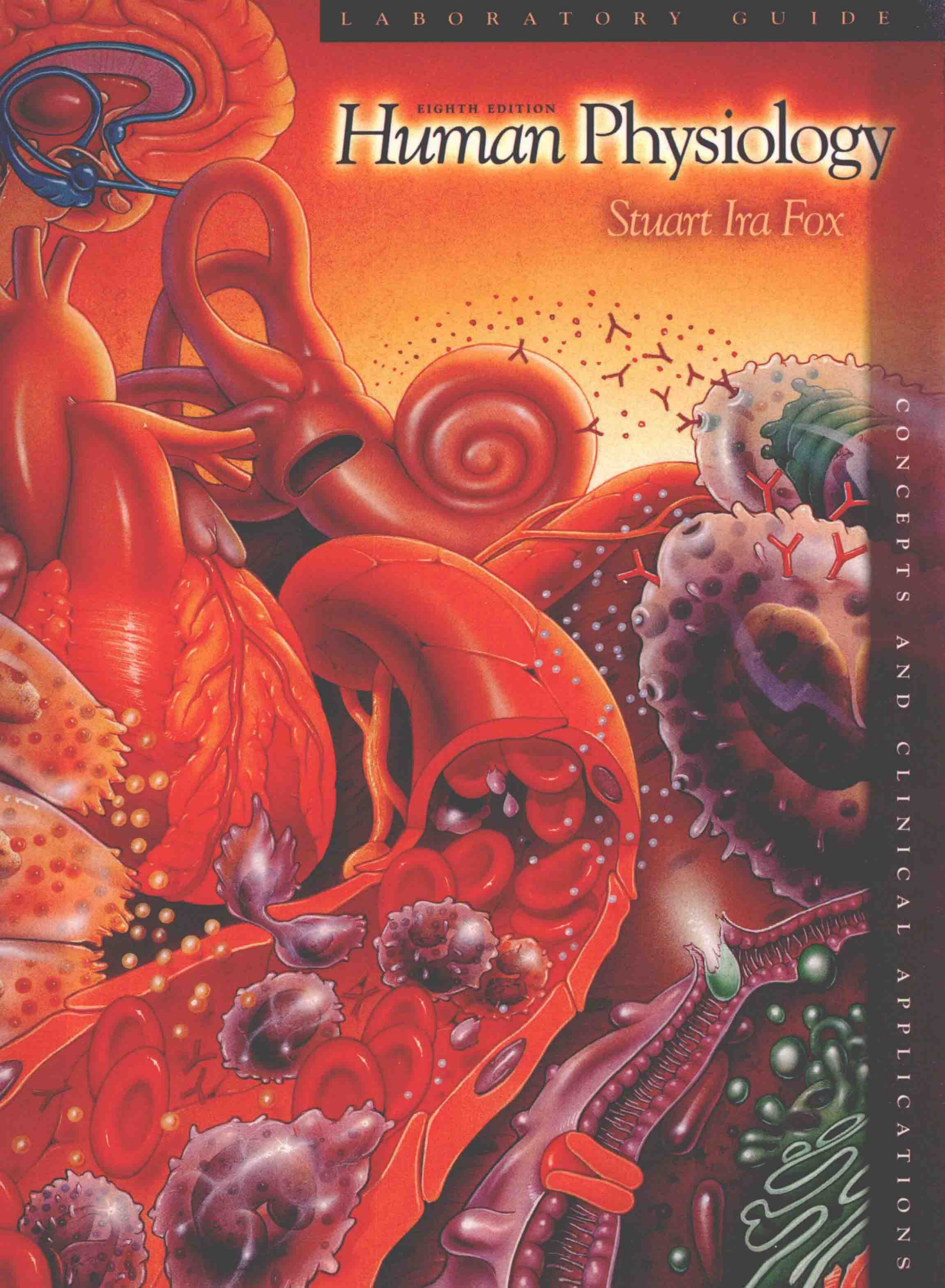
LABORATORY GUIDE

EIGHTH EDITION

Human Physiology

Stuart Ira Fox



CONCEPTS AND CLINICAL APPLICATIONS

LABORATORY GUIDE

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Stuart Ira Fox

PIERCE COLLEGE

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LABORATORY GUIDE HUMAN PHYSIOLOGY, EIGHTH EDITION


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1 2 3 4 5 6 7 8 9 0 QPD/QPD 9 3 2 1 0 9 8

ISBN 0-697-34218-2

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Typeface: *10/12 Goudy*
Printer: *Quebecor Printing Book Group/Dubuque, IA*

Cover/interior design: *Christopher E. Reese*
Cover illustration: *William B. Westwood*

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Preface

The eighth edition, like the previous editions, is a stand-alone human physiology manual that can be used in conjunction with any human physiology textbook. It includes a wide variety of exercises that support most areas covered in a human physiology course, allowing instructors the flexibility to choose those exercises best suited to meet their particular instructional goals. Background information that is needed to understand the principles and significance of each exercise is presented in a concise manner, so that little or no support is needed from the lecture text.

