

A LABORATORY GUIDE TO

Human Physiology

Concepts and Clinical Applications



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ELEVENTH EDITION

STUART IRA FOX

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ELEVENTH EDITION

Human Physiology

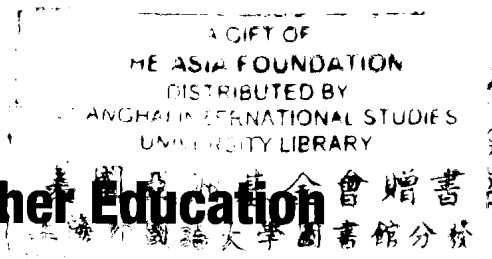
Concepts and Clinical Applications

Stuart Ira Fox

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A LABORATORY GUIDE TO HUMAN PHYSIOLOGY: CONCEPTS AND CLINICAL APPLICATIONS, ELEVENTH EDITION

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LABORATORY SAFETY GUIDELINES

Most of the reagents (chemicals) and equipment in a physiology laboratory are potentially dangerous. This circumstance will not detract from the enjoyment and efficacy of the laboratory learning experience providing all participants follow some commonsense rules of laboratory safety. Please read these laboratory safety guidelines carefully and practice them in the laboratory. In time, safe behavior will become routine.

1. Read all exercises **before** coming to the laboratory. Pay particular attention to the Materials section and note any chemicals, instruments, or equipment that might be hazardous if mishandled. Read all notes and cautions associated with the exercise. Disorganization and confusion in a laboratory can be dangerous. Proper preparation will increase your understanding, enjoyment, and safety during exercises.
2. With tremendous concern over the possibility of transferring viruses (such as AIDS and herpes), bacteria, or other pathogenic organisms from one person to another, it is strongly recommended that **each student handle only his or her own bodily fluids**. This warning is repeated in the appropriate exercises and is extended to include the cleanup of all spills and the proper disposal of all contaminated items in containers provided by the instructor. Some fluids, such as blood, can be purchased prescreened and "pathogen-free" from commercial life science laboratories.
3. Assume that all reagents are poisonous and act accordingly. **Do not** ingest any reagents; eat, drink, or smoke in the laboratory; carry reagent bottles around the room; or pipette anything by mouth unless specifically told to do so by your instructor. **Do** wash your hands thoroughly before leaving the laboratory; stopper all reagent bottles when they are not in use; thoroughly clean up spills; wash reagents

off yourself and your clothing; and, if you accidentally get any reagent in your mouth, immediately rinse your mouth thoroughly and inform the instructor.

4. Follow the procedures precisely as stated, or as modified by the instructor. **Do not** improvise unless the instructor specifically approves the change.
5. Clean glassware at the end of each exercise so that residue from one exercise does not carry over to the next exercise.
6. Keep your work area clean, neat, and organized. This will reduce the possibilities of error and help make your work safer and more accurate.
7. **Do not** operate any equipment until you are instructed in its proper use. If you are unsure of the procedures, ask the instructor.
8. Be careful about open flames in the laboratory. **Do not** leave a flame unattended; **do not** light a Bunsen burner near any gas tank or cylinder; and **do not** move a lit Bunsen burner around on the desk. Make sure that long hair or loose clothing is well out of the way of the flame.
9. Always make sure that gas jets are off when you are not operating the Bunsen burner.
10. Handle hot glassware with a test-tube clamp or tongs.
11. Note the location of an emergency first-aid kit, eyewash bottle, and fire extinguisher in the room. Report all accidents to the instructor immediately.
12. Wear safety glasses during those exercises in which glassware and solutions are heated with a Bunsen burner.

Remember, your safe behavior in the laboratory will serve as a model for others. It will also help you to experience the thrill of laboratory experimentation in a responsible manner and to take pride in your successful results.

