wall has two cellular layers, an **ectodermis** on the outside and an **endodermis** (sometimes called the gastrodermis) lining the gastrovascular

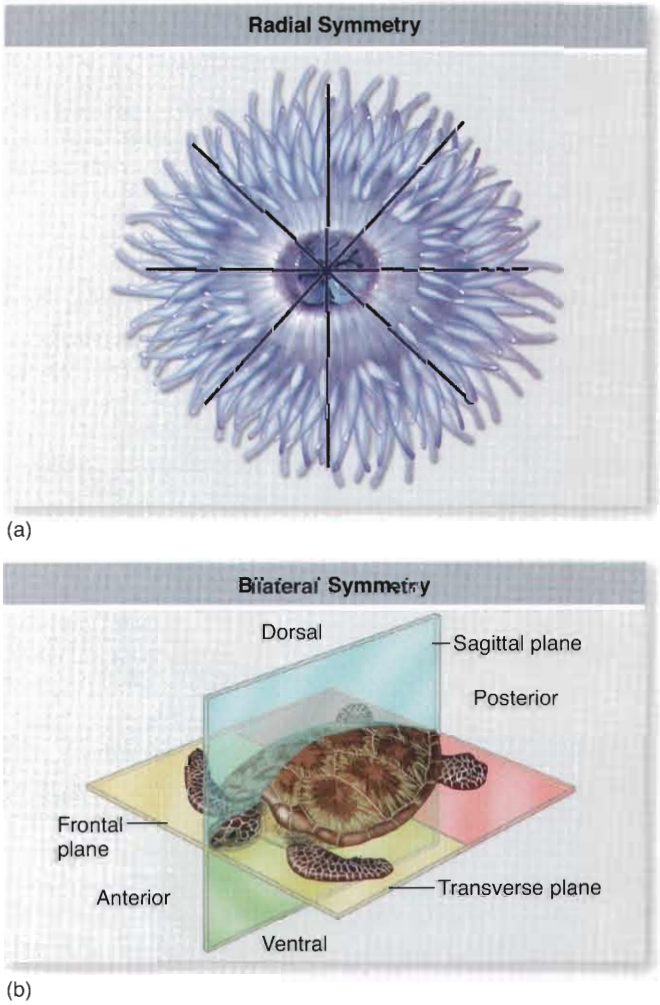


Figure 36.7

A comparison of radial and bilateral symmetry. (*a*) Radially symmetrical animals, such as this sea anemone, can be bisected into equal halves in any two-dimensional plane. (*b*) Bilaterally symmetrical animals, such as this turtle, can only be bisected into equal halves in one plane (the sagittal plane).

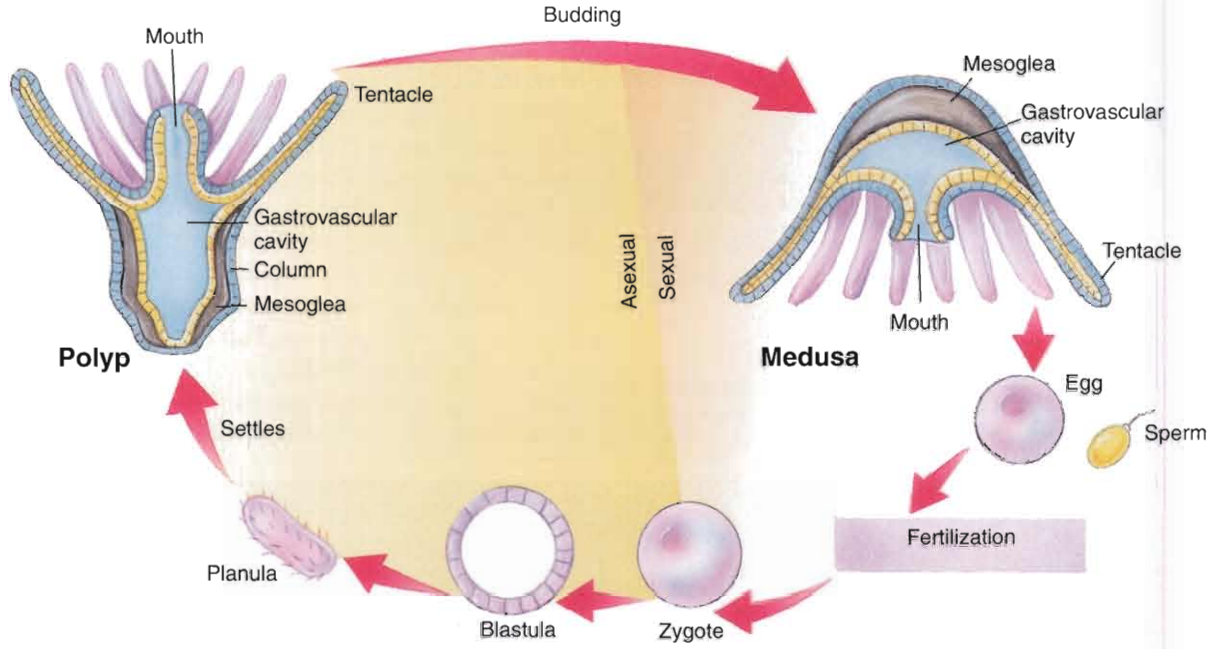


Figure 36.8

A generalized life cycle for Cnidaria. Cnidarians alternate between medusa and polyp forms. Male and female medusae use meiosis to produce gametes that, in water, are fertilized. These gametes are the only haploid (n) stage; all other stages are diploid ($2n$). After a short period of free swimming, the planula larva settles to the substrate and forms a polyp. When the polyp buds (an asexual process), additional polyps and medusa buds form. Medusae separate from the polyp and swim away. The polyp or medusa stage has been lost or reduced in many cnidarians, such as anemones and corals.

cavity. A gelatinous **mesoglea** separates the two true body layers. Cells of cnidarians are organized into true tissues (nervous, muscular, and reproductive) but not organs.

Cnidarians have two basic body plans: **polyps** and **medusae** (fig. 36.8). Polyps are cylindrical animals with a mouth surrounded by tentacles atop the cylinder (i.e., the end facing away from the substrate). Polyps are usually attached to the substrate and may be solitary or colonial. In contrast to polyps, medusae are usually free-floating and umbrella-shaped. Their mouths point downward and are surrounded by hanging tentacles. The classes of cnidarians are distinguished primarily by the relative dominance of the polyp stage or the medusa stage in the life cycle. Many cnidarians occur only as polyps, others only as medusae, and still others alternate between these two forms. This alternation is a form of **polymorphism**, which means "many forms."

The life cycle of many cnidarians is characterized by alternation between polyp and medusa (i.e., polymorphism; fig. 36.8). During the life cycle, medusae produce and release eggs and sperm into water for fertilization, although some species retain their eggs. After fertilization the zygote develops into a swimming mass of ciliated cells called a **planula larva**. A planula eventually attaches to the substrate and develops into a polyp. The polyp may reproduce asexually by budding other polyps or may continue the sexual cycle by budding immature medusae called **ephyrae**. An ephyra develops into a mature medusa.

All cnidarians are carnivores that capture their prey (small fishes and crustaceans) with tentacles that ring their mouth. Captured prey are pushed through the mouth into the **gastrovascular cavity** (GVC), where **extracellular digestion** occurs followed by phagocytosis of small food particles and some intracellular digestion (fig. 36.9).These tentacles are armed with stinging cells called **cnidocytes** containing small, barbed harpoonlike structures called **nematocysts** (fig 36.10).

Question 5

a. Consider objective 1 listed at the beginning of this exercise. Are cnidocytes significant to fundamental processes for cnidarians? In what ways?

b. Consider objective 1 listed at the beginning of this exercise. How could polymorphism contribute to the evolutionary success of cnidarians in their environment?

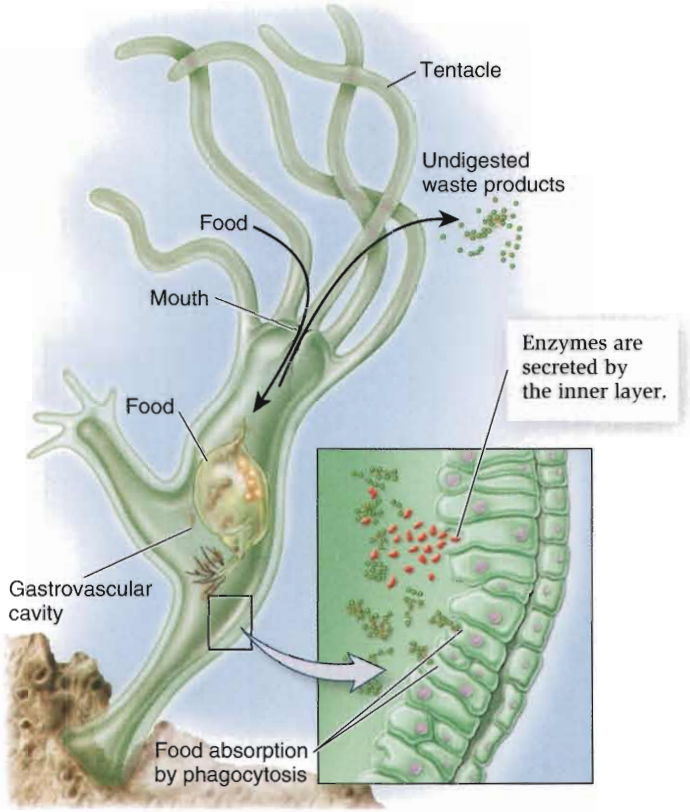


Figure 36.9

Extracellular digestion. In animals with gastrovascular cavities, such as the cnidarian Hydra, digestion occurs extracellularly. Food sources are trapped by tentacles and delivered to the mouth. Digestion occurs within the gastrovascular cavity. Digested food products are phagocytosed directly into the cells that line the cavity, and wastes are excreted out the same opening in which food entered.

Class Hydrozoa

The polyp stage dominates the hydrozoan life cycle, although both polyps and medusae occur in most species. The outer layer of cells, the ectoderm, and the inner layer, the endoderm, surround the gastrovascular cavity; these layers are separated by the gelatinous, acellular mesoglea. Amoeboid cells circulate in the mesoglea. Ectodermal cells include cnidocytes and muscular contractile cells. Endodermal and glandular cells secrete enzymes into the gastrovascular cavity for extracellular digestion. The gastrodermis lacks cnidocytes.

Hydra

Hydra are small, common hydrozoans that live in shallow, freshwater ponds. They are usually less than 1 cm tall and prey on smaller invertebrates among the filaments and leaves of freshwater algae and plants. *Hydra* have no medusa stage.

Procedure 36.2

Observe *Hydra*

1. Obtain a living *Hydra*; observe it in a small petri dish with a dissecting microscope. Polyps of *Hydra* are solitary and occasionally hang from the water's surface with their **basal disks** adhering to the surface of the water. More often, they attach to a hard substrate with their basal disk. However, *Hydra* can detach themselves and move, not by swimming but by somersaulting along their substrate.
2. Allow a few minutes for the animal to relax in the petri dish; then tap the edge and observe the animal's response.
3. If small, living crustaceans such as *Daphnia* or *Artemia* (brine shrimp) are available, place some of these organisms near the tentacles of a *Hydra*. When a prey item touches a tentacle it sticks tightly. The nematocysts help entrap the *Daphnia*.
4. Observe the cellular structure of *Hydra* by studying a prepared slide of a cross section of the organism (fig. 36.10, 36.11).

Question 6

a. How do *Hydra* respond to a tap on their substrate?

b. What tissues must exist for this response?

c. *Hydra* are predators. How actively do *Hydra* stalk their food?

d. What specialized cells of tentacles aid in capturing prey?

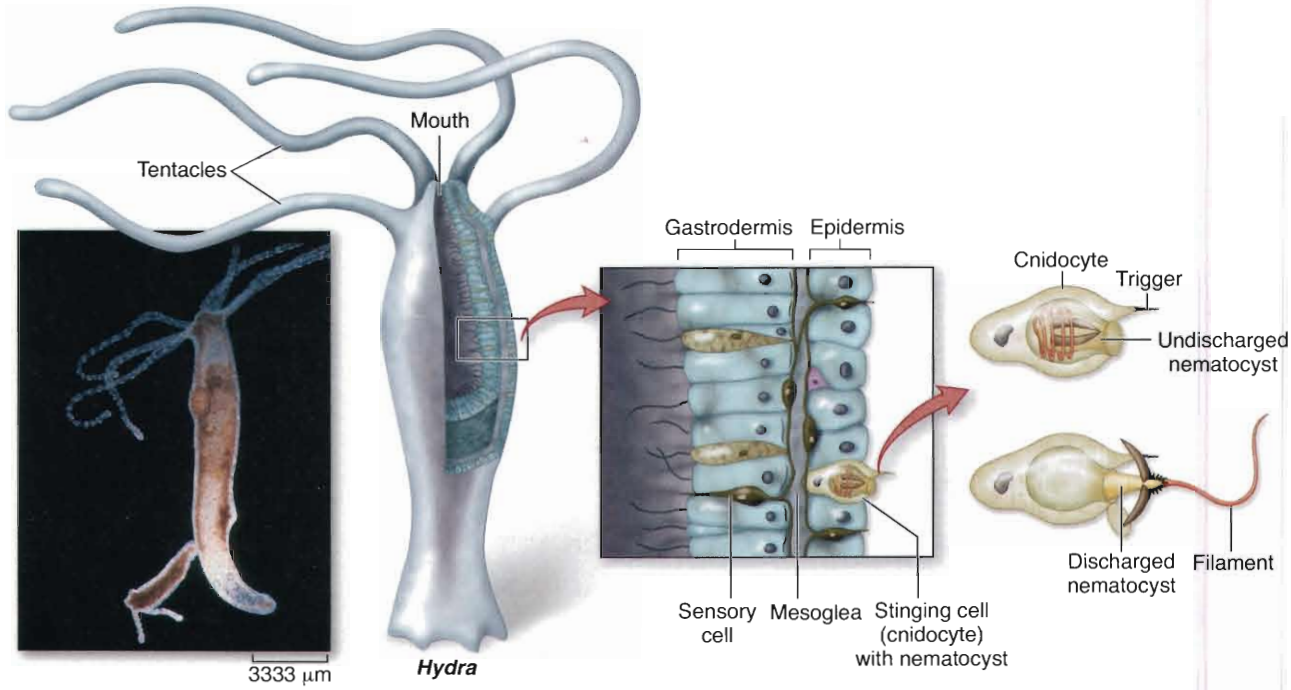


Figure 36.10

Phylum Cnidaria: cnidarians. The cells of a cnidarian such as this *Hydra* are organized into specialized tisues. The interior gut cavity is specialized for extracellular digestion—that is, digestion within a gut cavity rather than within individual cells. Cnidarians are radially symmetrical, with parts arranged around a central axis like the petals of a daisy. The epidermis includes stinging cells called cnidocytes, and each cnidocyte can discharge a harpoonlike nematocyst. Cnidocytes are scattered on the body wall and dense on the tentacles.

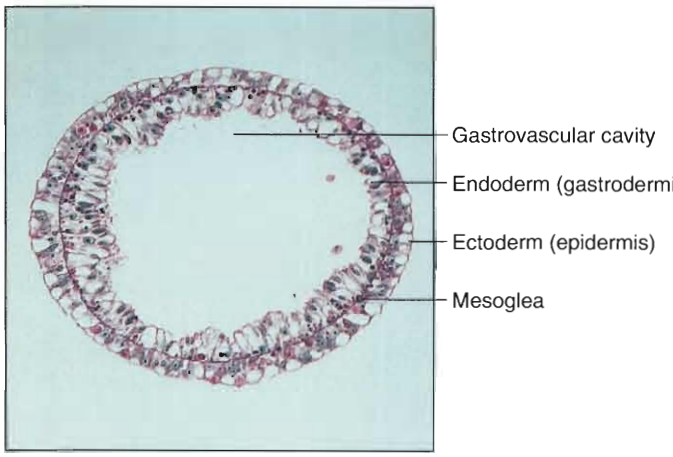


Figure 36.11

Micrograph of a cross section of a *Hydra*, a hydrozoan (100×).

Obelia

Examine a prepared slide of another hydrozoan, *Obelia*. Examine a prepared slide of the small medusae of *Obelia* in addition to the polyp colony. *Obelia* typifies most hydrozoans because it has colonial polyps and free-swimming medusae (fig. 36.12). These colonial polyps appear plantlike and branch from a tube. Polyps of *Obelia* are polymorphic because some are specialized feeding polyps called **gastrozooids**; others are reproductive polyps called **gonozoids**.

Question 7

a. What structures determine whether a polyp of *Obelia* is a gastrozooid (feeding polyp) rather than a gonozoid?

b. How do gonozoids obtain their food in this colonial organism?

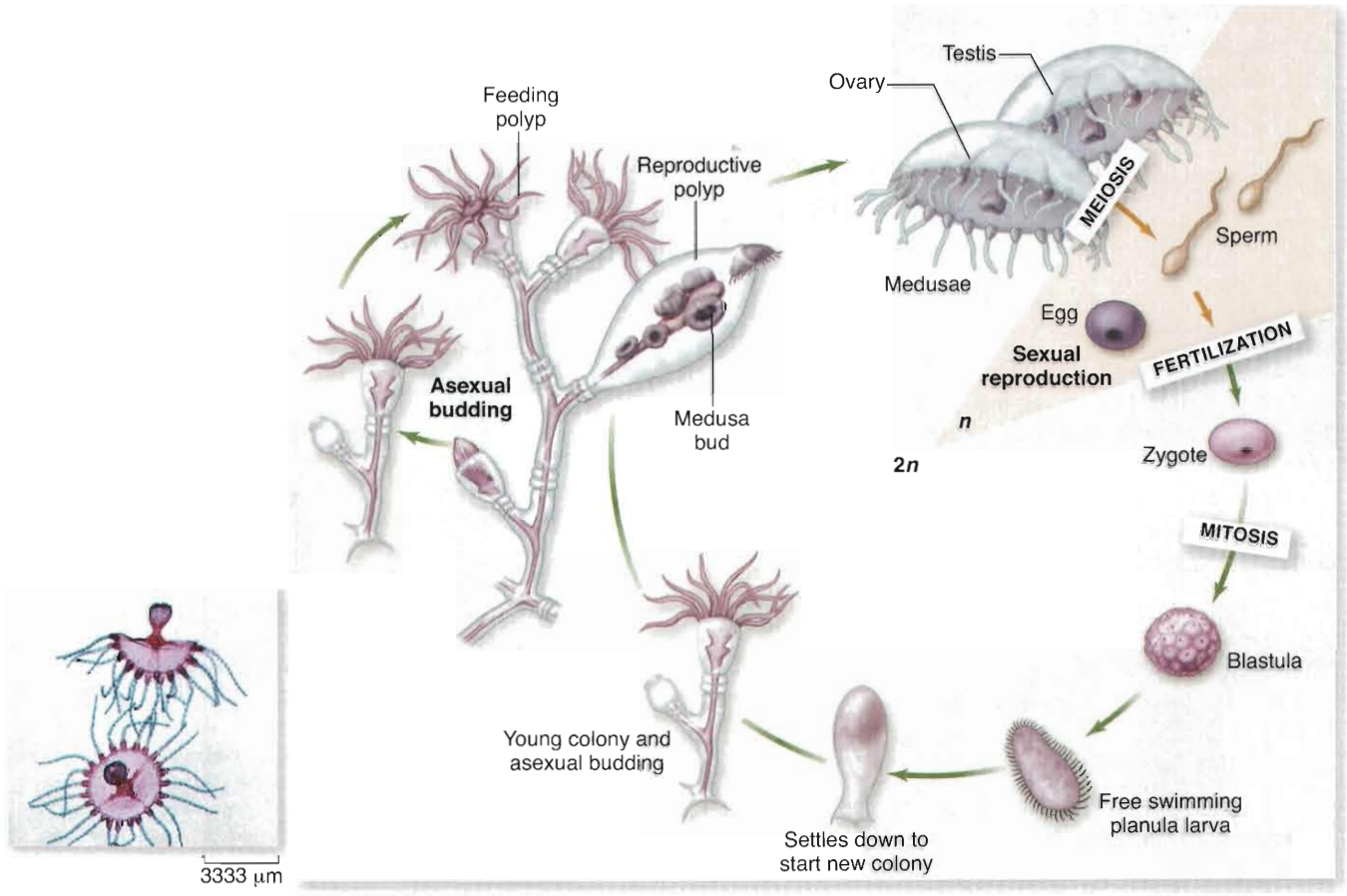


Figure 36.12

The life cycle of *Obelia*, a marine colonial hydroid. Polyps reproduce by asexual budding, forming colonies. Reproductive polyps may also give rise to medusae, which reproduce sexually via gametes. These gametes fuse, producing zygotes that develop into planulae, which in turn settle down to produce polyps.

INVESTIGATION

Prey Detection by Hydra

Observations: *Hydra* are predators capable of relatively complex behavior. They readily detect vibration as well as changes in water chemistry produced by their potential prey.

Question: How do *Hydra* sense their prey?

a. Establish a working lab group and obtain Investigation Worksheet 36 from your instructor.
b. Patiently observe *Hydra* movement and their responsiveness. Discuss with your group a well-defined question relevant to *Hydra* response to prey movement, to dissolved molecules from their prey, or both. Record the question on Worksheet 36.
c. Translate your question into a testable hypothesis and record it.
d. Devise a procedure to determine if *Hydra* are more responsive to prey movement, to dissolved molecules from their prey, or both.
e. Outline on Worksheet 36 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
f. Conduct your procedures.
g. Record your data, answer your question, and make relevant comments.
h. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

c. Gonozoids continue the reproductive cycle by budding medusae. About how many maturing medusae are visible in a typical gonozoid?

Physalia

Examine a preserved specimen of *Physalia*, a common hydrozoan better known as the Portuguese man-of-war (fig. 36.13). *Physalia* is a floating colony of polymorphic polyps. Some of the polyps form a gas-filled sac that floats and suspends the long tentacles of nutritive polyps. Touching the nematocysts on the dangling tentacles is lethal for small fish and painful (but rarely lethal) for swimmers.

Gonionemus

Examine a preserved specimen of *Gonionemus* with your dissecting microscope (fig. 36.14). Examine any other preserved hydrozoans on display. *Gonionemus* is a hydrozoan with large medusae. Medusae are more gelatinous than polyps because the mesoglea is more extensive. Locate the **velum** on the inner periphery of the medusae and the mouth at the end of the **manubrium**. The **gastrovascular cavity** radiates from the center as **ring canals** connected by a **circular canal** around the perimeter. The **gonads** (tissue that produces gametes) attach to the radial canals and

Figure 36.13

Portuguese man-of-war, *Physalia utriculus*. The Portuguese man-of-war is a colonial hydrozoan that has adopted the way of life characteristic of jellyfish. This highly integrated colonial organism can ensnare fish by using its painful stings and tentacles, sometimes over 15 m long.

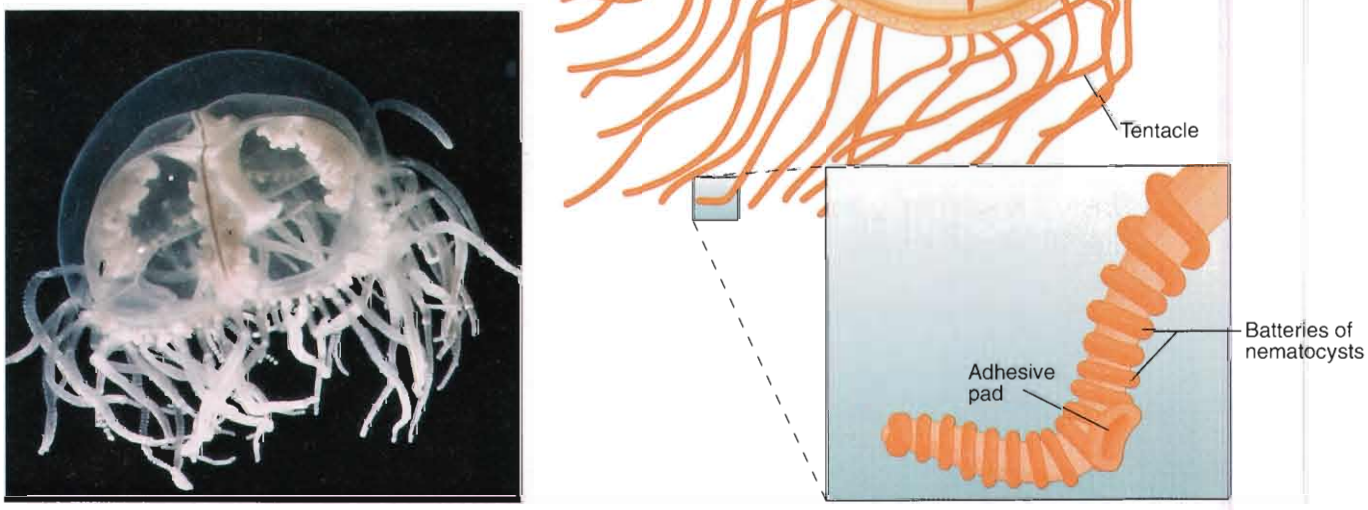


Figure 36.14

Gonionemus, a hydrozoan. (*a*) The medusa. (*b*) Diagram showing the structure of the medusa.

appear similar in males and females. The **tentacles** have a rough surface.

Question 8
Which cells give tentacles of *Gonionemus* their rough surface?

Class Scyphozoa

Observe preserved medusae of *Aurelia* and *Cassiopeia*. Scyphozoans are commonly called sea jellies (jellyfish) because the gelatinous medusa dominates their life cycle. The polyp is reduced to a small larval stage. The mesoglea has amoeboid cells. The GVC is divided into four radiating pouches, and the gastrodermis has cnidocytes. This group also includes one of the largest invertebrates in the world, *Cyanea capillota*. *Cyanea* lives in the North Sea and can exceed 2 m in diameter. *Aurelia* (2–6 cm) is a more typical sea jelly for you to examine (fig. 36.15).

Examine prepared slides of (1) planula larvae produced by sexual reproduction of medusae, (2) the polyp stage called a **scyphistoma**, and (3) ephyra (immature medusae) budded from the polyp. Review the life cycle of *Aurelia* (fig. 36.16).

Question 9
How do medusae of *Aurelia* and *Gonionemus* differ in size, arrangement of tentacles, and shape of manubrium?

Figure 36.15
Aurelia, the moon jellyfish. The gelatinous medusa is the dominant body form in the life cycle of these cnidarians.

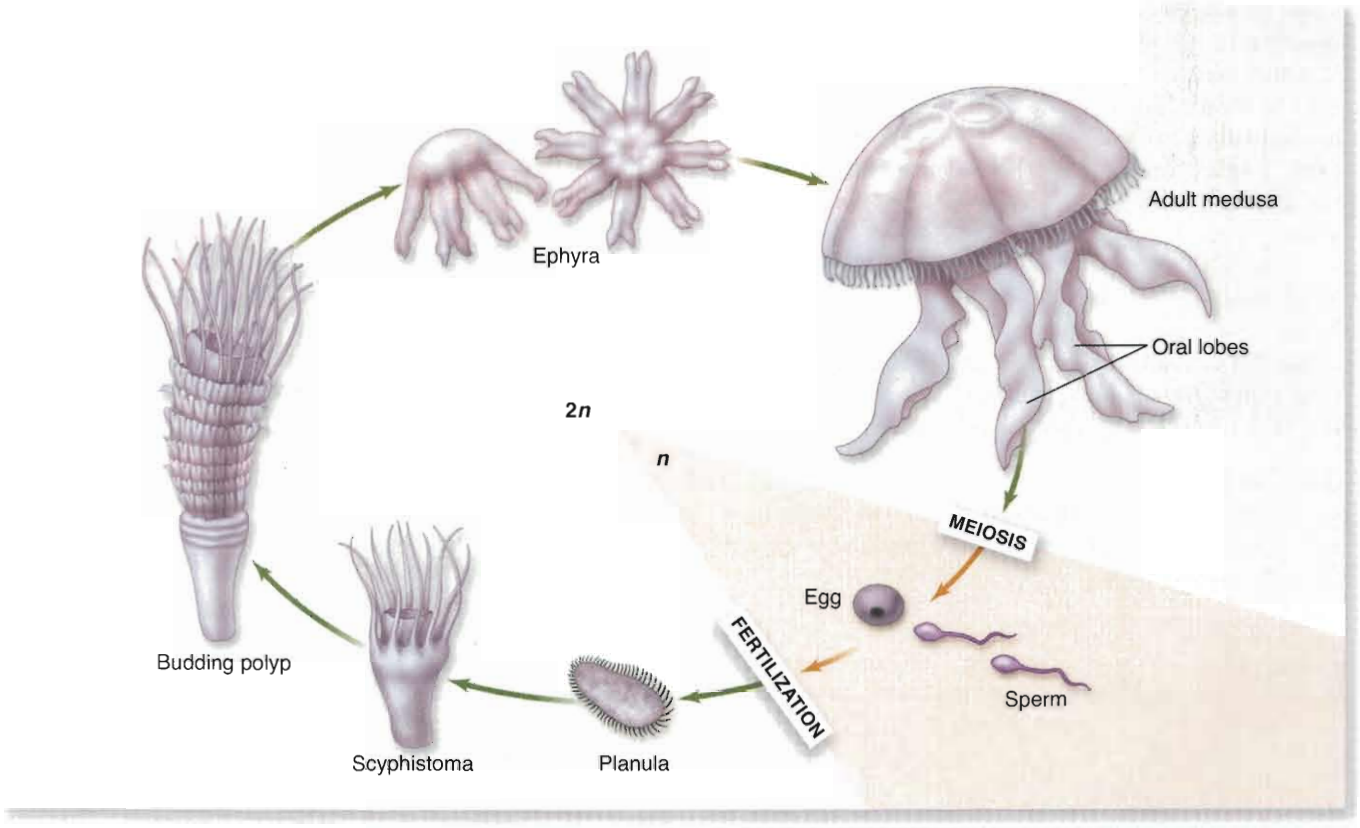


Figure 36.16
Aurelia life history. For the dioecious *Aurelia*, like all scyphozoans, the medusa stage dominates the life cycle. Male and female medusae produce gametes for fertilization in the water. These gametes are the only haploid (*n*) stage of the life cycle; all other stages are diploid (2*n*). The zygote develops into a planula larva that settles to the substrate and forms a scyphistoma (polyp) that produces ephyrae (immature medusae) by budding. Medusae separate from the polyp and swim away.

Class Anthozoa

Anthozoans (anemones and corals) form the largest class of cnidarians with more than 6000 species (fig. 36.17). Anthozoan polyps are solitary or colonial, and there is no medusa. The mouth leads to a tubular pharynx and to a GVC with septate compartments. Gonads are gastrodermal.

Metridium

Obtain a specimen of the common anemone (pronounced ah-neh-moh-nee), *Metridium*, and find its mouth and tentacles. Make a cross section through the body of *Metridium* and expose the gastrovascular cavity (this dissection may be on demonstration). Locate the structures shown in figure 36.18. Anemones are sessile and attach themselves to a substrate with their flat and sticky basal disk. However, this attachment is not permanent, and anemones can slowly slide on a film of mucus. When pieces of the basal disk tear away from a moving anemone the pieces form a new individual. This type of asexual reproduction is **fragmentation**.

The gastrovascular cavity of an anthozoan polyp is partitioned by thin septa. These septa distinguish anthozoan from scyphozoan and hydrozoan polyps.

Corals

Examine a piece of dry, calcareous coral and look for small depressions (fig. 36.19). Coral polyps are structurally similar to anemones, but corals are usually colonial and much smaller. Most corals secrete a hard skeleton of calcium carbonate with many small cups surrounding the polyps. The small, fragile polyps are probably absent from the specimen you are examining, but the depressions in which they lived are numerous.

Examine a piece of *Tubipora* (fig. 36.20) and any other anthozoans on display. The tropical organ pipe coral, *Tubipora*, is organized differently. Long parallel polyps are encased in calcareous tubes connected at intervals by transverse plates. The calcareous tubes are impregnated with iron salts that give the colony an attractive color.

Question 10

a. Locate the radial ridges within each depression on a piece of coral. What structures within the polyp did they support?

b. What is the advantage of a partitioned gastrovascular cavity?

c. Consider objective 1 listed at the beginning of this exercise. How does fragmentation contribute to the evolutionary success of anthozoans in their environment?