However, lecture and laboratory segments of a human physiology course are most effectively wedded when they cover topics in a similar manner and sequence. Thus, this laboratory guide is best used in conjunction with the textbook *Human Physiology*, sixth edition, by Stuart Ira Fox (WCB/McGraw-Hill, © 1999).

The laboratory experiences provided by this guide allow students to become familiar—in an intimate way that cannot be achieved by lecture and text alone—with many fundamental concepts of physiology. In addition to providing hands-on experience in applying physiological concepts, the laboratory sessions allow students to interact with the subject matter, with other students, and with the instructor in a personal, less formal way. Active participation is required to carry out the exercise procedures, collect data, and to complete the laboratory report. Critical thinking is necessary to answer all questions at the end of each exercise.

New to the Eighth Edition

Updated Information

The eighth edition is a thorough renovation of the seventh. Each exercise has been carefully refined and updated to keep pace with continual changes in laboratory technology, vendor supply sources, and to address updates in computer-assisted instruction and biohazard health concerns.

Clinically oriented laboratory exercises that heighten student interest and demonstrate the health applications of physiology have been a hallmark of previous editions and continue to be featured in this latest edition.

We are indebted to our colleagues and students for their suggestions and encouragement in the development of these exercises. Drawing on these recommendations, many of the laboratory procedures have been altered to accommodate both fluctuations in class size and laboratory time constraints. Some alterations were necessary since some of the sources of laboratory supplies and equipment have changed. New sources are indicated for some of the reagents, test strips, or kits required for certain exercises, reflecting changes made by the vendors.

Safety

Special effort has been made to address concerns about the safe use and disposal of body fluids. For example, normal and abnormal artificial serum can be used as a substitute for blood in Section 2 (plasma chemistry); artificial saliva is suggested in exercise 10.2 (digestion); and in Section 9 (renal function) both normal and abnormal artificial urine is now available. In the interest of safety, a substitute for the use of benzene (previously required in two exercises) is now provided.

The international symbol for caution is used throughout the laboratory guide to alert the reader when special attention is necessary while preparing for or performing a laboratory exercise. For reference, laboratory safety guidelines appear on the inside front cover.

Technology

Computer-assisted and computer-guided instruction in human physiology laboratories has greatly increased in recent years. Computer programs provide a number of benefits: some experiments that require animal sacrifice can be simulated; data can be analyzed against a data bank and displayed in an appealing and informative manner; class data records can be analyzed; and costs can be reduced by eliminating the use of some of the most expensive equipment.

This eighth edition continues to reference programs offered by Intelitool™, and new to this edition, A.D.A.M. Benjamin/Cummings *InterActive PHYSIOLOGY* Modules (800–755–2326; www.adam.com), and the ***Virtual Physiology Lab CD-ROM*** (ISBN 0–697–37994–9) by WCB/McGraw-Hill and Cypris Publishing.

Art Program

Almost every figure in this edition has been revised or improved, with a few deletions, and many new, exciting figures and tables added. These new figures enhance the

pedagogical value and add to the aesthetic appeal of the laboratory manual. Furthermore, the design was reworked, adding icons (such as the  balance icon for normal values), boxes, and shading to important concepts to enhance visual comprehension by students and to improve overall continuity.

Organization of the Laboratory Guide

The exercises in this guide are organized in the following manner:

1. Each exercise begins with a concise statement of the **concepts** illustrated by the exercise. This allows students to place the exercise in proper perspective.
2. **Learning objectives** are listed following the statement of concepts so that students can guide their learning while performing the exercise.
3.  **Materials** required for the laboratory exercise are listed next, before the exercise itself, to make setup easier. This section is now easily identifiable by the use of the materials icon.
4. A brief **introduction** to the exercise presents the essential information for understanding the physiological significance of the exercise. This concisely written section eliminates the need to consult the lecture text.
5. A boxed inset titled **"Clinical Significance"** emphasizes the practicality of the information presented.
6. The **procedure** is stated in the form of easy-to-follow steps. These directions are set off from the textual material through the use of a distinctive typeface, making it easier for students to locate them as they perform the exercise.
7. A **laboratory report** follows each exercise. Students enter data here when appropriate, and answer questions. The questions in the laboratory report begin with the most simple form (objective questions) in most exercises and progress to essay questions. The essay questions are designed to stimulate conceptual learning and to maximize the educational opportunity provided by the laboratory experience.

Supplemental Materials

Instructor's Manual for the Laboratory Guide to accompany Human Physiology, eighth edition, by Laurence G. Thouin, Jr. (ISBN 0–697–34221–2) provides a suggested correlation between the textbook and laboratory manual for *Human Physiology*, introductions, materials needed, approximate completion times, and solutions to the laboratory reports for each exercise, a listing of laboratory supply houses, and commonly used solutions.