Table 1 SI Unit Prefixes and Symbols

Prefix	Refers to Factor of	Symbol
Mega-	10^6 (one million)	M
Kilo-	10^3 (one thousand)	k
Hecto-	10^2 (one hundred)	h
Deka-	10^1 (ten)	da
Deci-	10^{-1} (one-tenth)	d
Centi-	10^{-2} (one-hundredth)	c
Milli-	10^{-3} (one-thousandth)	m
Micro-	10^{-6} (one-millionth)	μ
Nano-	10^{-9} (one-billionth)	n
Pico-	10^{-12} (one-trillionth)	p

Table 2 Greek Letters (Uppercase, Lowercase)

Greek Letter	Name	Greek Letter	Name
A, α	Alpha	N, ν	Nu
B, β	Beta	Ξ, ξ	Xi
Γ, γ	Gamma	O, \omicron	Omicron
Δ, δ	Delta	Π, π	Pi
E, ϵ	Epsilon	P, ρ	Rho
Z, ζ	Zeta	Σ, σ	Sigma
H, η	Eta	T, τ	Tau
Θ, θ	Theta	Y, υ	Upsilon
I, ι	Iota	Φ, ϕ	Phi
K, κ	Kappa	X, χ	Chi
Λ, λ	Lambda	Ψ, ψ	Psi
M, μ	Mu	Ω, ω	Omega

Table 3 Normal Values for Cardiac Function and Blood Gas Measurements

Measurement	Value
Ejection fraction (SV/EDV)*	0.55–0.78
End-diastolic volume	$75 \pm \text{mL/m}^2$ of body surface area
Cardiac output	$2,500\text{--}3,600 \text{ mL/min./m}^2$ of body surface area
Percent oxygen saturation	97% (artery); 60–85% (vein)
Arterial pH	7.38–7.44
Oxygen tension (P_{O_2})	80–100 mm Hg
Carbon dioxide tension (P_{CO_2})	35–45 mm Hg
Bicarbonate concentration	21–30 mEq/L

* SV = stroke volume; EDV = end-diastolic volume

Table 4 Normal Values for Renal Function Tests and Urine Constituents

Measurement	Value
<i>Renal Function Tests</i>	
Inulin clearance (GFR), males	$124 \pm 25.8 \text{ mL/min.}$
Inulin clearance (GFR), females	$119 \pm 12.8 \text{ mL/min.}$
Creatinine clearance	91–130 mL/min.
Urea clearance	60–100 mL/min.
<i>Urine Constituents</i>	
Specific gravity	1.002–1.028
Protein	under 150 mg/L
Potassium	25–100 mEq/L (varies)
Sodium	100–260 mEq/L (varies)
pH	5–7.5

Table 5 Normal Values for Erythrocyte and Leukocyte Measurements

Measurement	Value
Hemoglobin	13–18 g/dL (males); 12–16 g/dL (females)
Hematocrit	42–52% (males); 37–48% (females)
Erythrocyte count	$4.5\text{--}6.0 \times 10^6/\text{mm}^3$ (males); $4.0\text{--}5.5 \times 10^6/\text{mm}^3$ (females)
Leukocyte count	$5 \times 10^3\text{--}10 \times 10^3/\text{mm}^3$
<i>Differential Leukocyte Count</i>	
Neutrophils	55–75%
Eosinophils	2–4%
Basophils	0.5–1%
Lymphocytes	20–40%
Monocytes	3–8%

Table 6 Normal Values for Some Constituents of Blood Plasma

Measurement	Value
Cholesterol, bound to LDL	under 130 mg/dL
Cholesterol, total	under 200 mg/dL
Creatinine	under 1.5 mg/dL
<i>Enzymes</i>	
Amylase, serum	60–180 U/L
Creatine phosphokinase, serum	10–70 U/L (females); 25–90 U/L (males)
Lactate dehydrogenase, serum	25–100 units/L
Glucose, fasting	75–115 mg/dL
<i>Hormones</i>	
Aldosterone	under 8 ng/dL
Cortisol (8 a.m.)	5–25 $\mu\text{g/dL}$
Epinephrine	under 50 $\mu\text{g/dl}$
Estradiol, in women	20–60 pg/mL
Testosterone, in men	3–10 ng/mL
Insulin, fasting	6–26 $\mu\text{U/mL}$
Thyroxine	5–12 $\mu\text{g/dL}$
Osmolality, plasma	285–295 mOsm
Protein, total serum	5.5–8 g/dL
Triglycerides	under 160 mg/dL
Urea nitrogen	10–20 mg/dL