Figure 36.17
Sea anemone, a common anthozoan.

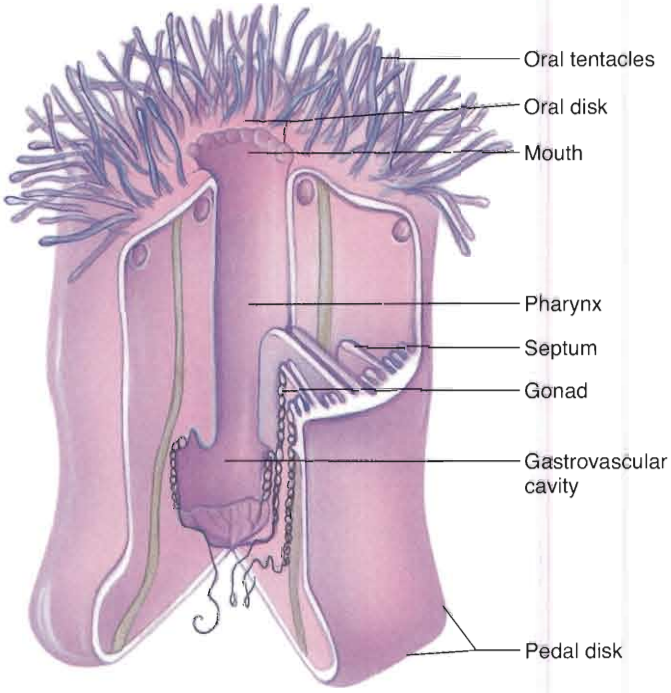


Figure 36.18
Class Anthozoa. The structure of the anemone, *Metridium*. Anthozoans have no medusa stage.

Question 11

a. In your notebook draw and describe the life cycle of a cnidarian.

b. Compare the feeding methods of sponges and sea jellies.

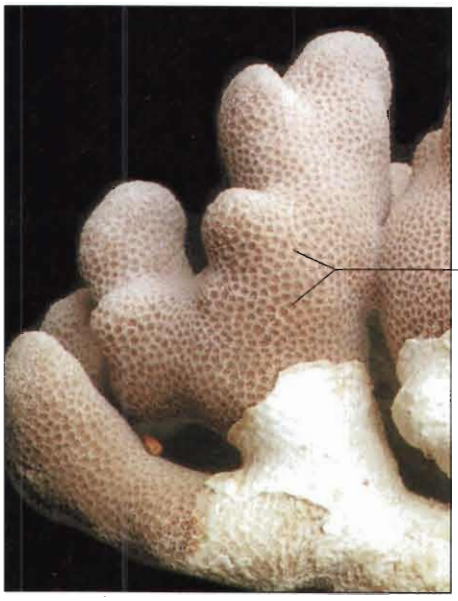


Figure 36.19
Calcium carbonate skeleton of a stony coral.

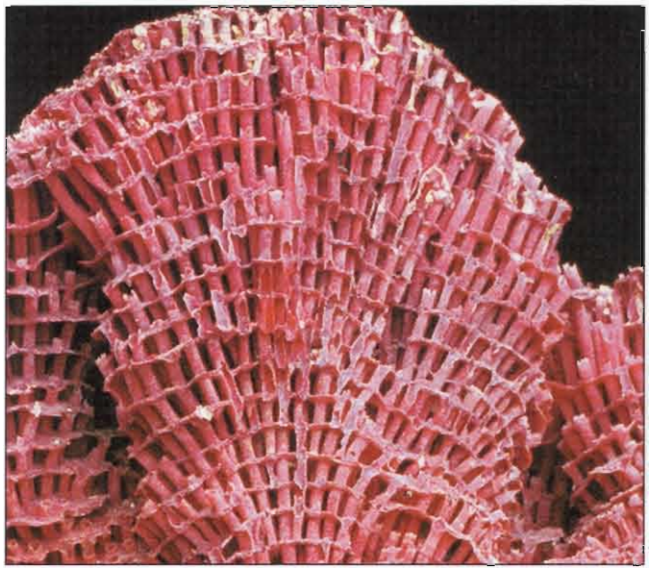


Figure 36.20
Tubipora, organ pipe coral.

Questions for Further Thought and Study

1. Which group within kingdom Protista probably gave rise to sponges? On what evidence do you base your answer?

2. Why are spicules used as a primary characteristic in the taxonomy of sponges?

3. Sponges and cnidarians have no lungs or gills. How do they exchange gases with the environment? Are humans better off having lungs? How so?

4. Why are sponges considered to be an evolutionary dead end?

5. Explain how cnidocytes with their nematocysts function in food capture and defense.

6. Discuss how polymorphism in the cnidarians might have influenced adaptive radiation of the group.

7. How does the digestive process in cnidarians differ from the digestive process in sponges?

8. Prepare a simple table of all of the taxonomic groups, their common names, their distinguishing characteristics, and all representative genera covered in this exercise. Keep this table with your study notes.

WRITING TO LEARN BIOLOGY

What are the advantages and disadvantages to a solitary versus colonial existence?

Survey of the Animal Kingdom
Phyla Platyhelminthes and Nematoda

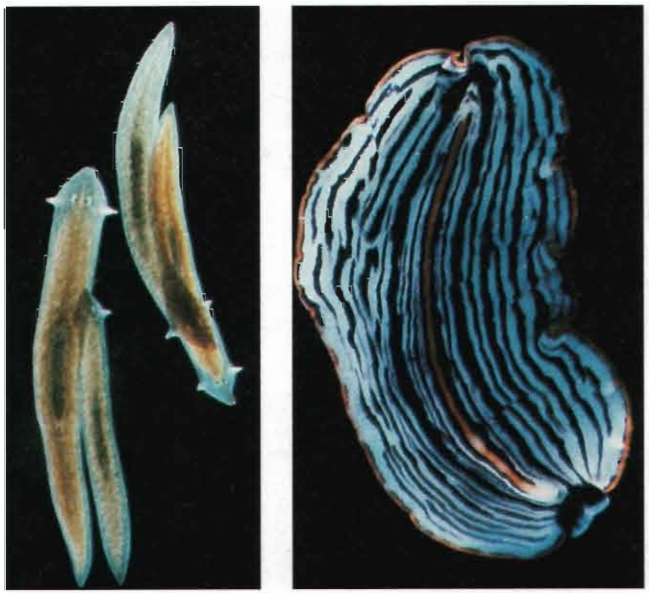
37

Objectives

By the end of this exercise you should be able to:

1. Describe how the prominent characteristics of platyhelminths and nematodes promote their survival and reproduction.
2. Describe the general morphology of flatworms in phylum Platyhelminthes and roundworms in phylum Nematoda.
3. List characteristics that phyla Platyhelminthes and Nematoda have in common with phyla Porifera and Cnidaria.
4. List characteristics of flatworms and roundworms more advanced than those of more primitive phyla.
5. List examples of roundworms and examples of each major class of flatworms.
6. Understand the differences between acoelomate, pseudocoelomate, and coelomate and know which phyla are associated with each.
7. Discuss characteristics that have most likely contributed to flatworm and roundworm success over millions of years and relate the discussion to objective 1.

Flatworms of **phylum Platyhelminthes** and **roundworms** of **phylum Nematoda** are successful—remarkably successful if we measure evolutionary success by high diversity, persistence in the environment for 400 million years, and radiation to wide-ranging habitats. Both phyla occur in marine, freshwater, terrestrial, and parasitic environments. Their morphology is more complex than that of sponges and sea jellies (table 37.1). For example, flatworms and roundworms have a cellular **mesoderm** in addition to ectoderm and endoderm. Flatworms are **acoelomate**, meaning that their mesoderm is a solid mass of tissue with no internal cavity surrounded by mesoderm (see fig. 38.1). Acoelomates have no spacious cavity for internal organs. **Coelomate** animals are discussed in the next three exercises and have major organs suspended in a coelomic cavity completely surrounded by mesoderm. Because flatworms have three germ layers, flatworms and roundworms are described as **triploblastic**. They also have **organs** made of interdependent tissues and are the simplest animals having **bilateral symmetry** with distinct anterior and posterior ends (see fig. 36.7).



(a) (b)

Figure 37.1
Flatworms (phylum Platyhelminthes). (*a*) A common freshwater flatworm. (*b*) A free-living marine flatworm.

PHYLUM PLATYHELMINTHES

Flatworms are dorsoventrally compressed, and free-living species have primitive sense organs (fig. 37.1). They also have a gastrovascular cavity with one opening that is both mouth and anus. Their nervous system is more advanced than that of cnidarians (see Exercise 36) and consists of a ladderlike arrangement of nerve cords extending the length of the body.

Class Turbellaria

Turbellarians (3000 species) are free-living flatworms inhabiting freshwater, saltwater, and moist terrestrial environments. Turbellarians scavenge and prey on small animals, and are **hermaphroditic**, meaning that individuals have both male and female sex organs.

Dugesia

Dugesia, often called planaria, is a common freshwater turbellarian with typical characteristics of flatworms (fig. 37.2). The head has lateral lobes and sensory organs

TABLE 37.1

PHYLA PLATYHELMINTHES AND NEMATODA

Phylum	Typical Examples	Key Characteristics	Approximate Number of Named Species
Platyhelminthes (flatworms)	*Planaria*, tapeworms, liver flukes	Solid, unsegmented, bilaterally symmetrical worms; no body cavity; digestive cavity, if present, has only one opening	20,000
Nematoda (roundworms)	*Ascaris*, pinworms, hookworms, *Filaria*	Pseudocoelomate, unsegmented, bilaterally symmetrical worms; tubular digestive tract passing from mouth to anus; tiny; without cilia; live in great numbers in soil and aquatic sediments; some are important animal parasites	12,000+

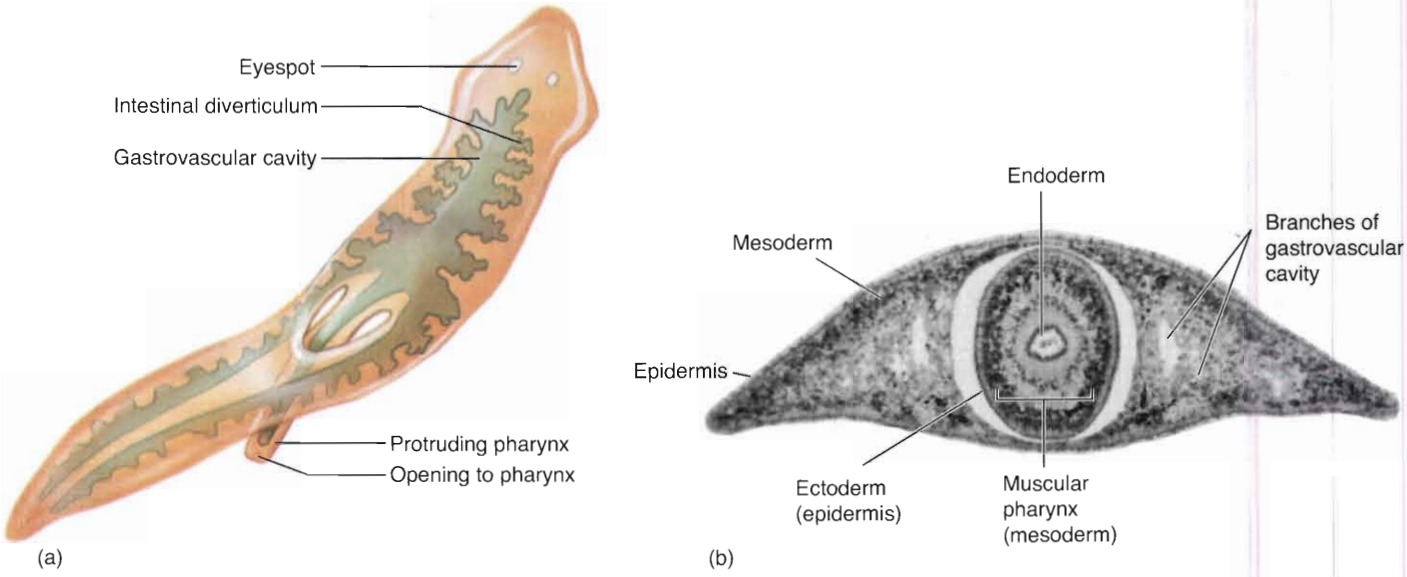


Figure 37.2

Anatomy of a free-living flatworm, *Dugesia*. (*a*) External structure. (*b*) Cross section of a planarian taken through the pharynx region.

called **eyespots**. *Dugesia* feeds by sucking food through its mouth and into a tubular **pharynx** leading to the gastrovascular cavity. The muscular pharynx is usually retracted in the body but can be everted through an opening in the midventral epidermis (middle of the lower surface). Most digestion in the gastrovascular cavity is extracellular, but phagocytic cells line the cavity and complete digestion of small particles intracellularly.

Planaria are simple organisms having no body cavity other than the digestive cavity. Planarias are acoelomate, a term that will be explained more fully in Exercise 38, figure 38.1. The mesodermal tissue includes the loose mass of cells between the ectoderm on the surface and the endoderm lining the gastrovascular cavity and pharynx. This body plan allows for remarkable powers of regeneration.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 37.1

Observe planaria

1. Obtain a living *Dugesia* and examine its morphology with a dissecting microscope.
2. Place the animal in the center of a petri dish and follow its movements for a few minutes.
3. Gently touch the animal with a probe and watch how it responds.
4. If a spotlight is available, determine how *Dugesia* may respond to strong light.
5. Offer *Dugesia* a small piece of liver, boiled egg, or cat food; watch it eat.
6. After a few minutes, gently roll the animal from the food and find its protruded pharynx.
7. Examine a whole mount of a stained planaria and a prepared slide of a cross section through a planaria. Specimens vary. Be sure to examine more than one slide, and do not rely on photographs.
8. Locate the structures shown in figure 37.2.
9. Examine cross sections taken through the region of the pharynx and away from the pharynx. Draw and label these cross sections, and have your instructor check them for accuracy.

Question 1

a. What features of *Dugesia* distinguish its head from its tail?

b. What is the difference between the eyes of most animals you are familiar with and the eyespots of *Dugesia*?

c. How does the head of *Dugesia* move differently from the tail?

d. Does *Dugesia* move randomly or in an apparent direction?

e. How is *Dugesia* adapted for directional movement?

f. How does a flatworm respond when touched with a probe?

g. Do the planaria move toward or away from light?

h. Where is the feeding tube located? Why is this unusual for bilaterally symmetrical organisms?

i. Is the gastrovascular cavity of *Dugesia* a simple sac? How is it divided and what advantage do these divisions offer?

j. Consider objective 1 listed at the beginning of this exercise. How could being monoecious contribute to evolutionary success of flatworms in their environment?

k. Planarians have a head. In biological terms, what constitutes a "head"? How does it relate to objectives 1, 4, and 7?

l. Planaria lack specialized gas-exchange organs. How do you think planaria accomplish this task?

Class Trematoda

Trematodes, commonly called flukes, are parasites, and their oval bodies are usually a few millimeters long. Flukes infect vertebrates and include both **endoparasites** (parasites inside their host) (fig. 37.3) and **ectoparasites** (parasites on the surface of their host). Trematodes lack an epidermis and are covered by an acellular but metabolically active **epicuticle**.

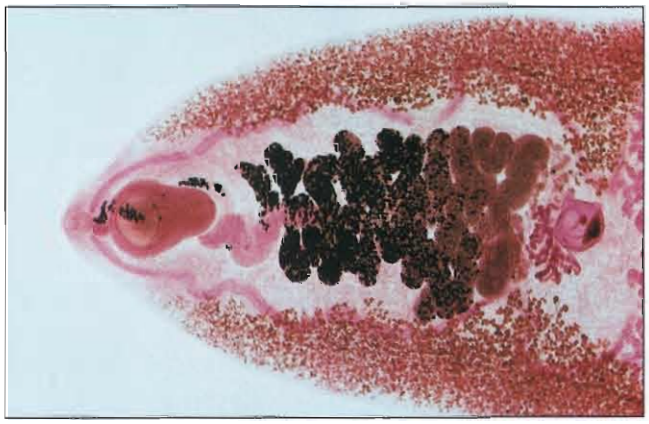


Figure 37.3

Fasciolopsis buski is a parasitic fluke that infects the small intestine of humans and pigs. Humans acquire the fluke by eating water chestnuts and other aquatic plants with larvae attached. The larvae develop in intermediate hosts such as snails and fish. A major adaptation of flukes and other parasites is the prolific production of eggs—as many as 3000 eggs per day for a lifetime of many years. The dark, coiled uterus shown here contains hundreds of eggs, but most of these eggs will never be engulfed by an intermediate host and hatch. To complete a complicated life cycle through multiple hosts, a fluke must produce immense numbers of eggs to ensure that one or two are successful. To appreciate the structure of the entire organism, see the related genus *Fasciola* in figure 37.5.

INVESTIGATION

Detection of Macromolecules by Planaria

Observations: Detecting food is crucial to success of planaria. They must "taste" the water for compounds released from nearby food. Some classes of macromolecules (fats, carbohydrates, proteins, etc.) dissolve and disperse more readily than others.

Question: What types of organic molecules does *Dugesia* respond to most readily?

a. Establish a working lab group and obtain Investigation Worksheet 37 from your instructor.

b. Patiently observe *Dugesia* movement and its responsiveness. Discuss with your group a well-defined question relevant to planarian response to dissolved molecules from their prey. Record the question on Worksheet 37.

c. Translate your question into a testable hypothesis and record it.

d. Devise a procedure to determine if *Dugesia* respond to some classes of dissolved nutrients more than other classes.

e. Outline on Worksheet 37 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

f. Conduct your procedures.

g. Record your data, answer your question, and make relevant comments.

h. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

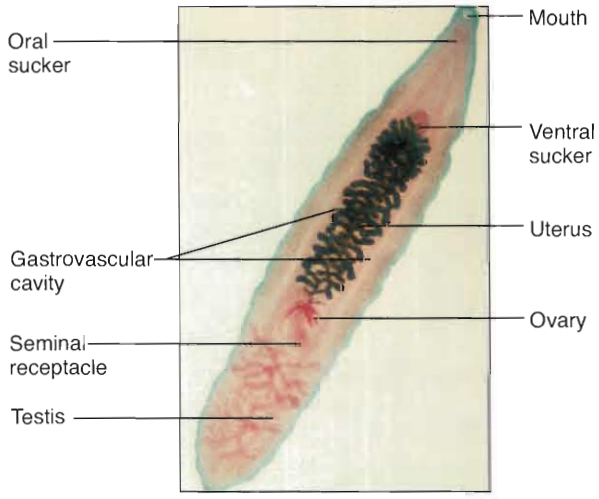


Figure 37.4
Internal structure of *Opisthorchis*, the Chinese liver fluke.

This epicuticle is made of protein and lipids secreted by mesodermal cells and resists digestive enzymes. The epicuticle helps in respiration and absorbing nutrients. The ventral surface of a fluke usually has two adhesive organs (suckers). The oral sucker surrounds the mouth.

Opisthorchis

Use a dissecting microscope to examine a prepared slide of *Opisthorchis* and locate the structures shown in figure 37.4. *Opisthorchis* (*Clonorchis*) *sinensis*, the Chinese liver fluke, often parasitizes humans in Japan and China. This hermaphroditic, adult fluke attaches to the bile duct and releases eggs that move through the digestive system of the host and exit with the feces. Larvae of flukes typically develop in snails and fish. Humans are infected when they eat raw or poorly cooked fish.

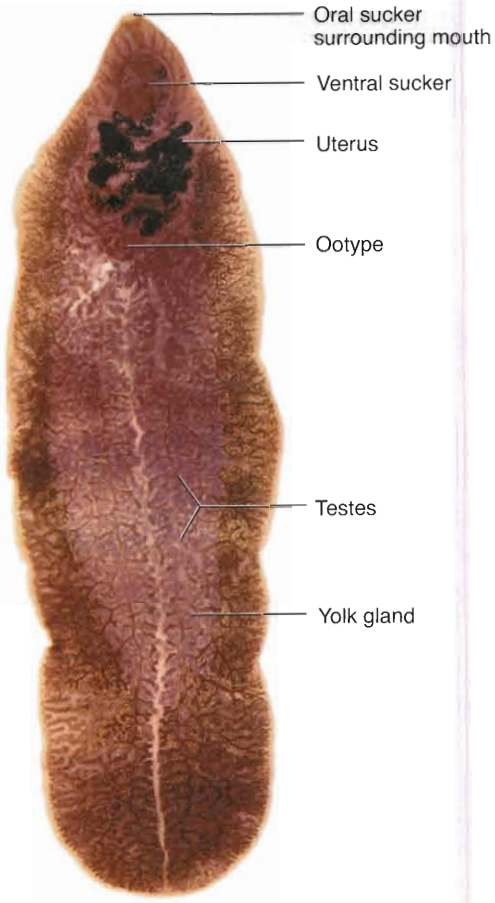


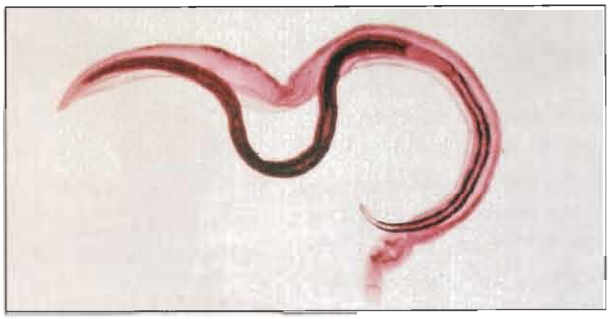
Figure 37.5
Internal structure of *Fasciola*, the sheep liver fluke.

Question 2

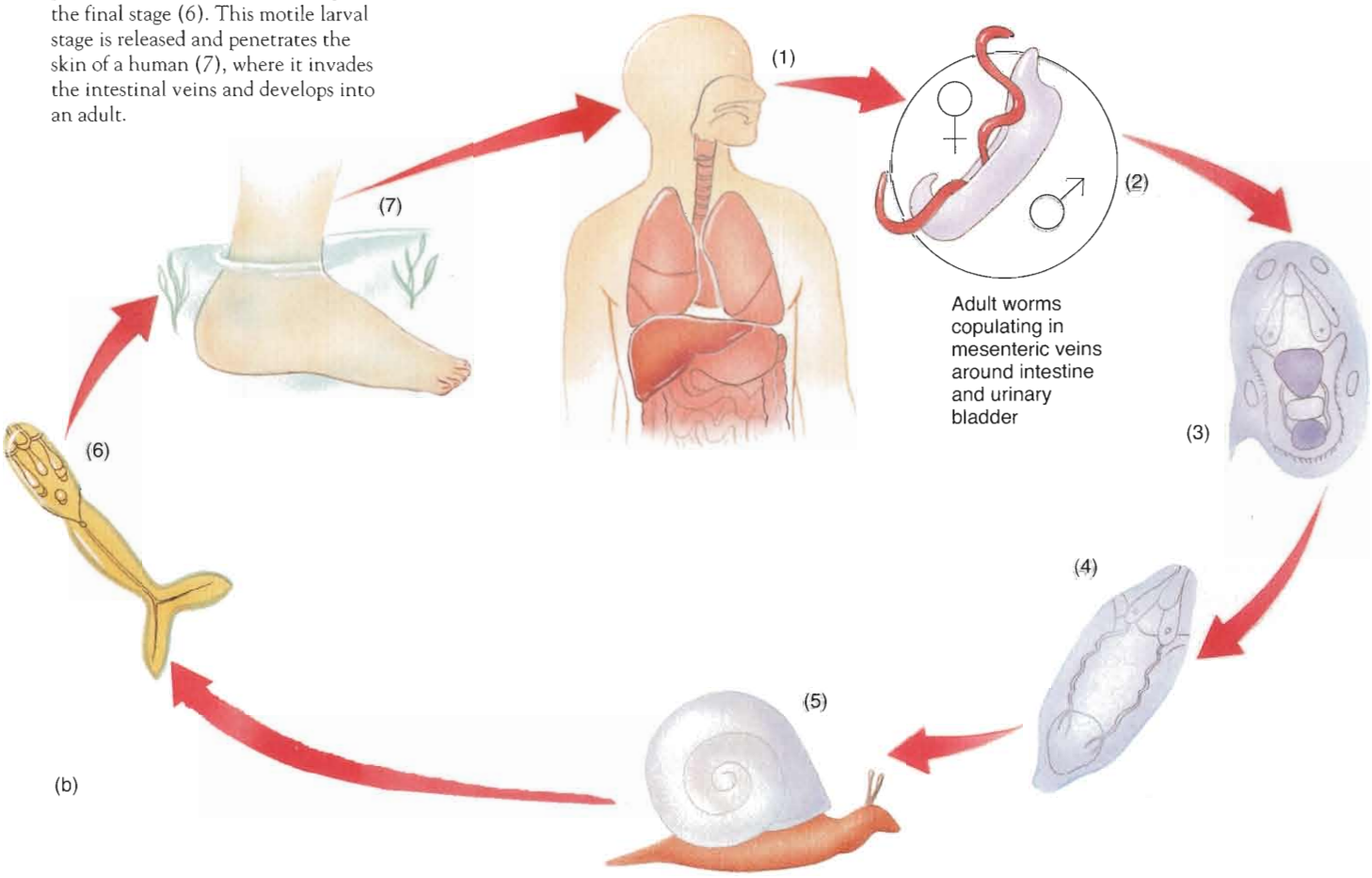
a. How does the shape of the digestive sac of *Opisthorchis* compare with that of *Dugesia*?

Figure 37.6

Schistosoma, the blood fluke that causes schistosomiasis. *Schistosoma* infects more than 200 million people. (*a*) Adult male and female *Schistosoma mansoni* in copulation. (*b*) Life cycle of *Schistosoma mansoni* begins in a human (*1*). Adult schistosomes are in permanent copulation (*2*) and produce thousands of eggs. Eggs leave the host with feces or urine and hatch as larvae (*3, 4*). A larval stage penetrates a snail (*5*) and develops the final stage (*6*). This motile larval stage is released and penetrates the skin of a human (*7*), where it invades the intestinal veins and develops into an adult.



(a)



(b)

b. How does the position of the mouth of *Dugesia* and flukes compare?

Fasciola

Examine figure 37.3 and read its caption carefully. Use a dissecting microscope to examine a prepared slide or plastic mount of *Fasciola* and locate the features shown in figure 37.5. *Fasciola hepatica*, the sheep liver fluke, infects sheep, other vertebrates, and (rarely) humans. It is much larger than *Opisthorchis* but similar in structure. *Fasciola* sucks food (blood, mucus, and cells) through a muscular pharynx located behind the mouth.

Question 3

Consider objective 1 listed at the beginning of this exercise. How could the production of large numbers of eggs contribute to the evolutionary success of flatworms in their environment?

Schistosoma

Examine a demonstration slide of *Schistosoma*, and draw its body shape. *Schistosoma*, a blood fluke, inhabits the intestinal veins and other organs of many vertebrates (including humans) and causes the disease schistosomiasis (fig. 37.6).

Schistosoma is socioeconomically important because it infects more than 200 million people in countries having tropical and temperate climates. Symptoms of infection include an enlarged liver, spleen, and bladder, as well as nutritional deficiency. Some of these symptoms, such as bleeding ulcers in the intestine and other organs, are enhanced by large deposits of eggs (up to 3500 per day from one female!).

Snails are **intermediate hosts** for *Schistosoma*. An intermediate host is an organism harboring immature stages of a parasite, whereas a **definitive host** contains sexually mature, egg-laying stages of the life cycle. Irrigation ditches with snail populations enhance the spread of schistosomiasis in underdeveloped agricultural areas. Immature larvae of blood flukes released from the snails burrow through skin and infect people wading in these ditches.

Unlike most trematodes, *Schistosoma* is dioecious. The male has a ventral groove along the length of his body into which the slender female cradles for copulation. Often the female remains in this groove for the remainder of her lifetime and extends slightly to lay eggs. Your prepared slide may include schistosomes in this position for copulation.

Question 4
How does the shape of *Schistosoma* differ from that of other flukes you have studied?

Class Cestoda

Cestodes, commonly called tapeworms, are the most specialized platyhelminths. They are endoparasites of the gut of vertebrates and are covered by a cuticle similar to that of trematodes (fig. 37.7). However, tapeworms lack a mouth or digestive tract and have a unique body plan (fig. 37.8). Their cuticle efficiently absorbs nutrients from their host. The anterior end, or **scolex**, adheres to the host's intestinal wall with hooks or suckers. Behind the scolex is the **neck** followed by a series of segments called **proglottids**. The scolex and neck are small, but the chain of proglottids may be 10–15 m long.

A tapeworm grows as the scolex and neck continually produce a chain of proglottids as self-contained packets of male and female reproductive organs. Self-fertilization occasionally occurs in a proglottid, but cross-fertilization by copulating proglottids of adjacent worms is more common. **Gravid** (egg-carrying) proglottids eventually break from the end of the worm and pass from the host with its feces.

Examine a prepared slide or whole specimen of a pork tapeworm, *Taenia solium*. Then examine a prepared slide of a scolex. Draw the basic shape of the scolex and note any hooks or suckers. Also examine and sketch from prepared slides young, mature, and gravid proglottids.

An adult *Taenia solium* may be up to 10 m long. Humans infect themselves with *Taenia* by eating uncooked meat from pigs, often intermediate hosts.

Compare a mature proglottid with that shown in figure 37.9. Although each proglottid has a complete reproductive system, the excretory ducts and longitudinal nerves are continuous between proglottids.

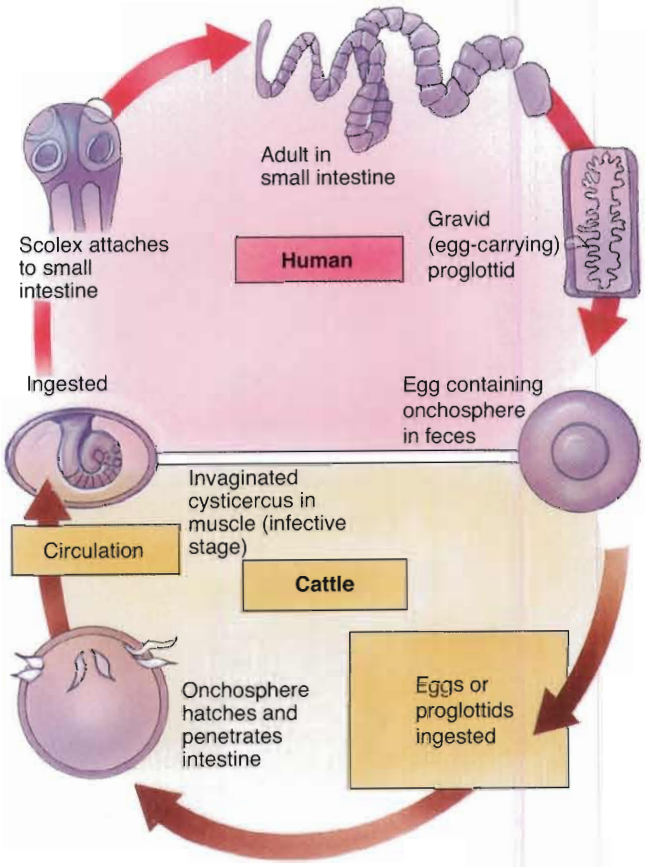


Figure 37.7
Life cycle of a beef tapeworm. Tapeworms, members of the class Cestoda, are the most specialized flatworms and require human and cattle hosts. The onchosphere and cysticercus are specialized stages of development.

Examine a demonstration specimen of *Dibothriocephalus latus* if it is available. *Dibothriocephalus latus* is the largest tapeworm to infect humans and can be up to 20 m long. Its intermediate hosts are small crustaceans and fish.

Question 5

a. How does a scolex compare in size to a proglottid near the scolex? Near the posterior end?

b. Tapeworms have no digestive system or mouth. How, then, do they obtain food?