Virtual Physiology Lab CD-ROM by WCB/McGraw-Hill and Cypris Publishing (ISBN 0–697–37994–9) features ten simulations of the most common and important animal-based experiments. The flexibility of this multimedia tool offers many pre-lab, actual lab, and post-lab options.

Laboratory Atlas of Anatomy and Physiology, second edition, by Douglas Eder et al. (ISBN 0–697–39480–8), is a full-color atlas including histology, skeletal and muscular anatomy, dissections, and reference tables.

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With ten simulations of the most common laboratory experiments, *Virtual Physiology Lab* lets you conduct lifelike research—without the animals or the lab.

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Each lab features: Objectives, Foundation, Experiment, Results, and Self-Testing.

Contents
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3 Frog Muscle
4 Effects of Drugs on the Frog Heart
5 Electrocardiogram
6 Pulmonary Function
7 Respiration and Exercise
8 Digestion of Fat
9 Diffusion, Osmosis, and Tonicity
10 Enzyme Characteristics

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To order a copy of the *Virtual Physiology Lab*, check your bookstore or call WCB/McGraw-Hill Customer Service at 1-800-338-3987.

Acknowledgments

The eighth edition owes its fresh perspective to my colleague Dr. Laurence G. Thouin, Jr. His numerous contributions include all of the organizational changes in this edition, the use of new icons, the updating of equipment and supply sources, and the substitution of updated illustrations. I am very grateful to him for undertaking this tremendously time-consuming task.

The shaping of the eighth edition was also aided by suggestions from other colleagues and students. Mr. Edmont Katz was particularly instrumental in checking laboratory sources for materials and reworking some of the procedures that are new to this edition. I greatly appreciate the support of the editors at WCB/McGraw-Hill,

Kelly Drapeau and Kristine Tibbetts; their contributions help to make this the best edition yet of the *Laboratory Guide to accompany Human Physiology*.

I wish to also express my gratitude to colleagues who formally reviewed the laboratory guide at different stages in its preparation. These reviewers are:

William Hamilton
Penn State University–New Kensington

Allan Scott Helgeson
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Contents

Introduction: Structure and Physiological Control Systems

Section 1

The cell is the basic unit of structure and function in the body. Each cell is surrounded by a *cell* (or *plasma*) *membrane* and contains specialized structures called *organelles* within the cell fluid, or *cytoplasm.* The structure and functions of a cell are largely determined by genetic information contained within the membrane-bound *nucleus.* This genetic information is coded by the specific chemical structure of *deoxyribonucleic acid (DNA)* molecules, the major component of *chromosomes.* Through genetic control of *ribonucleic acid (RNA)* and the synthesis of proteins (such as enzymes described in section 2), DNA within the cell nucleus directs the functions of the cell and, ultimately, those of the entire body.

Cells with similar specializations are grouped together to form **tissues,** and tissues are grouped together to form larger units of structure and function known as **organs.** Organs that are located in different parts of the body but that cooperate in the service of a common function are called **organ systems** (e.g., the cardiovascular system).

The complex activities of cells, tissues, organs, and systems are coordinated by a wide variety of regulatory mechanisms that act to maintain **homeostasis**—a state of dynamic constancy in the internal environment. **Physiology** is largely the study of the control mechanisms that participate in maintaining homeostasis.

Microscopic Examination of Cells

EXERCISE 1.1

The microscope and the metric system are important tools in the study of cells. Cells contain numerous organelles with specific functions and are capable of reproducing themselves by mitosis. However, there is also a special type of cell division called meiosis that is used in the gonads to produce sperm or ova.

OBJECTIVES

1. Identify the major parts of a microscope and demonstrate proper technique in the care and handling of this instrument.
2. Define and interconvert units of measure in the metric system; and estimate the size of microscopic objects.
3. Describe the general structure of a cell and the specific functions of the principal organelles.
4. Describe the processes of mitosis and meiosis and explain their significance.

Materials

1. Compound microscopes
2. Prepared microscope slides, including whitefish blastula (early embryo), clean slides, and cover slips (Note: Slides with dots, lines, or the letter *e* can be prepared with dry transfer patterns used in artwork.)
3. Lens paper
4. Methylene blue stain
5. Cotton-tipped applicator sticks

The **microscope** is the most basic and widely used instrument in the life science laboratory. The average binocular microscope for student use, as shown in figure 1.1, includes the following parts:

1. eyepieces each with an ocular lens (usually 10× magnification, and may have a pointer)
2. a stage platform with manual or mechanical stage controls
3. a substage condenser lens and iris diaphragm, each with controls
4. coarse focus and fine focus adjustment controls
5. objective lenses on a revolving nosepiece (usually include: a scanning lens, 4×; a low-power lens, 10×; and a high-power lens, 45×)

Care and Cleaning

The microscope is an expensive, delicate instrument. To maintain it in good condition, always take the following precautions:

1. Carry the microscope with two hands.
2. Use the *coarse* focus knob *only* with low power and always move the objective lens *away from the slide*, never toward the slide.
3. Clean the ocular and objective lenses with lens paper moistened with distilled water before and after use. (Use alcohol only if oil has been used with an oil-immersion, 100× lens.)
4. Always leave the lowest power objective lens (usually 4× or 10×) facing the stage before putting the microscope away.

