CHARLES F. LYTLE

twelfth edition

General ZOOLOGY LABORATORY GUIDE

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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

With this edition we continue the tradition of excellence begun by the late J. E. Wodsedalek of the University of Minnesota with the first edition of the General Zoology Laboratory Guide *in 1938. In recognition of his contributions as sole author of the first five editions of this book prior to his death, we continued to recognize Dr. Wodsedalek as coauthor of this book from the sixth through the eleventh editions.*

A solid foundation in basic zoology is essential for students who are preparing for careers in biology, zoology, genetics, physiology, medicine, veterinary medicine, agriculture, environmental science, conservation, and many other fields. Other students also benefit from the study of animals because animals are an important part of nature, which surrounds us, and also because animals have contributed to human life and welfare in innumerable ways since the dawn of civilization. Animals affect each of us every day whether or not we are aware of it. Some knowledge of zoology is therefore essential to every educated person.

Meaningful laboratory experiences are a vital part of learning zoology, and this book is designed to facilitate laboratory study of selected animals. In the laboratory students learn the importance of careful observation, of following specific instructions, and of seeing relationships of structure and function. By carrying out well-designed scientific experiments in the laboratory students learn to **do** science instead of merely listening to someone **talk** about science.

In writing this book I have tried to remember my own days as a student and the questions I had while studying various kinds of animals for the first time. I have attempted to provide descriptions, illustrations, and appropriate guidance for meaningful laboratory study of animals. Although I agree with the spirit of Louis Agassiz' admonition, "Study nature, not books," I believe that students can benefit most in their study of nature when aided by appropriate instructions and illustrations.

I can remember some of my own frustration in zoology labs when I attempted to follow some vague verbal description of anatomical structures with no illustrations or other visual aids to help me locate important structures or to give me some appropriate orientation. I have written the exercises in this book with the intention of reducing such frustration and with the intention of making student experiences in the zoology laboratory interesting, rewarding, and meaningful.

I continue to emphasize the study of living and anaesthetized animals whenever feasible because students should learn that zoology is the study of animal life rather than the study of dead animals. Live animals give students the opportunity to observe and experiment with behavior and to do simple physiological experiments as well as to see the natural color and texture of body parts. Preserved specimens serve well for many anatomical studies, but students should always have the opportunity to observe and to work with living animals whenever possible. Few of us would choose a stuffed or embalmed dog or cat for a pet if given the option.

This edition of the manual continues the tradition of more than fifty years of excellence in providing students with a comprehensive introduction to zoology and to the major animal phyla. This book is written to aid students and teachers in many colleges and universities operating with different schedules, resources, and preferences, so I have intentionally included more material than can reasonably be covered in the time available in a two-semester general zoology course. We expect instructors to select those parts of the guide and those animals they deem most appropriate for their own classes. With judicious selection of chapters and of animal types this book can also be used for one-semester and one-quarter zoology classes.

Changes in This Edition

A major innovation in the twelfth edition is the addition of full-color illustrations throughout the book. The success of the full-color illustrations included in several chapters of the last edition encouraged us to make more extensive use of color in this edition. Color photographs of dissections give students much more realistic views of what animals actually look like in the laboratory and help them to distinguish different organs and tissues.

We have found that adding color to drawings not only helps to differentiate various parts, but can also be very useful in showing relationships between different parts of an animal and, when properly done, can help students identify organs belonging to different organ systems.

We have added more than 150 new illustrations in this edition and have greatly increased the number of color photographs. Many of the photographs are from expert dissections done by Dr. Larry Grimes and photographed by Mr. Ken Taylor. Many other photographs are from the extensive collections of the Carolina Biological Supply Company.

Among the new material added in this edition is an exercise on the anatomy and dissection of the cockroach. Several teachers have told us that they prefer to use the cockroach rather than the grasshopper to introduce students to insect morphology. Since we are now including both insects in this edition, teachers now have an easy choice of forms. I am grateful to Dr. John Meyer for information on cockroach anatomy and to Christina Devorshak for her expert dissections.