Table 7 Normal Values for Pulmonary Function Tests

Measurement	Value
Vital capacity	4–5 L (men); 3–4 L (women)
Inspiratory capacity	2–4 L
Expiratory reserve volume	1–2 L
Residual volume	1–2 L
Functional residual capacity	2–3 L
Total lung capacity	6–7 L (men); 5–6 L (women)
Forced expiratory volume, 1 second ($FEV_{1.0}$)	over 3 L (men); over 2 L (women)
$FEV_{1.0}$ as a percent of vital capacity	over 60% (men); over 70% (women)
Arterial oxygen tension (Pa_{O_2})	$95 \pm 5 \text{ mmHg}$
Arterial carbon dioxide tension (Pa_{CO_2})	$40 \pm 2 \text{ mmHg}$
Arterial blood pH	7.40 ± 0.02

Preface

The eleventh edition, like the previous editions, is a standalone 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 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*, ninth edition, by Stuart Ira Fox (McGraw-Hill, © 2006).

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.

The questions in the laboratory reports, like those at the end of each chapter in the textbook *Human Physiology*, by Stuart Ira Fox, are organized into three levels. These are (1) *Test Your Knowledge of Terms and Facts*, (2) *Test Your Understanding of Concepts*, and (3) *Test Your Ability to Analyze and Apply Your Knowledge*. This organization promotes higher-order learning and understanding in the laboratory and helps to better integrate the laboratory with information learned in the lecture portion of the physiology course.

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. Change is required, however, because vendors change and available laboratory equipment and supplies change. This eleventh edition accommodates such changes and makes new advances in improving the ability of students to benefit from the physiology laboratory experience.

THE ELEVENTH EDITION

INTEGRATION OF THE LABORATORY GUIDE WITH THE TEXTBOOK

This laboratory guide contains all of the information students need to understand and perform the laboratory exercises. It is thus a self-contained, stand-alone laboratory guide. This benefits students because they don't have to (1) bring the larger and heavier textbook to the laboratory section and (2) sift through the textbook to find the information particularly relevant to the laboratory exercise.

However, students benefit when the laboratory is well integrated with the lecture portion of the physiology course. To facilitate the interaction between lecture and laboratory, this guide uses two devices to allow students to cross-reference the material in the laboratory to the information in the lecture textbook, *Human Physiology*, ninth edition, by Stuart Ira Fox:

1. **Textbook Correlations** boxes are found at the beginning of each laboratory exercise. These provide specific page numbers in the textbook that correspond to the laboratory exercise. Students don't need this information to answer the questions in their laboratory report, but will benefit from greater depth and wider perspective when their textbook is used in conjunction with the laboratory guide.
2. **Figure Cross-References** between the laboratory guide and the textbook are updated in the eleventh edition. Whenever a figure in this laboratory guide has a full-color counterpart in the textbook, the specific number of the full-color text figure is provided in the caption of the laboratory manual figure. This allows students to better integrate the laboratory exercise with the concepts discussed in the textbook and lecture portion of their course. A CD containing all of the figures in the textbook *Human Physiology*, ninth edition, by Stuart Ira Fox, together with a correlation list of these to figures in this laboratory guide, are available to instructors.

