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LABORATORY STUDIES
in Integrated Principles of
ZOOLOGY

Hickman • Hickman • Kats

Ninth Edition

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in Integrated Principles of
ZOOLOGY

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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Preface

We are pleased to present the ninth edition of *Laboratory Studies in Integrated Zoology*. This edition retains the organization of previous editions, with the addition of a new chapter of project exercises, many new color illustrations, and important revisions in existing chapters. This edition also represents a transition in authorship. We welcome the association of Lee B. Kats of Pepperdine University, who brings to the manual broad interests in zoology and extensive experience in the zoology teaching laboratory.

The changes most apparent to previous users will be the conversion of many existing illustrations to full color, and the addition of new color illustrations. These were prepared by William C. Ober and Claire W. Garrison who worked directly from dissections prepared by the senior author and the artists to produce artwork of the greatest possible accuracy.

In making revisions, we benefitted from the reviews of several professional zoologists commissioned by the publisher as well as from the numerous unsolicited comments of scientific acquaintances and users of the manual who kindly took the time to write to us with their suggestions. The following are the principal revisions and additions for this edition.

Part One is devoted to general principles: an exercise on the correct use of the microscope, and five exercises on cell biology, development, basic histology, and animal classification. Chapter 2, on cell structure and division, includes a reworked description of liver cells and new illustrations showing mitosis in fertilized eggs of *Ascaris*. In Chapter 4 we prepared new illustrations of meiosis, gametogenesis, and spiral and radial cleavage. The description of frog tadpole stages and metamorphosis in Exercise 4C was rewritten and expanded. We replaced several black and white illustrations of tissue types in Chapter 5 with color photomicrographs. In response to reviewer requests we replaced the key to the insect orders in Chapter 6 with a completely revised key to the major animal taxa. All characters used in this key are visible on adult specimens without dissection; they do not require previous knowledge of features not evident from external morphology (such as the presence or absence of a true coelom, or primary or secondary symmetry). The key to insect orders was moved to Exercise 16E.

Part Two is a survey of the protozoans and animal phyla. Through the study and dissection of selected specimens, students come to understand the common architectural themes and adaptations that unite groups of animals. To each of the classification sections in the invertebrate chapters and the chordate chapter we added a "pie" diagram showing the relative size of the classes of the phylum. The exercise examining the effects of temperature on the swimming speed of *Stentor* (formerly 7E) has been modified and moved to the last chapter. In addition to replacing many existing illustrations with full color illustrations in the invertebrate chapters, we added several new diagrams. These include a helpful interpretive diagram adjacent to the photomicrograph of the wall of *Sycon* in Chapter 8 and an illustration of the trilobite larvae of the horseshoe crab in Chapter 14. We added a new exercise on the external anatomy of the chiton (Exercise 12C) and rewrote, expanded, and reillustrated the section on internal anatomy of the squid. In Chapter 16 we separated the description of the grasshopper from that of the honeybee, as requested by several reviewers. New illustrations were prepared for the muscles of the frog (Exercise 20C). A complete new set of color illustrations drawn from specimens was prepared for the fetal pig (Chapter 23). The coverage of the fetal pig is, we believe, the clearest and most practical to be found in any introductory guide on the market today.

Throughout Part Two we have included the derivation of specialized biological terms and genera where first introduced. This is an important assist to the student in becoming familiar with the Latin and Greek roots from which technical terms are built. We repeatedly emphasize to students that the species is a binomial by spelling out the complete species binomial in the classification breakdown for each representative species in the exercises.

Part Three, which is on the activity of life, contains exercises on ecological relationships, and three project exercises. Exercise 24A, on population growth in flour beetles, was contributed by James Munger of Boise State University and Richard Inouye of Idaho State University. It is an excellent project, completely revised for this edition, that gives students valuable experience in experimental design and hypothesis testing. It can be started at the beginning of the term and completed with a report at term end. Exercise 24B offers field experience for students through a study of pond ecology. The illustrated key for this exercise was prepared by Deborah Kendall of Fort Lewis College. The study is applicable to almost any pond type found in North America.

