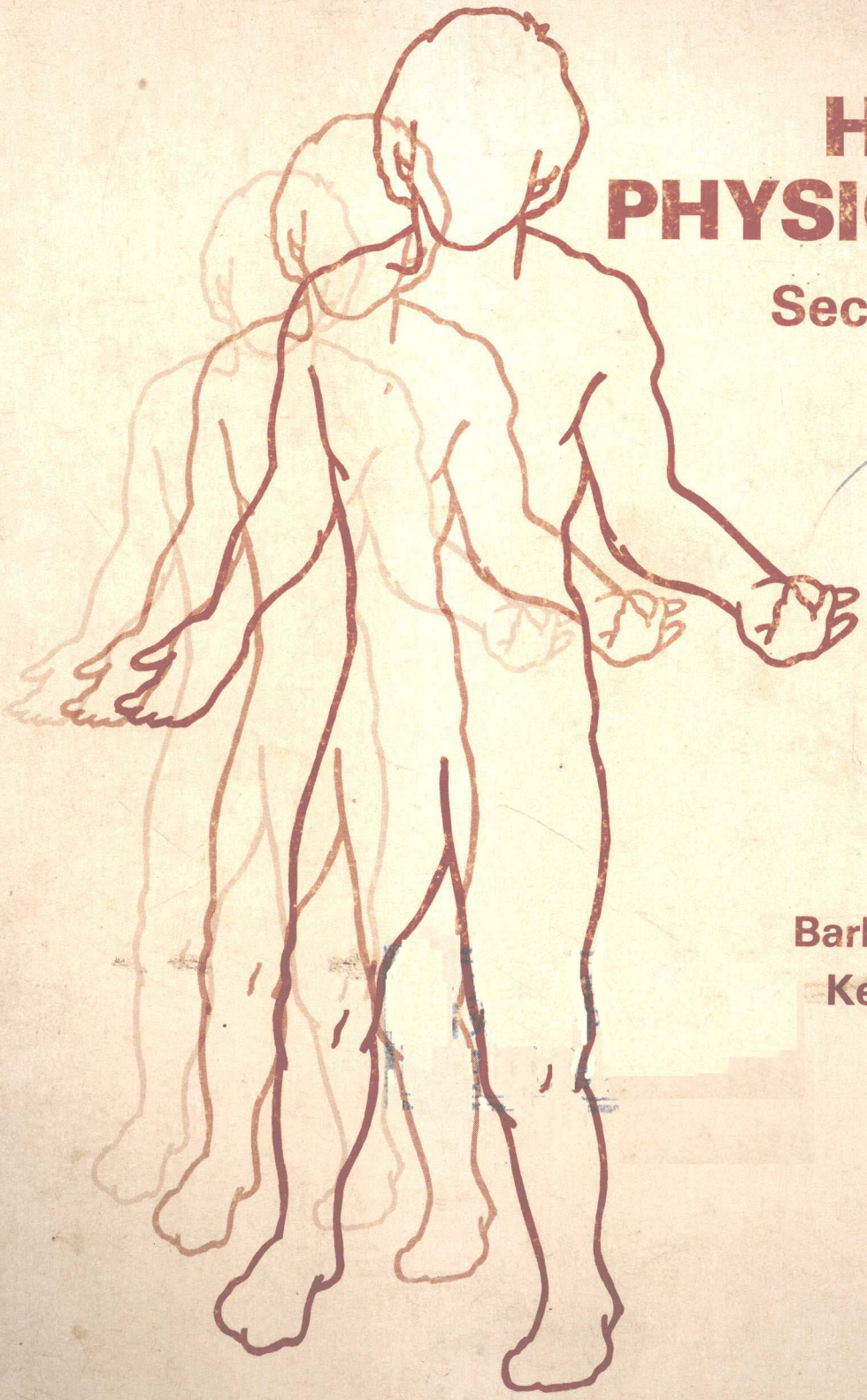
A Laboratory Manual for

HUMAN PHYSIOLOGY

Second Edition



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Preface

This laboratory manual was designed for a one-semester course in human physiology with 6 hours of laboratory per week. There are sufficient exercises for 30-33 (3-hour) laboratory periods. The manual may also be readily adapted for a course having only one laboratory period per week by appropriate selection of exercises.