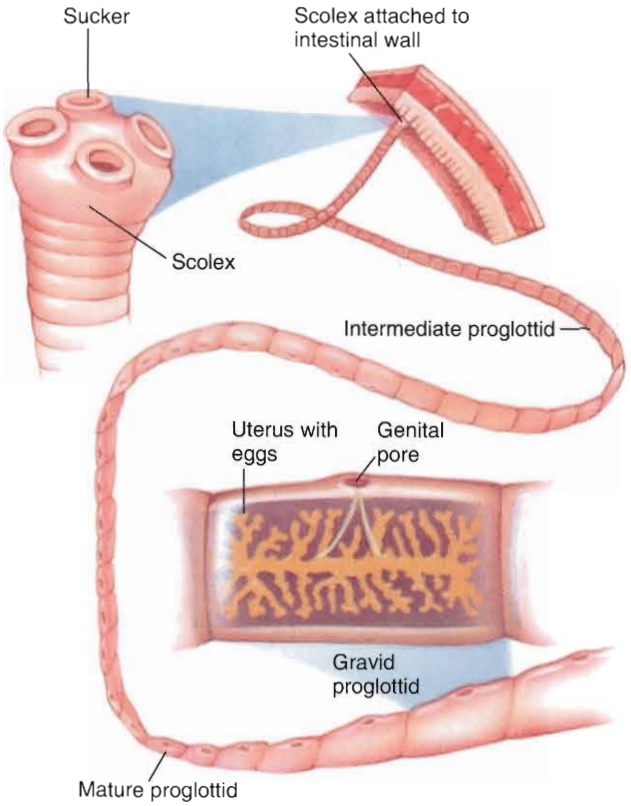


Figure 37.8
Body plan of a tapeworm (class Cestoda). A mature proglottid has mature reproductive organs; a gravid proglottid contains many eggs.

c. Examine figure 37.8 and its caption carefully. Which proglottids, mature or gravid, occur closest to the scolex?

d. What is the difference between a mature and gravid proglottid?

e. Consider objective 1 listed at the beginning of this exercise. Is having a scolex significant to a fundamental process for tapeworms? For which process, and how is it significant?

f. Tapeworms are specialized. Would you expect specialists to have a unique morphology? Why?

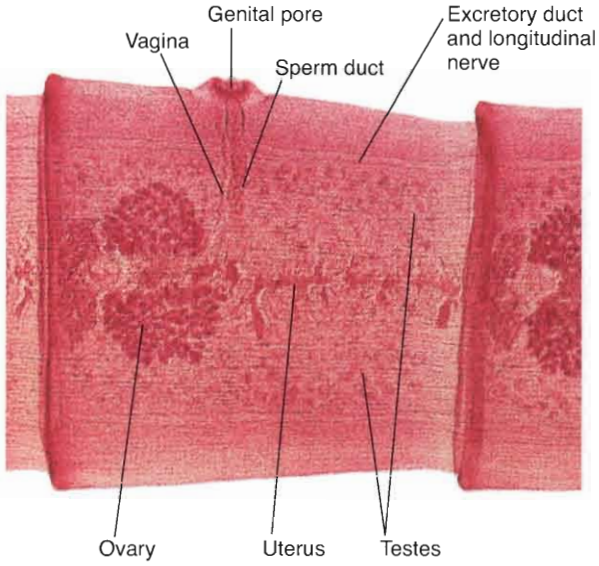


Figure 37.9
Mature proglottid of *Taenia pisiformis*, dog tapeworm. Portions of two other proglottids are also visible.

Figure 37.10
Ascaris lumbricoides, a common roundworm, inhabits the intestines of pigs and humans. Male ascarid worms are smaller than females and have a curved posterior end.

PHYLUM NEMATODA

Nematodes (fig. 37.10), commonly called roundworms, are everywhere and often occur in great numbers—a single decomposing apple may contain 100,000 nematodes of different species. If we removed everything but nematodes from

the environment, we would still see a ghostly outline of our entire biosphere. Estimates of the global number of species living in all aquatic, terrestrial, and parasitic environments often exceed one million. Most have not yet been formally described and named. Almost all feeding types (parasitic, predatory, etc.) are represented. Reproductive morphologies include dimorphic species, hermaphroditic species, and even species with males as well as hermaphrodites. Nematode diversity is extraordinary.

Many nematodes cause diseases in humans, other animals, and plants. One of the most serious of these diseases is elephantiasis, the grotesque swelling of an arm or leg resulting from nematodes (commonly *Filaria*) clogging the lymphatic system that drains the host's appendage (fig. 37.11). Fluid accumulates, and the appendage swells. Another parasitic nematode is the eye worm, *Loa loa*, which lives under the skin of humans and occasionally crawls across the surface of an eye (fig. 37.12).

Nematodes are slender and long with a rather featureless exterior. They lack flagella and cilia and are covered with a flexible and chemically complex **cuticle**. The cuticle resists digestive enzymes and is permeable only to water, dissolved gases, and some ions. Roundworms have two morphological advances absent in flatworms. First, in addition to their digestive cavity, roundworms have a body cavity called a **pseudocoelom** consisting of a fluid-filled space between the body wall and digestive tract (see fig. 38.1). Internal organs are suspended in this cavity. Second, nematodes have a **complete digestive tract** with a mouth and anus.

Question 6

a. How does the number of body cavities of nematodes compare with that of flatworms?

b. What are the advantages of a digestive tract having a separate entrance and exit?

c. Female *Ascaris* are more numerous than males. Why might this be adaptive?

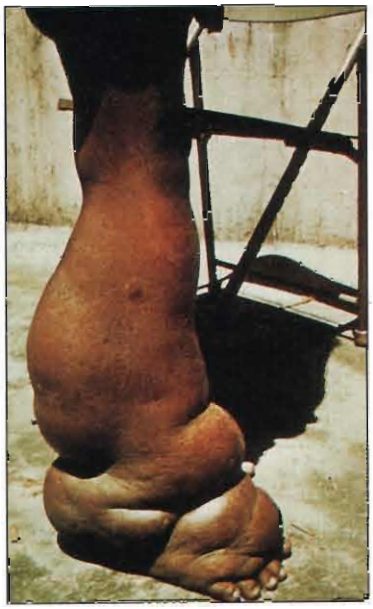


Figure 37.11

Elephantiasis of a leg caused by adult filarial worms that live in lymph passages and block the flow of lymph. Tiny juveniles, called microfilariae, are picked up in a blood meal of a mosquito where they develop to the infective stage and are transmitted to a new host.

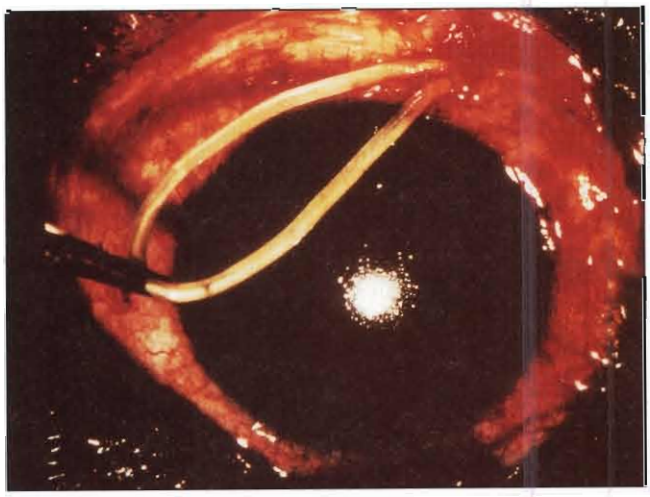


Figure 37.12

The nematode *Loa loa* appears in the eye of its host. This one is being surgically removed from someone's eye.

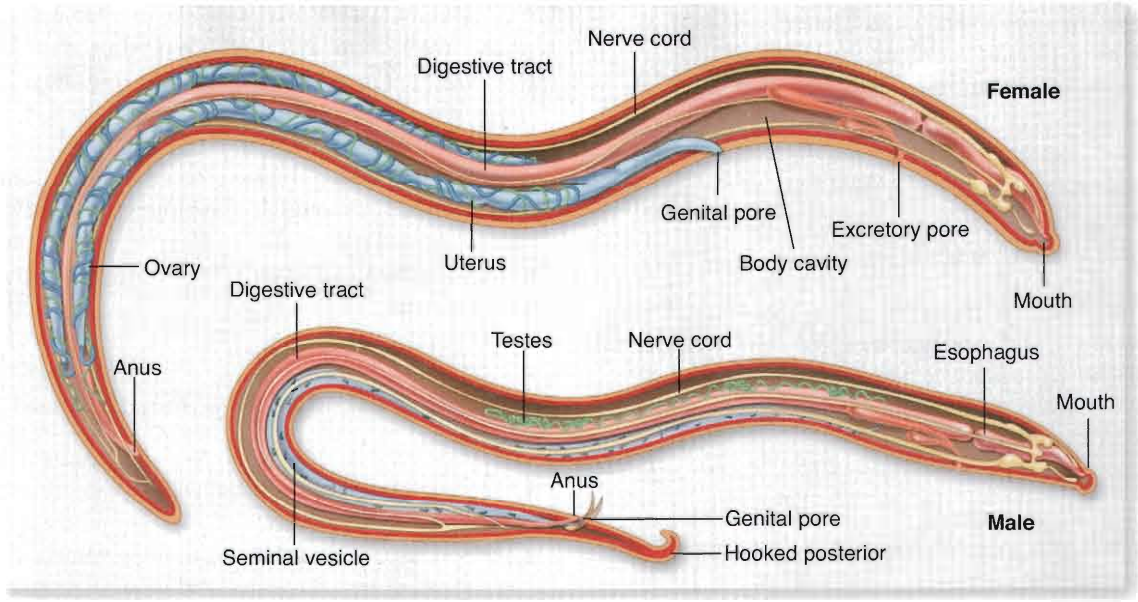


Figure 37.13
Internal anatomy of nematodes, commonly called roundworms.

Rhabditus and *Turbatrix*

Procedure 37.2

Examine living nematodes

1. Examine a culture of living *Rhabditus*, *Turbatrix* (the vinegar eel), or *Cephalobus*.
2. Using a toothpick, put a small piece of the culture medium on a slide with a drop of water and coverslip, and focus with low magnification of your microscope.
3. Watch the nematodes move.

The flexible cuticle and hydrostatic pressure of fluid in their pseudocoel aid locomotion by resisting antagonistic muscle contraction. Nematodes have only longitudinal muscles and lack circular or diagonal muscles. This combination of features produces a characteristic motion.

Question 7

a. How would you describe the motion of a nematode?

b. How is this movement related to the movement of its muscle layers?

Ascaris

Ascaris is a large nematode that infects the intestinal tract of humans and other vertebrates (fig. 37.10). Males are smaller than females and have a hooked posterior end. The opening in the posterior end is the anus.

Procedure 37.3

Dissect *Ascaris*

1. Obtain a preserved *Ascaris lumbricoides* and examine its external features using a dissecting microscope. Compare the external features of males and females.
2. At the anterior end, locate the mouth surrounded by three lobes of tissue.
3. Prick the cuticle with a dissecting probe (teasing needle) and determine its consistency.
4. Pin the ends of a female specimen near the edge of a dissecting pan to permit viewing with a dissecting microscope. Slit the body wall longitudinally with a dissecting needle or sharp-pointed scissors.
5. Pin the body wall open and locate the internal organs shown in figure 37.13. The excretory pore may be small and difficult to find. A dissection of a male may be on demonstration.
6. Examine a prepared slide of a cross section of both a male and female *Ascaris*.

7. Locate the features shown in figure 37.14 and determine from where along the length of your dissected specimen this section was taken.
8. Examine cross sections from different areas of the body if appropriate slides are available. Gametes mature as they move along the length of the tubular reproductive organs.
9. Dispose of waste in labeled containers.

Question 8

a. The cuticle of *Ascaris* is flaky and tough. Consider objective 1 listed at the beginning of this exercise. What might be an adaptive advantage of a thick and tough cuticle?

b. Where do the internal organs of *Ascaris* attach to the body wall?

c. How does the diameter of the female reproductive tract change?

d. Are any sensory organs evident in *Ascaris*? Why would this be adaptive?

Trichinella

Trichinella spiralis causes the disease trichinosis. Adult females of *Trichinella* live in the intestine of their host and release larvae. These larvae migrate through the body to striated muscles, especially in the diaphragm and tongue, where they form calcified cysts that can be painful (fig. 37.15). The larvae remain encysted until the muscle tissue is eaten by another host, where the larvae mature. Humans infect themselves by eating poorly cooked pork containing encysted larvae. However, the occurrence of trichinosis is decreasing. In the United States fewer than 50 cases of human trichinosis are reported annually to the Centers for Disease Control and Prevention, compared with 500 cases a year in the 1940s.

Procedure 37.4

Examine *Trichinella, Necator, Enterobius,* and *Dirofilaria*

1. Examine a slide of muscle tissue containing encysted larvae of *Trichinella*.
2. If slides are available, examine specimens of *Necator* (hookworm), *Enterobius* (pinworm), and *Dirofilaria* (heartworm).
3. Note variation in the morphology of the anterior and posterior ends of the organisms.
4. Sketch and note the body sizes of *Trichinella*, *Necator*, *Enterobius*, and larval *Dirofilaria*.

Hookworm causes anemia in infected livestock, which causes great economic losses. Pinworms, although less dangerous, irritate many children because the pinworms infect the intestine and inflame the anus (fig. 37.16). Children often scratch themselves, put their fingers in their mouths, and thereby reinfect themselves. Pinworms infect about 30% of children and 16% of adults in the United States. Heartworms (*Dirofilaria immitis*) are among the most significant parasites of dogs and cats. The larvae are transmitted by mosquitoes and large, mature stages (9 to 16 inches long) reside in the heart and lungs where they disrupt blood flow.

Figure 37.14
Cross sections of male and female *Ascaris*, a large nematode that infects the intestinal tract of a variety of vertebrates.

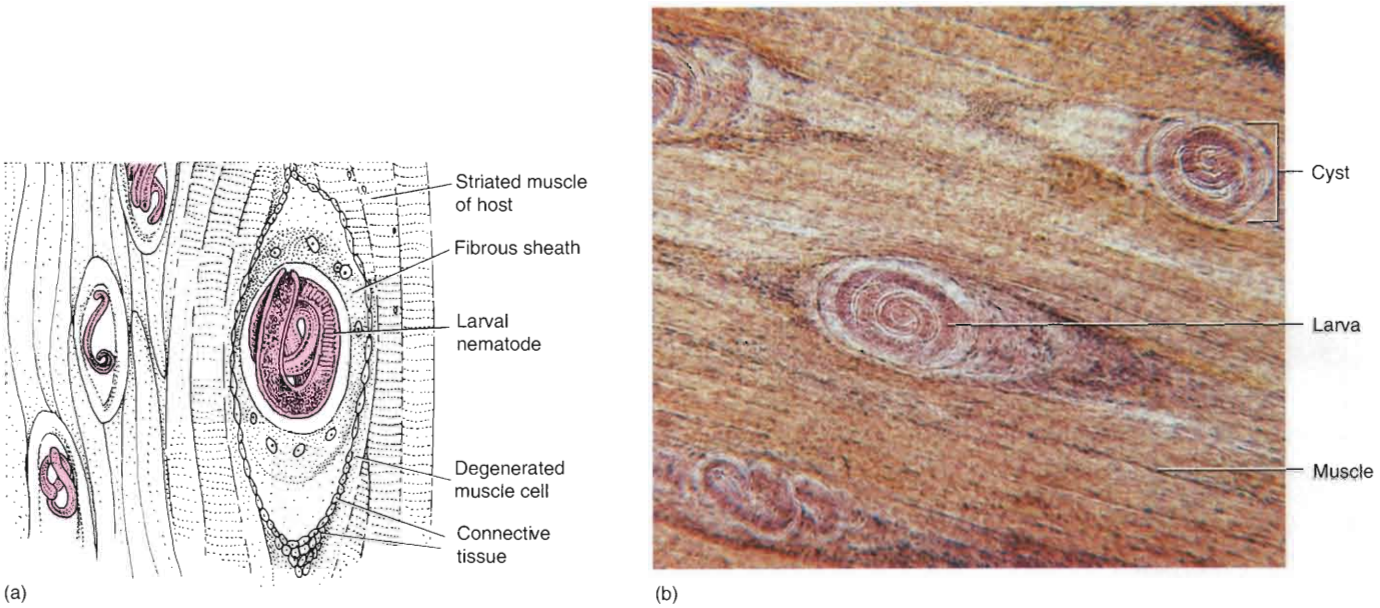


Figure 37.15

Trichinella spiralis. (*a*) Anatomy of larvae encrusted in muscle. (*b*) Larvae in muscle section (400×). The disease trichinosis is acquired by eating poorly cooked meat that contains encysted larvae.

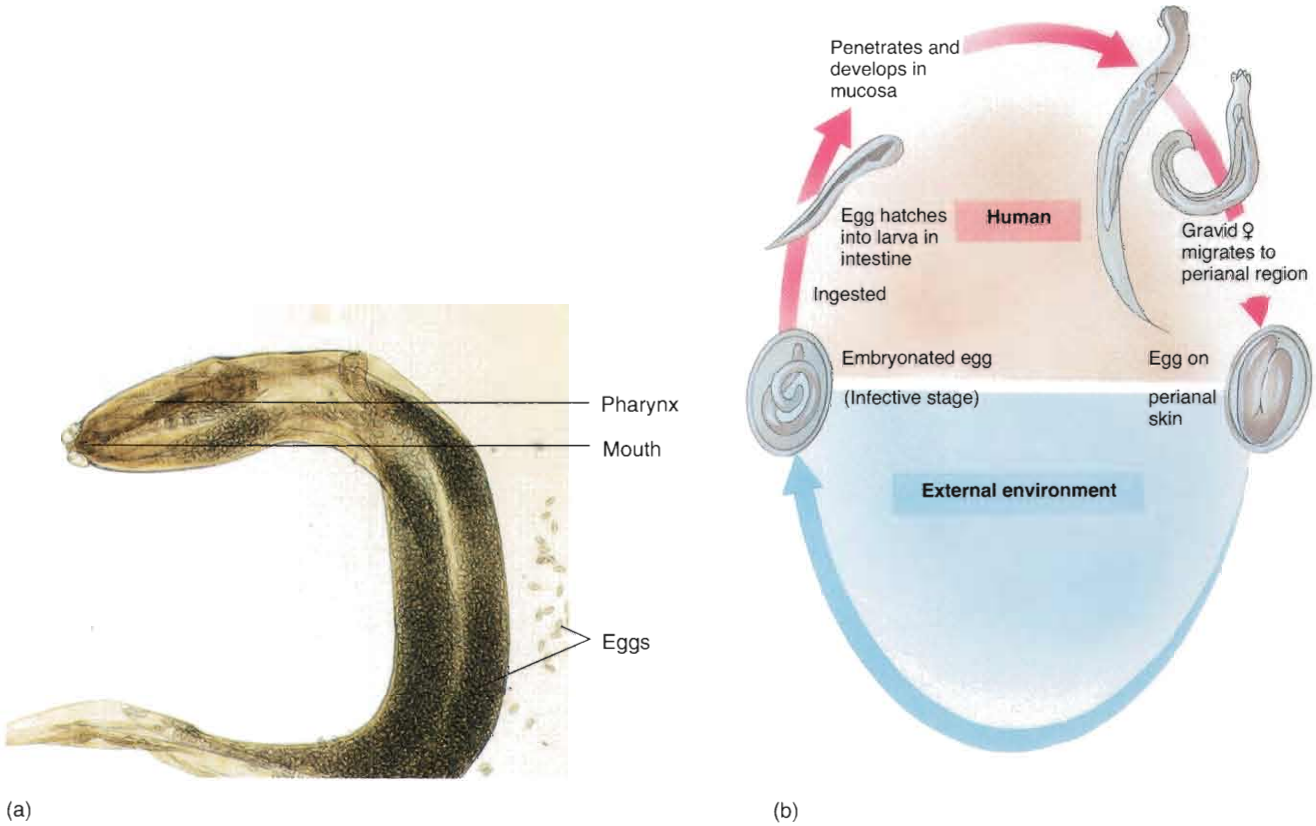


Figure 37.16

(*a*) *Enterobius*, pinworm (40×). Pinworms are the most common parasite in the United States—30% of children and 16% of adults are infected. (*b*) The life cycle of the pinworm, *Enterobius vermicularis*. The adult crawls from the intestine and lays eggs on the skin just outside the anus. The host, usually a human child, is irritated by the pinworms and scratches. Eggs are then unknowingly eaten and the larvae hatch in the intestine to complete the life cycle.

Questions for Further Thought and Study

1. Flatworms are the first organisms we have discussed with an anterior-posterior orientation. How does this affect their movement compared to the movement of more primitive organisms?

2. What are the disadvantages of a flatworm's digestive system having only one opening?

3. The complete digestive tract of nematodes and other phyla allows functional specialization. What specializations are common in the digestive tract of higher organisms such as humans?

4. Prepare a simple table of all of the taxonomic groups, their common names, their distinguishing characteristics, and all representative genera covered in this exercise. Keep this table with your study notes.

5. What is the advantage of radial symmetry for sessile animals such as hydras and bilateral symmetry for mobile animals such as planaria? What major evolutionary trends accompany bilateral symmetry?

Survey of the Animal Kingdom
Phyla Mollusca and Annelida

38

Objectives

By the end of this exercise you should be able to:

1. Describe how structures specific to mollusks and annelids help them survive in their environment and promote their evolutionary persistence.
2. Describe the general morphology of organisms of phylum Mollusca and phylum Annelida.
3. List the characteristics that phyla Mollusca and Annelida have in common with phyla Platyhelminthes and Nematoda.
4. Discuss those characteristics unique to mollusks and annelids newly derived from those of their ancestral phyla.
5. List examples of each of the major classes of mollusks and annelids.

Phylum Mollusca and **phylum Annelida** are the first **coelomate** organisms that we have discussed (table 38.1, fig. 38.1). They have a coelomic body cavity surrounded by mesoderm and containing complex systems of organs and compartments. Coelomates are further divided into **protostomes** and **deuterostomes**. Protostomes include phyla Mollusca, Annelida, and Arthropoda (see Exercise 39) and have well-developed nervous, circulatory, excretory, reproductive, and digestive systems. Deuterostome phyla include Echinodermata, Hemichordata, and Chordata (see Exercise 40). A detailed comparison of protostomes and deuterostomes is presented in Exercise 40.

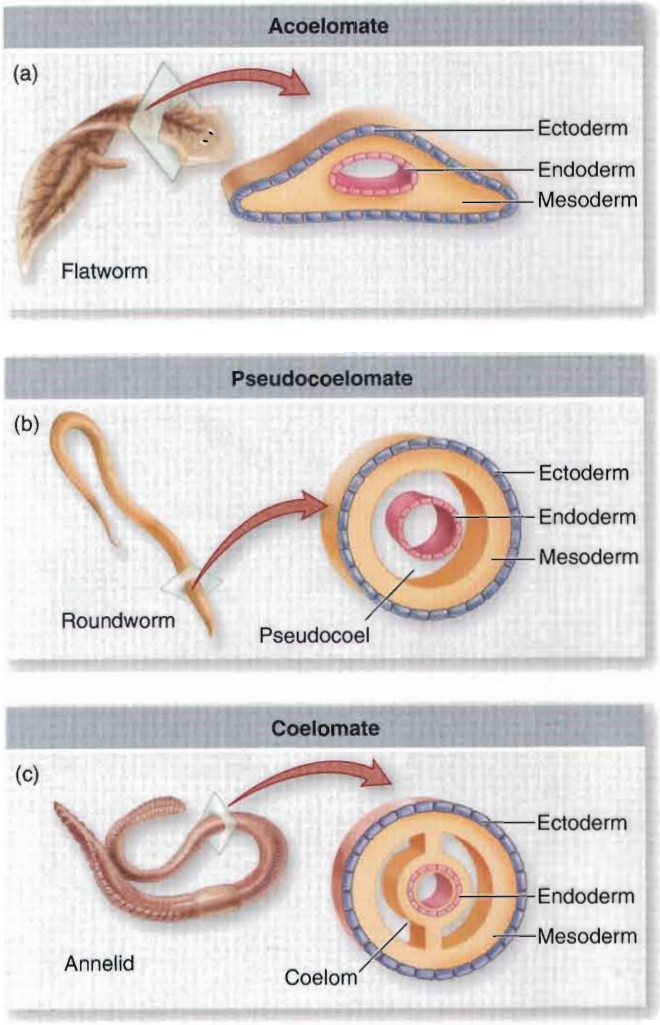


Figure 38.1
Three body plans for bilaterally symmetrical animals. (*a*) Acoelomates (including flatworms) have no body cavity. (*b*) Pseudocoelomates (including nematodes) develop a body cavity between the mesoderm and endoderm. (*c*) Coelomates (including annelids, mollusks, and more advanced phyla) have a body cavity bounded by mesoderm.

PHYLUM MOLLUSCA

Mollusks such as snails, clams, octopuses, and squids are soft-bodied animals with a specialized layer of epidermal cells called a **mantle** that secretes a **shell** (fig. 38.2). They have evolved a remarkable diversity of forms built on a consistent ancestral body plan (fig. 38.2). Molluskan forms are so diverse that a clam and a squid belong to the same phylum!

Mollusks produce many kinds of external shells—some mollusks have only a remnant internal shell, whereas others have no shell at all (fig. 38.3c). This phylum's diversity of 110,000 species is surpassed only by arthropods and probably nematodes. Although mollusks are coelomate, their coelom is often reduced to a small chamber surrounding the heart. The circulatory system is open (except in cephalopods), meaning that blood pools in sinuses and bathes the organs directly. **Open circulatory systems** have

TABLE 38.1

PHYLA MOLLUSCA AND ANNELIDA

Phylum	Typical Examples		Key Characteristics	Approximate Number of Named Species
Mollusca (mollusks)	Snails, oysters, octopuses, nudibranchs		Soft-bodied coelomates whose bodies are divided into three parts: head-foot, visceral mass, and mantle; many have shells; almost all possess a unique rasping tongue, called a radula; 35,000 species are terrestrial.	110,000
Annelida (segmented worms)	Earthworms, polychaetes, beach tube worms, leeches		Coelomate, serially segmented, bilaterally symmetrical worms; complete digestive tract; most have bristles called setae on each segment that anchor them during crawling.	12,000

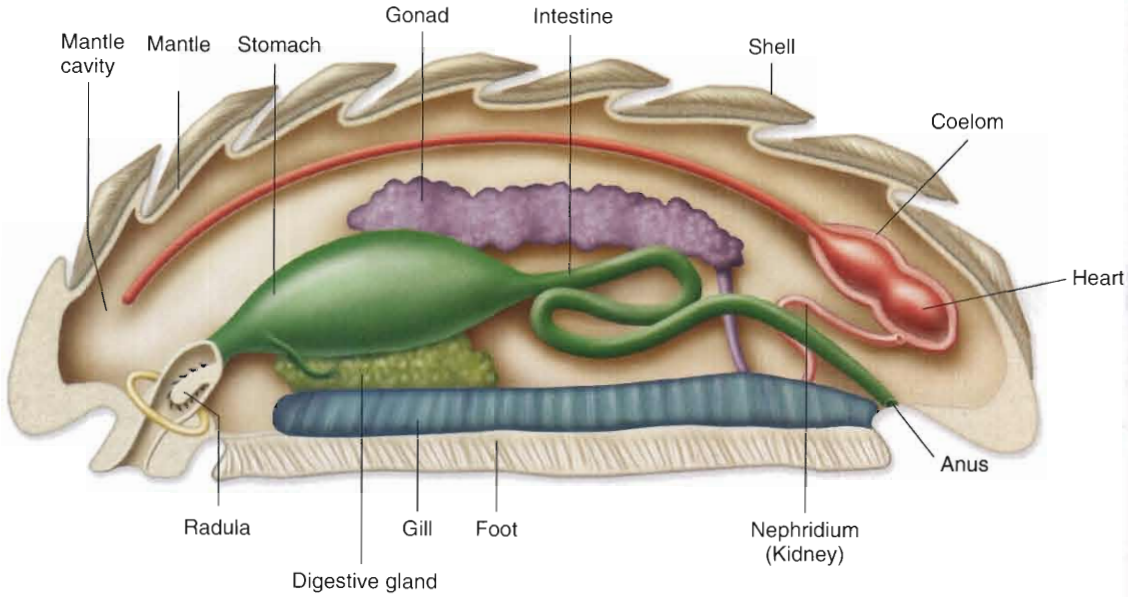


Figure 38.2
Generalized molluscan body plan.

a few large vessels and a heart but no smaller vessels and capillaries.

The basic body plan of a mollusk shows little segmentation, but consistently includes (1) a **visceral mass** of organ systems (digestion, excretion, and reproduction) and sensory structures; (2) a ventral, muscular, and often highly modified **foot** used for locomotion; (3) a calcium-based shell, though occasionally absent; and (4) a mantle that secretes the shell and may aid in respiration and locomotion in some species (fig. 38.3). Some mollusks also have a differentiated **head**.

Class Polyplacophora

Obtain a preserved chiton and examine its external features. Polyplacophorans (*poly* = many, *placo* = plate, *phora* = move), commonly called chitons, are exclusively marine and have a primitive molluskan structure (fig. 38.4). The dorsal shell is divided into eight plates embedded in the mantle. The ventral foot is a broad oval muscle used to propel chitons slowly over the surface of rocks. The **radula**, a horny-toothed organ in the mouth, scrapes food (algae) from rocks.

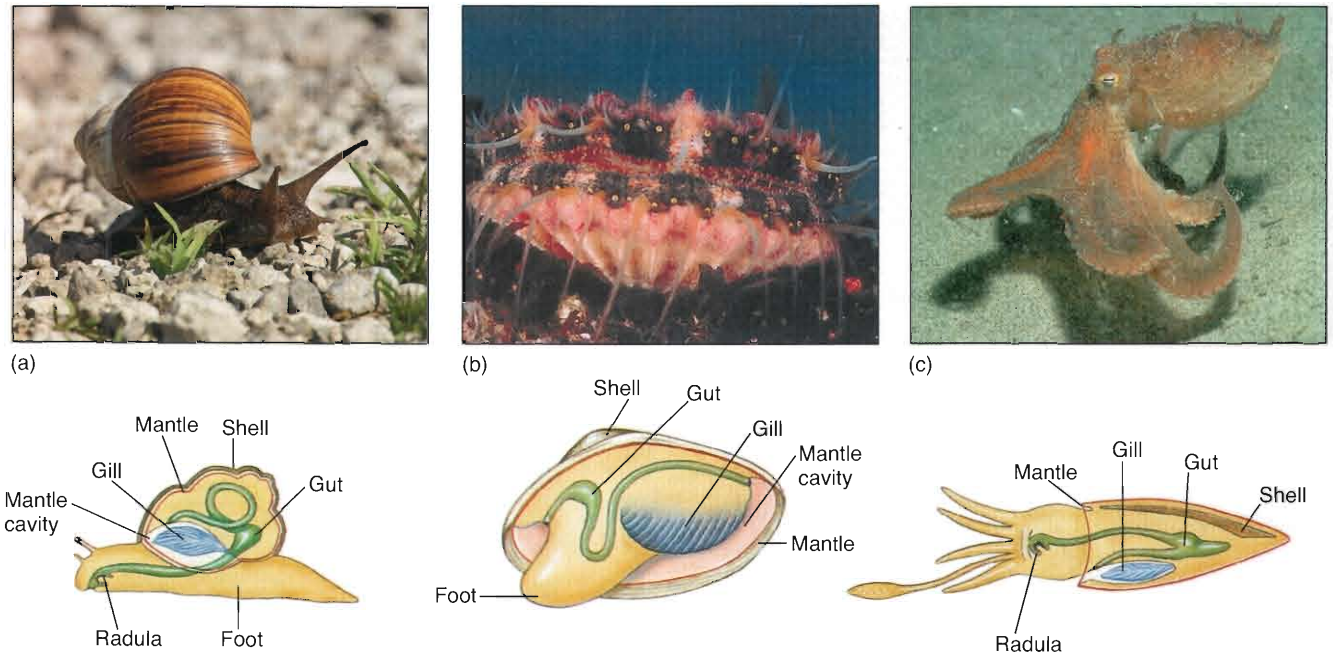


Figure 38.3

The body plans of the three major classes of mollusks: (*a*) Gastropoda, (*b*) Bivalvia, and (*c*) Cephalopoda. A mollusk's shell has an outer layer overlying layers of densely packed crystals of calcium carbonate. A visceral mass includes organs of digestion, excretion, and reproduction and extends as a muscular foot adapted for locomotion, attachment, or food capture (in squids and octopuses). The radula is a scraping and feeding organ characteristic of mollusks (except bivalves, which obtain their food by filter feeding). Folds of tissue called the mantle arise from the dorsal body wall, line the shell, and enclose a cavity adjacent to the visceral mass. Within the mantle cavity are gills or lungs.