CHANGES IN THE LABORATORY EXERCISES

The eleventh edition retains the new procedures using the Biopac, Intelitool, and iWorx systems, where appropriate. These are systems for performing computerized

data acquisition and analysis that can be adapted for use with this laboratory guide. Also retained from the previous editions are the multimedia correlations for the exercises. These multimedia correlations include those using computerized data acquisition and analysis (the Biopac, Iworx, and Intelitool systems), as well as *MediaPhys*, a supplementary CD that supports concepts presented in lecture and laboratory. Also, new to the eleventh edition of this laboratory guide, is the inclusion of *Physiology Interactive Lab Simulations* (Ph.I.L.S.). This CD contains simulated lab exercises that can be performed in addition to (or instead of) the noted exercises in this laboratory guide.

Here are some additional changes that are specific for the exercises:

- **Exercise 1.1** New instructions for use of the oil-immersion lens
- **Exercise 1.3** New discussion of positive feedback added
- **Exercise 3.2** Crossed-extensor reflex figure and Biopac photo added
- **Exercise 3.3** Added description of flexors and extensors
- **Exercise 3.4** Photo of referred pain procedure added
- **Exercise 3.5** Visual accommodation figure added
- **Exercise 3.6** Photos of Rinne's and Weber's test procedures added
- **Exercise 4.1** Added explanation and new figure of negative feedback and positive feedback regulation of the anterior pituitary
- **Exercise 5.1** More detailed steps and figures added for Biopac procedure; *Physiology Interactive Lab Simulations* exercises 1, 2, 3 correlation added
- **Exercise 5.2** More detailed steps added for Biopac procedure; *Physiology Interactive Lab Simulations* exercises 1, 2, 3 correlation added
- **Exercise 5.3** *Physiology Interactive Lab Simulations* exercises 1, 2, 3 correlation added
- **Exercise 6.4** New information and figure on platelet aggregation added
- **Exercise 7.1** Expanded procedure of use of Biopac system in recording frog heart exercise; revised description of digitalis action
- **Exercise 7.2** New table of blood pressure classifications added; *Physiology Interactive Lab Simulations* exercises 4 and 6 correlation added
- **Exercise 8.1** *Physiology Interactive Lab Simulations* exercises 7, 8, 9, 10 correlation added
- **Exercise 8.2** Expanded discussion and new figure of gas exchange; revised discussion and new figure of chemoreceptor regulation of breathing; *Physiology Interactive Lab Simulations* exercises 7, 8, 9, 10 added
- **Exercise 8.3** *Physiology Interactive Lab Simulations* exercises 7, 8, 9, 10 correlation added
- **Exercise 8.4** Added discussion of the role of CO₂ in the regulation of breathing; *Physiology Interactive Lab Simulations* exercises 7, 8, 9, 10 correlation added
- **Exercise 10.3** Reference information updated; added information about metabolism; *Physiology Interactive Lab Simulations* exercise 11 correlation added
- **Exercise 11.1** Revised procedure of rat vaginal smear
- **Appendix 2** *Sources of Equipment and Solutions* extensively updated to reflect changes in exercises listed here, changes in company ownership, and changes of phone numbers and Web sites
- **Appendix 3** *Multimedia Correlations to the Laboratory Exercises* reorganized and updated to include correlations of lab exercises in this guide with the new *Physiology Interactive Lab Simulations*, as well as with *MediaPhys* sections,

NEW AND REVISED FIGURES IN THE ELEVENTH EDITION

The eleventh edition contains 16 figures that are new to this edition. Some of these depict newer laboratory equipment or techniques. Most, however, are figures that help students to better understand the physiological concepts related to the exercises. These improve the self-contained aspect of this laboratory guide while also increasing the degree of cross-referencing between lecture and laboratory.

In addition to figures unique to the eleventh edition, this laboratory guide contains many figures that have been revised from previous editions. This was done to improve the usefulness of these figures to students studying the laboratory exercises, and to increase the correspondence between figures in the laboratory guide and lecture textbook.

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 edition continues to reference programs offered by Biopac, Intelitool, and iWorx. In addition, correlations to McGraw-Hill's *MediaPhys* and to the *Physiology Interactive Lab Simulations* have been incorporated into the eleventh edition.