A. The Inverted Image

Obtain a slide with the letter *e* mounted on it. Place the slide on the microscope stage, and rotate the nosepiece until the 10× objective clicks into the down position. Using the coarse adjustment, carefully lower the objective lens until it almost touches the slide. Now, looking through the ocular lens, slowly raise the objective lens until the letter *e* comes into focus.

PROCEDURE

1. If the visual field is dark, increase the light by adjusting the lever that opens (and closes) the iris diaphragm. If there is still not enough light, move the substage condenser lens closer to the slide by rotating its control knob. Bring the image into sharp focus using the fine focus control. Now, draw the letter *e* as it appears in the microscope.

_____

2. While looking through the ocular lens, rotate the mechanical stage controls so that the mechanical stage moves to the *right*. In which direction does the *e* move?

_____

3. While looking through the ocular lens, rotate the mechanical stage controls so that the mechanical stage moves *toward* you. In which direction does the *e* move?

_____

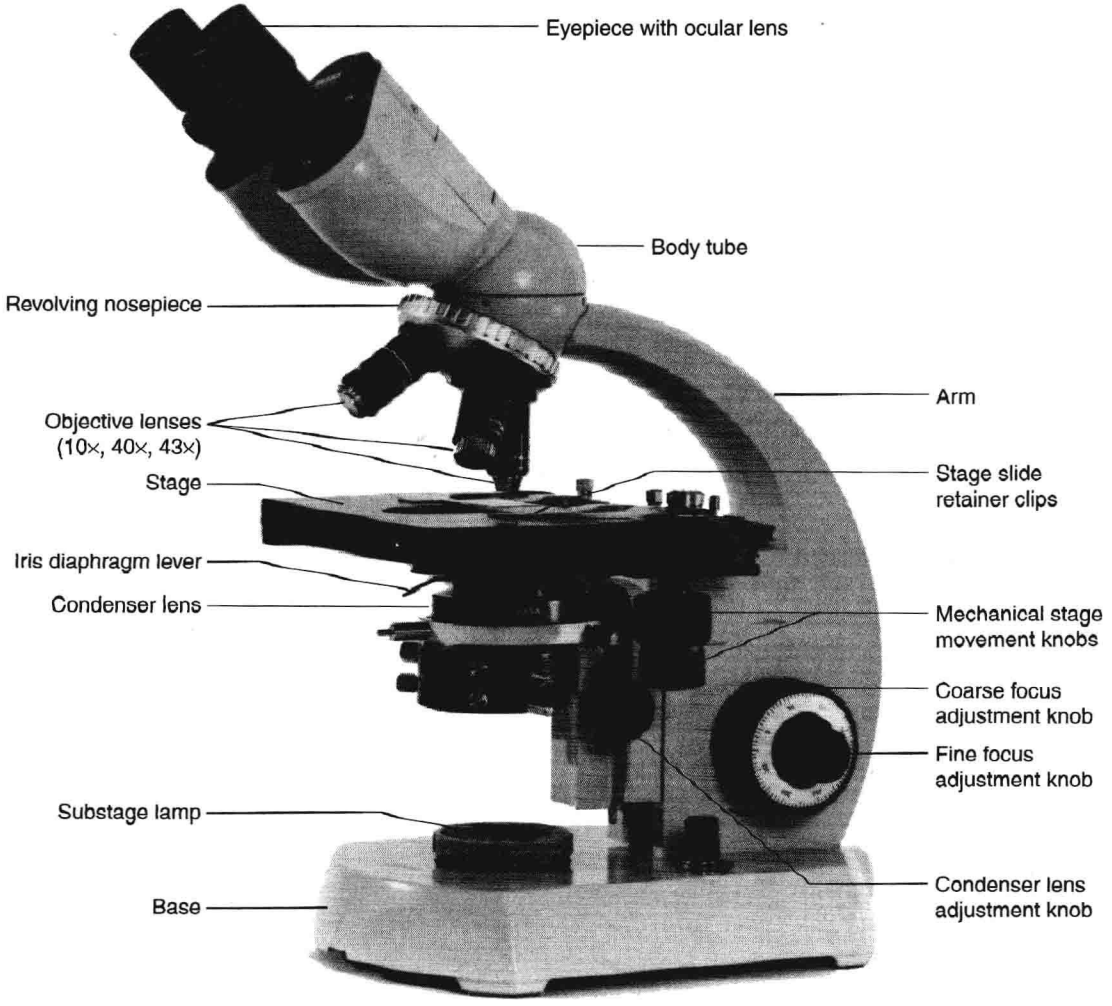


Figure 1.1 The parts of a compound microscope.