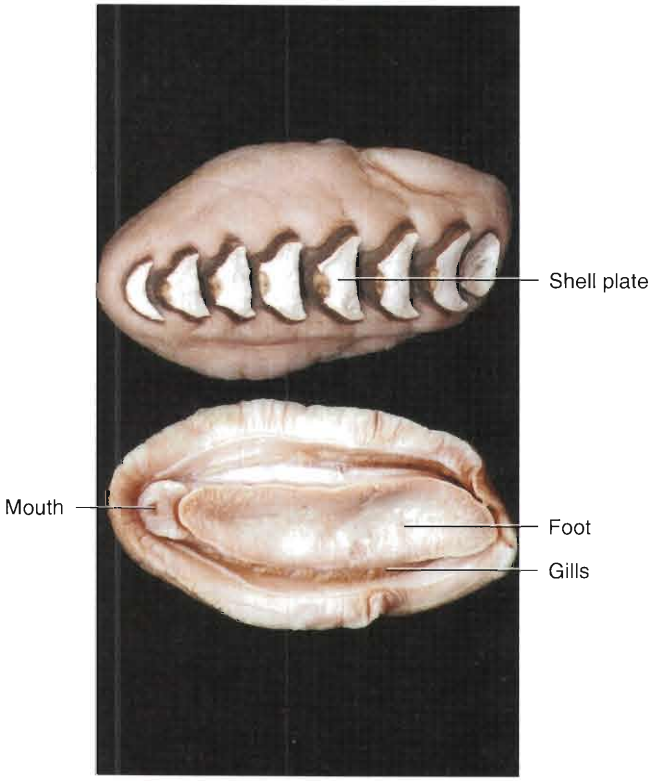


Figure 38.4

Chiton, *Chiton* (class Polyplacophora). Dorsal view (*above*), and ventral view (*below*). The shell of chitons has eight plates atop a mantle; visceral mass; and large, oval foot.

Class Gastropoda

Most gastropods (*gastro* = stomach, *poda* = foot) (snails) have a single shell that is often coiled and elaborate. Some gastropods, such as marine nudibranchs and the common garden slug, do not produce a shell (fig. 38.5). Most species are marine, but freshwater snails and land snails are common. Gastropods also feed with a rasping band of teeth called a radula (fig. 38.6).

Examine preserved gastropods and aquarium snails and compare the relative spiraling of the shells of different species. This spiral growth is common in mollusks and results from unequal growth of the two halves of the larva and mantle.

Class Bivalvia

Clams, oysters, scallops, and mussels are bivalves (*bi* = two, *valve* = door or shell) having a dorsally hinged shell in two parts (figs. 38.3*b*, 38.7). The mantles of the left and right valves join posteriorly to form a ventral **incurrent siphon** and a dorsal **excurrent siphon** that direct water through the clam. When a clam burrows in sediment the siphons extend to the water. The space between the mantle and visceral mass is the **mantle cavity**. Water flows into the mantle cavity through the incurrent siphon, over the gills, and then

Figure 38.5

Colorful nudibranchs, such as *Flabellina iodinae*, are gastropod mollusks without a shell. They have a rather mysterious defensive strategy—nudibranchs use the weapons of their prey. They eat sea jellies, hydrozoans, and corals, all of which have stinging structures called nematocysts. When a nudibranch attacks and eats sea jellies, it can swallow and digest these nematocysts without discharging them. The stingers pass through the digestive tract and are stored in feathery projections on the dorsal surface of the nudibranch. A predator taking a mouthful of nudibranch gets a nasty taste.

dorsally into a space between the visceral mass where gills attach to the mantle. Water exits this chamber posteriorly through the excurrent siphon. As water flows over gills, suspended food particles are filtered by cilia and swept along grooves on the gills to the ventral edge. Cilia at the ventral edge move food to the labial palps that surround and direct food to the **mouth**.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 38.1

Examine bivalve anatomy

1. Obtain a preserved freshwater bivalve, such as *Anodonta*. The valves should be slightly separated by a wooden peg. Rinse the specimen thoroughly before dissection.
2. Locate the two valves and the anterior, posterior, dorsal, and ventral regions of the clam. The **hinge** and the **umbo** are on the dorsal surface (fig. 38.8*b*). The umbo is toward the anterior end.

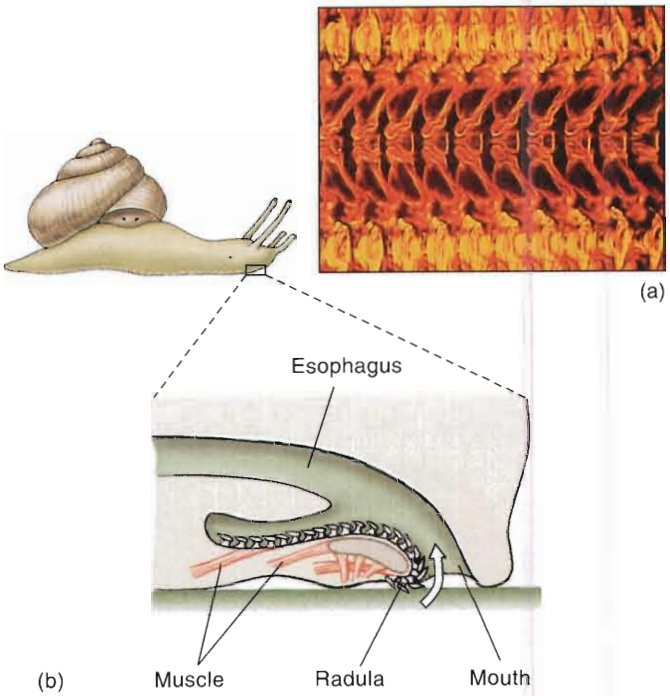


Figure 38.6

A radula is a unique rasping tongue of mollusks with rows of chitinous teeth covering its surface. (*a*) These rows of teeth are part of a snail's radula. (*b*) The membranous radula is stretched over a rigid cartilaginous rod pressed against a surface to be scraped. Snails use their radula to scrape algae from rocks or tear pieces from plant leaves.

Figure 38.7

A giant clam (class Bivalvia, *Tridacna* spp.) of the Indopacific may weigh up to 900 lb (400 kg). Its mantle is often intensely colored by blue, green, and brown pigments, and it harbors rich and colorful colonies of symbiotic algae in the blood sinuses of the tissue. Photosynthesis by this algae provides extra food for the clam. Some clams have lenslike structures that focus light deep within their tissues and promote photosynthesis by the algae.

3. Place the organism vertically and dorsal side (umbo) down on a dissecting pan. To further separate and loosen the valves, slip the flat end of a dull kitchen knife between the valves and rotate the knife. Be careful. Notice that a layer of mantle lies against the inside of each of the two valves.

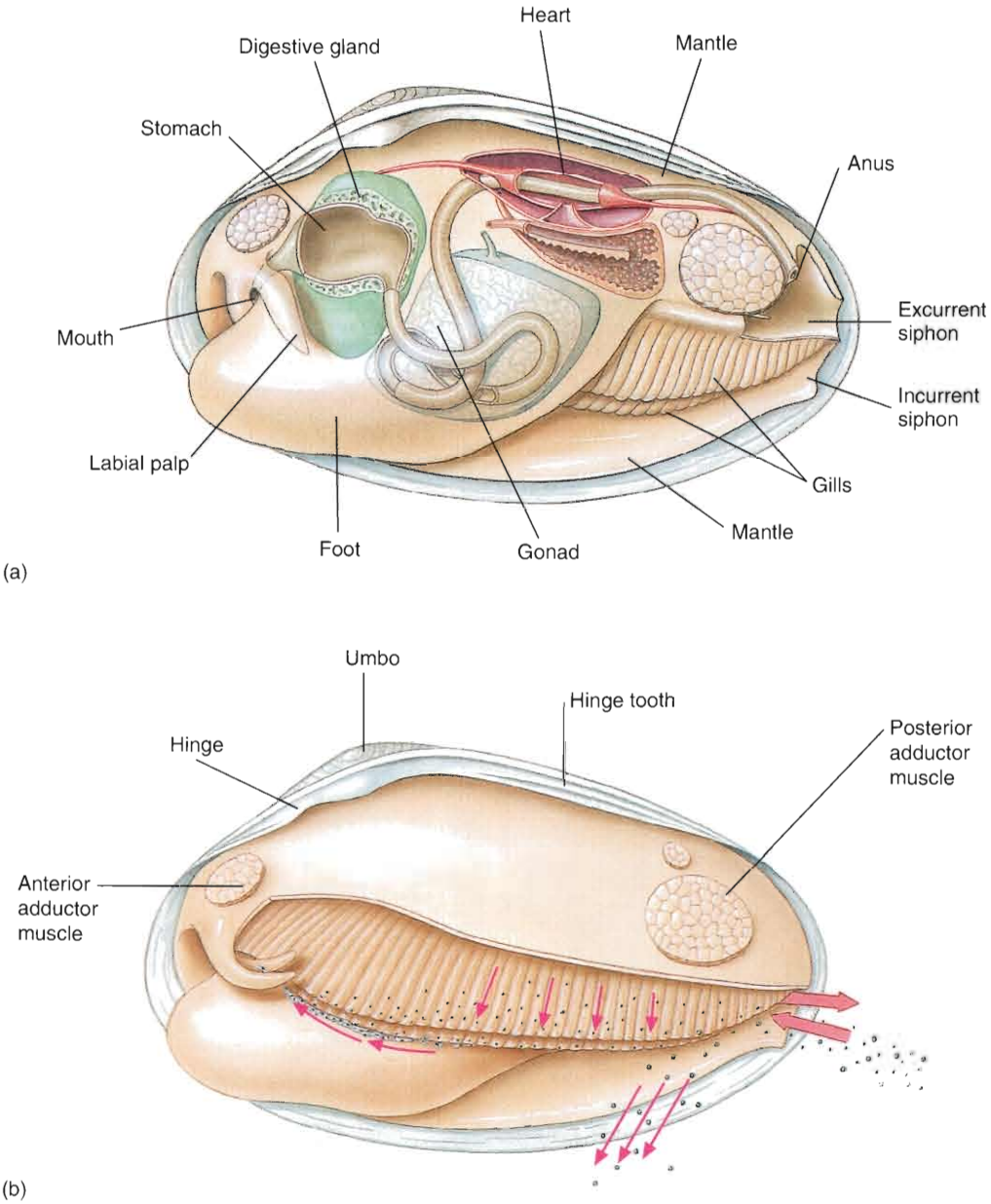


Figure 38.8

(*a*) Clam (*Anodonta*) anatomy. The left valve and mantle are removed. (*b*) During filter feeding, water enters the mantle cavity posteriorly and is drawn forward by ciliary action to the gills and palps. As water enters the tiny openings of the gills, food particles are filtered out and caught in strings of mucus carried by cilia to the palps and directed to the mouth. Arrows show the path of food particles being moved toward the mouth by cilia on the gills. Sand and debris drop into the mantle cavity and are removed by cilia.

4. Review figure 38.8 to find the general position of the **adductor muscles**.
5. When the valves are loose enough to be held slightly open (about 1.0 cm), slip a scalpel blade between the mantle tissue and shell of one side. When you have felt resistance of an adductor muscle against the tip of the blade, draw the blade snugly along the shell to cut the posterior and anterior adductor muscles. Try to cut them as close to the inner shell surface as possible. Always cut down toward the dissecting pan.
6. Pull the valves apart so that the organism lies on one side and the other valve lifts away. The mantle that once lined the removed shell now covers the visceral mass. Cut the mantle away to expose the visceral mass.
7. On the separated and empty valve notice the muscle's scars; hinge teeth; mantle line; and inner, iridescent surface of shell called **mother of pearl**.

8. Locate the **gills**; **labial palps**; tough muscular **foot**; and the softer, more dorsal visceral mass. The labial palps channel food to the open mouth.
9. To expose the internal organs of the visceral mass, slice the foot and visceral mass longitudinally (anterior-posterior plane) from the ventral surface toward the dorsal surface. This will produce left and right halves. Make the cut slightly off center to avoid cutting through all of the tough foot tissue.
10. Locate evidence of the coiled intestine, the green **digestive gland**, brown **gonad** tissue, and the dorsal **heart**. This sac is thought to be a remnant of the ancestral coelomic cavity. The intestine runs through it, and the sac contains the heart tissue.
11. When you finish your observations, dispose of the preserved tissue and shells in appropriate containers.

Question 1

a. What is the texture of the mantle of *Anodonta*?

b. Are siphons in *Anodonta* as obvious as they are in figure 38.8?

c. Consider objective 1 listed at the beginning of this exercise. In what ways would having a shell contribute to the survival and reproductive success of mollusks in their environment?

Examine a cluster of oyster shells and note how they are stuck together. Mature oysters attach permanently to their substrates. Most bivalves produce pearls, but the finest pearls are made by *Pinctada* in the warm waters of the Pacific. Natural pearls form in oysters when an irritant such as a grain of sand lodges between the mantle and the shell. The mantle responds by surrounding the irritant with layers of the same crystalline material used for the shell. Cultured pearls are produced by oysters about 3 years after aquaculturists introduce an irritant to their mantle.

Question 2

a. How does the foot of a bivalve differ from that of snails or chitons?

b. How could predators attack an animal closed "tight as a clam"?

c. Is immobility a problem for filter feeders such as oysters? Why or why not?

Class Cephalopoda

Examine a preserved specimen of the common squid, *Loligo*. Note the number and arrangement of the tentacles and the pointed, hardened beak surrounding the mouth. Most cephalopods (*cephalo* = head, *poda* = foot) (e.g., squid, octopuses, nautilus, and cuttlefish) do not resemble their molluskan relatives because cephalopod shells may be absent or reduced to an internal remnant (fig. 38.3). For example, a cuttlebone is actually the internal shell of a mollusk called a cuttlefish. You may have offered your pet parakeet a cuttlebone to sharpen its beak. The foot of a cephalopod is modified into tentacles. Squid and other cephalopods are predatory, and their external features are appropriately adapted. Unusual among mollusks is the relatively closed circulatory system of cephalopods.

Examine the eyes of *Loligo* closely. Also examine other displayed cephalopods such as the chambered nautilus; its external shell is beautiful and unusual for a cephalopod (fig. 38.9). Review mollusks by completing table 38.2 with brief descriptions of basic molluskan features. The eyes are probably the most surprising feature of a squid. The largest eyes in kingdom Animalia belong to the giant squid; they resemble mammalian eyes and use a lens to form clear images.

Question 3

a. What features of squid and octopuses are adaptations for predation?

b. Do all of the tentacles of a squid have suckers?

c. What are some functions of suckers?

d. Find the mouth at the base of the tentacles. What is the shape and consistency of the jaws? You may need to make an incision to expose the mouth and jaws.

e. Why are sensory organs more prominent in cephalopods than in other classes of mollusks?

f. Consider objective 1 listed at the beginning of this exercise. In what ways are image-forming eyes significant to fundamental processes for cephalopods?

PHYLUM ANNELIDA

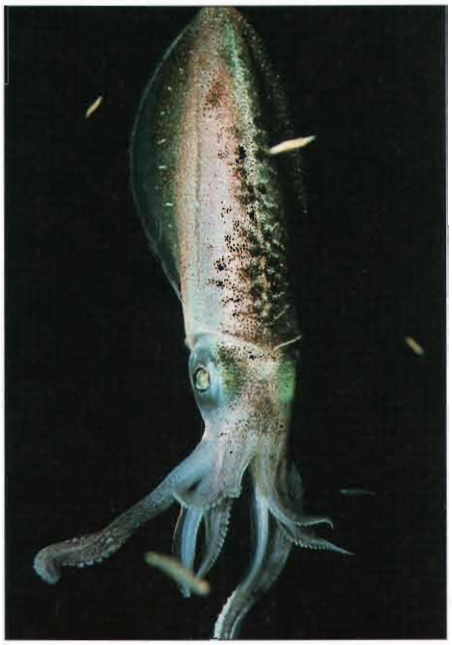
Annelids include earthworms, leeches, and many less familiar marine and freshwater species. The most distinctive char-

TABLE 38.2

A COMPARISON OF MAJOR CHARACTERISTICS OF FOUR CLASSES OF MOLLUSKS

	Polyplacophora	Gastropoda	Bivalvia	Cephalopoda
Shell				
Mantle				
Foot				
Locomotion				
Feeding				
Sensory structures				

(a)



(b)

(c)

Figure 38.9

Cephalopod diversity. (*a*) An octopus. Octopuses generally move slowly along the bottom of the sea. (*b*) A squid. Squids are active predators, competing effectively with fish for prey. (*c*) Pearly nautilus, *Nautilus pompilius*.

acteristic of this phylum is **segmentation**. The body of an annelid is divided into repetitive segments (sometimes called metameres) arranged on a longitudinal axis and divided by **septa**. Each segment contains parts of the circulatory, digestive, nervous, and excretory systems. The circulatory system of annelids is closed, meaning that blood is always retained in vessels. Annelids also have **setae**, small, bristlelike appendages often occurring in pairs on lateral and ventral surfaces. The degree of setal development is distinctive for each of the three classes of annelids.

Class Polychaeta

Most polychaetes, such as the clam worm *Nereis*, are marine worms living in sediment (fig. 38.10). *Nereis* is distinctly segmented and each segment bears a pair of fleshy appendages called **parapodia**. These appendages have a large surface area, are highly vascularized with blood vessels, and help the polychaete move and respire. Protruding from the fleshy parapodia are many setae from which the class derives its name (*poly* = many, *chaeta* = setae). In some species the

brittle, tubular setae are filled with poison and used for defense; in others the setae help filter food from the water.

Procedure 38.2

Examine polychaetes

1. Examine a preserved *Nereis* and a prepared slide of a parapodium. Sketch the parapodium. Note the tufts of bristles and lobes of tissue.
2. Examine the well-developed head of *Nereis* and locate the features shown in figure 38.10*b*. The mouth and jaws of polychaetes are retractile, so you may have to pull out the jaws with a pair of forceps.
3. Examine other displayed polychaetes such as *Aphrodita*, the sea mouse, and *Chaetopterus*, the parchment worm. These worms have many setae and highly modified parapodia. The parchment worm gets its common name from the paperlike tube it builds.

Question 4

a. The common name of the sea mouse refers to what external feature characteristic of polychaetes?

b. List several functions of parapodia and setae.

c. What is the probable function of the tentacles shown in figure 38.10*b*?

d. Consider objective 1 listed at the beginning of this exercise. Are parapodia significant to fundamental processes for polychaetes? In what ways?

e. What features of *Nereis* indicate that it is an annelid?

Class Oligochaeta

A common oligochaete is *Lumbricus terrestris*, the familiar earthworm.

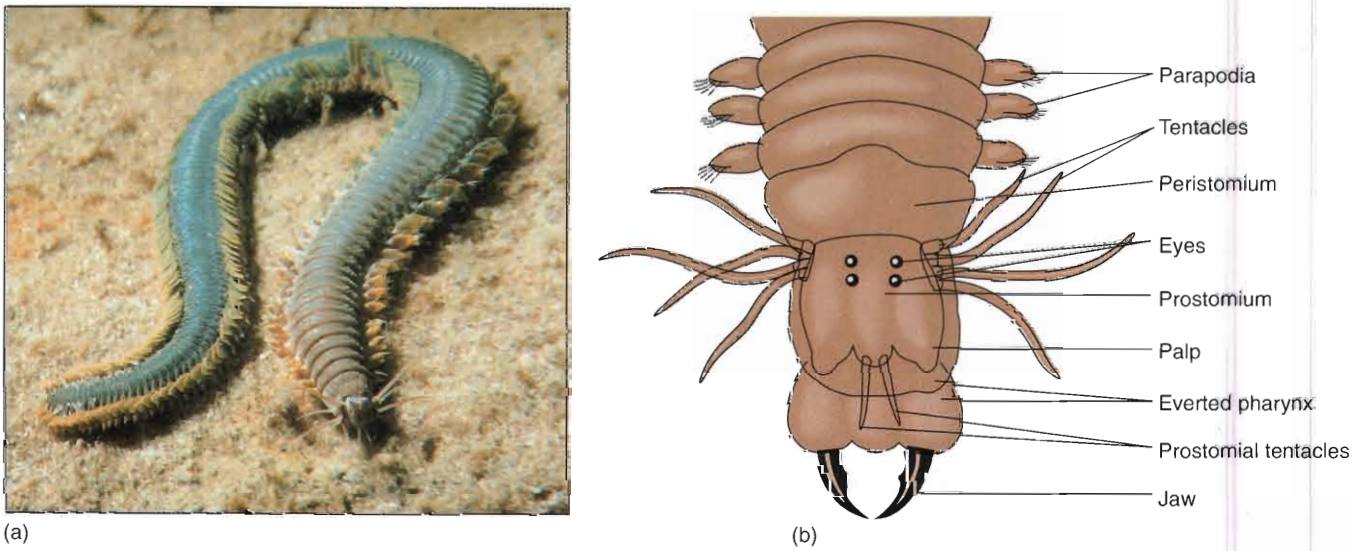
Earthworm Locomotion

Movement of oligochaetes is not undulatory—rather, movement involves extension, anchoring, and contraction. These motions occur by alternating contractions of circular and longitudinal muscles against **hydrostatic** (water) pressure within each segment (fig. 38.11).

Procedure 38.3

Examine locomotion in earthworms

1. Watch a living earthworm move on the hard surface of a pan, then compare and contrast this motion with that of a snake and a vinegar eel.
2. Place the worm on some loose soil, and describe its burrowing motion.



(a) (b)

Figure 38.10

Nereis virens, a common polychaete (phylum Annelida). (*a*) External structure. (*b*) Anatomy of the anterior end.

Question 5

a. Does the earthworm move randomly?

b. What do you suppose the worm is seeking or avoiding?

c. What muscles allow the worm to change its length and thickness?

d. How does an earthworm's motion differ from that of a snake and nematode?

e. What features of *Lumbricus* indicate that it is an annelid?

External Anatomy of Earthworms

Oligochaetes (*oligo* = few or small, *chaeta* = setae), besides the common earthworm, include many freshwater species. They lack parapodia and have few setae. The anus is on the terminal segment, but the mouth is preceded by a fleshy lobe called the **prostomium** (*pro* = before, *stoma* = mouth). Posterior to the mouth is the first body segment, the **peristomium** (*peri* = around, *stoma* = mouth).

The most obvious external feature of an earthworm is the **clitellum**, a series of swollen segments at the anterior third of the body. Copulating worms attach at their clitella and exchange sperm (fig. 38.12). Earthworms are hermaphroditic, and each of the copulating worms produces egg and sperm cells. Sperm mature in **seminal vesicles** and exit the worm through **male gonopores** on segment 15 (fig. 38.13). Sperm then pass to the adjacent worm and move along its body surface to openings of the **seminal receptacles** (each side of segment 10), where they are stored temporarily. After copulation, the worms separate. A few days later the clitellum secretes a mucous band that slides anteriorly and picks up eggs from the **female gonopores** (segment 14) and stored

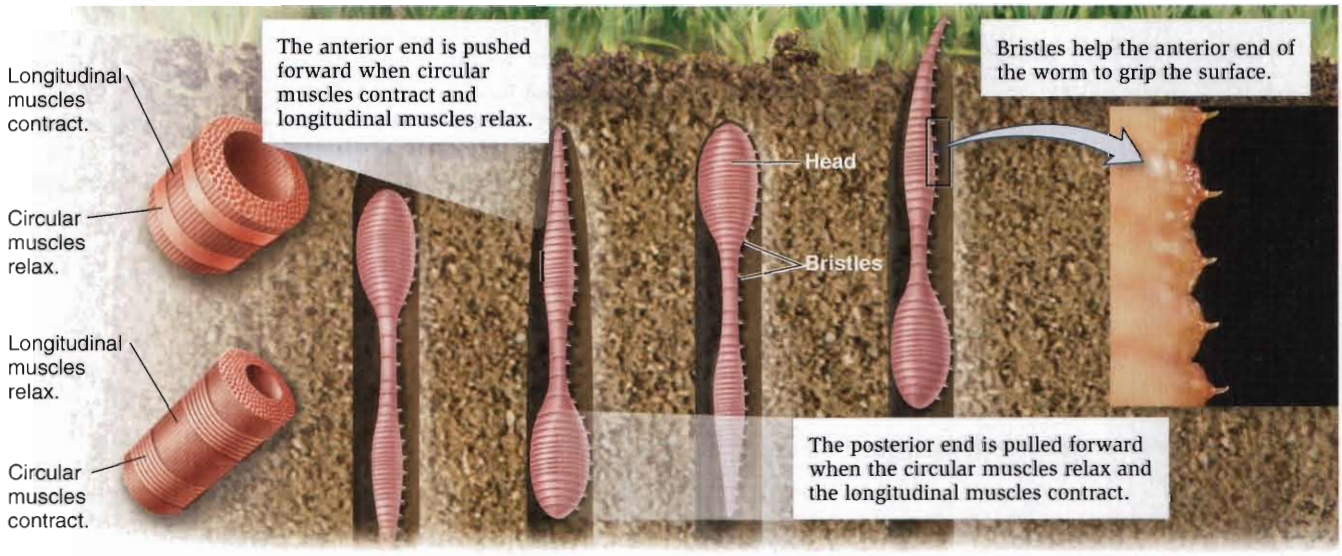


Figure 38.11

Hydrostatic skeleton in a worm. By alternately contracting and relaxing circular and longitudinal muscles, earthworms use hydrostatic pressure to achieve locomotion. Clinging bristles along the body surface help prevent backsliding.

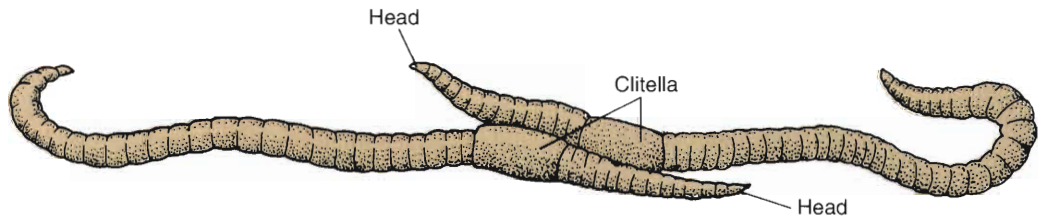


Figure 38.12

Position for copulation and transfer of sperm in earthworms (class Oligochaeta).

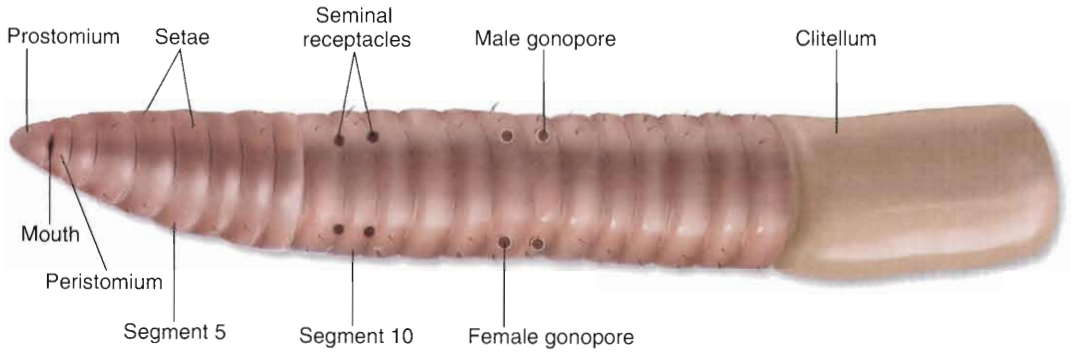


Figure 38.13
External features of an earthworm's ventral surface.

sperm from seminal receptacles. After the eggs are fertilized in the mucous band, the worm releases it as a **cocoon**.

Procedure 38.4

Examine the external features of an earthworm

1. Examine the external features of a preserved *Lumbricus*, and notice its segmentation.
2. Although the body lacks parapodia, if you touch the smooth epidermis and cuticle you can feel short setae on the ventral surface.
3. Use a dissecting microscope to determine the number and arrangement of these setae.
4. Locate the mouth and anus of the earthworm.
5. Use a dissecting microscope to locate the male gonopores and seminal receptacle openings on your specimen (fig. 38.13).
6. Locate the female gonopores on your specimen.

Question 6

a. How many setae are on each segment of the earthworm?

b. Are they paired?

Internal Anatomy of Earthworms

Segmentation is best appreciated by looking at internal anatomy (fig. 38.14). Some of the repeating segments are not identical. Segments of the digestive tract are fused and specialized to form a muscular **pharynx** (for suction and ingestion of food), **esophagus** (for transport of food), **crop** (for food storage and some digestion), **gizzard** (for maceration or crushing of food), and **intestine** (for absorption of nutrients). Recall that nematodes also have a linear digestive tract, but it lacks the specialization of that of annelids. Reproductive organs cluster around the anterior segments. Locating small structures such as the female gonads and seminal receptacles will require careful work. A rudimentary **brain** is just anterior and dorsal to the pharynx and is continuous with the **ventral nerve cord**.

Some structures such as lateral branches of the ventral nerve cord and paired **nephridia** occur in each segment (fig. 38.15). Nephridia, which are small, white, convoluted tubes, are found on the inner surface of each segment. Nephridia function like kidneys—they collect and release excretory wastes. Ciliated, funnel-shaped **nephrostomes** on the ends of the nephridia gather waste products that are released through external pores called **nephridiopores**.

The **dorsal blood vessel** and **ventral blood vessel** (above and below the intestine) are the main vessels of the closed circulatory system. They are connected by five lateral "**hearts**." However, these hearts promote blood circulation no more than the dorsal and ventral vessels.

The digestive tract has an internal fold of tissue called the **typhlosole** arising from the dorsal wall. This creates a U-shaped intestinal lumen and doubles the surface area for absorption.

Procedure 38.5

Examine the internal features of an earthworm

1. Pin the terminal segments of a preserved *Lumbricus*, dorsal side up, near the edge of a dissecting pan.
2. Open the body with a shallow, longitudinal incision. Use sharp-pointed scissors, and be careful not to cut too deeply. Hold the skin up with forceps to prevent damage to internal organs.
3. Pin the skin back on both sides of the incision, and expose the organs lying in the coelomic cavity. You may have to cut each septum so the body wall lies flat when pinned.
4. When you have completed the dissection, pour a small amount of water into the pan to cover the exposed worm.

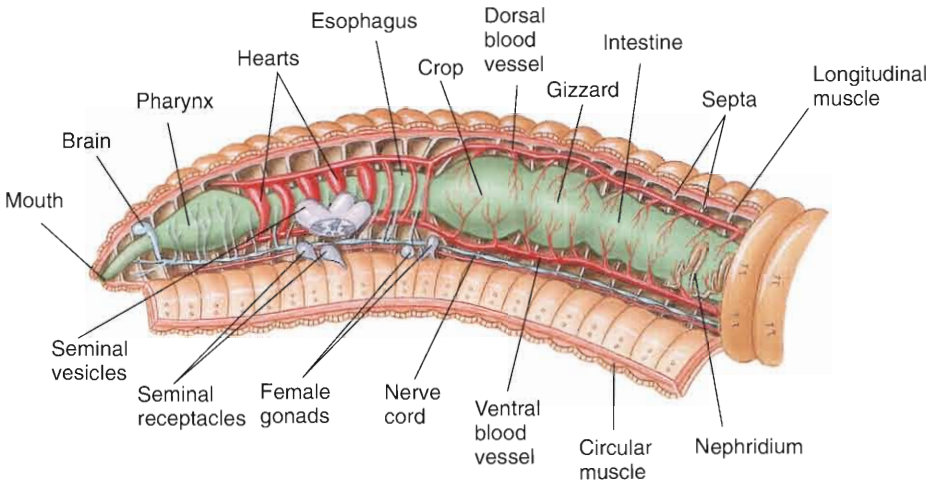


Figure 38.14
Internal anatomy of an earthworm.

5. Locate the structures shown in figure 38.14. You may need to use a dissecting microscope for close examination.
6. Locate the hearts, dorsal blood vessel, and ventral blood vessel.
7. Locate the organs of the digestive system. Slit open the digestive tract and observe its contents.
8. Locate a nephridium in each segment of your dissected specimen.
9. When you have completed your observations, dispose of your specimen in an appropriate container.
10. Examine a prepared slide of a cross section of an earthworm; locate the structures in figure 38.15.