The exercises are suitable for students majoring in science, nursing, or physical education, and for those students for whom physiology is a terminal course.

The experiments, for the most part, are not new; however, many of the standard physiology experiments have been rewritten for use with the physiograph. Special features of the manual are the inclusion of experiments involving the use of the electrocardiograph and respirometer, and the inclusion of a Results and Questions section which is directly related to each exercise. These sections are arranged so that they may be removed and handed in.

The number of laboratory periods devoted to each topic in the authors' course is as follows:

Chapter	Number of Periods
I	1
II	1 - 2
III	2
IV	2
V	1
VI	2 - 3
VII	2
VIII	2
IX	2
X	1
XI	2
XII	2
XIII	2 - 3
XIV	3
XV	2
XVI	2
XVII	1
XVIII	2

Acknowledgements

We would like to acknowledge our deep appreciation for the long hours spent by Robert Bush in the preparation of most of the illustrations used in this manual.

All illustrations used in this manual are the copyrighted property of ELOT PUBLISHING CO., INC. (P.O. Box 8294, Long Beach, CA 90808). Our thanks to ELOT Publishing Company for granting us permission to use the illustrations in this publication.

Our thanks also to the many students who aided us in improving the quality of the manual with a number of worthwhile suggestions.

The Authors

Introduction

In the laboratory the student has an opportunity to study the physiological processes described in the text and in lecture. To assist you in learning physiology and laboratory procedure, a diversity of experiments will be conducted which will introduce a number of modern techniques and instruments used by physiologists in their investigation of physiological processes. Perform all experiments with care. Your own observations, along with the text and lecture material, should provide answers to most of the questions that arise in the laboratory. Learn to think independently. Your instructor will offer helpful suggestions, but should not be expected to constitute a source of ready answers to all your questions.

Laboratory work (and all experimental writeups) must be done on an individual basis unless you are specifically instructed otherwise. Usually three students will work together on experiments involving the kymograph or the frog. Discussion with other students of the results of your laboratory work or problems that arise is ordinarily permitted. One of the most important rules for success in the laboratory is to read through the directions for an experiment prior to each laboratory period.

A. Laboratory Supplies Furnished by the Student:

Place a check mark in the margin next to those items your instructor indicates you must furnish.

_____ 1. dissecting kit (should include scissors, probe, scalpel, and forceps)
_____ 2. roll of transparent tape
_____ 3. 6-inch ruler (marked in centimeters)
_____ 4. hard bound notebook (7-1/2" x 10-3/4") with lines
_____ 5. 1 package of graph paper (10 squares per inch)
_____ 6. felt-tip (wedge-shaped) pen of any color
_____ 7. 3H drawing pencil
_____ 8. eraser
_____ 9. biology drawing paper
_____ 10. 4-6 glass slides (for use with microscope)
_____ 11. 4-6 cover glasses
_____ 12. 1 package of lens paper
_____ 13. 2 manila folders
_____ 14. _____

B. Laboratory Supplies Contained in Desk Locker:

_____ 1 kymograph with drum and supporting rod (Position the drum carefully in the locket to prevent it from rolling. Use the wooden support provided for this purpose.)
_____ 2 dry cells
_____ 1 tin can containing wires, weight pan, 10-gram weight, empty ink bottle, and 3 two-way clamps. Coil all wires around a pencil and then slide out the pencil (this action keeps the wires neat and manageable).
_____ 1 stand with semicircular base
_____ 1 induction stimulator
_____ 1 frog board
_____ 1 electronic stimulator

_____ 1 frog web board
_____ 1 moist chamber
_____ 1 signal magnet box containing signal magnet, short ink pen, ink bottle, and clamp
_____ 1 box containing heart lever (with long ink pen, ink bottle, and clamp), tuning fork, and tuning fork starter
_____ 1 muscle lever box containing muscle lever, intermediate length ink pen, ink bottle, and clamp
_____ 1 box containing pneumograph
_____ 1 Marey tambour box containing Marey tambour, Marey tambour pen, ink bottle, and clamp
_____ 1 box containing platinum electrodes
_____ 1 box containing muscle warmer
_____ 2 keys
_____ 1 50-ml beaker
_____ 1 watch glass
_____ 1 medicine dropper