ORGANIZATION OF THE LABORATORY GUIDE

The exercises in this guide are organized in this manner:

-  Each exercise begins with a list of **materials** needed to perform the exercise, so that it is easier to set up the laboratory. This section is identified by a materials icon.
- Following the materials section is an overview paragraph describing the **concept** behind the laboratory exercise.
- Following the concept paragraph is a list of **learning objectives**, to help students guide their learning while performing the exercise.
-  A box providing **textbook correlations** is placed near the beginning of each exercise. This section can be used to help integrate the lecture textbook (*Human Physiology*, ninth edition, by Stuart Ira Fox) with the laboratory material.
- 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.
-  Boxed information, set off as screened insets, provides the **clinical significance** of different aspects of the laboratory exercise. This approach was pioneered by this laboratory manual and the current edition continues that tradition.
- 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.
-  **Normal values** boxes are placed following the procedures, in cases where students obtain measurements that are clinically applicable. These boxes are indicated with a scales icon to emphasize the relationship between clinical measurements for diagnosis and physiological regulation of homeostasis.
- 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

In addition to this laboratory manual, a comprehensive selection of supplemental materials is available for use in conjunction with *Human Physiology*, ninth edition, by Stuart Ira Fox. Students can order supplemental study materials by contacting their campus bookstore. Instructors can obtain teaching aids by calling the McGraw-Hill Customer Service Department at (800)338-3987, visiting our A&P website at www.mhhe.com/ap, or contacting a local McGraw-Hill sales representative.

ONLINE LEARNING CENTER

The *Human Physiology*, ninth edition, Online Learning Center (OLC) at www.mhhe.com/fox9 offers an extensive array of learning and teaching tools.

Essential Study Partner A collection of interactive study modules that contains hundreds of animations, learning activities, and quizzes designed to help students grasp complex concepts.

Monitored News Feeds Online access to course-specific current articles refereed by content experts, course-specific real-time news, weekly course updates, refereed and updated research links, and daily news.

Online Tutoring A 24-hour tutorial service moderated by qualified instructors. Help with difficult concepts is only an e-mail away.

Along with these outstanding online tools, the OLC features specialized content for both students and instructors using the ninth edition of *Human Physiology*. The Student Center of the OLC features quizzes, interactive learning games, and study tools tailored to coincide with each chapter of the textbook. The Instructor Center is an online repository of teaching aids. It houses downloadable and printable versions of traditional ancillaries plus a wealth of online content.

INSTRUCTOR'S MANUAL FOR THE LABORATORY GUIDE

Accessed via the Instructor Center of the *Human Physiology* OLC (www.mhhe.com/fox9), this helpful manual created by Laurence G. Thouin, Jr. of Pierce College includes suggestions for coordinating lab exercises with the textbook, introductions to each exercise, materials lists, approximate completion times, and solutions to the laboratory reports for each exercise. A listing of laboratory supply houses and instructions for mixing commonly used solutions are also provided.

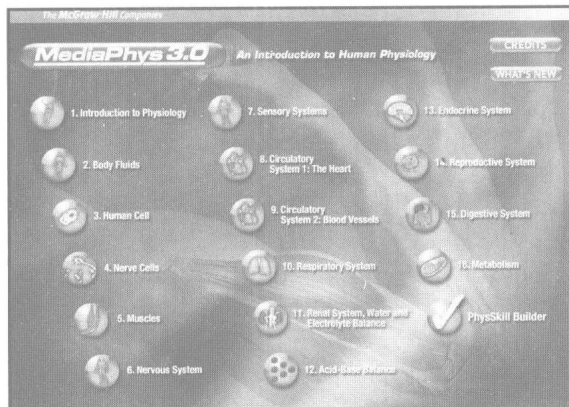
Instructors using this lab manual independently of *Human Physiology*, ninth edition, by Stuart Ira Fox, can access the Instructor's Manual on McGraw-Hill's Lab Central at www.mhhe.com/biosci/ap/labcentral.