We have also added a new exercise on the anatomy and biology of the nematode *Caenorhabditis elegans. C. elegans* has been an important research animal for many genetic studies in recent years. More than 50 genes have been identified in this species, and it will continue to be a useful research animal with many important applications in research. Students should learn about such important species in an introductory zoology course.

Another important addition is a new introductory chapter on animal morphology (chapter 9). In this chapter we discuss some basic features of the organization of the animal body that should help students compare different types of animals and better understand some of the morphological features important in determining possible phylogenetic relationships among animals.

We have made significant changes and additions in nearly every chapter of the book. New information, improved explanations, and additional illustrations have been added to many chapters, including the chapter dealing with microscopy. We have expanded and updated the material on laboratory safety and on the care and handling of animals in the laboratory. Both topics are extremely important and have received much attention in the past few years. We hope that these changes and additions will make this lab guide more helpful. As always, we will welcome your comments and suggestions for further improvement.

Basic Features of This Manual

In this edition we have continued the basic organization and pedagogical features of the previous edition. A **Correlation Chart** is provided at the beginning of the book to help teachers and students coordinate the laboratory exercises with appropriate chapters in several of the popular zoology textbooks in current use. Important pedagogical features of the book include **boldface headings** within each chapter to indicate the major divisions of each exercise.

We also use **boldface** in the text to identify important terms (ideas, structures, processes) that students should remember and understand. The most important of these boldface terms are included in the list of **key terms** at the end of the chapter in which they are first introduced.

Several chapters provide space for students to add their own drawings of particular animals or structures to aid them in learning and remembering things observed during their laboratory study. The book also provides several blank tables and pages of graph paper for students to record and plot data from their laboratory observations and experiments.

Each chapter begins with a list of specific **objectives** that identifies important principles, concepts, and facts that students should learn as a result of their laboratory study. I have found that a specific list of laboratory objectives helps students focus their attention on the important material in each lab. I also suggest that instructors modify and add to these lists of objectives as appropriate for their own classes. Such lists of objectives can be most helpful in ensuring that students understand what they will be tested on and that the tests actually focus on students' understanding of the important principles, concepts, and processes.

Most chapters in this book start with a brief **introduction** with pertinent background material to help orient students for the exercises to follow. A **materials list** is provided showing the specimens and other materials needed for each exercise. Most chapters have one or more lists of suggested **demonstrations,** which are suggested to supplement the main studies of each exercise.

At the end of each chapter is a list of **key terms** introduced in that chapter. Chapters also have blank pages provided for students to add their own notes and sketches. If students use these pages to record their observations they will have a consolidated record of their laboratory work bound in a single place instead of a scattered bunch of papers and drawings likely to be lost.

I have tried to make this laboratory manual a convenient, user-friendly companion for laboratory study. I hope every student has as much fun and satisfaction in zoology lab as I have had.

Anatomy Films

From my many years of teaching zoology laboratories I have learned that it is very helpful to give students an overview of the anatomy of an animal to be studied and/or dissected before they undertake the anatomical study on an actual specimen themselves. It's a lot like football players viewing game films before facing a major rival football team. They might do all right without knowing what kinds of plays the opposition typically runs and who their key players are, but they are not likely to win the championship without some good scouting information.

An excellent way to prepare for a serious anatomical study of an animal is to view a good film or video of the anatomy of that animal prior to beginning work with an actual specimen. Such preparation for the lab study gives students a better perspective and orientation and greatly increases their confidence. It also aids in their identification of anatomical structures, facilitates their recognition of relationships among various organs, and assists them in relating structure and function. Good films or videos also help students review their laboratory work in preparation for a test and in comparing the anatomy of different animals.

I have collaborated with the staff of Carolina Biological Supply Company in the development of a series of videos specifically designed to aid in the study of nine of the more complex animals included in this manual. These videos illustrate the anatomy and dissection of these nine animals and parallel the descriptions of those animals in this book.

Each video illustrates the anatomy of the animal in detail, discusses the functions of various organs and systems, and demonstrates good dissection techniques. Each video is divided into sections according to organ system so that each system can be located easily and viewed separately if desired. Several of the longer videos are too long to be productively viewed in a single session.