C. General Laboratory Rules:

1. Instructions are given at the beginning of the laboratory. It is your responsibility to be present to receive them. After a student is tardy twice, one point will be deducted from laboratory points for each additional tardy.
2. Bring your text book, laboratory manual, and laboratory notebook to each laboratory.
3. Visitors are not allowed in the laboratory except by permission of the instructor.
4. There is no laboratory fee; however, broken, damaged, or wet equipment will be charged against the liable student or students.
5. Do not remove any chemicals or any other materials from the areas where they have been placed. Either bring your test tube to the area where the materials are located, or pour a small amount into a beaker and take it to your desk (read the experiment to determine the amount of material needed).
6. Always return a reagent to its correct position (*i.e.,* the place where it has been put by the instructor or the laboratory assistant).
7. When pouring chemicals, pour on the side away from the label (to preserve the label).
8. Do not set the bottle tops on the laboratory table. Always hold the top of corrosive substances as directed.
9. Use care to avoid spilling chemicals. If an accident occurs, wipe up the area immediately with water and paper towels. Wipe the bottom of all bottles before returning them to their appropriate locations.
10. All equipment in trays must be returned clean and dry to the stockroom before the end of the period.
11. Clean your deck, sink, and all equipment, at the end of the period. Do not put solid material in the sinks. Waste baskets are for solids, sinks are for liquids. Leave no debris of any kind on top of the decks.
12. Before leaving the laboratory, return all equipment to its proper location. Have the instructor check the locker, then lock it and return the key to the laboratory assistant or the instructor.

D. Laboratory Notebook:

1. Your instructor will specify the exact manner in which you are to write up your experiments. This may involve either removing the Results and Questions section from your laboratory manual at the conclusion of each exercise and turning this in to the instructor for evaluation, or keeping a formal notebook, or a combination of both methods.

2. If you are instructed to keep a formal notebook, write up each experiment using the following form:

   Ex. No._____Title of Experiment

   a. Objective:
   The objective(s) will be found in the laboratory manual. This should be a statement of the purpose of the experiment.

   b. Results:
   It is important to carefully record all results during the performance of the experiment. Include all results in this portion of your report, even if you know they are erroneous. When writing results of an experiment, try to make this section concise. Tabulate when possible. Include any kymograph records labeled and briefly described, or drawings in this portion of the writeup.

   c. Discussion:
   Thoroughly discuss and interpret your results. The discussion will show whether you understand the experiment. Use complete sentences. A good discussion will include answers to the questions integrated throughout this portion of the report. If erroneous results were obtained, explain any possible causes and discuss what the correct results should have been.

   d. Conclusion:
   Briefly summarize the results. The conclusion should answer the object rather than being a further discussion of your results. The conclusion will usually be no longer than one or two sentences.