OTHER OFFERINGS

In addition to the materials specifically designed to accompany *Human Physiology*, McGraw-Hill offers these supplemental resources to enrich the study and instruction of human physiology.

Digital Content Manager for the textbook *Human Physiology*, ninth edition, by Stuart Ira Fox. This CD contains all of the figures in the textbook and can be used to demonstrate full-color versions of those figures in this laboratory guide that correspond to textbook figures. A correspondence list is provided in the Instructor's Manual, and the corresponding figures are also provided at the bottom of the figure legends in this laboratory guide.

MediaPhys McGraw-Hill's *MediaPhys* is an interactive CD-ROM that offers 13 complete modules (including Muscular, Nervous, Cardiovascular, Respiratory, Urinary, Digestive, Endocrine, and Reproductive systems) that feature detailed explanations, high-quality illustrations, and animations to provide students with a thorough introduction to the world of physiology. *MediaPhys* is filled with interactive activities and quizzes to help reinforce concepts that are often difficult to grasp, making *MediaPhys* a tool that helps students truly understand the concepts of the human body rather than simply memorize them. Contact your campus bookstore to order.



Physiology Interactive Lab Simulations (Ph.I.L.S.) CD-ROM provides simulated laboratory exercises that can be used to supplement the exercises in this laboratory guide. In some cases, where unavailable equipment prevents the performance of an exercise, or where the use of frogs for an exercise isn't desired, the simulated exercises may substitute for the laboratory exercises. The simulated exercises on this CD relevant to particular exercises in this laboratory guide are referenced where appropriate.

Dynamic Human Version 2.0 This set of two interactive CD-ROMs covers each body system and demonstrates clinical concepts, histology, and physiology with animated three-dimensional and other images.

Case Histories in Human Physiology, third edition, by Donna Van Wynsberghe and Gregory Cooley (print or Internet-based), stimulates analytical thinking using case studies and problem solving. Includes an instructor's answer key.

Life Science Animations Library CD-ROM offers more than 400 animations in an easy-to-use program that enables instructors to quickly view the animations and import them into multimedia classroom presentations or Web-based course materials.

Laboratory Atlas of Anatomy and Physiology, third edition, by Eder et al. is a comprehensive full-color atlas that covers histology, human skeletal anatomy, and human muscular anatomy using dissections and reference tables.

ACKNOWLEDGMENTS

The eleventh edition was greatly benefited by input from my colleague Dr. Laurence G. Thouin, Jr. His numerous suggestions helped to make the eleventh edition more accurate and student-friendly.

The shaping of the eleventh edition was also aided by suggestions from other colleagues and students. Ms. Karen Gebhardt was particularly instrumental in checking laboratory sources for materials and reworking some of the procedures new to this edition. I greatly appreciate the support of the editors at McGraw-Hill. Their contributions helped make this the best edition yet of *A Laboratory Guide to Human Physiology: Concepts and Clinical Applications*.

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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 *plasma* (or *cell*) *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.

- | | |
|---------------------|---|
| Exercise 1.1 | Microscopic Examination of Cells |
| Exercise 1.2 | Microscopic Examination of Tissues and Organs |
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Microscopic Examination of Cells

EXERCISE

1.1



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 or toothpicks

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.

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 these parts:

1. eyepieces, each with an ocular lens (usually 10 \times magnification, and may have a pointer)
2. a stage platform with manual or mechanical stage controls



Textbook Correlations

Before performing this exercise, you should study the introductory material presented here. Further information relating to this exercise can be found in these pages of *Human Physiology*, ninth edition, by Stuart I. Fox:

- *Cytoplasm and Its Organelles*. Chapter 3, pp. 57–63.
- *DNA Synthesis and Cell Division*. Chapter 3, pp. 72–81.