B. The Metric System: Estimating the Size of Microscopic Objects

It is important in microscopy, as in other fields of science, that units of measure are standardized and easy to use. The **metric system** (from the Greek word *metrikos*, meaning "measure") first developed in late eighteenth-century France, is the most commonly used measurement system in scientific literature. The modern definitions of the units used in the metric system are those adopted by the General Conference on Weights and Measures, which in 1960 established the International System of Units, also known (in French) as Système International d'Unités, and abbreviated SI (in all languages). The definitions for the metric units of *length*, *mass*, *volume*, and *temperature* are as follows:

meter (m)—unit of length equal to 1,650,763.73 wavelengths in a vacuum of the orange-red line of the spectrum of krypton-86

gram (g)—unit of mass based on the mass of 1 cubic centimeter (cm^3) of water at the temperature (4°C) of its maximum density

liter (L)—unit of volume equal to 1 cubic decimeter (dm^3) or 0.001 cubic meter (m^3)

Celsius (C)—temperature scale in which 0° is the freezing point of water and 100° is the boiling point of water; this is equivalent to the centigrade scale

Conversions between different orders of magnitude in the metric system are based on powers of ten (table 1.1). Therefore, you can convert from one order of magnitude to another simply by moving the decimal point the correct number of places to the right (for multiplying by whole numbers) or to the left (for multiplying by decimal fractions). Sample conversions are illustrated in table 1.2.

Dimensional Analysis

If you are unsure about the proper factor for making a metric conversion, you can use a technique called *dimensional analysis*. This technique is based on two principles:

1. Multiplying a number by 1 does not change the value of that number.
2. A number divided by itself is equal to 1.

Table 1.1 International System of Metric Units, Prefixes, and Symbols

Multiplication Factor	Prefix	Symbol	Term
$1{,}000{,}000 = 10^6$	Mega	M	One million
$1{,}000 = 10^3$	Kilo	k	One thousand
$100 = 10^2$	Hecto	h	One hundred
$10 = 10^1$	Deka	da	Ten
$1 = 10^0$			
$0.1 = 10^{-1}$	Deci	d	One-tenth
$0.01 = 10^{-2}$	Centi	c	One-hundredth
$0.001 = 10^{-3}$	Milli	m	One-thousandth
$0.000001 = 10^{-6}$	Micro	μ	One-millionth
$0.000000001 = 10^{-9}$	Nano	n	One-billionth
$0.000000000001 = 10^{-12}$	Pico	p	One-trillionth
$0.000000000000001 = 10^{-15}$	Femto	f	One-quadrillionth

Table 1.2 Sample Metric Conversions

To Convert From	To	Factor	Move Decimal Point
Meter (Liter, gram)	Milli-	× 1,000 (10^3)	3 places to right
Meter (Liter, gram)	Micro-	× 1,000,000 (10^6)	6 places to right
Milli-	Meter (Liter, gram)	÷ 1,000 (10^{-3})	3 places to left
Micro-	Meter (Liter, gram)	÷ 1,000,000 (10^{-6})	6 places to left
Milli-	Micro-	× 1,000 (10^3)	3 places to right
Micro-	Milli-	÷ 1,000 (10^{-3})	3 places to left

These principles can be used to change the units of any measurement.

Example

Since 1 meter (m) is equivalent to 1,000 millimeters (mm),

$$\frac{1\text{ m}}{1{,}000\text{ mm}} = 1 \text{ and } \frac{1{,}000\text{ mm}}{1\text{ m}} = 1$$

Suppose you want to convert 0.032 meter to millimeters:

$$0.032\text{ m} \times \frac{1{,}000\text{ mm}}{1\text{ m}} = 32.0\text{ mm}$$

Notice that in dimensional analysis the problem is set up so that the unwanted units (meter, *m* in this example) cancel each other. This technique is particularly useful when the conversion is more complex or when some of the conversion factors are unknown.

Example

Suppose you want to convert 0.1 milliliter (mL) to microliter (μL) units. If you remember that 1 mL = 1,000 μL, you can set up the problem as follows:

$$0.1\text{ mL} \times \frac{1{,}000\ \mu\text{L}}{1\text{ mL}} = 100\ \mu\text{L}$$

If you remember that a milliliter is one-thousandth of a liter and that a microliter is one-millionth of a liter, you can set up the problem in this way:

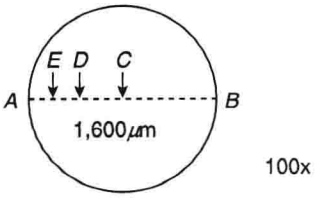
$$0.1\text{ mL} \times \frac{1.0\text{ L}}{1{,}000\text{ mL}} \times \frac{1{,}000{,}000\ \mu\text{L}}{1.0\text{ L}} = 100\ \mu\text{L}$$

Visual Field and the Estimation of Microscopic Size

If the magnification power of your ocular lens is 10× and you use the 10× objective lens, the total magnification of the visual field will be 100×. At this magnification, the diameter of the visual field is approximately 1,600 micrometers (μm).