The final chapter is new with this edition. In "Experimenting in Zoology" we describe project laboratories that can be completed within a single laboratory session. The student handles living organisms, follows experimental procedures, records and analyzes quantitative data, and draws conclusions from the results. Students are encouraged to use the basic methodology of the exercise to pursue independent investigations. Suggestions for further investigation are given at the end of each exercise. Many instructors will want their students to gain additional experience by writing a laboratory report in which the student states the objectives, methods followed, results obtained, and conclusions that can be drawn from the results.

Directions for preparing the exercises in this manual are found in Appendix A. For each exercise we have listed the materials required, directions for preparing solutions, suggestions for maintaining and working with living materials, suggestions for demonstrations, and a listing of appropriate references, most of which are annotated. This information, previously placed in a separate handbook, is convenient to the instructor as well as to students who may later wish to consult or implement an exercise, but who would not have a separate handbook available to them. To provide space for Appendix A without increasing the length of the manual, we have deleted several exercises on animal physiology and genetics. However, these exercises are available to any instructor who wishes to use them.

There are many aids for the student in this laboratory manual. Throughout the exercises, working instructions are clearly set off from the descriptive material. Classifications, where appropriate, are included with the text. Function is explained along with anatomy. Topic headings help the student mentally organize the material. Metric tables and definitions are placed on the inside front and back covers for convenient use. Much of the artwork was designed to assist the student with difficult dissections. The popular taxonomic chart, completely redesigned and updated for the previous edition, serves as a concise and conveniently arranged review of the animal phyla. It is available free of charge for those instructors who wish to order it for themselves and/or their students.

New Technology

The Virtual Physiology Lab CD-ROM for Macintosh and Windows features 10 physiological simulations of the most common animal experiments. This CD offers students the opportunity to work at their own pace, using software that realistically duplicates the experience of working with actual specimens. For more information, contact your WCBP sales representative. (ISBN: 0–697–36286–8)

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We wish to thank the following reviewers who provided numerous suggestions for improvements to the manual.

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Cleveland P. Hickman, Jr.
Frances M. Hickman
Lee B. Kats

Laboratory Safety Procedures

1. Keep your work area uncluttered. Unnecessary books, backpacks, purses, etc. should be placed somewhere other than on your desktop.
2. Avoid contact with embalming fluids. Wear rubber or disposable plastic gloves when working with preserved specimens.
3. Wear eyeglasses or safety glasses to protect your eyes from splattered embalming fluid.
4. Keep your hands away from mouth and face while in the laboratory. Moisten labels with tap water, not your tongue.
5. Sponge down your work area and wash all laboratory instruments at the end of the period.
6. Wash your hands with soap and water at the end of the laboratory period.

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General Instructions

Equipment

Each student will need to supply the following equipment:

Laboratory manual and textbook
Dissecting kit containing scissors, forceps, scalpel, dissecting needles, pipette (medicine dropper), probe, and ruler, graduated in millimeters
Drawing pencils, 3H or 4H
Eraser, preferably kneaded rubber
Colored pencils—red, yellow, blue, and green
Box of cleansing tissues
Loose-leaf notebook for notes and corrected drawings

The department will furnish each student with all other supplies and equipment needed during the course.

Aim and Purpose of Laboratory Work

The zoology laboratory will provide your "hands-on" experience in zoology. It is the place where you will see, touch, hear, smell—but perhaps not taste—living organisms. You will become acquainted with the major animal groups, make dissections of preserved or anesthetized specimens to study how animals are constructed, ask questions about how animals and their parts function, and gain an appreciation of some of the architectural themes and adaptations that emphasize the unity of life.

General Instructions for Laboratory Work

Prepare for the Laboratory. Before coming to the laboratory, read the entire exercise to familiarize yourself with the subject matter and procedures. Read also the appropriate sections in your textbook. Good preparation can make the difference between a frustrating afternoon of confusion and mistakes and an experience that is pleasant, meaningful, and interesting.

Follow the Manual Instructions Carefully. It is your guide to exploring and understanding the organisms or functions you are investigating. Its instructions have been written with care and with you in mind, to help you do the work (1) in logical sequence, (2) with economy of time, and (3) to arouse a questioning attitude that will stimulate interest and curiosity.

Use Particular Care in Making Animal Dissections. A glossary of directional terms used in dissections will be found inside the front cover. The object in dissections is to separate or expose parts or organs so as to see their relationships. Working blindly without the manual instructions may result in the destruction of parts before you have had an opportunity to identify them. **Learn the functions** of all the organs you dissect.