3. Students are not permitted to make up laboratory work.

Contents

Tables

CHAPTER I

Basic Microscopy

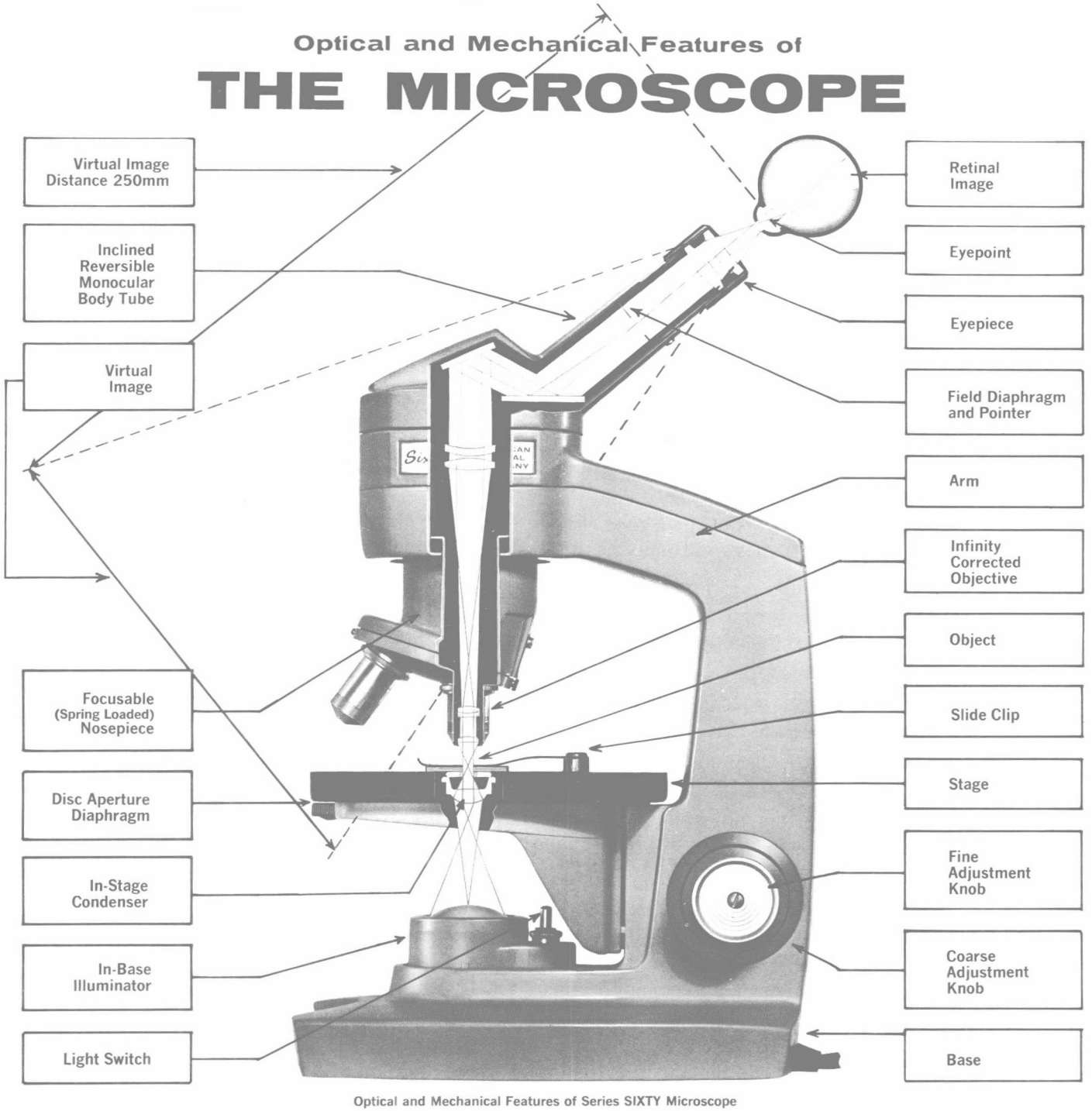
EX. I-A. STRUCTURE OF THE MICROSCOPE

Objectives: To become familiar with the functions of the parts of the microscope; to become acquainted with the procedure for focusing.

Materials: Microscope, microscope lamp, and practice slide.

Procedure:

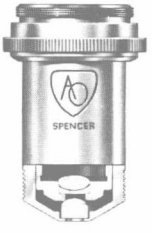
1. Examine the drawing of the microscope (Figure I-1). Compare this drawing with your microscope and learn the names and functions of all parts indicated by the instructor.
2. Use of the microscope:
   a. When it is necessary to lift the microscope, hold the **arm** of the microscope with one hand and add support at the **base** with the other hand.
   b. Place the microscope on the desk with the arm facing you.
   c. Since tilting results in poor light regulation and unwanted movement in fresh preparations, do not tilt the instrument unless instructed to do so.
   d. Always begin by cleaning the **ocular** (eyepiece), **objectives**, **slide**, and **mirror** (when present), with **lens paper**.
   e. To avoid eyestrain and subsequent headaches, it is best to look through the microscope with both eyes open. Practice makes this a simple procedure.
   f. Focusing procedure:
      (1) Turn the **revolving nosepiece** until the **low power objective** (10X) clicks into position over the hole in the stage of the microscope. Always begin with the low power objective.
      (2) Open the **iris diaphragm** approximately halfway or turn the disc diaphragm to the III setting.
      (3) If your microscope is equipped with a mirror, arrange the microscope lamp and concave side of the mirror so that a maximum amount of light will be reflected into the condenser. This adjustment is made while you are looking through the eyepiece (ocular). To regulate light intensity adjust the iris or disc diaphragm (not the mirror) when the slide is in focus. If your microscope is equipped with an in-base illuminator, turn on the illuminator.
      (4) Use the **coarse adjustment knob** to raise the nosepiece for easier access to the stage and then place a slide on the stage of the microscope. Position the specimen to be observed directly above the center of the condenser.
      (5) Usually a stop is present on the microscope which will prevent the low power objective from striking the slide. Determine if this is true of your instrument by lowering the 10X objective as close to the slide as possible without touching it, or until the tube can be lowered no farther.
      (6) If the microscope is equipped with an autofocus, when the low power objective is as close to the slide as possible, the specimen can be brought into sharp focus by



Optical and Mechanical Features of Series SIXTY Microscope

Cross section of scanning objective, 4X.

Cross section of low power objective, 10X.



Cross section of "high dry" objective, 43X.

Cross section of oil immersion objective,100X.

Figure I-1. The Microscope (courtesy of American Optical Corporation, Scientific Instrument Division, Buffalo, N.Y. 14215)

slowly rotating the **fine adjustment knob** either down or up. If the microscope is not equipped with an autofocus, look through the eyepiece and turn the coarse adjustment to slowly raise the objective from the slide until the specimen comes into focus. The image should then be brought into sharp focus with the fine adjustment knob. This knob should also be used for observing depth in a specimen.

(7) Adjust the amount of light to the optimum when the slide is in focus. If there is too much light, so that the specimen cannot be seen clearly against the glare, rotate the disc diaphragm to a smaller opening. If a condenser with an iris diaphragm is being used, close down the diaphragm. When using a mirror, if the field of view is not uniformly illuminated, adjust the mirror or the position of the illuminator so that the specimen field is evenly illuminated.

(8) Move the slide to find the specific area you desire to view in more detail. Since the quality of slides and individual portions of the slides may vary, always select a slide (or area of the slide) that seems typical.

(9) Once the object is in sharp focus and centered with the 10X objective, rotate the the revolving nosepiece to the **high power objective** without changing the position of the coarse adjustment knob. (The microscope should be parfocal, meaning that when the high power objective is brought into position in place of the low power objective, the object will be in focus, or almost in focus, provided you make certain that the object to be viewed is centered and in sharp focus before turning from low power.)

(10) Adjust the fine adjustment knob to bring the specimen into sharp focus with the high power objective. Never focus downward with the coarse adjustment knob when using high power. Each time a different objective is used, the disc or iris diaphragm setting must be changed.

(11) Unless otherwise directed, drawings should be made using the high power objective.

(12) Always return the slide to the correct position in the slide box after use.

3. Estimates of size of cells made with the microscope involve the use of the metric system. The base metric units of length, weight, and volume are included in Appendix IV.

EX. I-B. MICROSCOPIC DRAWINGS

Objectives: To become familiar with basic microscope technique and the procedure to be used for drawings.

Materials: Plain white paper (or Biology Filler paper), 3H pencil, and practice slides.

Discussion: Drawings are a valuable means of recording and checking the results of your observations. A drawing should indicate the accuracy and detail of all observations made. Properly completed drawings provide a basis for reviewing material you have observed under the microscope. They also provide evidence to the instructor that you have seen the significant structures.

The practice slides used in this exercise may consist of assorted tissues on damaged slides. It is not the purpose of this exercise to identify the various tissues; the student should use these slides to learn how to make a drawing.

Procedure:

1. Select one or more of the practice slides and, using the procedure indicated in Ex. I-A, practice focusing (on both low and high power) until you are thoroughly familiar with the technique.
2. On one of the practice slides, locate two or three cells and draw them in accordance with the following specifications (this is the procedure to be used for all drawings, unless instructions