Question 7

a. How many segments of an earthworm have a heart?

b. Does the ventral nerve cord traverse the entire length of the body?

c. Is the inside of the digestive tract the same from the pharynx to the end of the intestine? Explain.

d. How could absorption in the intestine be increased without increasing the intestine's length?

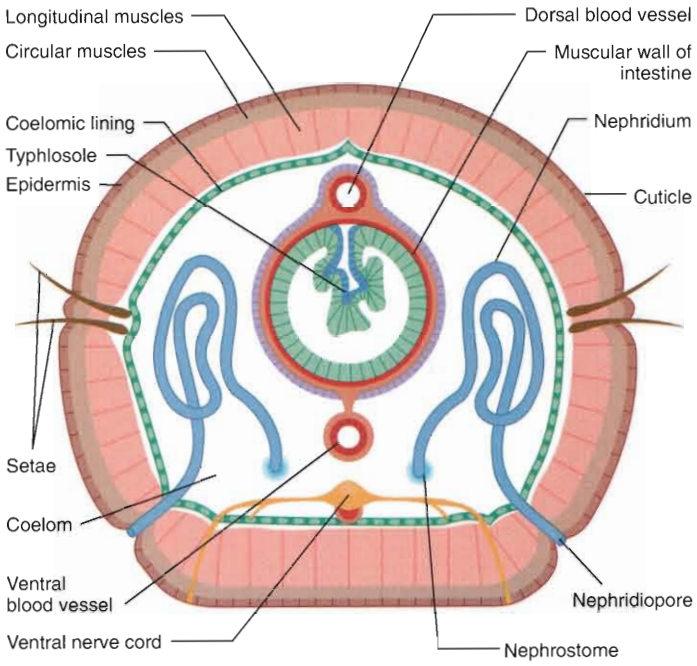


Figure 38.15
Cross section and internal anatomy of an earthworm.

e. How do the layers of musculature in an earthworm differ from those of a nematode?

f. List two or three features of an earthworm cross section that distinguish the dorsal and ventral surfaces.

Class Hirudinea

Hirudineans include leeches, which are primarily freshwater ectoparasites (fig. 38.16). Some leeches eat detritus and small invertebrates such as worms, snails, and insect larvae. Leeches are not segmented as distinctly as are other annelids. Leeches lack setae, are dorsoventrally flattened, and have anterior and posterior suckers that hold prey. Blood sucking leeches eat infrequently, but species such as *Hirudo medicinal* can quickly consume five to ten times their body weight in blood. Many years ago physicians used these leeches for bloodletting from sick patients thought to have "too much blood." Today, laboratory-grown leeches are occasionally used to extract fluid that has accumulated around injuries and the incisions of microsurgery to enhance healing. Leeches extract fluid more efficiently and with less damage than does hypodermic suction (fig. 38.17).

Leeches reproduce sexually and individuals are hermaphroditic. Two leeches intertwine, and species with copulatory organs inject a packet of sperm called a **spermatophore** into the female gonopore. Other species have no copulatory organ; these leeches copulate by injecting a spermatophore directly through the epidermis of their partner (the spermatophore may have tissue-dissolving enzymes to aid penetration). The introduced spermatophore releases sperm into the coelomic cavity, and the cells move to the ovaries to fertilize the eggs. In 2 days to many months after copulation, the leech secretes a nutrient-rich cocoon to protect the eggs, and eggs are extruded into the cocoon. The cocoon is brooded by the leech or attached to submerged objects or vegetation.

Procedure 38.6

Observe leeches

1. Observe living leeches; compare their movement with that of earthworms.
2. Examine the external anatomy of a preserved leech and locate the two suckers.
3. Open the body with a pair of scissors and look for signs of segmentation. A dissected leech may be on demonstration.

Question 8

a. What is the difference in general body shape of leeches compared to oligochaetes or polychaetes?

b. What function other than feeding do suckers serve?

c. Are setae visible on a leech?

d. Is internal segmentation of a leech as distinct as that of an oligochaete?

e. Consider objective 1 listed at the beginning of this exercise. How could production of a packetlike spermatophore contribute to the evolutionary success of leeches in their environment?

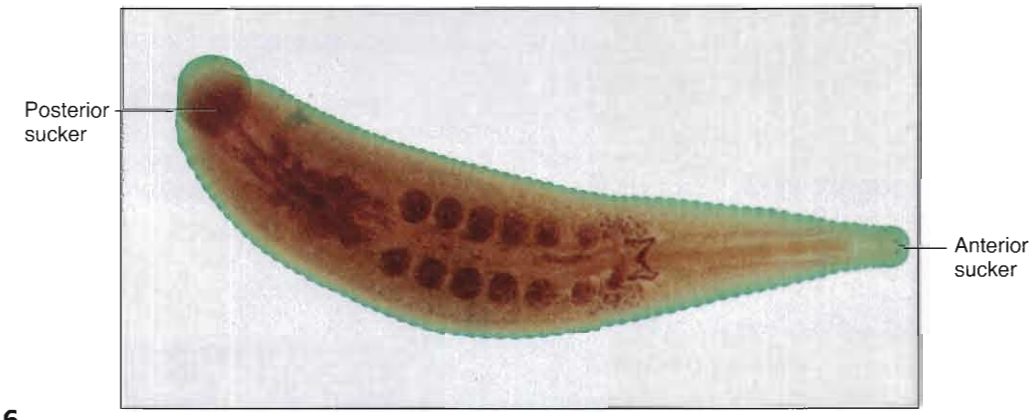


Figure 38.16
External view of a leech. Leeches are primarily freshwater ectoparasites. The mouth is within the anterior sucker.

Figure 38.17
Leech. These advanced annelids have external signs of segmentation but have little repetition of internal parts or internal compartments.

Question 9

a. Now that you have examined the unifying characteristics of mollusks and annelids, list three or four characteristics that they share with flatworms and nematodes as their close ancestors.

b. Draw and label three coelomic body plans and list which of the phyla in Exercises 37 and 38 are associated with each plan.

INVESTIGATION

The Use of Leeches in Medicine

Observations: Many years ago, physicians used leeches for "bloodletting" from sick patients thought to have "too much blood." Today, laboratory-grown leeches are occasionally used to enhance healing by extracting fluid that accumulates around incisions following microsurgery and injuries. Leeches extract fluid more efficiently and with less damage than hypodermic suction (fig. 38.16). Medicine is a business as well as a science that must be effective and delivered safely to large populations.

Question: Are leeches appropriate as a viable medical treatment?

a. Establish a literature search group according to your instructor's guidelines, and obtain Investigation Worksheet 38 from your instructor.

b. Discuss among your group and record on Worksheet 38 the requirements of effective use of leeches in medicine. Include necessary characteristics of the organism and the logistics of delivering treatment.

c. Determine with your group the specific questions that doctors must answer concerning leeches and their effective, safe use. Record these questions on Worksheet 38.

d. Research the current literature and outline information about the use of leeches in medicine.

e. Record your findings on Worksheet 38.

f. Discuss your findings with your instructor and with other students.

Questions for Further Thought and Study

1. Mollusks exhibit a variety of feeding methods. List at least four and discuss adaptations and examples for each type.

2. A snail shell is quite different from the familiar bony skeleton of a mammal. In what ways does a shell function as a skeleton?

3. Some land snails have formed a lunglike structure from a major layer of tissue. What is that layer?

4. You have examined at least three phyla commonly referred to as "worms." How would you define this term?

5. Of what economic importance are earthworms?

6. Earthworms have no lungs or gills. Do they "breathe"? If not, how do they gain oxygen to survive?

7. Do you suppose bloodletting by leeches was a good technique to cure psychosomatic illnesses? Why or why not?

8. Prepare a simple table of all of the taxonomic groups, their common names, their distinguishing characteristics, and all representative genera covered in this exercise. Keep this table with your study notes.

9. Cephalopods are considered by many to be the most distinctive class of mollusks. What makes them seem almost out of place?

WRITING TO LEARN BIOLOGY

Are leeches predators or parasites? What reasons would you give for both possibilities?

Survey of the Animal Kingdom
Phylum Arthropoda

39

Objectives

By the end of this exercise you should be able to:

1. Describe the structures that contribute significantly to the survival of arthropods in their environments.
2. Describe the general morphology of organisms of phylum Arthropoda.
3. List characteristics that arthropods share with the phyla discussed previously.
4. Discuss those characteristics of arthropods that were newly derived from those of their ancestral phyla.
5. List examples of the major classes of arthropods.
6. Describe modifications of the exoskeleton and paired appendages of arthropods.

Figure 39.1

Scorpions are the oldest known terrestrial arthropods. Typical of all arthropods, scorpion bodies are segmented, have jointed appendages, and are covered with a hard, chitinous exoskeleton. Shown here is the world's largest scorpion (18 cm), the Emperor scorpion (*Pandinus emperator*) from west Africa. Some extinct species were five times larger than the Emperor. The sting and neurotoxic venom of most species are equivalent to those of a hornet sting. The most notorious stingers are *Androctonus* of North Africa and species of *Centruroides* in Mexico, Arizona, and New Mexico. Children and weak adults may die from the neurotoxin of a scorpion in 6–7 hours by paralysis of respiratory muscles or cardiac failure. Scorpions locate their prey by detecting vibrations, often through sand rather than air. Desert species can locate and dig out a burrowing cockroach in just a few seconds. Desert species can also withstand temperatures of 46°C (115°F) and tolerate water loss equivalent to 40% of their body weight.

The success and predominance of the arthropod body plan in marine, freshwater, and terrestrial environments is extraordinary. **Phylum Arthropoda** is the most diverse as well as most abundant phylum of animals—estimates range from 2–10 million species. Their tremendous success in all major habitats is due mainly to a rigid external skeleton and **jointed appendages** (*arthro* = jointed, *poda* = foot or appendage) (table 39.1, fig. 39.1). Their appendages are extensions of the main body and are highly adapted for locomotion, feeding, reproduction, defense, and sensing the environment. Bodies of arthropods are segmented, although some segments may fuse during development. The arrangement of these segments and the structure of appendages are often used to classify arthropods. Arthropods are coelomate, their circulatory system is open, and all organ systems are well developed.

The **exoskeleton** of arthropods is made of **chitin**, a long chain of nitrogen-containing sugar molecules arranged in strong fibers. Chitin may be as soft as the body of a butterfly or, if impregnated with calcium carbonate, as hard as the shell of a lobster. This exoskeleton provides protection, a moisture barrier, and a place for muscle attachment. Although this tough covering limits growth, arthropods periodically shed their exoskeleton and quickly enlarge before the new exoskeleton hardens.

Each body segment of ancestral arthropods had a single pair of appendages. Recall that this condition of paired appendages also occurs in annelids. In modern arthropods there are extensive variation and elaboration of these appendages, which are covered by the rigid exoskeleton and usually have flexible joints. These jointed appendages provide arthropods with such strength and flexibility that the variation in structure and function of appendages is enormous. It is so great that a survey of the phylum and its diversity is primarily a study of variation in appendages.

The prominent arthropod characteristics, such as an exoskeleton and jointed appendages, were adaptive during their evolution and contributed to their remarkable success. Their adaptive characteristics probably allowed them to replace other species that weren't equipped to deal as well with their environment, and also allowed them to exploit new environments, microhabitats, and niches. Review the introductory information in this exercise (as well as in your

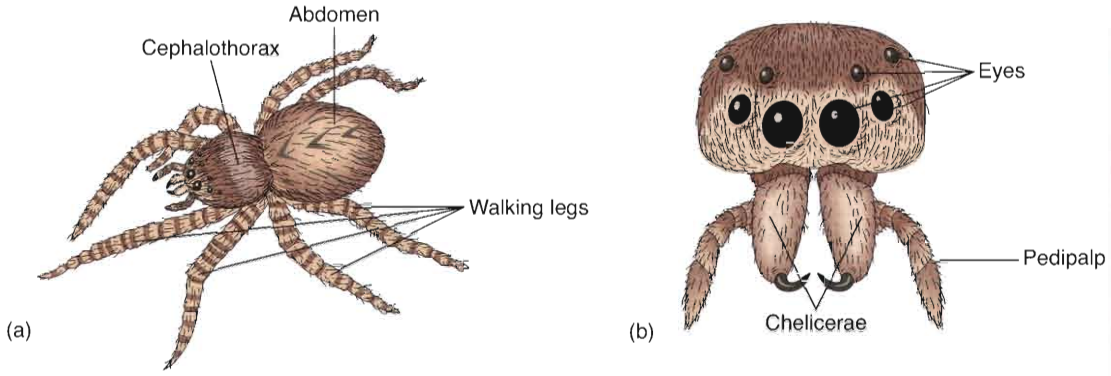


Figure 39.3
Anatomy of a jumping spider. (*a*) External view. (*b*) Anterior view of head.

Figure 39.4
Tarantulas, sometimes called bird spiders, may have a 25-cm leg span. They live on the ground, dwell in trees, and sometimes burrow. The hairy body of this brown tarantula is extremely sensitive to vibration. Many hairs contain chemoreceptors as well as mechanoreceptors, and hairs on the tips of the feet are often iridescent. Captive tarantulas can live 20 years or more.

Figure 39.5
Black widow spiders (*Latrodectus mactans*) are shiny black with a red hourglass on the abdomen's ventral surface. As with other spiders, their chelicerae have poison glands opening at the tip of the fangs. Toxins and enzymes are injected into prey, liquefying the prey's tissue. The nutritious broth is then eaten. Black widows live in most parts of the world, and their venom causes nausea, muscular spasms, respiratory paralysis, and pain in the abdomen and legs. Human death from their bite is rare. Black widows, like other spiders, can withstand months of starvation by reducing their metabolic rate up to 40%. A male is only half the size of the female and is killed soon after mating.

c. Why are appendages of abdominal segments called "book" gills?

Class Arachnida (Scorpions, Ticks, Mites, Daddy Longlegs, Spiders)

Arachnids (57,000 species) are the most diverse class of chelicerates and are mostly terrestrial. The cephalothorax has chelicerae modified as fangs to pierce prey, has pedipalps to manipulate food and sense the environment, and has four pairs of walking legs.

Examine a preserved scorpion, noting the many distinct segments of its thorax and abdomen. Compare the segmentation of scorpions with that of other available preserved chelicerates such as mites, ticks, and daddy longlegs. Scorpions were the first terrestrial arthropods and have been around since the Silurian period (425 million years ago). Their lack of fused segments is unusual for chelicerates and indicates the scorpion's ancient origins. These secretive carnivores are 1–8 cm long. Stings of *Centruroides*, a common scorpion in Mexico, Arizona, and New Mexico, have killed many humans, mostly children (fig. 39.1). Its venom is neurotoxic and causes convulsions, paralysis of respiratory muscles, and heart failure. However, most scorpion stings are not fatal.

Examine the external features of a preserved spider and compare them with the features shown in figure 39.3. Spiders are the most familiar arachnids and comprise 30,000 species. They are terrestrial and prey mostly on insects and other small invertebrates (figs. 39.4, 39.5). Spiders are often confused with insects, but spiders have two body regions and eight legs. Insects have three body regions and six legs. The

spinnerets of spiders are independently moving nozzles that release silk from internal silk glands. Web silk is made primarily of polypeptides of the amino acids glycine, alanine, and serine. When this fluid is emitted it hardens, not from exposure to air but from polypeptide cross-linkages that form during release. The fluid is not forced out under pressure; rather, it is drawn out by the hind legs or by the weight of the body falling through the air. Spiders can produce silk as strong as nylon, highly elastic and dry or sticky depending on the construction of the web.

Question 2

a. What external features make scorpions appear so menacing?

b. Which are larger, a scorpion's chelicerae or pedipalps?

c. What is the shape of a spider's chelicerae?

d. Do you see any evidence that a spider's body is segmented?

e. How many eyes do most spiders have? Are they paired and similar in size?

f. Many spiders are hairy. How might this feature be adaptive?

SUBPHYLUM CRUSTACEA

Class Crustacea (Crayfish, Crabs, Shrimps)

Crustaceans (35,000 species) live in marine and freshwater. Only a few species are terrestrial. Crustaceans differ from the other subphyla because they have fundamentally **biramous** or double-branched appendages (fig. 39.6). Crustaceans have two pairs of antennae and usually have **compound eyes** with multiple lenses. Crustaceans along with insects are commonly called mandibulates because they have opposing mandibles derived from an anterior pair of appendages.

Crustacean Anatomy

The body of a crustacean such as a crayfish usually has two regions: a cephalothorax covered by a carapace and an abdomen (fig. 39.7). The five anterior pairs of crustacean appendages are modified into **first antennae**, **second antennae**, **mandibles**, **maxillae**, and **maxillipeds**.

Obtain and examine a preserved crayfish. Appendages 3 (**mandible**) through 9 (**cheliped**) are used for feeding. These appendages have different shapes. The shape of some appendages such as the cheliped indicate an obvious function.

Question 3

How might mouthparts of various shapes be adaptive for crayfish?

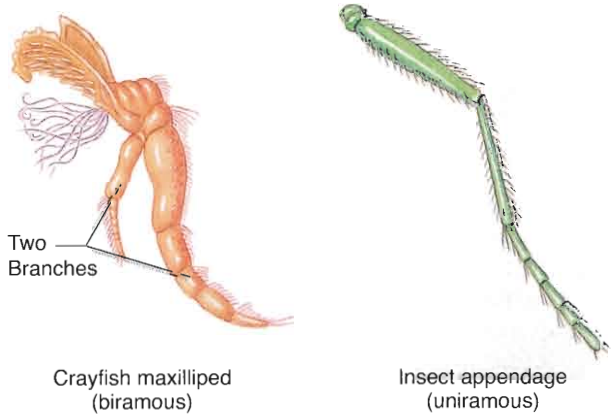
The four pairs of walking legs attach to the thorax at the ventral edge of the carapace. Abdominal appendages are much smaller than walking legs and are called **swimmerets** or **pleopods**. If you study a male crayfish you'll notice that each of the first pair of swimmerets has an odd spatulate shape, modified to transfer packets of sperm to a female during reproduction. The most posterior pair of appendages are broad, flat **uropods**. They surround the terminal abdominal segment called the **telson**. All crustacean appendages are fundamentally biramous (i.e., double-branched).

By now you've probably guessed that studying arthropods consists mostly of examining segments and their associated paired appendages. Appendages of two different species may have similar functions but different embryological origin. Such structures are **analogous**. For example, chelicerae of a spider are analogous to mandibles of a crayfish because they have similar functions even though they are not derived from the same body segment. In contrast, **homologous** structures of two different species have similar developmental origin but may or may not serve the same function. Chelicerae of a spider are homologous to the antennae of a crayfish.

Procedure 39.1

Study the external anatomy of a crayfish

1. Obtain a preserved *Cambarus* (the common crayfish) and locate the external features shown in figure 39.7.
2. To appreciate the variation in appendages in *Cambarus*, use forceps to remove one of the first



Crayfish maxilliped (biramous)

Insect appendage (uniramous)

Figure 39.6

Branched and single appendages. A biramous leg in a crustacean (crayfish) and a uniramous leg in an insect.

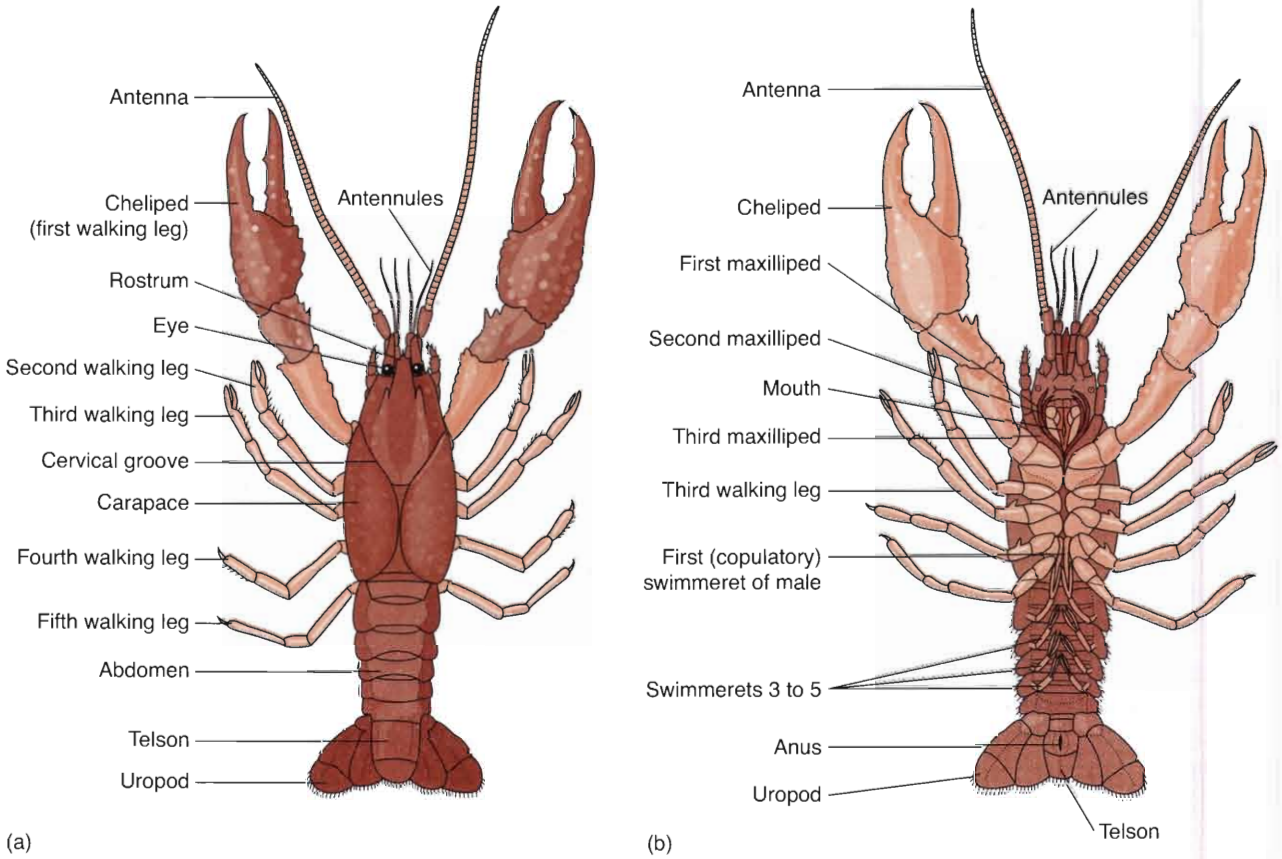


Figure 39.7

External structure of the crayfish (class Crustacea). (*a*) Dorsal view. (*b*) Ventral view.

antennae, a cheliped, a walking leg, an anterior swimmeret, a posterior swimmeret, and a uropod.

3. Arrange them in order in a dissecting pan or on a piece of paper and relate each one's structure to its function.
4. Examine the appendages you've removed and note which ones are biramous.
5. Observe a live crayfish (if one is available) and determine its two major methods of locomotion.
6. Examine other available crustaceans such as a crab. The cephalothorax of a crab is obvious, but the abdomen is highly modified.

Question 4

a. Which body region of a crayfish is most obviously segmented?

b. What structures are located just under the carapace and attached to each leg of a crayfish? What is the adaptive advantage of these structures being attached to legs?

c. How many legs are **chelate** (pincerlike with opposing claws)?

d. What is the function of the uropods and telson, and what feature indicates this function?

e. Are the anterior swimmerets different from the posterior pair? Is your crayfish a male or female?

f. Which set of legs (swimmerets or walking legs) appears best adapted to carry an incubating egg mass delicately and in a protected place on the body?

g. Crayfish can walk. Can they also "swim"? How would the uropods and telson help them do this?

h. How does the shape of a crab's abdomen differ from that of a crayfish?

The internal anatomy of a crayfish features a diamond-shaped **heart** surrounded by a thin **pericardial sac**. The heart lies on the dorsal midline just anterior to the abdomi-

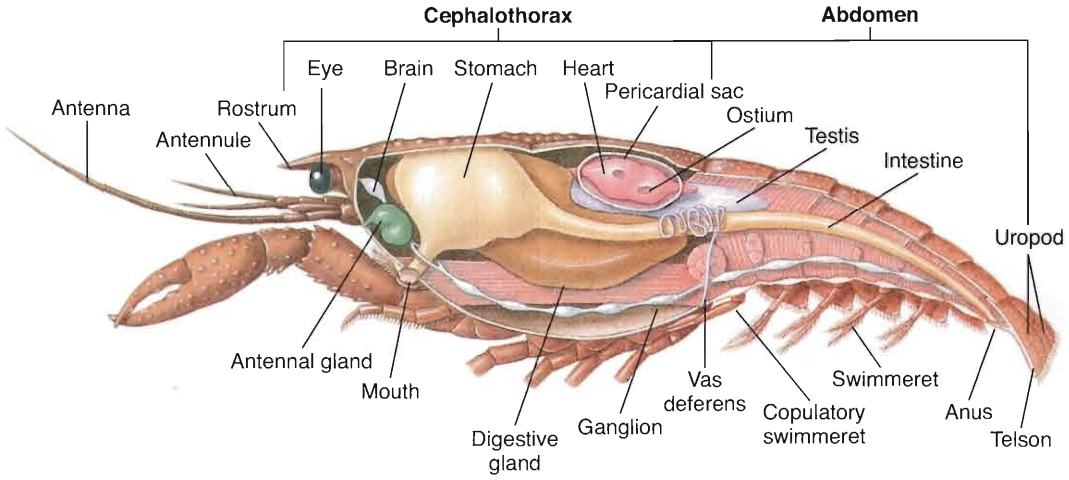


Figure 39.8
Internal structure of a male crayfish.

nal segments (fig. 39.8). In the open circulatory system of a crayfish, blood flows from the heart through large arteries to the gills and to sinuses surrounding and bathing the internal organs. Blood returns to the pericardial sac and enters the heart through small openings called **ostia**.

The **gonads** (testes or ovaries) are lateral and just anterior to the heart. Testes are usually white, and ovaries are orange. Sperm from testes exit the body through a pore at the base of the fifth pair of walking legs, and eggs are released at the base of the third pair of walking legs. During reproduction, males and females copulate and sperm cells are passed to the genital openings of the female. Fertilization is internal, and fertilized eggs are extruded and retained for maturation on swimmerets of the female.

The stomach is a continuous, membranous structure along the dorsal midline of the cephalothorax. It is surrounded by muscle, reinforced with ridges of tissue, and receives food from the **esophagus** and **mouth**. Food moves from the stomach, through the intestine, and out through the anus at the base of the telson. A large **digestive gland** that secretes enzymes and stores food lies just posterior and laterally to the stomach.

Beneath the internal organs lies the anterior part of the **ventral nerve cord**. A pair of nerves from the ventral nerve cord pass around the esophagus and come together anteriorly as a **brain** between and beneath the eyestalks. Posterior to the esophagus the nerve cord extends the length of the body as a series of swellings or **ganglia**, each of which controls organs in the immediate segment.

Procedure 39.2

Study the internal anatomy of a crayfish

1. Obtain a preserved crayfish.
2. Make an incision on each side of the cephalothorax. To make this incision, cut with scissors beginning at the posterior edge of the carapace halfway up the side (i.e., midlateral). Cut along the side toward the eye. Finish each lateral incision on each side of the rostrum.
3. Cut across the base of the rostrum and carefully remove the dorsal portion of the carapace.
4. Locate three pairs of ostia on the heart. Remove the heart.
5. Locate the stomach and digestive gland.
6. Carefully remove the internal organs (stomach, digestive gland, gonads). This exposes the end of the torn esophagus and the anterior part of the ventral nerve cord.
7. Remove a considerable amount of musculature on the floor of the abdomen (tail) to see the nerve cord more clearly.
8. Locate the **antennal glands**. They are excretory organs opening at the base of each antennae.
9. When you have finished examining your specimen, dispose of the material as directed by your instructor.

SUBPHYLUM UNIRAMIA

This subphylum of mandibulates includes centipedes, millipedes, and insects. They have **uniramous**, or single-branched, appendages.

Class Chilopoda (Centipedes)

Centipedes (3000 species) live in soil under logs and stones where they prey on small arthropods. Centipedes move rapidly and are dorsoventrally flattened. Some of the larger centipedes such as *Scolopendra* can inflict a painful bite but are not lethal to humans (fig. 39.9).

Examine a preserved centipede. Each body segment bears a pair of legs. The large fangs (sometimes called poison claws) on the head are not mandibles but are maxillipeds, appendages modified for feeding. The mandibles are smaller and lie between the maxillipeds.

INVESTIGATION

Variation in Crustacean Appendages

Observations: Crustaceans are highly diverse but share fundamental similarities retained throughout their evolution. Much diversity among crustaceans stems from variation in their appendages. Yet, there are consistent patterns in appendage morphology and body plan.

Question: What variations and similarities are apparent among crustaceans?

a. Establish a working lab group and obtain Investigation Worksheet 39 from your instructor.
b. Discuss with your group and instructor specific observations to be made during dissection of two closely related arthropods relevant to the preceding observations and question. Record them on Worksheet 39.
c. Translate your proposed observations into a testable hypothesis and record them on Worksheet 39.
d. Obtain a large, whole shrimp and crayfish for dissection.
e. Carefully remove appendages from one side of the specimens and tape the appendages side by side for comparison.
f. Complete Worksheet 39.

Question 5

a. Do centipedes have 100 legs as their name suggests?

b. What structures of chelicerates are analogous to the antennae of chilopods (centipedes) and other mandibulates?

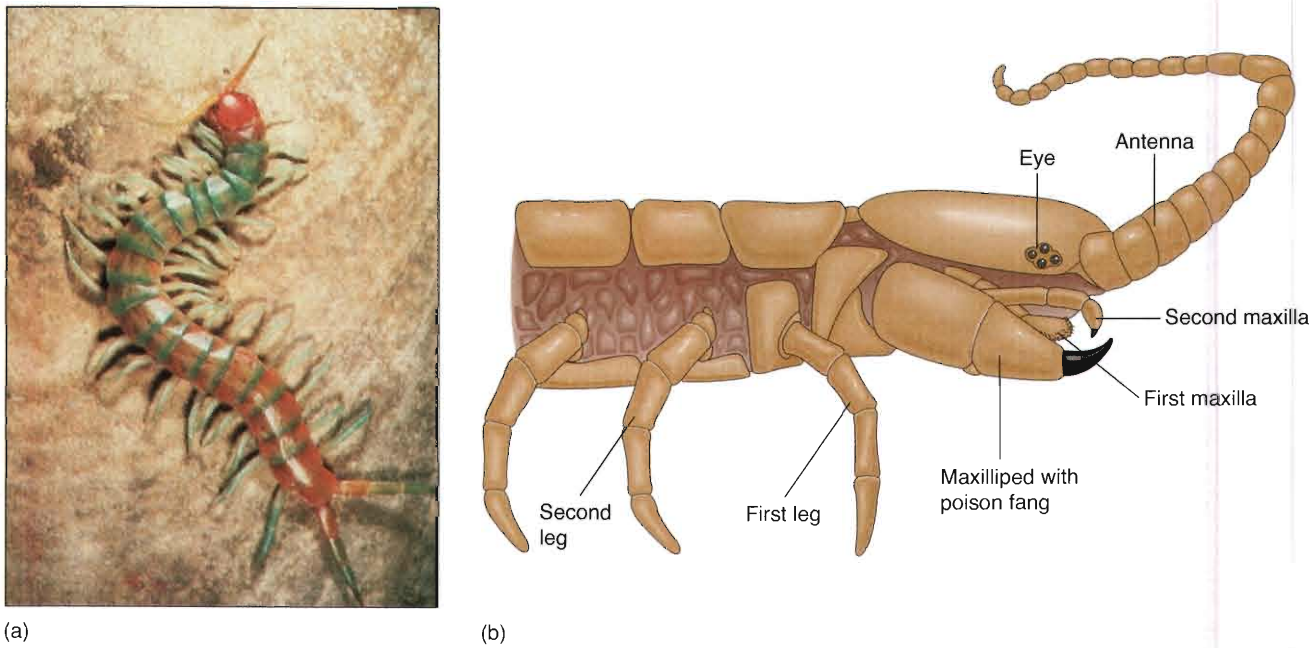
Class Diplopoda (Millipedes)

Examine a preserved millipede. Millipedes (8000 species) live in the same environment as centipedes but feed mainly on decaying plant material (fig. 39.10). Millipedes move slowly and are round in cross section. A disturbed millipede will frequently roll up to protect its soft underside. The number of legs on each segment distinguishes millipedes from centipedes.