Record Your Observations. Keep a personal record in a notebook of everything that is pertinent, including the laboratory instructor's preliminary instruction and all experimental observations. Do not record data on scraps of paper with the intention of recopying later; record directly into a notebook. The notes are for your own use in preparing the laboratory report later.

Take Care of Equipment. Glassware and other apparatus should be washed and dried after use. Metal instruments in particular should be thoroughly dried to prevent rust or corrosion. Put away all materials and equipment in their proper places at the end of the period.

Tips on Making Drawings

You need not be an artist to make laboratory drawings. You do, however, need to be **observant.** Study your specimen carefully. Your simple line drawing is a record of your observations.

Before you draw, locate on the specimen all the structures or parts indicated in the manual instructions. Study their relationships to each other. Measure the specimen. Decide where the drawing should be placed and how much it must be enlarged or reduced to fit the page (read further for estimation of magnification). Leave ample space for labels.

When ready to draw, you may want first to rule in faint lines to represent the main axes, and then sketch the general outlines lightly. When you have the outlines you want, draw them in with firm dark lines, erasing unnecessary sketch lines. Then fill in details. Do not make overlapping, fuzzy, indistinct, or unnecessary lines. Indicate differences in texture and color by stippling. Stipple deliberately, holding the pencil vertically and making a neat round dot each time you touch the paper. Placing the dots close together or farther apart will give a variety of shading. Avoid line shading unless you are very skilled. Use color only when asked for it in the directions.

Label the Drawing Completely. Print labels neatly in lowercase letters and align them vertically and horizontally. Plan the labels so that there will be no crossed label lines. If there are to be many labels, center the drawing and label on both sides.

Indicate the magnification in size beneath the drawing, for instance, "×3" if the drawing is three times the length and width of the specimen. In the case of objects viewed through a microscope, indicate also the magnification at which you viewed the subject, for example, 430× (43× objective used with a 10× ocular).

Estimating the Magnification of a Drawing

A simple method for determining the magnification of a drawing is to find the ratio between the size of the drawing and the actual size of the object you have drawn. The magnification of the drawing can be expressed in the following formula:

$$\times = \frac{\text{Size of drawing}}{\text{Size of object}}$$

If your drawing of the specimen is 12 cm (120 mm) long, and you have estimated the specimen to be 0.8 mm long, then $\times = 120 \div 0.8$, or 150. The drawing, then, is ×150, or 150 times the length of the object drawn.

This same formula will hold good whether the drawing is an enlargement or a reduction. If, for example, the specimen is 480 mm long, and the drawing is 120 mm, then $\times = 120/480$, or 1/4.

Statement on the Use of Living and Preserved Animals in the Zoology Laboratory

Congress has probably received more mail on the topic of animal research in universities and business firms than on any other subject. Do humans have the right to experiment on other living creatures to support their own medical, pharmaceutical, and commercial needs? A few years ago, Congress passed a series of amendments to the Federal Animal Welfare Act, a body of laws covering animal care in laboratories and other facilities. These amendments have become known as the three R's: **r**eduction in the number of animals needed for research; **r**efinement of techniques that might cause stress or suffering; and **r**eplacement of live animals with simulations or cell cultures whenever possible. As a result, the total number of animals used each year in research and in commercial product testing has declined steadily as scientists and businesses have become more concerned and more accountable. The animal rights movement, largely comprising vocal antivivisectionists, has helped to create an awareness of the needs of laboratory research animals and has stretched the resources and creativity of the researchers to discover cheaper and more humane alternatives to animal experimentation.

However, computers and cell cultures—the alternatives—can only simulate the effects on organismal systems of, for instance, drugs, when the principles are well acknowledged. When the principles are themselves being scrutinized and tested, computer modeling is insufficient. Nor can a movie or computer simulation match the visual and tactile comprehension of anatomical relationships provided by direct dissection of preserved or anesthetized animals. Medical and veterinarian progress depends on animal research. Every drug and every vaccine that you and your family have ever taken has first been tested on an animal. Animal research has wiped out smallpox and polio; has provided immunization against diseases previously common and often deadly, such as diphtheria, mumps, and rubella; has helped create treatments for cancer, diabetes, heart disease, and manic depression; and has been used in the development of surgical procedures such as heart surgery, blood transfusions, and cataract removal.