The videos are available from Carolina Biological Supply Company, 2700 York Road, Burlington, North Carolina, 27215. The videos and the corresponding chapters in this manual containing the exercises for the study of these animals are listed in the following table.

Chapter		Video
11	Mollusca	The Anatomy of the Freshwater Mussel (49-2365V)
12	Annelida	The Anatomy of the Earthworm (49-2372V)
13	Arthropoda	The Anatomy of the Crayfish (49-2403V)
		The Anatomy of the Grasshopper (49-2404V)
14	Echinodermata	The Anatomy of the Starfish (49-2369V)
16	Shark Anatomy	The Anatomy of the Shark (49-2655V)
17	Perch Anatomy	The Anatomy of the Perch (49-2662V)
18	Frog Anatomy	The Anatomy of the Frog (49-2704V)
19	Fetal Pig Anatomy	The Anatomy of the Fetal Pig (49-3075V)

Acknowledgements

I am grateful to all those who have contributed to the improvement of this edition of the manual. Many teachers and several students have made suggestions and comments based on their experiences with the book. Such assistance is particularly helpful since it helps us understand the changing interests and needs of teachers and students elsewhere. We encourage you to continue sending us your suggestions and comments and to help us identify any errors in the book.

I also wish to thank several colleagues who have aided in the preparation of this edition. My good friends at the Carolina Biological Supply Company have been most helpful throughout this revision providing encouragement, supplying information, and lending materials, photographs and other illustrations. I greatly value my continued association with such a fine group of professionals. I wish especially to thank Roger Phillips, Bill West, Dr. Raymond Flagg, Dan James, Harry Hollowell, and Wes Reynolds for their interest and assistance.

Dr. Robin Leech of the University of Alberta and Dr. Edward Lyke of California State University at Hayward provided careful reviews of the previous edition and important suggestions for the revision. Dr. Colin Hermans of California State University at Sonoma gave many helpful suggestions and corrections. Gwilym S. Jones of Northeastern University, Henry Kirkland of Southwestern Oklahoma State University, and Stanley L. Sissom of Southwest Texas State University, as well as several other anonymous reviewers, also provided helpful reviews.

Ms. Patty Aune, Dr. Phyllis Bradbury, Dr. John Roberts, Dr. C. M. Williams, and Dr. John Meyer of North Carolina State University reviewed portions of the manuscript. Also, Dr. Larry Grimes of Meredith College, Dr. Frederick Harrison of Western Carolina University, and Dr. Dean Decker of the University of Richmond contributed valuable reviews of specific chapters. Ms. Karen Hoffman of the North Carolina Association for Biomedical Research provided information on the use of animals in research.

My former student, Carol Majors, did several new drawings for this edition, adding to the many fine drawings she has contributed to earlier editions. Ms. Peggy Holliday of Laboratory and Science Education Consultants Inc. gave valuable assistance in the review and preparation of the section on laboratory safety. Ms. Patty Aune and Dr. C. M. Williams also provided helpful reviews of this section.

I am also grateful to Dr. Larry Grimes for his suggestions and expert dissections of several animals and to Mr. Ken Taylor of Wildlife Images for some outstanding new photographs of Larry's dissections. Ms. Katy Rushnov of Southern Micro Instruments, Dr. Barbara Grimes and Dr. JoAnn Burkeholder of North Carolina State University, and Dr. Charles Wyttenbach of the University of Kansas provided additional photographs to enhance this edition.

Finally, I wish to thank my wife, Carol Lytle, for her patience as well as her continued editorial and secretarial assistance with the manuscript. I sincerely appreciate the aid of all those who have assisted in the preparation of this edition but must point out that any errors or omissions remaining are my own responsibility. I hope that this edition will continue to stimulate student interest in the study of zoology and lead students to the rewards of a better understanding of animal structure and function and an improved perspective on the evolutionary relationships of animals.