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 \times ; a low-power lens, 10 \times ; a high-power lens, 45 \times ; and often an even more powerful oil-immersion lens, 100 \times).

CARE AND CLEANING

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

1. Carry the microscope with two hands.
2. Use the *coarse focus* knob *only* with low power; after focusing on low power with the coarse focus and *fine focus* knob, you can change to higher magnification objective lenses and focus using *only* the fine focus knob.
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 \times lens.)
4. Always leave the lowest power objective lens (usually 4 \times or 10 \times) 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 \times objective clicks into the down position. Using the coarse adjustment, carefully lower the objective

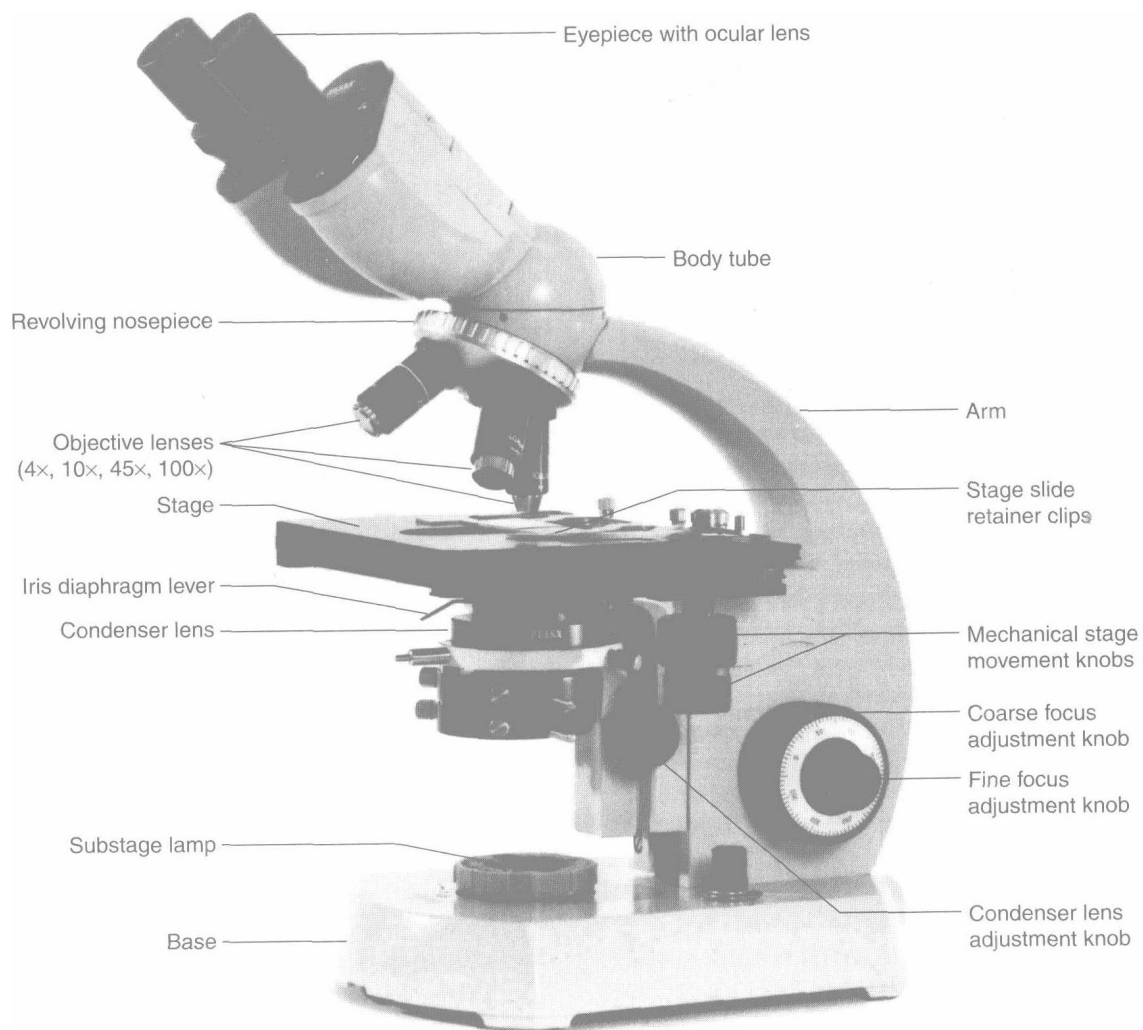


Figure 1.1 The parts of a compound microscope.