You can estimate the size of an object in the visual field by comparing it with the total diameter (line *AB*) of the visual field. Using the diagram below:

How long is line *AC* in micrometers (μm)? _____
How long is line *AD* in micrometers (μm)? _____
How long is line *AE* in micrometers (μm)? _____



The diameter of the field of vision using the 45× objective lens (total magnification 450×) is approximately 356 micrometers. Using the diagram above and applying the same technique, answer the following questions assuming use of a 45× objective lens:

How long is line AC in micrometers (μm)? _____
How long is line AD in nanometers (nm)? _____

PROCEDURE

From your instructor, obtain a slide that contains a pattern of small dots and a pattern of thin lines.

1. Using the 10× objective lens:
   (a) estimate the diameter of one dot:____m
   (b) estimate the distance between the *nearest* edges of two adjacent dots:____m
2. Using the 45× objective lens:
   (a) estimate the width of one line:____m
   (b) estimate the distance between the *nearest* edges of two adjacent lines:____m

C. Microscopic Examination of Cheek Cells

The surfaces of the body are covered and lined with *epithelial* membranes (one of the primary tissues described in exercise 1.2). In membranes that are several cell layers thick, such as the membrane lining of the cheeks, cells are continuously lost from the surface and replaced through cell division in deeper layers. In contrast to cells in the outer layer of the epidermis of the skin, which die before they are lost, the cells in the outer layer of epithelial tissue in the cheeks are still alive. You can therefore easily collect and observe living human cells by simply rubbing the inside of the cheeks.

Most living cells are difficult to observe under the microscope unless they are stained. In this exercise, the stain *methylene blue* will be used. Methylene blue is positively charged and combines with negative charges in the chromosomes to stain the nucleus blue. The cytoplasm contains a lower concentration of negatively charged organic molecules, and so appears almost clear.

PROCEDURE

1. Rub the inside of one cheek with the cotton tip of an applicator stick.
2. Press the cotton tip of the applicator stick against a clean glass slide. Maintaining pressure, rotate the cotton tip against the slide and then push the cheek smear across the slide about 1/2 inch.
3. Observe the *unstained* cells under 100× and 450× total magnification.
4. Remove the slide from the microscope. Holding it over a sink or special receptacle, place a drop of methylene blue stain on the smear.
5. Place a cover slip over the stained smear and again observe the *stained* cheek cells at 100× and 450× total magnification.
6. Using the procedure described in the previous section, estimate the size of the average cheek cell using both 100× and 450× total magnification.
   100× _____ μm; 450× _____ μm
   Are they the same?

D. Cell Structure and Cell Division

Cells vary greatly in size and shape. The largest cell, an *ovum* (egg cell), can barely be seen with the unaided eye; other cells can be observed only through a microscope. Each cell has an outer *plasma membrane* (or cell membrane) and generally one *nucleus*, surrounded by a fluid matrix, or *cytoplasm*. Within the nucleus and the cytoplasm are a variety of subcellular structures, called **organelles** (fig. 1.2). The structures and principal functions of important organelles and other cellular components are listed in table 1.3.

The process of cell division, or replication, is called **mitosis** (fig. 1.3). This process allows new cells to be formed to replace those that are dying and also permits body growth. Mitosis consists of a continuous sequence of four stages (table 1.4 and fig. 1.3) in which both the nucleus and cytoplasm of a cell split to form two **identical** *daughter cells*. During mitotic cell division, the chromosomes (which had been duplicated earlier) separate, and one of the duplicate sets of chromosomes goes to each daughter cell. The two daughter cells therefore have the *same* number of chromosomes as the parent cell.

The forty-six chromosomes present in most human cells actually represent twenty-three *pairs* of chromosomes; one set of twenty-three was inherited from the mother and the other set of twenty-three from the father. A cell with forty-six chromosomes is said to be *diploid*, or *2n*.

In the process of *gamete* (sperm and ova) production in the *gonads* (testes and ovaries), specialized germinal cells undergo a type of division called **meiosis** (fig. 1.3). During meiosis, each germinal cell divides twice, and the daughter cells (the gametes) get only one set of twenty-three chromosomes; they are said to be *haploid*, or *1n*. In this way the original diploid number of forty-six chromosomes can be restored when the sperm and egg unite in the process of fertilization.

PROCEDURE

1. Study figure 1.2. Cover the labels with a blank sheet of paper and try to write them in (watch spelling!).
2. Examine a slide of a whitefish blastula (or similar early embryo) and observe the different stages of mitosis as shown in figure 1.3.