Question 6

a. How many pairs of legs does a millipede have?

b. Each apparent segment is actually two fused segments. How many legs are on each apparent segment?



(a) (b)

Figure 39.9

Centipede, *Scolopendra* (class Chilopoda). (*a*) Most segments have one pair of appendages each. The first segment bears a pair of poison claws (maxillipeds), which in some species can inflict serious wounds. Centipedes are carnivorous. (*b*) The head of a centipede.

Class Insecta (Flies, Grasshoppers, Butterflies, Beetles, and Others)

Insects are by far the largest group of organisms on Earth and probably include 1 to 10 million species. At least 70% of all known species of animals are insects, and they dominate virtually all terrestrial habitats. Three separate body regions and six thoracic legs are the major diagnostic features of insects. Insects possess all the major characteristics of arthropods, but their ability to fly is probably the key to their great success. Although other groups of organisms can fly, insects were the first fliers. There are many advantages to flying, such as avoiding predators. Can you name some others?

Most insects have two pairs of wings. However, insect wings are not modified appendages. Rather, they are evaginations (outgrowths) of the thoracic exoskeleton.

The success of insects on land is further enhanced by an efficient respiratory system of tubes called **tracheae** that conduct air throughout the body. Insects also have highly modified mouthparts. For example, mouthparts of grasshoppers grind coarse plant tissue, whereas mosquito mouthparts puncture and suck fluid from animals.

External Anatomy of a Grasshopper

The grasshopper, *Romalea*, has characteristics typical of insects (fig. 39.11). Insects have three body regions: head, thorax, and abdomen, one pair of antennae, and six legs. The thorax is divided into the **pro-**, **meso-**, and **metathorax**, each having a pair of legs. Mouthparts are covered by the **labrum**, an extension of the head. Beneath the labrum are the **mandibles**, followed by a pair of **maxillae** with segmented extensions called **palps**, and then the **labium** with palps (fig. 39.12).

Romalea has 10 abdominal segments, each with a **spiracle** or breathing pore opening to the respiratory system of tracheal tubes (fig. 39.13). The terminal abdominal segment bears the reproductive genitalia. The terminal segment of

Figure 39.10
Millipede, *Sigmoria*, in North Carolina. Apparent body segments are actually fused pairs of body segments. Each apparent segment has two pairs of legs.

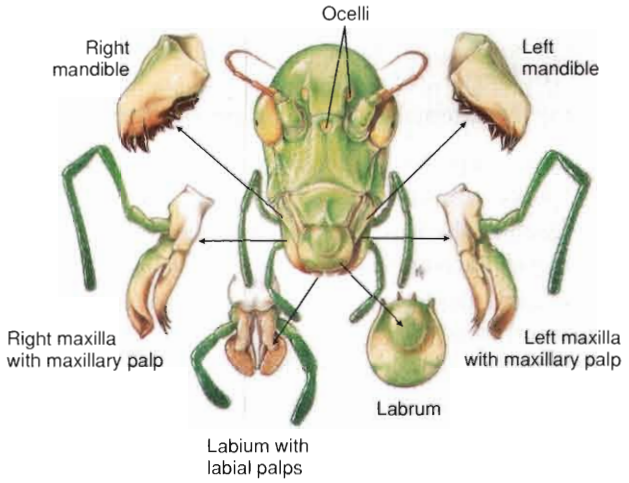


Figure 39.12
Head and mouthparts of a grasshopper.

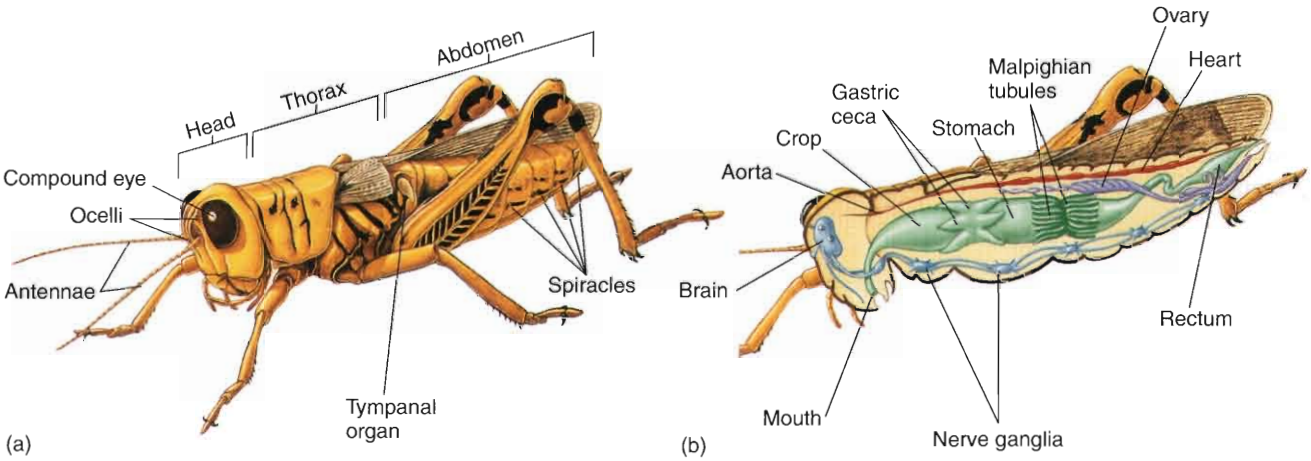


Figure 39.11
Grasshopper, *Romalea*, a member of class Insecta. (*a*) External anatomy. (*b*) Internal anatomy.

DICHOTOMOUS KEY TO SOME MAJOR ORDERS OF INSECTS

1. Insects with two wings . . . . . **(flies) Diptera**
   Insects with four wings, a pair of forewings, and a pair of hindwings 2
2. Fore- and hindwings are not alike in texture and color. One pair may be hard and dense while the other may be light and transparent 3
   Fore- and hindwings similar, usually clear, thin, and transparent 5
3. Forewings thick and leatherlike at base, tips much thinner and may be transparent; mouthparts pointed and beaklike to puncture prey and suck body fluids . . . . . **(bugs) Hemiptera**
   Forewings same texture throughout, biting mouthparts with opposing mandibles 4
4. Forewings leathery and with veins . . . . . **(grasshoppers, crickets) Orthoptera**
   Forewings hard, without veins . . . . . **(beetles) Coleoptera**
5. Wings of same length, antennae usually shorter than head 6
   Wings not of same length, antennae long or enlarged toward end 7
6. Large insects (usually > 3 cm), wings long, transparent, and with many strong veins; abdomen long and slender . . . . . **(dragonflies) Odonata**
   Smaller insects, wing venation faint, wings extending posterior to the abdomen . . . . . **(termites) Isoptera**
7. Wings covered with fine, opaque scales; tubular, coiled, sucking mouthparts . . . . . **(butterflies, moths) Lepidoptera**
   Wings thin, transparent, and not covered with scales; mandibles well developed . . . . . **(ants, bees, wasps) Hymenoptera**

Questions for Further Thought and Study

1. Arthropods usually have a distinct head. How would you define a "head"? What are the advantages and disadvantages of such a body region?

2. Does an insect's exoskeleton limit growth? Why or why not?

3. Diagram the arrangement of muscles necessary to bend a joint with an exoskeleton versus a joint supported by an endoskeleton.

4. Arthropod body segments are sometimes distinct, sometimes indistinct, and sometimes fused as groups to form body regions. Which groups of arthropods appear the most distinctly segmented? Which appear the least segmented?

5. What effect would 2.5 million spiders per acre have on the insect community?

6. Do you suspect that each eye of a spider provides the same sensory input to the brain? Why or why not?

7. What activities and body functions of arthropods require the most specialized appendages?

8. Do beetles have wings? If so, where are they?

9. What other group of organisms you have studied thus far has chitin as part of its outer covering?

10. What class of arthropods dominates the sea?

11. Prepare a simple table of all of the taxonomic groups, their common names, their distinguishing characteristics, and all representative genera covered in this exercise. Keep this table with your study notes.

12. Does a crayfish have an open or closed circulatory system? Review detailed references and summarize how the circulatory systems vary among animal phyla.

13. A major feature of annelids and arthropods is segmentation. Speculate on the adaptive advantages of segmentation.

WRITING TO LEARN BIOLOGY

Do you think that arthropods constitute a single phylum, or should they be divided into multiple phyla? Describe what divisions you would make and your reasons for them.

Survey of the Animal Kingdom
Phyla Echinodermata, Hemichordata, and Chordata

40

Objectives

By the end of this exercise you should be able to:

1. List echinoderm and chordate characteristics closely associated with successful food gathering and survival.
2. Describe the morphology of organisms of phyla Echinodermata, Hemichordata, and Chordata.
3. List characteristics that echinoderms and chordates share with phyla discussed previously.
4. Discuss characteristics of echinoderms and chordates that are unique or advanced compared to more primitive phyla.
5. Describe the water vascular system of echinoderms.
6. Discuss embryological characteristics that distinguish deuterostomes from protostomes.
7. Understand which phyla are protostomes and which are deuterostomes.

Members of the three phyla remaining in our survey of animals—that is, Echinodermata, Hemichordata, and Chordata—are **deuterostomes**. Deuterostomes are a major departure from the phylogenetic line of **protostomes** such as annelids, mollusks, and arthropods. The fundamental basis for the separation of deuterostomes from protostomes involves different morphological patterns of embryonic development (table 40.1); most notably, the blastopore of deuterostomes gives rise to an anus rather than the mouth. The blastopore is the opening to the first cavity formed in a developing embryo and is discussed more in Exercise 50.

Read table 40.1 carefully. Refer to your textbook for a more detailed comparison of protostomes and deuterostomes.

Question 1
Describe three major differences between deuterostome and protostome development.

PHYLUM ECHINODERMATA

Echinoderms (6000 species) are marine bottom-dwellers and include sea stars, brittle stars, sea urchins, sand dollars, sea cucumbers, and crinoids (table 40.2, fig. 40.1). These organisms are called echinoderms (*echino* = spiny, *derm* = skin) because their internal skeleton of calcareous plates, called **ossicles**, usually has spines protruding through a thin layer of skin. The five classes of echinoderms are distinguished primarily by the arrangement of their ossicles.

TABLE 40.1
A COMPARISON OF FOUR MAJOR FEATURES OF DEVELOPING EMBRYOS OR PROTOSTOMES AND DEUTEROSTOMES

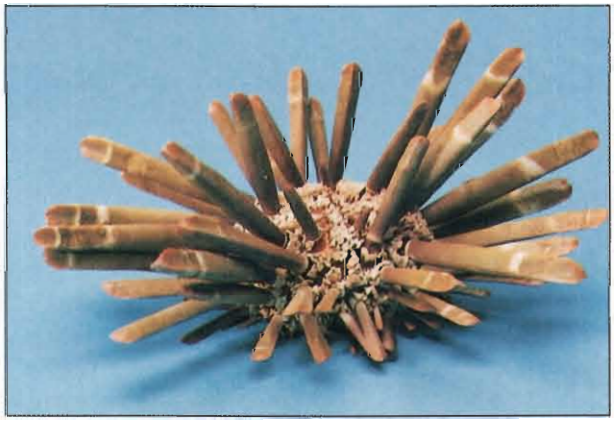
Feature	Protostomes	Deuterostomes
1. Fate of the first opening (blastopore) to the digestive cavity	Becomes the mouth	Becomes the anus
2. Pattern of early cell division	Spiral	Radial
3. Fate of cells in the early embryo	Determinate, fate is fixed during early development	Indeterminate, fate is not fixed until late development
4. Mesoderm formation	From endodermal cells near the blastopore	From endodermal cells opposite the blastopore
Major phyla	Annelida, Mollusca, Arthropoda	Echinodermata, Hemichordata, Chordata

(a)

(b)

(c)

(d)



(e)

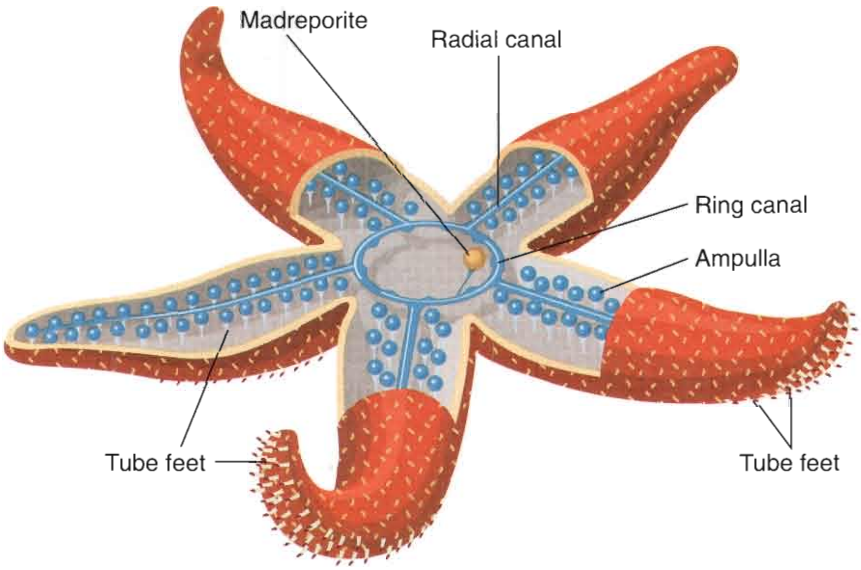
Figure 40.1

Diversity in echinoderms (phylum Echinodermata). (*a*) Sea star, *Oreaster occidentalis* (class Asteroidea), in the Gulf of California. (*b*) Sea cucumber, *Strichopus* (class Holothuroidea), in the Philippines. (*c*) Feather star, *Comanthina* (class Crinoidea), uses its highly branched arms in filter feeding. Although this probably reflects the original use of echinoderm appendages, most modern echinoderms use their arms for locomotion, capturing prey, and scavenging the substrate for food. (*d*) Brittle star, *Ophiothrix* (class Ophiuroidea). (*e*) Sea urchin, class Echinoidea. Urchins defend themselves with long and sometimes barbed spines, often filled with toxins. Spines of this pencil urchin (*Heterocentrotus* sp.) are particularly robust and blunt.

TABLE 40.2

PHYLA ECHINODERMATA AND CHORDATA

Phylum	Typical Examples		Key Characteristics	Approximate Number of Named Species
Echinodermata (echinoderms)	Sea stars, sea urchins, sand dollars, sea cucumbers		Deuterostomes with radially symmetrical adult bodies; endoskeleton of calcium plates; five-part body plan and unique water vascular system with tube feet; able to regenerate lost body parts; marine	6000
Chordata (chordates)	Mammals, fish, reptiles, birds, amphibians		Segmented coelomates with a notochord; possess a dorsal nerve cord, pharyngeal slits, and a tail at some stage of life; in vertebrates, the notochord is replaced during development by the spinal column; 20,000 species are terrestrial; deuterostomes	42,500



(a)

(b)

Figure 40.2

Echinoderms. (*a*) The echinoderm body plan of a sea star, emphasizing the water vascular system. (*b*) The extended tube feet of a sea star, *Ludia magnifica*. Tube feet are used for locomotion and to grip and pull apart prey such as clams.

Adult echinoderms are radially symmetrical, and their bodies typically consist of a ring of five repetitive parts (that is, they are pentaradial). In contrast, larvae of echinoderms are bilaterally symmetrical. This indicates that radial symmetry is secondarily derived and not directly related to the symmetry of more ancient phyla such as Cnidaria.

Echinoderms have a unique **water vascular system** consisting of a series of coelomic water-filled canals with hollow projections called **tube feet**. Muscle contractions and hydrostatic pressure in the water vascular system extend and move the tube feet and other parts of the system and thereby move the animal (fig. 40.2).

6. Cut around the perimeter of the central disk. Then cut from the perimeter up to and around the madreporite. This incision will allow you to remove the upper body wall without tearing away the madreporite.
7. Remove the upper body wall. As you lift it, try to see the delicate connection between the anus on the surface of the thick-walled pyloric stomach below.
8. Locate the structures shown in figure 40.6.

Question 3

a. How many tube feet would you estimate are in one arm?

b. What part of the water vascular system extends into each arm?

c. What other phyla that you have examined rely on "hydraulics" as part of their locomotion system?

d. Does the stomach wall appear highly folded and extensible? How does that relate to the feeding method of most sea stars?

Class Ophiuroidea (Brittle Stars)

Examine a preserved brittle star such as *Ophioderma*. Brittle stars have slender, sometimes branched arms clearly demarcated from the central disk (fig. 40.1*d*). Ossicles of brittle stars are typically thick and have attached musculature. They may form "shields" on the surface. As the name "brittle" star implies, their arms detach easily, allowing escape from predators.

The ambulacral grooves are closed in brittle stars, and the reduced tube feet are not used for locomotion. The thin flexible arms of brittle stars allow them to crawl rapidly like an octopus rather than creep slowly like sea stars. Brittle stars eat suspended food particles captured with their tube feet and passed to their mouth.

Question 4

a. Between brittle stars and sea stars, which have the most apparent ossicles? Do they overlap?

b. Are tube feet visible in *Ophioderma*?

Class Crinoidea (Sea Lilies and Feather Stars)

Examine a preserved crinoid. Crinoids are the most ancient echinoderms; only a few genera live today (figs. 40.1*c*, 40.7). They differ from other living echinoderms because their oral surface (mouth and anus) usually faces up. Their ossicles are well developed and give the animal a coarse, jointed appearance. Highly branched and feathery arms surround the mouth and anus. Most ancient crinoids were attached to the substrate by a stalk and appeared to be plants. However, most modern species are not stalked or permanently attached. Crinoids filter feed by capturing food particles on the mucus of their tube feet.

Question 5

How does the position of the mouth and anus of a crinoid relate to a primitive sessile existence?

Class Echinoidea (Sea Urchins and Sand Dollars)

Examine an urchin such as *Arbacia* and locate the features shown in figure 40.8. Also examine a dissected Aristotle's lantern if one is available. In addition, examine a sand dollar and compare its test of fused ossicles to an urchin's test. Urchins lack distinct arms, and their ossicles are fused into a solid shell called a **test** (fig. 40.1*e*). Holes in the test allow long tube feet to protrude. Spines of sea urchins are jointed, movable, and longer than those of other classes of echinoderms. These spines and long tube feet control locomotion of urchins. The mouth contains five ossified plates, or teeth, used to scavenge and scrape surfaces of rocks and gather algae for food. This small internal structure of five teeth is called **Aristotle's lantern**.

Question 6

a. Is an urchin's test pentaradially symmetrical?

b. Urchins and sand dollars lack arms. How do they move?

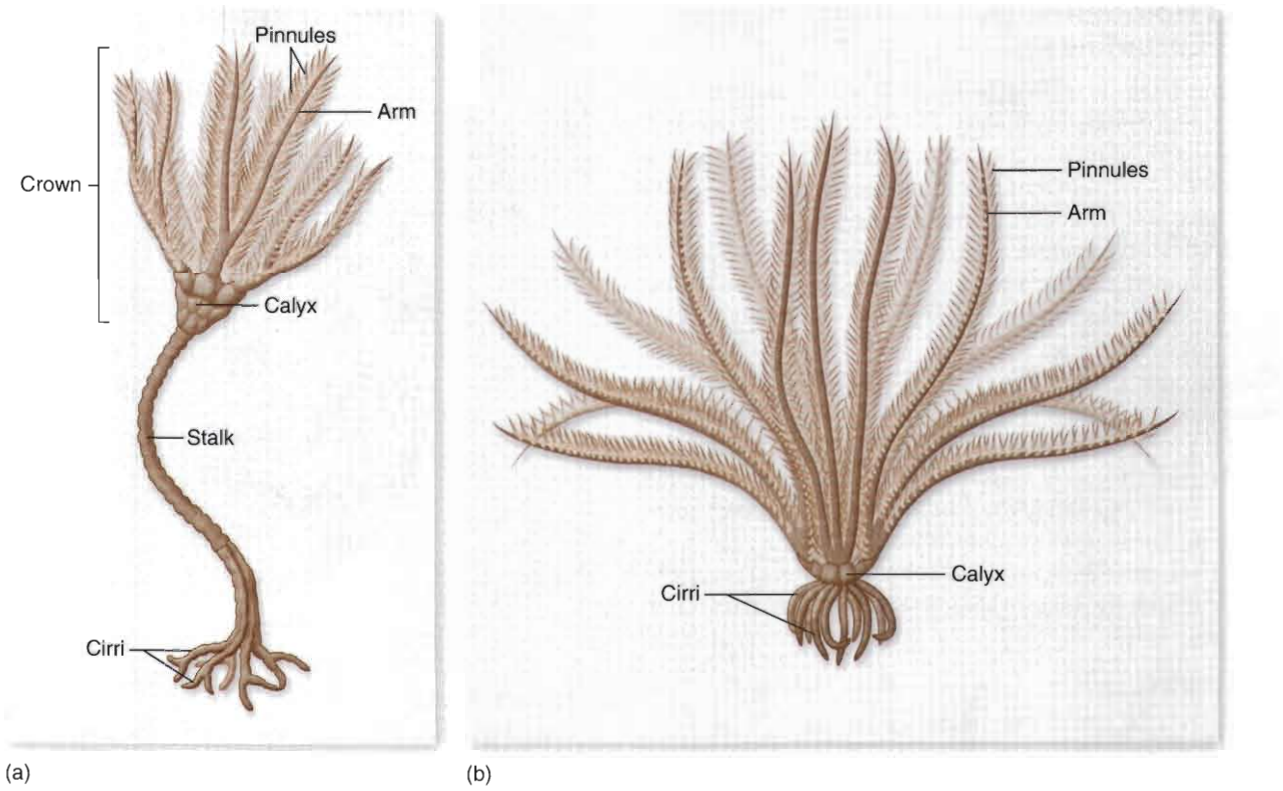


Figure 40.7
Class Crinoidea. (*a*) A sea lily (*Ptilocrinus*). (*b*) A feather star (*Neometra*).

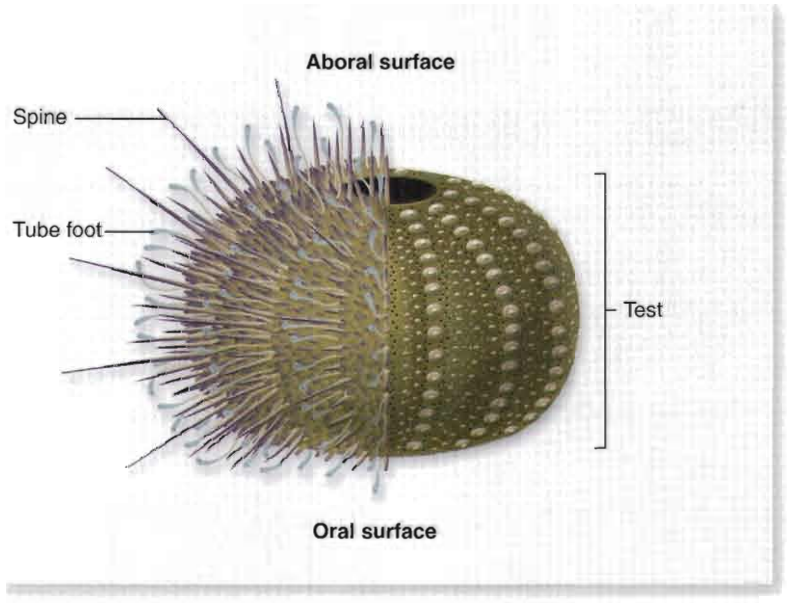


Figure 40.8
External anatomy of a sea urchin. Spines and tube feet are removed on the right half of the diagram to show the test.

Class Holothuroidea (Sea Cucumbers)

Examine a sea cucumber *Cucumaria* and determine the orientation of its radial symmetry. Find the mouth at one end; it is surrounded by modified tube feet called **tentacles**. Sea cucumbers look different from other echinoderms because they have soft bodies with reduced ossicles and few if any spines (figs. 40.1*b*, 40.9). Radial symmetry is less evident in sea cucumbers and their body axis is oriented horizontally. This orientation gives sea cucumbers a semblance of cephalization. The tentacles secrete a mucus that captures small floating organisms, which they eat. Interestingly, some sea cucumbers respond to stress by rupturing anteriorly and rapidly expelling their pharynx, digestive tract, and other organs. This process is called evisceration; because of it, the animal must regenerate the lost parts of the organs. Some gourmets consider sea cucumbers a delicacy.

Examine other preserved echinoderms and review in your textbook the major characteristics of each class. Then complete table 40.3.

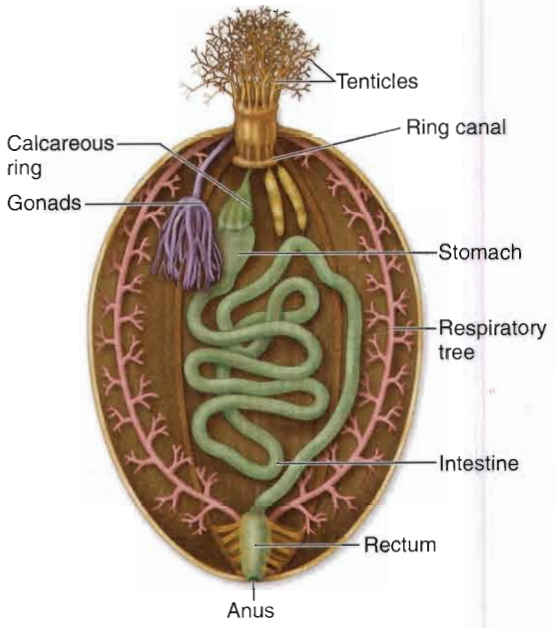


Figure 40.9

Internal structure of a sea cucumber, *Thyone*. The mouth leads to a stomach supported by a calcareous ring. The calcareous ring is also the attachment site for longitudinal retractor muscles of the body. Contractions of these muscles pull the tentacles into the anterior end of the body. The stomach leads to a looped intestine.

Question 7

a. Are tube feet visible on the sea cucumber?

b. Hydras, octopuses, and sea cucumbers have tentacles. Do tentacles have a single universal function, or varied functions? What functions are common?

TABLE 40.3

A COMPARISON OF THE MAJOR CHARACTERISTICS OF THE CLASSES OF ECHINODERMS

	Class				
	Asteroidea	Ophiuroidea	Crinoidea	Echinoidea	Holothuroidea
Shape of arms					
Development of tube feet					
Development of ossicles					
Feeding method					
Spine structure					

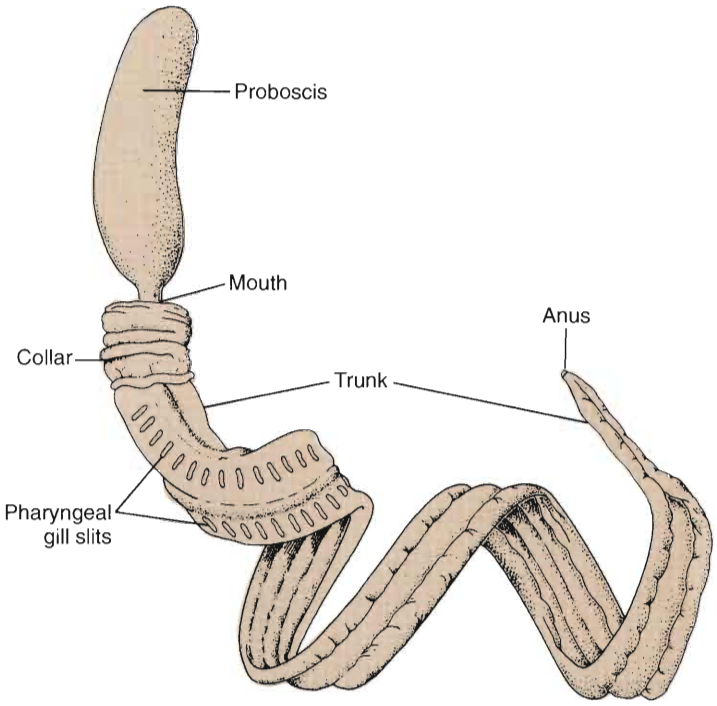


Figure 40.10
External lateral view of an acorn worm (phylum Hemichordata). Acorn worms are marine animals that burrow in sand or mud.

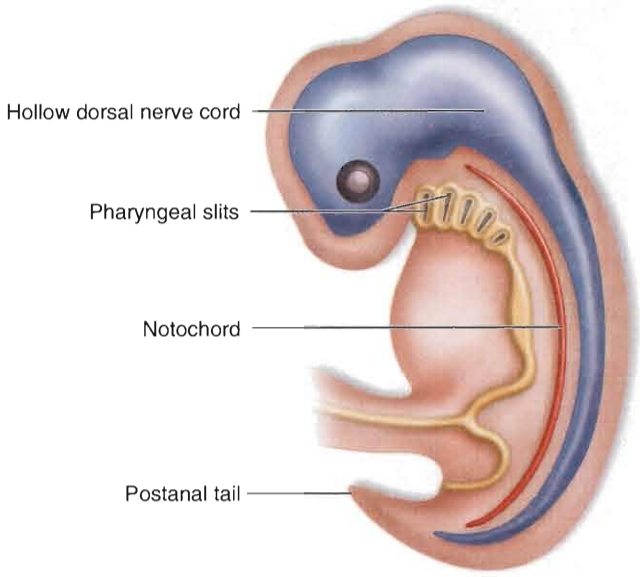


Figure 40.11
The four principal features of the chordates, shown in a generalized embryo.

c. Describe the functions of: pedicellariae, madreporite, dermal gills, Aristotle's lantern, tube feet, water vascular system.

PHYLUM HEMICHORDATA

Examine a preserved acorn worm, such as *Balanoglossus* or *Saccoglossus*, and compare it with figure 40.10. Hemichordates such as acorn worms are inconspicuous animals and include only 90 species. Members of this phylum share two important features with phylum Chordata: (1) a **dorsal nerve cord**, part of which is hollow, and (2) **pharyngeal slits**, openings in the throat that filter water that has entered through the mouth. Hemichordates do not, however, have a notochord as once thought or even possess half a notochord as their name implies.

Acorn worms are soft-bodied marine animals that burrow in sand or mud. Their bodies are fleshy and contractile and consist of a **proboscis**, a **collar**, and a **trunk**.

Question 8

a. Are pharyngeal gill slits readily apparent?

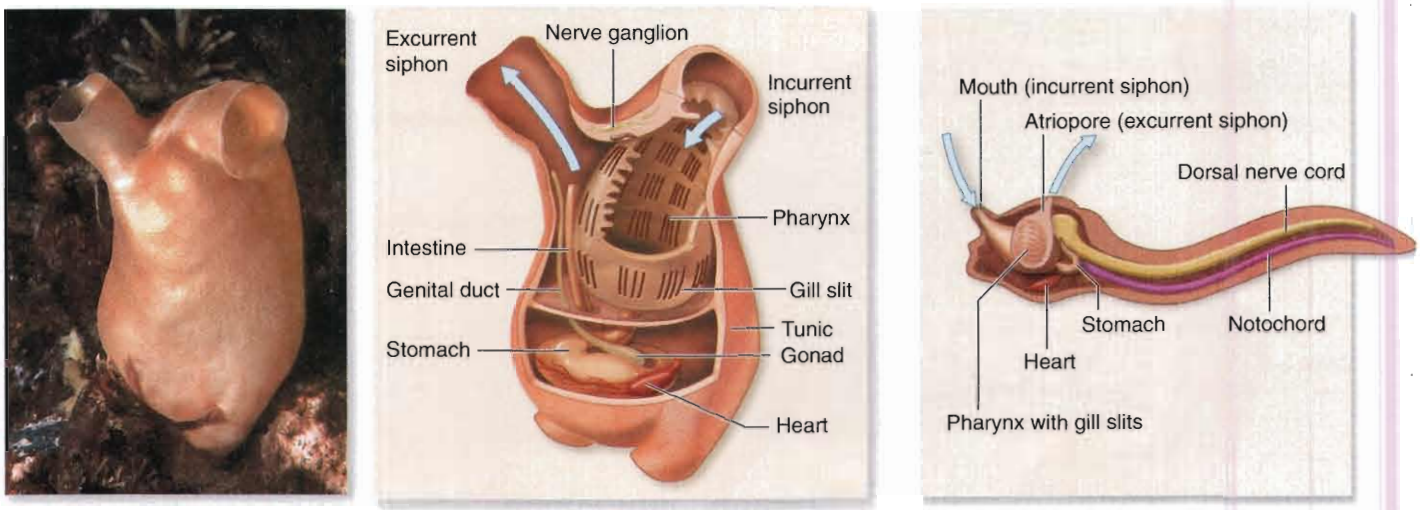
b. Do the shape and flexibility of the acorn worm body appear to be adapted for burrowing? How so?