Animal research has also benefited other animals for veterinary cures. The vaccine for feline leukemia that could threaten the life of your cat, as well as the parvo vaccine given to your puppy, were first introduced to other cats and dogs. Many other vaccinations for serious animal diseases were developed through animal research; for example, rabies, distemper, anthrax, hepatitis, and tetanus.

The animal models used by the artist for the illustrations in the exercises of this laboratory manual, and the animals you will dissect in this laboratory course, were prepared for educational use following strict humane procedures. No endangered species have been used. No living vertebrate organisms will be harmed in this laboratory setting. Invertebrate animals that are to be dissected while alive are anesthetized before the procedure. The experiments selected are unoffensive, are respectful of the integrity of the animal's evolutionary contributions, and often require only close observation. The experiments closely follow the tenets of the scientific method, which cannot dictate ethical decisions but can provide the structure for common sense. Do not be wasteful. Share the animals with the other students as often as possible. At the same time, you are encouraged to observe the live animal in its natural setting and its relationships to other species, for only in this manner will you gain a full appreciation of the unique evolutionary position and special structure and systems of each animal.

Notes

PART

I

Introduction to the Living Animal

Notes

1

The Microscope

Exercise 1A: Compound Light Microscope
Understanding the parts and operation of the microscope
Getting acquainted with your microscope
Taking control of your microscope
Magnification in the microscope
How to measure size of microscopic objects
Exercises with the compound light microscope
Exercise 1B: Stereoscopic Dissecting Microscope
Exercises with the dissecting microscope
Exercise 1C: Electron Microscope

For a biologist the compound microscope is probably the most important tool ever invented. It is indispensable not only in biology but also in the fields of medicine, biochemistry, and geology; in industry; and even in crime detection and many hobbies. Yet even though the microscope is one of the most common tools in the biologist's laboratory, too frequently it is used without any effective understanding of its construction and operation. The results may be poor illumination, badly focused optics, and misleading interpretations of what is (barely) seen.

Both the compound microscope and the binocular dissecting microscope (stereoscopic microscope) will open up a whole new world for you if you make the effort to become proficient in their use. Learn their possibilities and use them to the greatest advantage. Take good care of the microscope. Microscopes are expensive instruments and, although they are sturdily built and will stand many years of use, they are precision instruments that require careful treatment.

Exercise 1A
Compound Light Microscope

The compound light microscope may be either monocular or binocular, with either vertical or inclined oculars.

☞ Use both hands to carry a microscope. Grasp it firmly by the arm with one hand and support the **base** with the other. Carry it in a fully upright position.

Understanding the Parts and Operation of the Microscope

If you are not familiar with the parts of the microscope, please study Figures 1-1 and 1-2.

The **image-forming optics** consist of (1) a set of **objectives** screwed into a **revolving nosepiece,** and (2) a **body tube,** or head, with one or two **oculars (eyepieces).**

Each **objective** is a complex set of tiny lenses that provide most of the magnification. Your microscope may have two, three, or four objectives, each with its magnification, or power, engraved on the side. For example, if the objective magnifies an object 10 times, the magnification is said to be 10 diameters and is commonly written simply as 10×. Most microscopes include a **scanning objective** (3.5× or 4.5×), a **low-power objective** (10×), and a **high-power objective** (40×, 43×, or 45×). Some microscopes also carry an **oil-immersion objective** (95×, 97×, or 100×), which must always be used with a drop of oil to form a liquid bridge between itself and the surface of the slide being viewed.

☞ Revolve the nosepiece and note the clicking sound when an objective swings into place under the tube.

The lenses in the **ocular** further magnify the image formed by the objective. The ocular most often used is the 10×. A 6× or a 15× ocular may also be provided. Often a pointer is mounted into the ocular.

☞ If there is a pointer in your microscope, rotate the ocular and note the movement of the pointer. Remember that if you look through a binocular scope with only one eye you may miss the pointer. Always use both eyes.