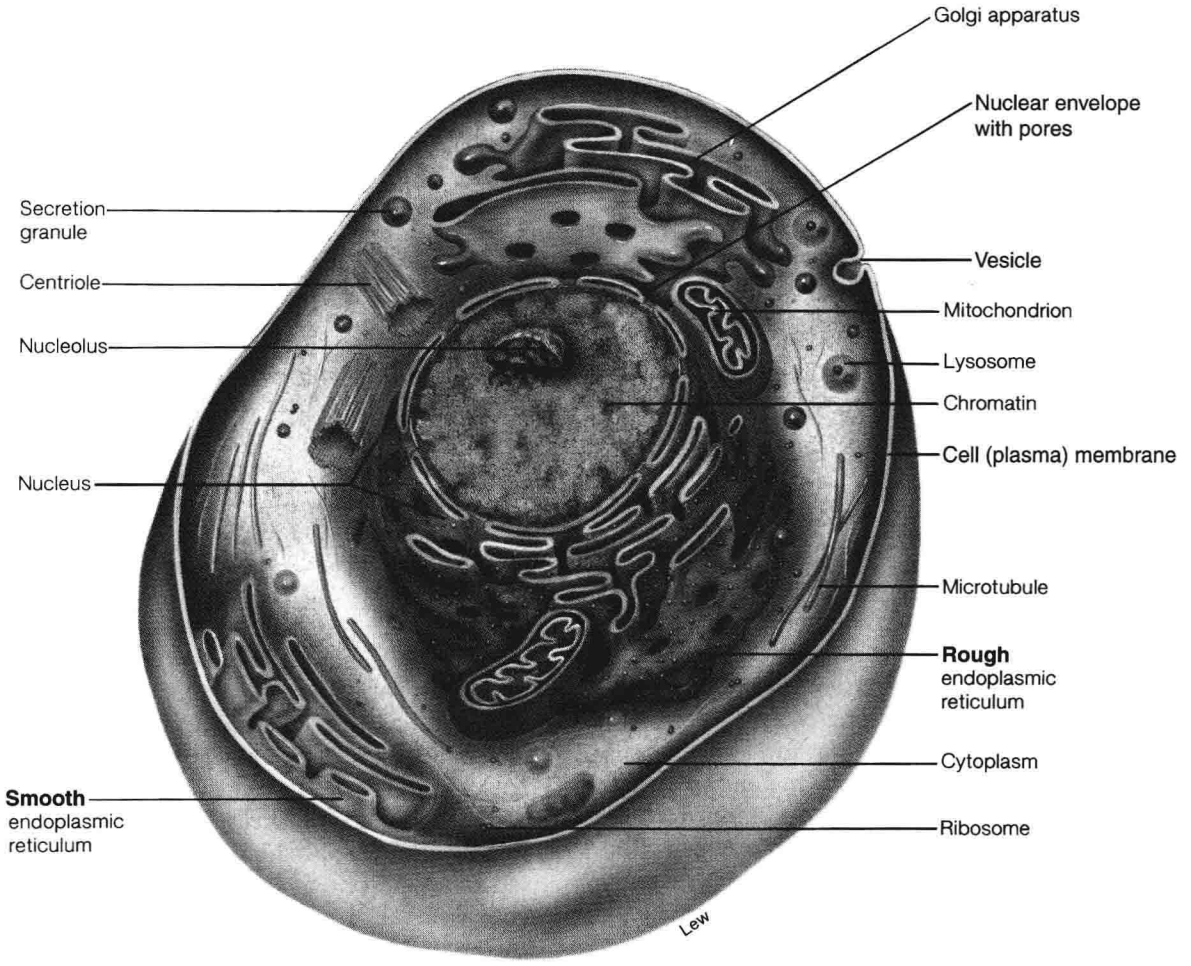


Figure 1.2 **Generalized cell.** Most cells have the principal organelles shown here.