PHYLUM CHORDATA

Chordates include 42,500 species of fish, amphibians, reptiles, birds, and mammals. They all are characterized by (1) a dorsal hollow nerve cord; (2) a **notochord**, a cartilaginous rod that forms on the dorsal side of the gut in the embryo; (3) pharyngeal slits; and (4) a **postanal tail** (fig. 40.11). An internal, bony skeleton is also common and provides sites for muscle attachment for efficient movement.

Subphylum Urochordata (Tunicates or Sea Squirts)

Examine a preserved adult tunicate. Urochordates, sometimes called tunicates, are sessile or planktonic marine organisms whose larvae possess the general chordate form—that is, they are elongated with a notochord and dorsal nerve cord. In contrast, the structure of an adult is highly modified to include a sievelike basket perforated with pharyngeal gill slits and surrounded by a cellulose sac called a **tunic**. Water enters through an incurrent siphon, is filtered by the pharyngeal basket, and exits through an excurrent siphon (fig. 40.12). Water is actively filtered; some tunicates



(a) (b) (c)

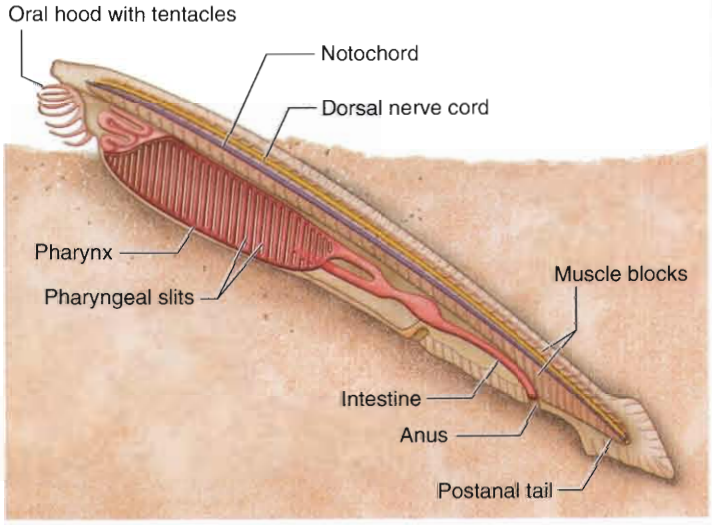
Figure 40.12

Tunicates (phylum Chordata, subphylum Urochordata). (*a*) Living adult. (*b*) Structure of adult tunicate. (*c*) Larval structure.

Figure 40.13

(*a*) Two lancelets, *Branchiostoma lanceolatum* (phylum Chordata, subphylum Cephalochordata), partly buried in shell gravel, with their anterior ends protruding. The muscle segments are visible; the square, pale yellow objects along the side of the body are gonads, indicating that these are male lancelets. (*b*) Internal structure of amphioxus. This bottom-dwelling cephalochordate has the four distinctive features of chordates: notochord, dorsal nerve cord, pharyngeal gill slits, and a postanal tail. The vertebrate ancestor probably had a similar body plan.

(a)



(b)

only a few centimeters long can filter 170 liters of water per day. Food collected by mucus on the pharyngeal basket is moved by cilia to the stomach and intestine. The intestine empties into the body cavity near the excurrent siphon.

Examine a prepared slide of larval tunicates. A larval tunicate has bilateral symmetry, a dorsal nerve cord, a notochord, and a postanal tail but loses these features when it settles for adult life (fig. 40.12*c*).

Question 9
What other group of organisms has cellulose in its supporting structures? Does this shared feature surprise you?

Subphylum Cephalochordata (Lancelets)

Examine either a preserved lancelet or a slide of a whole mount and compare the specimen with that shown in figure 40.13. Also examine a cross section through the pharynx and try to visualize the paths of food and water (fig. 40.14). Lancelets are small, fishlike, marine chordates that burrow in sand or mud. They are commonly called amphioxus, but the most common genus is *Branchiostoma*.

The dorsal nerve cord and notochord extend the length of the animal. The buccal cavity surrounds the mouth followed by a long pharynx with many gill slits (openings) separated by gill arches of reinforced tissue. As seawater enters the mouth and exits through the slits it must pass over the surfaces of the arches that form the sides of the slits. As this occurs, food particles are caught on the arches, and are eventually swept to the intestine. The anus is not terminal. Lancelets and vertebrates have a postanal tail, another diagnostic trait of chordates. After water passes by the arches it moves into a surrounding chamber called an **atrium** and then leaves the body through the **atriopore**.

Subphylum Vertebrata (Fish, Birds, Amphibians, Reptiles, and Mammals)

Vertebrates have a vertebral column that replaces the notochord in adults and surrounds the dorsal nerve cord (fig. 40.15). Vertebrates also have a distinct head. There are seven classes of living vertebrates, three of them fishes and four of them terrestrial tetrapods.

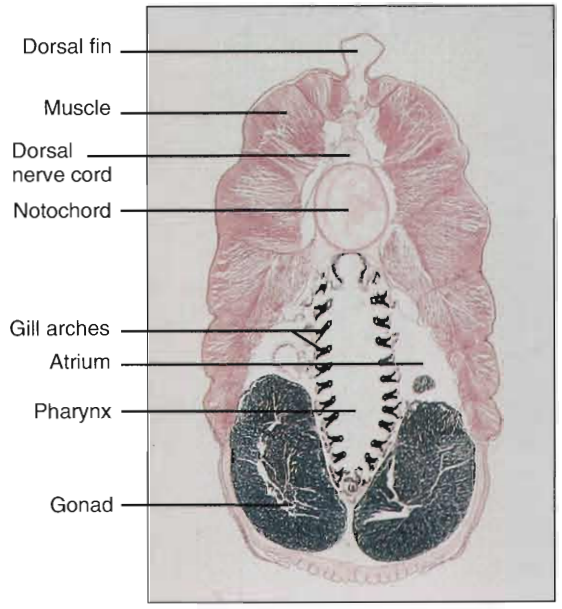


Figure 40.14
Cross section through the pharynx of a lancelet.

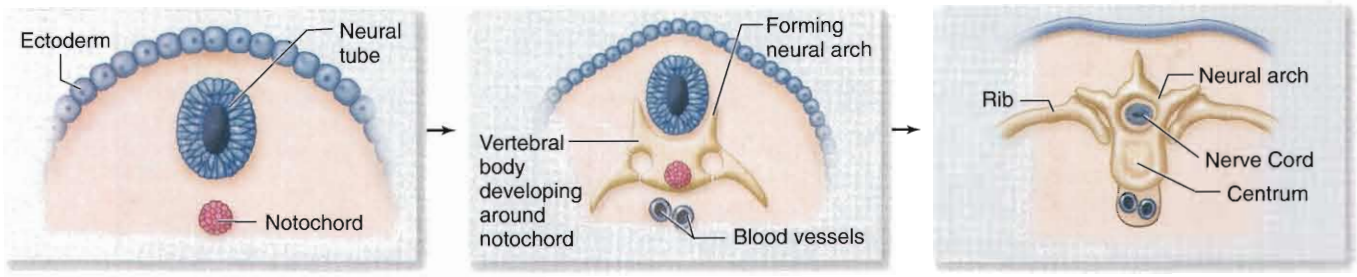


Figure 40.15
Embryonic development of a vertebra. During the course of evolution, or of development, the flexible notochord is surrounded and eventually replaced by a cartilaginous or bony covering, the centrum. The neural tube is protected by an arch above the centrum. The vertebral column is a strong, flexible rod that the muscles pull against when the animal swims or moves.

Class Agnatha (Lampreys and Hagfishes)

Examine a preserved lamprey, *Petromyzon* (fig. 40.16). Also examine a prepared slide of an **ammocoete**, the larva of a lamprey. Living agnathans descended from representatives of the earliest stages in the evolution of vertebrates. They lack jaws typical of other vertebrates but have a cartilaginous endoskeleton and a notochord. Seven pharyngeal gill slits are evident near the head. The gill arches separating the gill slits are reinforced with cartilage. The mouth is at the center of the round **buccal funnel** and is armed with horny teeth and a rasping tongue. Most lampreys are parasites. They attach their buccal funnel to the side of a fish, rasp a hole in the body with their tongue, and feed on the body fluids of the fish.

Question 10
Which closely related subphylum of chordates does an ammocoete resemble?

Class Chondrichthyes (Sharks, Skates, and Rays)

Sharks and their relatives are abundant in oceans as predators and scavengers. Their **endoskeleton** is cartilaginous and the anterior gill arches are modified into jaws. Jaws are a significant adaptation and modification of strong, anterior gill arches to process food (fig. 40.17). Like agnathans, their cartilaginous skeleton is not necessarily primitive but is probably derived secondarily from an ancestral bony skeleton.

Examine a preserved specimen of *Squalus*, the dogfish shark (fig. 40.18). Its external anatomy illustrates some advanced features appropriate for a predator. Fin structure includes paired pelvic fins (on the ventral surface near the anus) and pectoral fins (behind the gill slits) for stabilization and maneuvering. Jaws are large and powerful, and receptors in the nostrils and epidermis are sensitive to smells and electrical currents. A **lateral line** runs along each side of the body and contains sensory cells to detect slight vibrations.

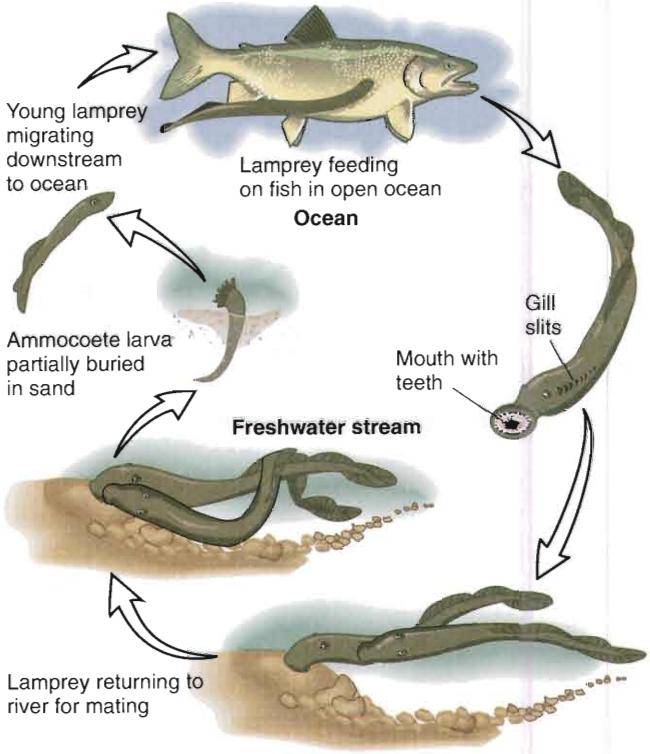
Question 11

a. Which fins of sharks provide power and speed?

b. Why is the number of pharyngeal gill slits in sharks fewer than that in lampreys?

c. Consider objective 1 listed at the beginning of this exercise. Is a lateral line system significant to fundamental processes for sharks and bony fish? How so?

(a)



(b)

Figure 40.16
Lamprey (class Agnatha, *Petromyzon marinus*). (*a*) Note the sucking mouth attached to aquarium glass and teeth used to feed on other fish. (*b*) External structure and life history of a sea lamprey. Sea lampreys feed in the open sea; toward the end of their lives lampreys migrate into freshwater streams, where they mate. Females deposit eggs in nests on the stream bottom, and the young larvae hatch 3 weeks later.

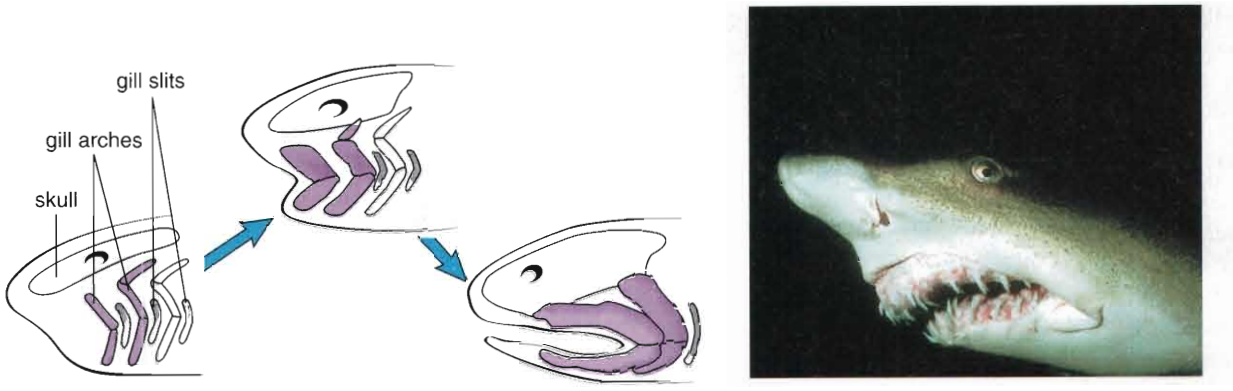


Figure 40.17

(*a*) Jaws are believed to have evolved from the first pair of gill arches of agnathans. The second pair of gill arches became support structures for the jaws. (*b*) Head of sand tiger shark, *Carcharias* sp., showing a series of successional teeth on strong jaws.

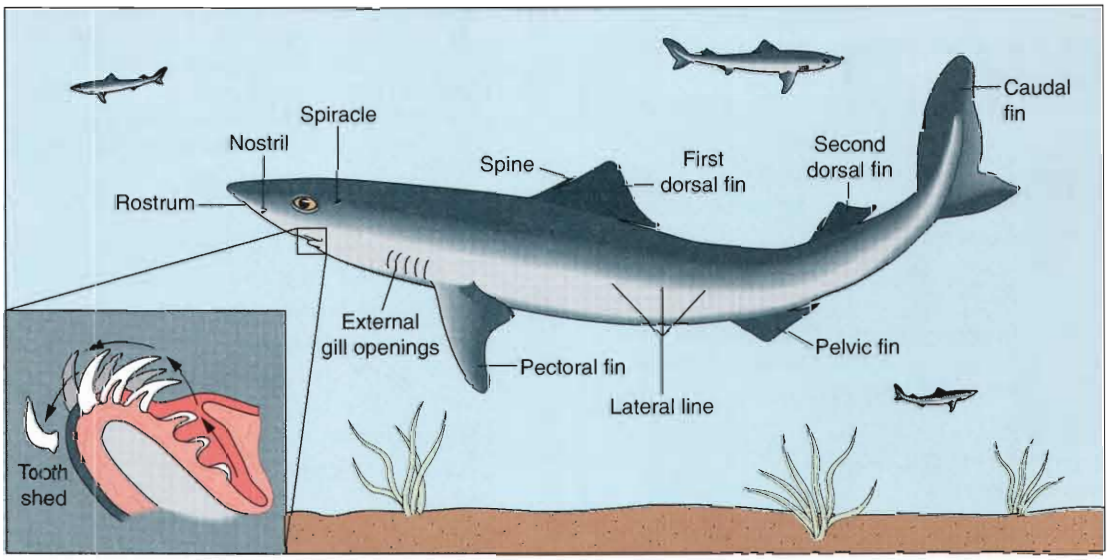


Figure 40.18

Sharks (class Chondrichthyes). Dogfish shark, *Squalus acanthias*. Section of lower jaw (inset) shows new teeth developing inside the jaw. These teeth move forward to replace lost teeth. The rate of replacement varies in different species.

Class Osteichthyes (Bony Fish)

Procedure 40.3

Examine the anatomy of a bony fish

1. Examine the external anatomy of a preserved fish (fig. 40.19).
2. Although sharks are built for speed, the maneuverability of bony fishes is much greater. If living fish are available, observe their swimming and "breathing."
2. Examine a dissected perch and locate the structures shown in figure 40.19.

Bony fish are the most diverse class of vertebrates (20,000 species). Advanced features of bony fish include a bony endoskeleton, modified gill arches, and internal air bladders for balance and buoyancy. Gills are protected by a movable gill cover called an **operculum**. Along each side and

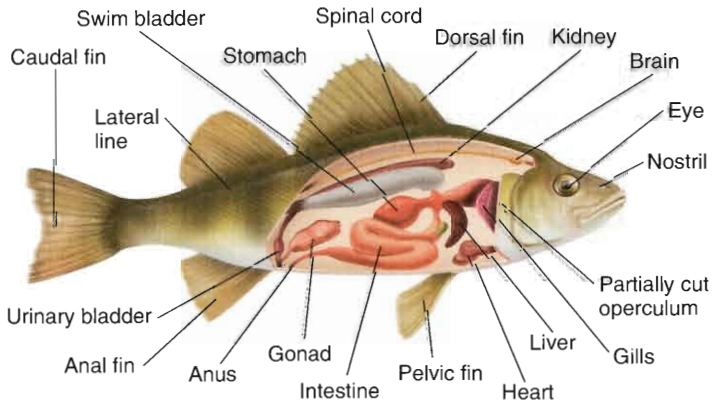


Figure 40.19

Anatomy of a bony fish, class Osteichthyes.

branching over the head of most fishes is a **lateral-line system** consisting of sensory pits in the skin. These pits detect water currents and predators or prey that may be moving near the fish.

Question 12

a. How do the number and shape of fins of a bony fish differ from those of a shark?

b. Fins of a bony fish are flexible and diverse in shape. Describe the location of a fin present in bony fish but not in sharks.

c. How does the symmetry of the tail of a fish compare with that of a shark?

d. Does most of the power for movement by a fish come from the tail or from other fins?

e. Can fish move water over their gills without moving through the water? What role does the operculum play in this movement?

f. How does the buoyancy of an air bladder affect the motion of a fish compared to that of a shark?

Class Amphibia (Frogs, Toads, and Salamanders)

Examine preserved amphibians on display. Amphibians were the first land vertebrates, arising from fish with stout, fleshy fins. Most amphibian adults are terrestrial, but they lay eggs in water (fig. 40.20). The eggs are fertilized externally and each hatches into an aquatic larval stage called a **tadpole**. Tadpoles undergo a dramatic metamorphosis of body shape as they become adults.

Development of legs and the development of lungs in amphibians were major evolutionary events. However, primitive lungs had already developed in some fish. In addition to lungs, the soft moist skin of some amphibians is highly vascularized and accounts for as much oxygen diffusion as the lungs.

Question 13

How are the legs of a frog different from the fins of a fish to enable movement on land?

Class Reptilia (Turtles, Snakes, and Lizards)

Examine preserved reptiles and note their morphological diversity (fig. 40.21). Reptiles, unlike their ancestors, are independent of aquatic environments and have developed structures for internal fertilization (fig. 40.22). Most reptiles also lay watertight eggs that contain a food source (the yolk) and a series of four membranes—the chorion, the amnion, the yolk sac, and the allantois (fig. 40.23). Each membrane plays a role in making the egg an independent life-support system. The outermost membrane of the egg, the **chorion**, allows oxygen to enter the porous shell but retains water within the egg. The **amnion** encases the developing embryo within a fluid-filled cavity. The **yolk sac** provides food from the yolk for the embryo via blood vessels connecting to the embryo's gut. The **allantois** surrounds a cavity into which waste products from the embryo are excreted.

Figure 40.20

A frog (class Amphibia). This poison arrow tree frog (*Dendrobates* sp.) exhibits strong coloration. These colors advertise its powerfully toxic secretions to predators, which quickly learn the frog's noxious taste. Natives of South America use the frog's toxins as a weapon. They kill the frog by piercing it with a sharp stick and holding it over a fire. The heat causes the cutaneous glands to secrete drops of venom, which are scraped into a container and allowed to ferment. Arrows dipped into the poison can paralyze birds and small monkeys. One poison arrow tree frog contains enough toxin to kill about 20,000 mice.

Reptiles have a dry skin covered with scales that retard water loss. This dry skin does not aid respiration, but the lungs are well developed. Reptiles, fish, and amphibians are *poikilothermic*, meaning that their body temperature depends on the environment.

Question 14

a. What is the adaptive significance of internal fertilization and a watertight egg?

b. How do the legs of different reptiles vary in number, size, and function?

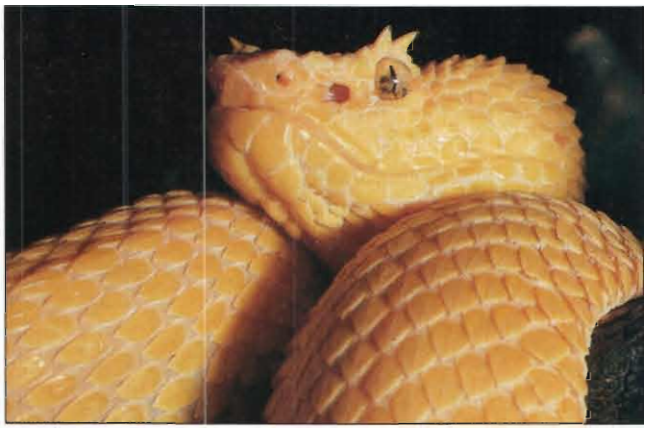


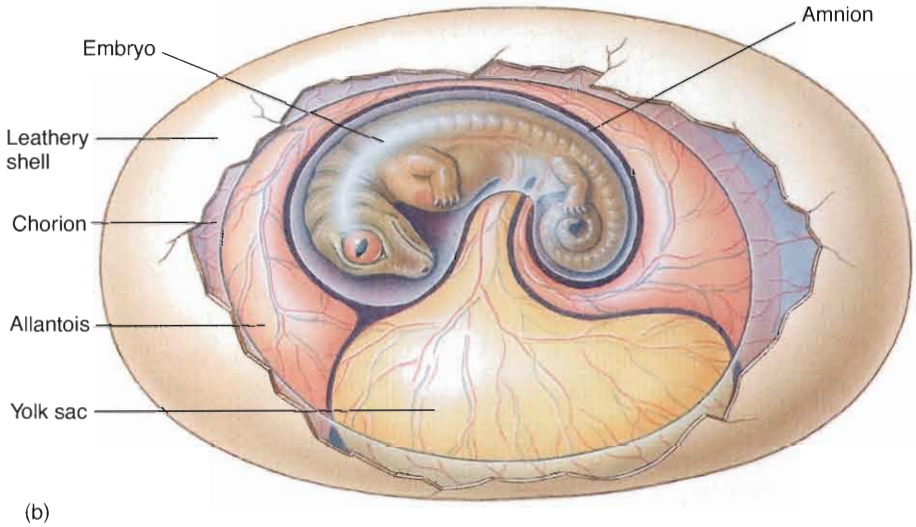
Figure 40.21

Pit vipers are venomous reptiles; they have a pair of heat-detecting pit organs on each side of the head. Pit organs are visible between the eye and the nostril of this golden eyelash viper (*Bothrops schlegeli*). These vipers can locate and strike a motionless warm animal in total darkness by sensing heat from its body. Pit organs are highly sensitive to infrared wavelengths and are especially sensitive to sudden changes of temperature. Pit organs can detect temperature differences of 0.2°C or less, allowing effective hunting of small animals at night.

Figure 40.22

Internal fertilization. The male injects sperm-containing semen into the female's body during copulation. Reptiles such as these turtles were the first terrestrial vertebrates to develop this form of reproduction, particularly suited to terrestrial existence.

(a)



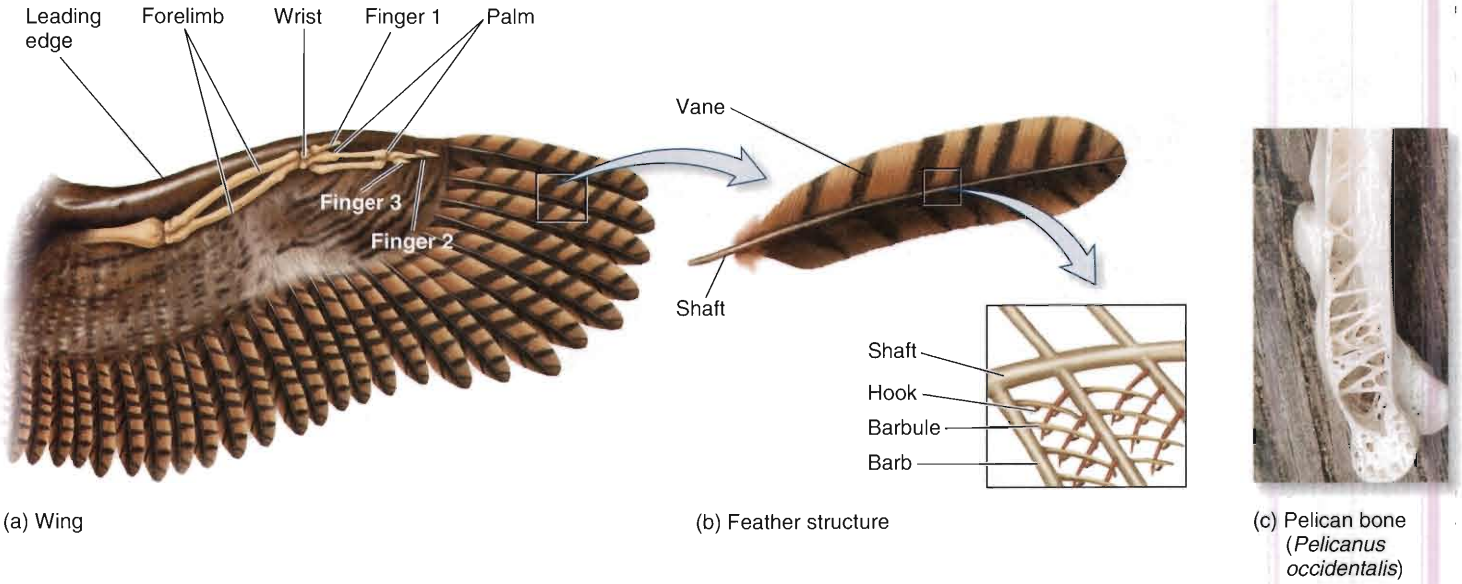
(b)

Figure 40.23

The watertight amniotic egg enables reptiles to live in a wide variety of habitats. (*a*) This green iguana from South America is hatching from a typical reptilian egg. Reptiles are the most primitive vertebrates to produce terrestrial eggs. The shell of reptile eggs can be leathery or rigid. (*b*) The major functional membranes of a reptilian egg.

c. Would you expect the legs of a terrestrial tetrapod to be more robust than those of an aquatic organism? Why or why not? Is this true for the reptiles and amphibians that you examined?

d. Consider objective 1 listed at the beginning of this exercise. How could poikilothermy contribute to the evolutionary success of reptiles in their environment?



(a) Wing (b) Feather structure (c) Pelican bone (*Pelicanus occidentalis*)

Figure 40.24

Features of the bird wing and feather. (*a*) The wing is supported by an elongated and modified forelimb with extended fingers. (*b*) Each feather has a hollow shaft that supports many barbs, which in turn support barbules that interlock with hooks to give the feather its form. (*c*) The bones of a pelican (*Pelicanus occidentalis*) are hollow but criscrossed with a honeycomb structure that provides added strength.

Source: Darrell Vodopich

(a)

(b)

Figure 40.25

Birds (class Aves). (*a*) The flightless cormorant, *Phalacrocorax harrisi*, lives only on the Galápagos islands and is the only cormorant in the world (out of 30 species) that cannot fly. Ancestors arriving at the islands had no predators and little competition for their feeding niche of bottom-fish, eels, and octopuses. Flight was no longer adaptive. Over time, natural selection favored a streamlined body and strong legs for swimming. Flight muscles atrophied through the generations, and their sparsely feathered wings have become vestigial. (*b*) The California condor (*Gymnogyps californianus*) is the largest land bird in North America. Young condors acquire full adult plumage after 6 years and may live 50 years. They are efficiently adapted to soaring effortlessly in search of carrion. Their bald heads are adapted for reaching deep within the carcass and tearing pieces of meat. Unfortunately, they are in danger of becoming extinct. The remaining three or four wild individuals were captured in 1987. Offspring have been raised in captivity and were reintroduced into their dwindling habitat in 1991. Even efficient survival adaptations of the condor have not prevented a dramatic population decline. Condors are extremely sensitive to human disturbances, and we have steadily encroached on their habitat.

Class Aves (Birds)

Examine a prepared slide and whole mount of a feather (fig. 40.24). Notice the interlocking structures. Also examine specimens of birds (fig. 40.25). Birds are the only animals with feathers, and they share the ability to fly with only a few groups. Eyes of birds are always prominent, and vision is one of their most highly developed senses. Birds are *homeothermic*, meaning that they maintain a constant body temperature. Other adaptations to flight include a high body temperature for high metabolism, a lightweight skeleton, an efficient respiratory system, and heavy musculature at the breast to move the wings.

Question 15

a. What are wings of flying animals other than birds made of?

b. Why might birds use keen vision more than reptiles or amphibians do?

c. Consider what you have learned about enzymes in Exercise 11. What might be the adaptive advantage of homeothermy?

d. Describe six adaptations of birds to flight.

Class Mammalia

Examine some preserved mammals. Mammals are covered with insulating body fat and **hair** and maintain a constant body temperature as birds do (fig. 40.26). Mammals are active and have a well-developed circulatory system with a four-chambered heart. The circulatory system distributes oxygen, nutrients, and heat. Mammals nourish their young with milk produced by the mother's **mammary glands** (fig. 40.27).

Although you are already familiar with the external anatomy of *Homo sapiens*, you will study the anatomy of a rat, another representative mammal, in later exercises. As you examine preserved and living mammals, search for common features such as hair distribution, body orientation, and structures for locomotion.

Figure 40.26

This tarsier (*Tarsius syrichia*) is a primitive primate (class Mammalia) found in the Philippines. It is the size of a rat, lives in a tree, and eats insects. The position of its eyes in the front of its head allows full stereoscopic vision. It has nails instead of claws, which indicates a common ancestry with higher primates. Its large eyes are efficient adaptations for nocturnal activity. The retinas lack cones for detecting color but are extra rich in rods for black/white sensitivity.

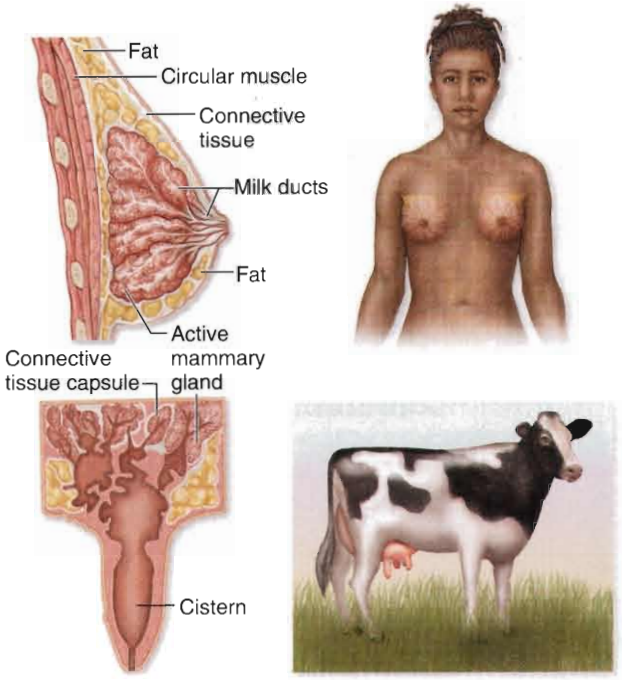


Figure 40.27

Mammary glands are specialized to secrete milk following the birth of young. (*a*) Many ducts lead from the glands to a nipple. Parts of the duct system are enlarged to store milk. Suckling by an infant initiates a hormonal response that causes the mammary glands to release milk. (*b*) Some mammals (e.g., cattle) have teats formed by the extension of a collar of skin around the opening of mammary ducts. Milk collects in a large cistern prior to its release.

Question 16

a. What factors govern the distribution of hair on species such as the human or rat?

b. How do the mammals that you are examining vary in body orientation (resting stance and position during movement)?

c. What charateristics of mammals help explain how they can occupy a variety of habitats?