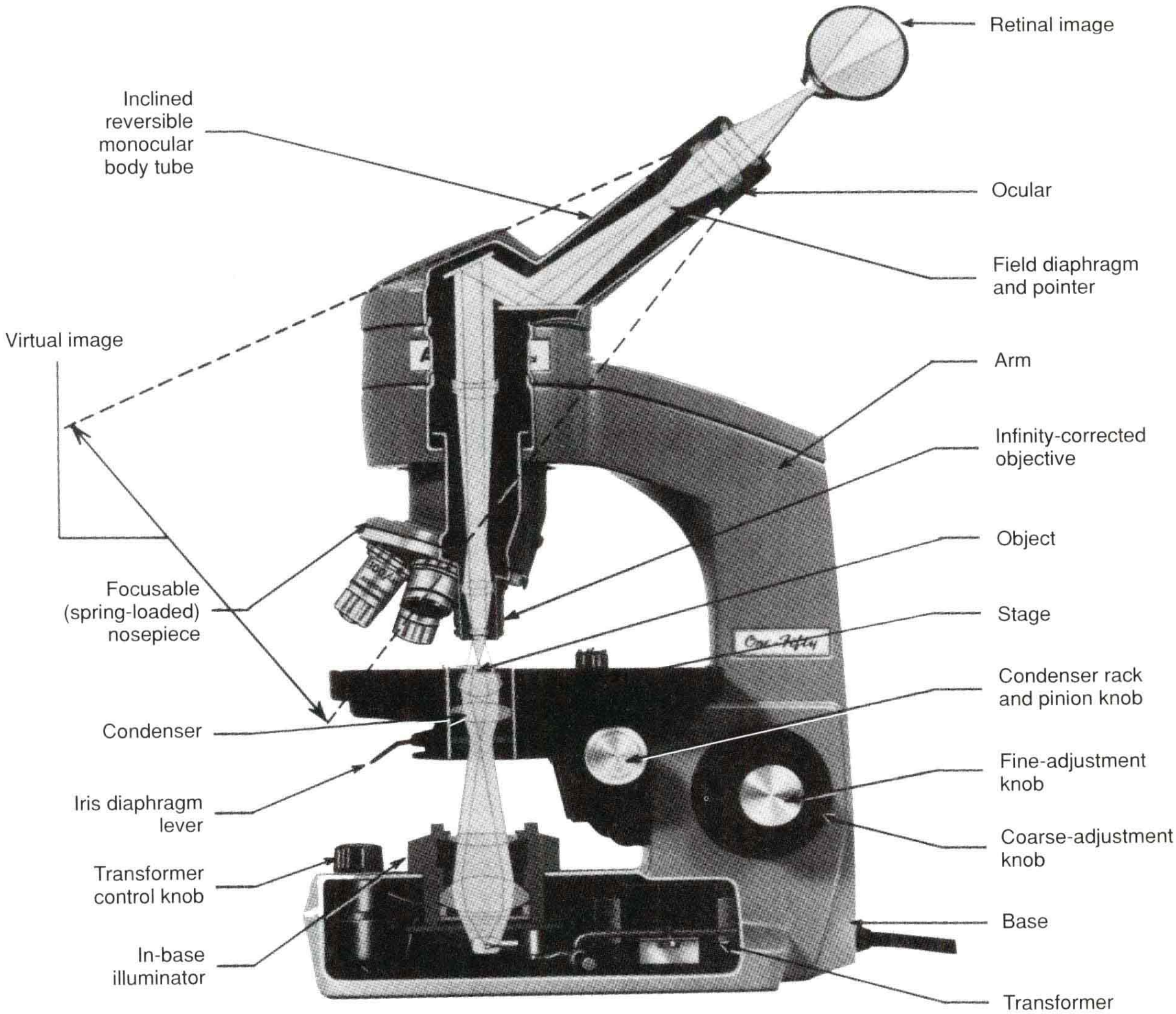

Figure 1-1

Optical and mechanical features of a compound microscope.

The **stage** is the platform with clips to hold the slide in place. Some microscopes have a mechanical stage possessing knobs for moving the slide back and forth or up and down.

Directly beneath the stage of most microscopes is a **substage condenser,** a system of enclosed lenses that concentrates the light on the specimen above. The condenser may have a knob that allows you to move the condenser up and down.

Beneath the condenser, many microscopes have a built-in, low-voltage **substage illuminator.** Microscopes lacking a substage illuminator employ an adjustable **reflecting mirror** that reflects natural light or light from a microscope lamp up into the optical system. The **concave surface** of the mirror is used with natural light or with a separate microscope lamp when there is no condenser on the microscope. The **plane (flat) surface** of the mirror is used with a substage condenser.