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?

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*,

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-	$\times 1,000 (10^3)$	3 places to right
Meter (Liter, gram)	Micro-	$\times 1,000,000 (10^6)$	6 places to right
Milli-	Meter (Liter, gram)	$\div 1,000 (10^{-3})$	3 places to left
Micro-	Meter (Liter, gram)	$\div 1,000,000 (10^{-6})$	6 places to left
Milli-	Micro-	$\times 1,000 (10^3)$	3 places to right
Micro-	Milli-	$\div 1,000 (10^{-3})$	3 places to left

and abbreviated SI (in all languages). The definitions for the metric units of *length*, *mass*, *volume*, and *temperature* are as listed here:

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 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.

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:

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

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 micro-liter (μL) units. If you remember that $1 \text{ mL} = 1,000 \mu\text{L}$, you can set up the problem as shown here:

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

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:

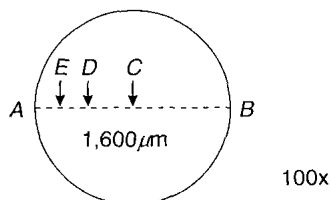
$$0.1 \text{ mL} \times \frac{1.0 \text{ L}}{1,000 \text{ mL}} \times \frac{1,000,000 \text{ }\mu\text{L}}{1.0 \text{ L}} = 100 \text{ }\mu\text{L}$$

VISUAL FIELD AND THE ESTIMATION OF MICROSCOPIC SIZE

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

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 this diagram:

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 \times objective lens (total magnification 450 \times) is approximately 356 micrometers. Using the diagram above and applying the same technique, answer these questions assuming use of a 45 \times objective lens:

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

Since the diameter of the visual field is inversely proportional to the magnification, when the 4 \times , scanning objective lens is used (total magnification 40 \times), the visual field is 4,000 μm ; when the 100 \times , oil-immersion lens is used (total magnification 1,000 \times), the visual field is 160 μm .

PROCEDURE

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

- Using the 10 \times objective lens:
 - estimate the diameter of one dot: _____ m
 - estimate the distance between the nearest edges of two adjacent dots: _____ m
- Using the 45 \times objective lens:
 - estimate the width of one line: _____ m
 - 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 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

- Rub the inside of one cheek with the cotton tip of an applicator stick (or a toothpick).
- Press the cotton tip of the applicator stick (or the end of the toothpick) 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.
- Observe the *unstained* cells under 100 \times and 450 \times total magnification.
- Remove the slide from the microscope. Holding it over a sink or special receptacle, place a drop of methylene blue stain on the smear.
- Place a cover slip over the stained smear and observe the stained cheek cells at 100 \times and 450 \times total magnification.
- Using the procedure described in the previous section, estimate the size of the average cheek cell using both 100 \times and 450 \times total magnification. 100 \times _____ μm ; 450 \times _____ μm . Are they the same? _____
- If you have an oil-immersion lens, you can observe the cheek cells under a total magnification of 1,000 \times . Follow these steps:
 - Focus the cells using the 45 \times objective lens, as before.
 - Move that objective lens out of the way, but do not yet place a different lens over the slide.
 - Place a drop of immersion oil on the spot of the slide where the light shines through.
 - Turn the 100 \times objective lens until it clicks into position; the lens should be immersed in the oil drop but not touching the slide.
 - Wait a moment for movement of the oil to subside, and then adjust the focusing using only the fine focus knob.