INVESTIGATION

Adaptations of Vertebrate Skeletons

Observations: Natural selection shapes available genetic variation into adaptations that boost fitness. Over many generations, characteristics with no adaptive advantage for survival and reproduction may decrease in frequency, while those that confer reproductive advantages become increasingly frequent. External features interface an organism with its environment and are subject to strong selective pressures. External features serving multiple functions also vary a great deal among the various classes of vertebrates.

Question: To what extent are vertebrate external morphologies adapted for various functions?

a. Establish a working lab group and obtain Investigation Worksheet 40 from your instructor.
b. Obtain and examine the external features of a preserved fish, amphibian, bird, reptile, and mammal.
c. Discuss with your group and instructor specific comparisons to make among these vertebrates. Record them on Worksheet 40.
d. The table on Worksheet 40 lists four broad functions of an adaptation. Can you think of others? List at least two other functions in the worksheet table.
e. Complete the investigation directed by Worksheet 40.

Questions for Further Thought and Study

1. Does it surprise you that echinoderms are more closely related to our own phylum (Chordata) than are other phyla? Why would you have thought otherwise?

2. Why are embryological features important for distinguishing the major groups of phyla?

3. Echinoderms lack cephalization. What characteristics of this group deemphasize the need for a head?

4. What problems were associated with colonizing land during the evolution of vertebrates?

5. Why do you suppose four rather than five or six appendages is the rule for vertebrates?

6. A cuticle occurs on the surface of organisms of many phyla and appears to be an advantageous feature. Why have higher organisms not retained this structure?

7. What is the difference in the developmental derivation of mandibles among insects, jaws of vertebrates, and the beak of an octopus?

8. Although other groups of vertebrates are more numerous and have existed longer than mammals, mammals are often called the most advanced form of life. Why?

9. Locomotion in mammals is varied. Do you believe that their powers of locomotion are superior to those of birds? Why or why not?

10. Compare the origin and function of reptile scales, bird feathers, and mammal hair. How are they similar? How do they differ?

11. Prepare a simple table of all of the taxonomic groups, their common names, their distinguishing characteristics, and all representative genera covered in this exercise. Keep this table with your study notes.

12. Vertebrates have a closed circulatory system, meaning that the blood is always enclosed within vessels and does not fill body cavities. Mollusks (Exercise 38) and arthropods (Exercise 39) have open circulatory systems, meaning that blood is pumped by a heart into body cavities, where tissues are surrounded by the blood. What are the advantages and disadvantages of each type of circulatory system?

13. Two classes of vertebrates (Aves and Mammalia) are *endothermic*. What is meant by this term? Hypothesize some evolutionary advantages of being endothermic. What are some of the costs?

WRITING TO LEARN BIOLOGY

What external anatomical features of amphibians are associated with their dual life on land and in water?

Dissection of a Fetal Pig

I

Objectives

By the end of this exercise you should be able to:

1. Perform a whole-body dissection of a vertebrate animal.
2. Identify the major anatomical features of the vertebrate body in a dissected specimen.

Fetal pig anatomy provides an excellent model of general mammalian anatomy. Although there are some significant differences, the body plan is the same, and the functional relationships within and between the anatomical systems model that of humans as well as other commonly studied mammals.

MATERIALS NEEDED

- Preserved (plain or double-injected) fetal pig
- Dissection tools and trays
- Mounted fetal pig skeleton (optional)
- Storage container (if specimen is to be reused)

Read the directions and safety tips for this exercise carefully before starting any procedure. In a short course, a well-preserved specimen can be used from time to time throughout your studies. If you will be looking at your dissected specimen from time to time during the next several months, make sure that it is kept in the appropriate container under conditions suggested by your instructor.

THE EXTERNAL ANATOMY

Observe the usual precautions when working with a preserved or fresh specimen. Heed the safety advice accompanying preservatives used with your specimen. Use protective gloves when you handle your specimen. Avoid injury with dissection tools and dispose of your specimen as instructed.

Procedure A.1

Examine the external characteristics of your specimen

1. Determine the anatomical orientation of the specimen. Which direction is anterior? Posterior? Which direction is ventral? Dorsal? Identify sagittal, transverse, and frontal planes in your specimen.
2. Identify these externally visible features:
   pinna (auricle)
   external nares (nostrils)
   umbilicus (umbilical cord)
   forelimbs
   hind limbs
   thoracic region
   abdominal region
   nipples
   anus
   tail
3. Determine the sex of your specimen by examining the external genitals:
   Female—Immediately anterior to the anus, on the ventral surface, is the vulva with an opening to the vagina and the urethra. The vulva is also called the urogenital opening.
   Male—In the male fetal pig, there is a rather loose area of skin immediately posterior to the anus, perhaps even hiding the anus from view. Around the time of birth, each testis will descend from its position inside the body into the space under this skin. The skin will pouch out to form the outer wall of the scrotum. Just posterior to the umbilical cord is the distal end of the penis with its prepuce, or skin-fold covering. Locate the opening of the urethra in the penis.

SKIN, BONES, AND MUSCLES

Procedure A.2

Remove the skin from the specimen

1. Place the animal in a tray; make sure the animal's ventral surface is facing you.
2. Pull up on the skin over the neck and puncture it with the tip of a scissors. Slide the bottom tip of the scissors into the subcutaneous area under the skin.
3. Begin cutting along the lines indicated in figure A.1*a*. The posterior cuts are different for male and female specimens.
4. Be careful not to cut into the skeletal muscles under the skin or through the base of the umbilical cord.
5. With your forceps, pull the two flaps of skin over the neck away from the animal's body. Notice the areolar tissue under the skin that is pulled apart as you remove the skin. Sometimes it helps if you scrape at the loose connective tissue under the skin with your scalpel as you peel the skin away. The skinning process is difficult unless you have patience and proceed slowly. Pull the flaps of skin over the abdomen and over the groin area away in a similar fashion. If you have a male pig, leave the skin posterior to the umbilical cord in place for now.
6. Pin the flaps of skin to the floor of the dissection tray or cut them away from the body entirely. If you plan to study the musculature of the dorsum, you must similarly remove the skin from the animal's back.
7. Now examine the skin, identifying these features of the integument:
   - Dermis—the thick inner layer of the skin
   - Epidermis—the thinner outer layer of the skin
   - Hypodermis—the subcutaneous tissue under the skin proper, made of areolar and adipose tissue

Explore the shape of the skinned fetal pig body. How many bones of the fetal pig's skeleton can you see or feel? If you have a mounted fetal pig skeleton available, identify as many of the bones of the skeleton as you can. If you become stumped, refer to figure A.1*a*, *b*. Notice the similarity between the fetal pig's skeletal plan and that of a human. One difference easy to see is in the vertebral column: The pig has a different number of each type of vertebral bone compared with a human and has numerous caudal vertebrae instead of a single coccyx. Also, because the fetal pig's skeleton is just beginning its development, many of the bones are cartilaginous rather than bony.

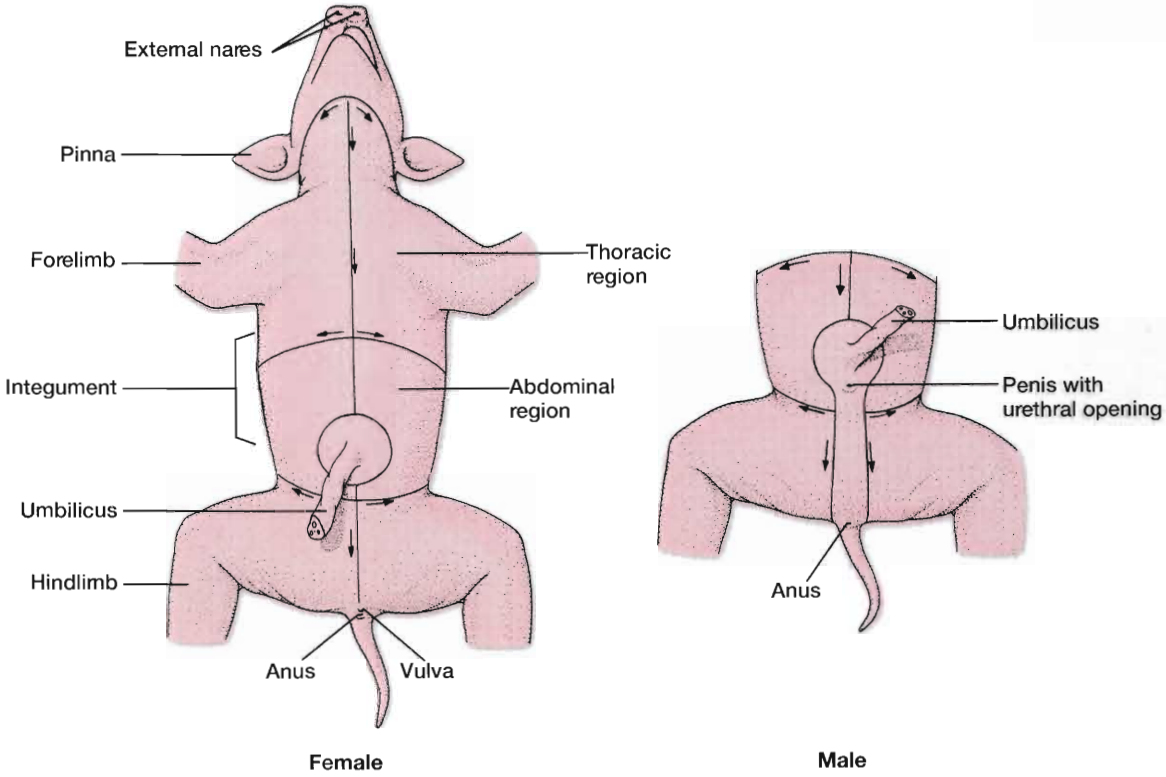
Observe the fetal pig's musculature. Some of the external muscles of the torso can be separated from each other for easier viewing. Slide a probe into the loose connective tissue joining adjacent muscles and run the probe along their margins. Using figure A.2 as a guide, try to identify the major skeletal muscles of the fetal pig's body. Because the animal was not yet active when the specimen was prepared, the muscles appear underdeveloped and make muscle identification difficult.

CARDIOVASCULAR STRUCTURES

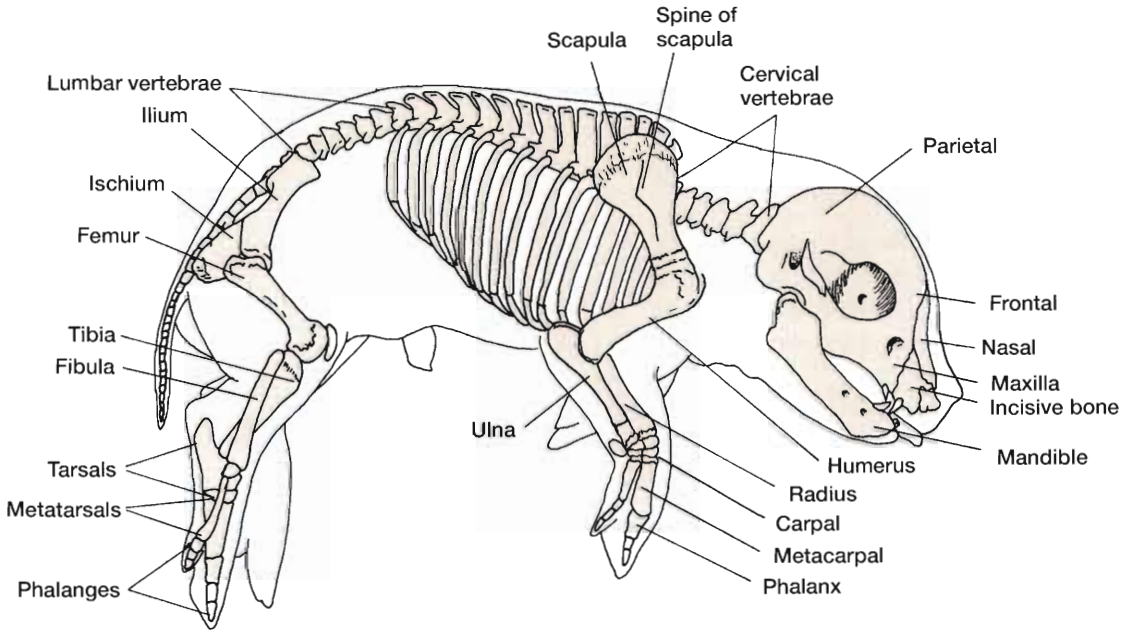
Procedure A.3

Examine cardiovascular structures

1. Cut flaps in the neck, abdomen, and groin areas as you did with the skin. Be careful not to damage any visceral organs with your scissors as you cut. Likewise, avoid injuring the internal structures associated with the umbilicus. Fold back the flaps and anchor them with pins or remove them.
2. Open the ventral body cavity by cutting into its muscular wall in a manner similar to your earlier cut into the skin over the abdomen.
3. Locate the heart near the middle of the thoracic cavity, in the mediastinum. Can you identify the four chambers? After identifying the major vessels (see steps 4 and 5), you may want to remove the heart and dissect it.
4. Locate the aorta, which is the large artery leaving the heart and arching posteriorly. If you have a double-injected preserved specimen, the systemic arteries are filled with red latex and the systemic veins are filled with blue latex. If not, the arteries usually can be distinguished from veins because they are stiffer and lighter in color than veins. Trace the major branches of the aorta, naming them if you can. Use figure A.3 if you need help.
5. Locate the anterior vena cava and note where it drains into the heart. It is sometimes called the precava and is analogous to the superior vena cava in a human. Follow its tributary veins and identify them with the help of figure A.3. Locate the posterior vena cava (postcava) and trace its tributaries. Once you have cut into the ventral body cavity, you may be tempted to cut and remove organs. It is important that you keep everything as intact as possible. You may pull organs to the side to view deeper structures, but avoid making cuts.



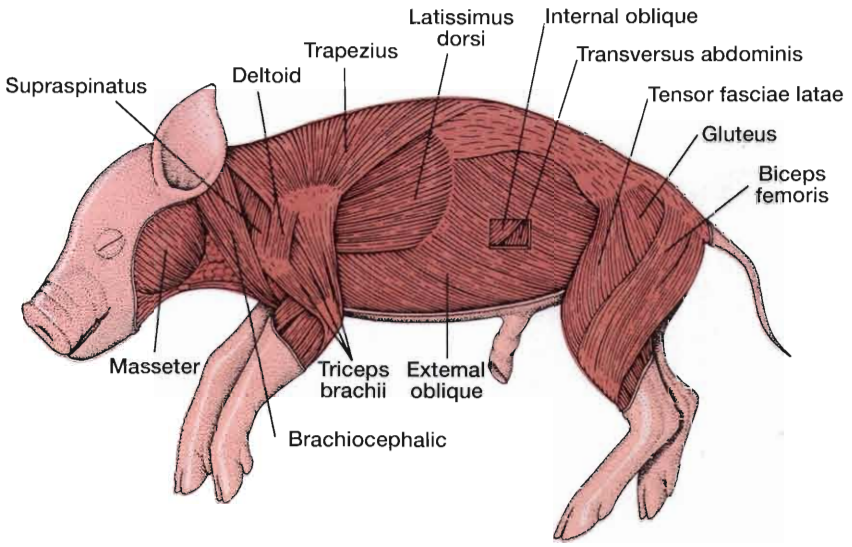
(a)



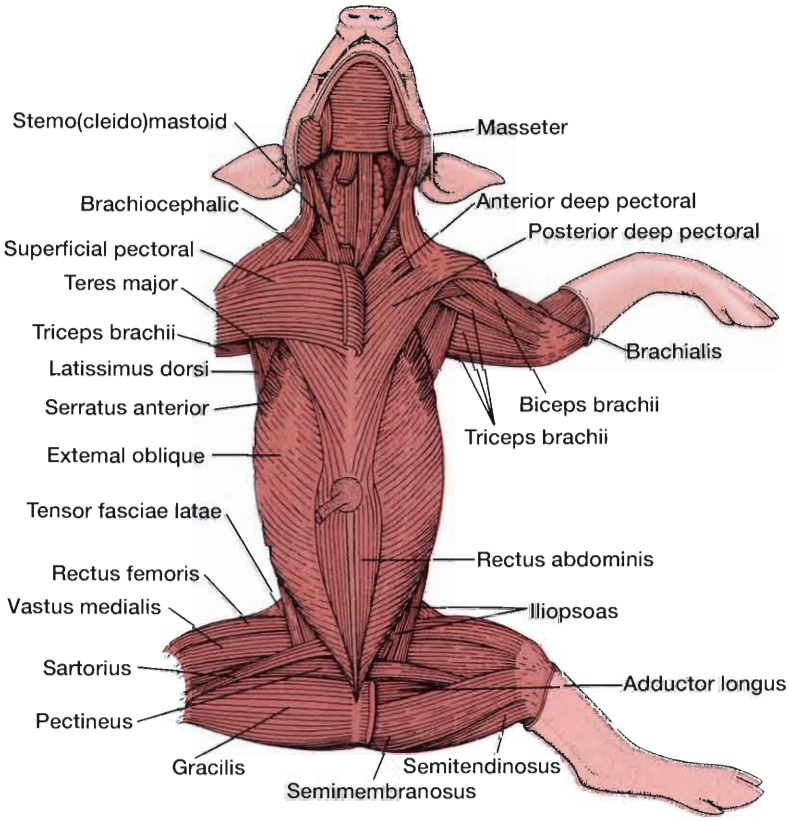
(b)

Figure A.1

(*a*) External aspect of a female (*above left*) and male (*above right*) pig. (*b*) Fetal pig skeleton.



(a)



(b)

Figure A.2

(*a*) Lateral view of fetal pig musculature. (*b*) Ventral view of fetal pig musculature.

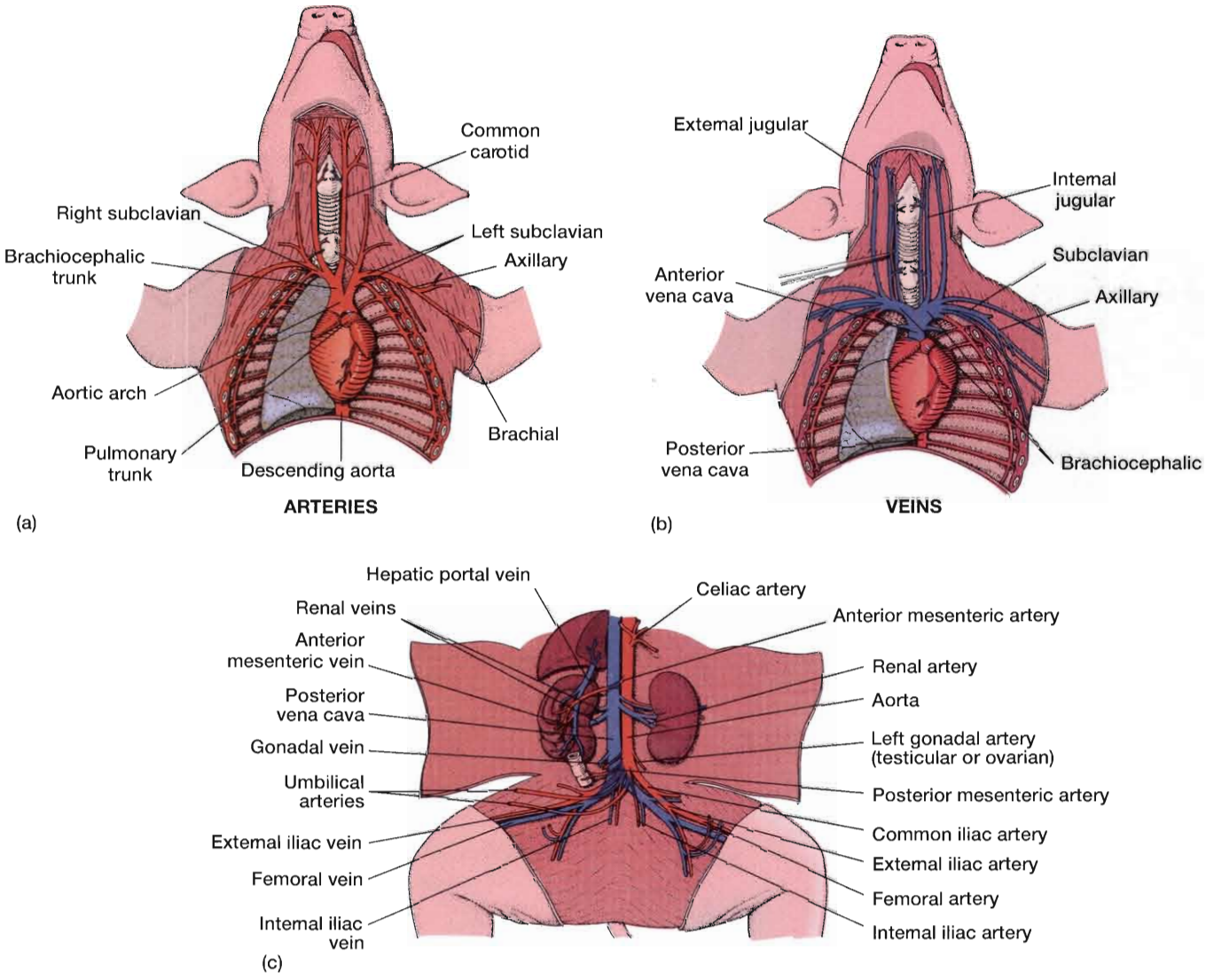


Figure A.3

(*a*) Arteries and (*b*) veins of the anterior body. (*c*) Major vessels of the posterior body.

THE VISCERA

The viscera, or major internal organs, can be seen within the ventral body cavity. Use figures A.4 and A.5 to guide you in locating the following:

1. Locate some of these features of the lower respiratory system:
   - larynx
   - trachea
   - primary bronchi
   - lungs (Can you distinguish the parietal and visceral pleurae?)
   - diaphragm
2. Locate these structures of the digestive system:
   - esophagus
   - stomach
   - liver (four separate lobes)
   - gallbladder
   - pancreas
   - small intestine
   - mesentery
   - large intestine (spiral colon)
3. Locate these lymphatic organs:
   - spleen
   - thymus
4. Locate these features of the urinary system:
   - kidney
   - renal cortex
   - renal pyramid
   - renal pelvis
   - renal calyx
   - ureter
   - urinary bladder
   - urethra
5. Try to locate these endocrine glands in your specimen:
   - thyroid gland
   - thymus gland
   - pancreas
   - adrenal glands
   - testes
   - ovaries
6. Identify these structures associated with the male reproductive system:
   - testes
   - epididymis
   - ductus deferens
   - seminal vesicle
   - penis
7. Find these female reproductive system structures:
   - ovaries
   - oviducts (fallopian tubes)
   - uterus (The fetal pig uterus has a Y shape, with right and left uterine horns.)
   - vagina
8. Carefully examine the umbilical cord, noting these structures:
   - umbilical vein
   - umbilical arteries
9. In the following space, sketch a cross section of the umbilical cord and label all identifiable structures. Unless your lab group has both a male and a female specimen, you may want to temporarily trade specimens with a group that has a pig of the opposite gender from yours. By doing so, you will be able to find the features of both reproductive systems.

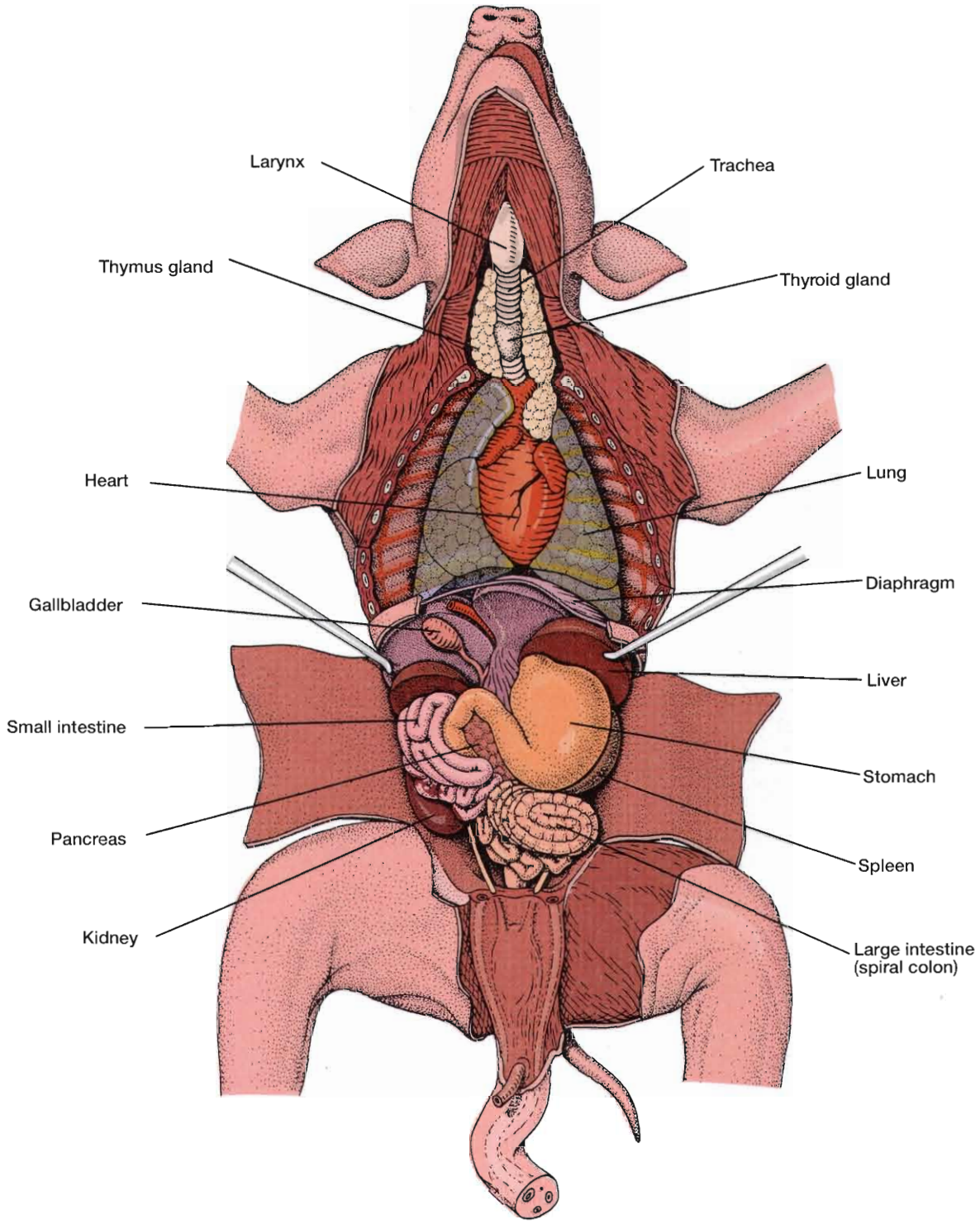


Figure A.4
Major visceral organs of a fetal pig.

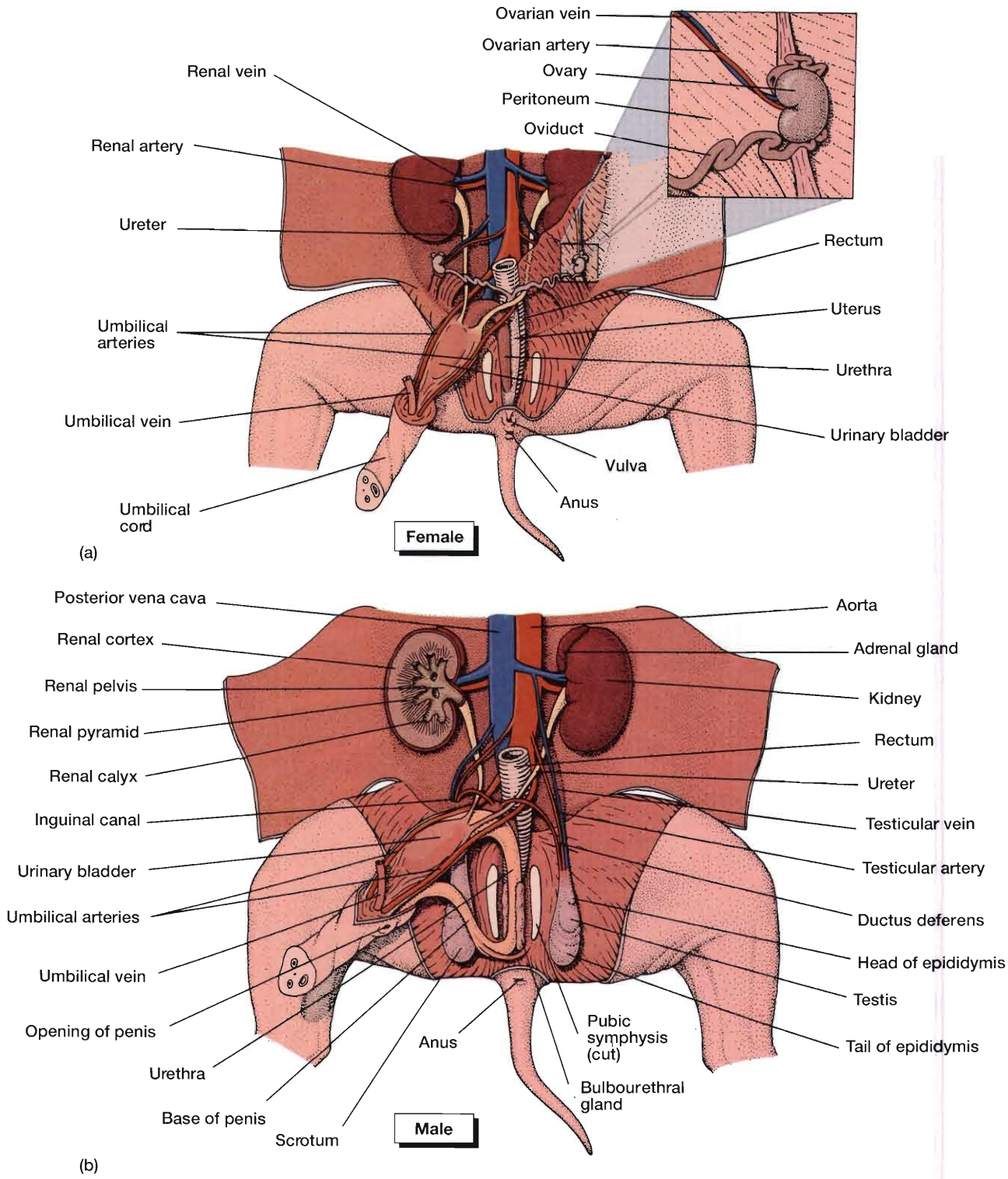


Figure A.5

Urinary and reproductive organs of (*a*) a female and (*b*) a male fetal pig.

II

Conversion of Metric Units to English Units

Units of Length

The meter (m) is the basic unit of length.

1 m = 39.4 inches (in)
= 1.1 yard (yd)
= 3.28 feet (ft)

1 km = 1000 m = 10^3 m
= 0.62 miles (mi)

1 cm = 0.01 m = 10^{-2} m
= 0.39 in = 10 mm

1 nm = 10^{-9} m = 10^{-6} mm
= 10 angstroms (Å)

1 in = 2.54 cm

1 ft = 30.5 cm
= .305 m

1 yd = 0.91 m

1 mi = 1.61 km

Units of area are squared (two-dimensional) units of length.

1 m^2 = 1.20 yd^2 = 1550 in^2 = 1.550 × 10^3 in^2

1 hectare = 10,000 square meters (m^2) = 2.47 acres

Measurements of area and volume can use the same units.

1 m^3 = 35.314 ft^3 = 1.31 yd^3

1 cm^3 (cc) = 0.000001 m^3 = 0.061 in^3

Units of Mass

The gram (g) is the basic unit of mass.

1 g = mass of 1 cm^3 of water at 4°C = 0.035 oz

1 kg = 1000 g = 10^3 g = 2.2 lb

Units of Volume

The liter (L) is the basic unit of volume. Units of volume are cubed (three-dimensional) units of length.

1 liter = 1000 cm^3

1 liter = 2.1 pints = 1.06 qt

1 liter = 0.26 gal = 1 dm^3

1 mL = 0.034 fl oz

1 cup = 240 mL

Units of Temperature

5 × degrees Fahrenheit = (9 × degrees Celsius) + 160

For example:

40°C = 104°F (a hot summer day)

75°C = 167°F (hot coffee)

−5°C = 23°F (coldest area of freezer)

37°C = 98.6°F (human body temperature)

Credits

LINE ART

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