☞ Turn on the substage illuminator. If your microscope lacks one, use a microscope lamp or position your microscope and mirror to take advantage of natural light. With the low-power objective in place, adjust the mirror to bring a bright, evenly distributed circle of light through the lens.

An adjustable **iris diaphragm** under the stage regulates the amount of light passing through the **aperture** of the stage. On some microscopes the light is regulated by revolving a **disc diaphragm,** which has holes of various sizes.

☞ Raise or lower the substage condenser to adjust the light to the desired intensity. Usually the best condenser position is near its uppermost limit, where it gives maximum illumination. Then close down the iris or disc diaphragm gradually until all glare is gone (but not too far; dark halos will

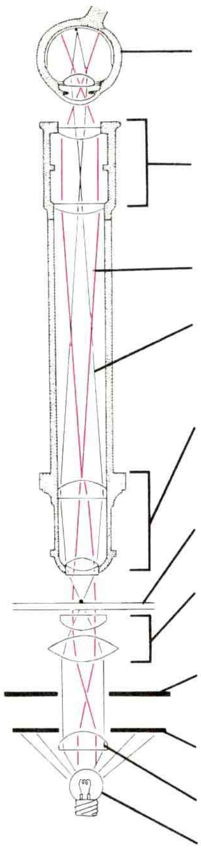

Figure 1-2
Optical path of light through a microscope.

appear around objects if you step down too much). You can check for the best diaphragm setting by removing the ocular and looking down the body tube. Adjust the diaphragm so that you have reduced the disc of light on the objective lens to about three-quarters of its fully illuminated setting. Replace the ocular lens.

The **body tube** is raised and lowered by two sets of adjustment knobs. The **coarse-adjustment knob** is for low-power work and for initial focusing. The **fine-adjustment knob** is for final adjustment and varying the plane of focus for viewing an object at different depths. Note: Microscopes with an inclined tube (like the one pictured in Figure 1-1) are focused by **moving the stage** rather than the body tube up and down. If you are using an inclined microscope, read "lower the stage" when the directions say "raise the objective" in the following exercise. In either case the distance between objective lens and the object is increased.

☞ Turn the coarse-adjustment knob and note how it moves the body tube (or stage). Find out which way to turn the knob to raise and lower the tube.

Never use the coarse-adjustment knob when a high-power objective is in place. Turn the fine-adjustment knob. This moves the tube so slightly that you cannot detect it unless you are examining an object through the ocular. The fine-adjustment knob works the same as the coarse-adjustment knob. To focus downward, turn the knob in the same direction as you would to focus down with the coarse adjustment. Practice this. **Always use the fine adjustment when the high-power objective is in place.**

Getting Acquainted with Your Microscope

You need not wear glasses when using the microscope unless they correct a severe astigmatism. Nearsightedness and farsightedness can be corrected by adjusting the microscope.

First clean the lenses of both the ocular and the objectives by wiping them gently with a clean sheet of lens paper. Do this each time you use the microscope. **Never touch the lenses with anything except clean lens paper.** Do not remove an ocular or objective unless told to do so by the instructor. Dust the mirror with a cleansing tissue.

Keep both eyes open while using a monocular microscope. If this seems difficult at first, hold a piece of paper over one eye while viewing the object with the other. Should one eye become tired, shift to the other one.

How to Focus with Low Power. Turn the low-power (10×) objective until it clicks in place over the aperture. Adjust the condenser and iris diaphragm for optimal illumination as already described.

Obtain a slide containing the letter *e* (or *a, b,* or *k*). Place it, coverslip up, on the stage with the letter centered under the objective lens. *While watching from the side,* lower the objective with the coarse adjustment until it is close to the slide surface.

Now look through the ocular and slowly raise the objective by turning the coarse adjustment toward you until the object on the slide is in sharp focus. Is the image upside down? Is it reversed; that is, does the left side of the letter appear on the right, and the right side on the left? On a separate sheet of paper, draw the letter as it appears.

Shift the slide very slightly to the right while viewing it through the ocular. In what direction does the image move? Move the slide away from you. What happens to the image? Turn the fine-adjustment knob toward and then away from you and observe the effect on the image.

How to Focus with High Power. Focus the object first with low power; then slowly rotate the high-power objective into position. If the microscope