Table 1.3 Structure and Function of Cellular Components

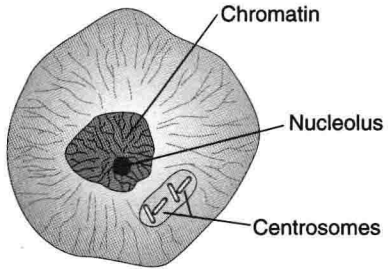
Component	Structure	Function
Cell (plasma) membrane	Membrane composed of phospholipid and protein molecules	Gives form to cell and controls passage of materials in and out of cell
Cytoplasm	Fluid, jellylike substance between the cell membrane and the nucleus in which organelles are suspended	Serves as matrix substance in which chemical reactions occur
Endoplasmic reticulum	System of interconnected membrane-forming canals with (rough) or without (smooth) attached ribosomes	Smooth endoplasmic reticulum metabolizes nonpolar compounds and stores Ca^{++} in straited muscle cells; rough endoplasmic reticulum assists in protein synthesis
Ribosomes	Granular particles composed of protein and RNA	Synthesize proteins
Golgi apparatus	Cluster of flattened, membranous sacs	Synthesizes carbohydrates and packages protein and lipid molecules for secretion
Mitochondria	Double-walled membranous sacs with folded inner partitions	Release energy from food molecules and transform energy into usable ATP
Lysosomes	Single-walled membranous sacs	Digest foreign molecules and worn and damaged cells
Peroxisomes	Spherical membranous vesicles	Contain enzymes that produce hydrogen peroxide and use this for various oxidation reactions
Centrosome	Nonmembranous mass of two rodlike centrioles	Helps organize spindle fibers and distribute chromosomes during mitosis
Vacuoles	Membranous sacs	Store and excrete various cytoplasmic substances
Fibrils and microtubules	Thin, rodlike, or hollow tubes of varying lengths	Support cytoplasm and transport materials within the cytoplasm (e.g., cytoskeleton)
Cilia and flagella	Small cytoplasmic projections containing microtubules	Move particles along surface of cell and enable sperm to migrate
Nuclear membrane	Porous, double membrane surrounding nucleus composed of protein and lipid molecules	Supports nucleus and controls passage of materials between nucleus and cytoplasm
Nucleolus	Dense, nonmembranous mass composed of protein and RNA molecules	Forms ribosomes
Chromatin	Fibrous strands composed of DNA molecules and protein	Controls cellular activity for carrying on life processes, such as protein synthesis

Table 1.4 Major Events in Mitosis

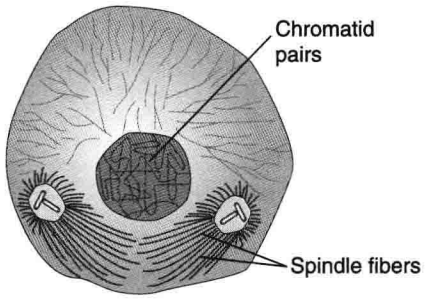
Stage	Major Events
Prophase	Chromosomes form from the chromatin material, centrioles migrate to opposite sides of the nucleus, the nucleolus and nuclear membrane disappear, and spindles appear and become associated with centrioles and centromeres.
Metaphase	Duplicated chromosomes align themselves on the equatorial plane of the cell between the centrioles, and spindle fibers become attached to duplicate parts of chromosomes.
Anaphase	Duplicated chromosomes separate, and spindles shorten and pull individual chromosomes toward the centrioles.
Telophase	Chromosomes elongate and form chromatin threads, nucleoli and nuclear membranes appear for each chromosome mass, and spindles disappear.

(a) Mitosis

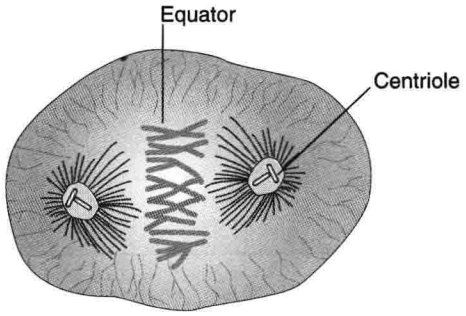
Interphase
- The chromosomes are in extended form and seen as chromatin in the electron microscope.
- The nucleus is visible.



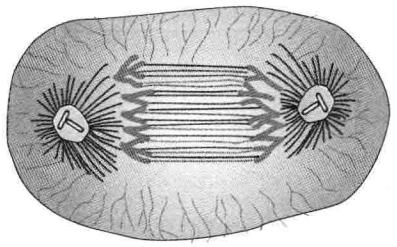
Prophase
- The chromosomes are seen to consist of two chromatids joined by a centromere.
- The centrioles move apart toward opposite poles of the cell.
- Spindle fibers are produced and extend from each centrosome.
- The nuclear membrane starts to disappear.
- The nucleolus is no longer visible.



Metaphase
- The chromosomes line up at the equator of the cell.
- The spindle fibers from each centriole are attached to the centromeres of the chromosomes.
- The nuclear membrane has disappeared.



Anaphase
- The centromeres split, and the sister chromatids separate as each is pulled to an opposite pole.



Telophase
- The chromosomes become longer, thinner, and less distinct.
- New nuclear membranes form.
- The nucleolus reappears.
- Cell division is nearly complete.

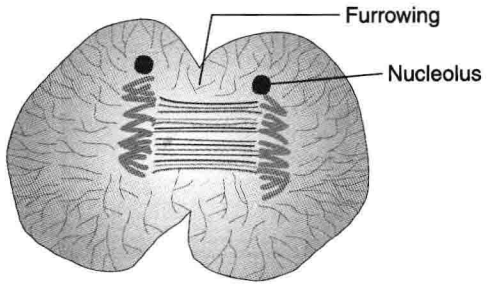


Figure 1.3 Cell division. (a) The stages of mitosis. (b) The stages of meiosis. Note that meiosis occurs only in the cells of the gonads that produce the gametes (sperm and ova).