Laboratory Safety

A zoology laboratory is a place for serious scientific work and study. Students and teachers must recognize that a number of potential safety hazards are present in all science laboratories. In a zoology laboratory, the principal safety hazards are electrical circuits, potentially dangerous chemicals, hot liquids and heat sources, broken glass, live animals, and sometimes infectious agents (pathogenic bacteria, viruses, and parasites). Achieving safety in the laboratory, as in other places, requires paying attention to potential hazards and observing appropriate safety measures. Nothing we do is without some degree of risk. For example, people sometimes fall out of bed and injure themselves; many people also drown in swimming pools each year. But science laboratories can provide a safe environment when both students and teachers are aware of potential hazards and follow appropriate safety procedures.

The following list of safety rules is offered as a good start toward safe practices in the lab. This is not a complete list of safety rules, and it is certainly not a substitute for common sense. Common sense comes with an awareness of the need and importance of safety. All actions in a laboratory have consequences. Be protected by paying attention, listening to your instructors, and knowing about the materials and procedures necessary to perform each laboratory investigation. You should also be mindful of the activities of other students around you. Frequently it is an accident caused by one person that endangers other people in a laboratory.

Some Basic Rules of Safety for the Laboratory

1. Use common sense.
2. Avoid horseplay in the laboratory.
3. Never eat, drink, or smoke in the laboratory.
4. Always wash your hands for at least fifteen (15) seconds and rinse them well after handling chemicals or live or preserved animals.
5. Always wear close-toed shoes in the laboratory. Sandals or open-toed shoes are not appropriate.
6. Be familiar with the location, operation and proper use of fire extinguishers, eyewash fountains, safety showers, and other safety equipment in the laboratory.
7. Know the location of emergency exits and the evacuation routes to be used in case of an emergency.
8. Always be cautious when using electric hot plates and gas burners. You can get a serious burn by touching a hot surface or by spilling a hot liquid.
9. Use protective mittens or tongs to handle hot objects.
10. Be cautious when transferring liquids because aerosols may be formed, which can be dangerous to your eyes and lungs.
11. Wear safety goggles or other appropriate protective eye gear when performing or observing experiments or demonstrations.
12. Be familiar with the properties of, and hazards associated with, all chemicals used in the laboratory exercises. When you are in doubt about the hazards associated with any chemical, consult the Material Safety Data Sheets (MSDS) provided by the manufacturer of every chemical substance. Your laboratory instructor should provide you with appropriate warnings for the materials to be used, but you may also ask to see the MSDS for any chemical to be used if you need further information.
13. Beware of electrical equipment with frayed or bare wires or with faulty switches or plugs. Report such damaged items to your instructor.
14. Always work in a well-ventilated area when studying preserved specimens.
15. Make sure that all specimens you dissect are properly secured in a dissecting pan or appropriate surface. A specimen not properly secured might slip and lead to an injury from a scalpel or other sharp dissecting instrument.
16. Keep scalpel blades sharp to avoid slipping and possible injury.
17. All broken glass should be placed in a container designated as a glass receptacle or sharps container.
18. Any contact with human blood should be reported promptly to your instructor to limit your exposure to possible infection.
19. Clean all laboratory tables and other work surfaces after each use.
20. USE COMMON SENSE, and remember that you are responsible for the safety of yourself and your coworkers.

Safety Precautions When Using Preserved Animals

The chemicals used to preserve animals and parts of animals can be toxic, flammable, and/or dangerous if used improperly or under improper conditions. Ethanol, isopropanol, formaldehyde, phenol, and ethylene glycol are commonly used preservatives. Combinations of these and other solvents are contained in embalming fluids used to preserve larger animals.

It is very important for students and instructors working with preserved specimens to understand the proper precautions and conditions for safe usage of such materials. All instructors are responsible for implementing proper safety procedures when students will be using potentially hazardous chemicals and for communicating appropriate information about these materials to their students in accordance with applicable federal, state, and local regulations. In recent years these regulations have greatly increased in complexity as a result of increased public concern about environmental health and safety.

The following information, supplied through the courtesy of the Carolina Biological Supply Company, provides some excellent safety guidelines to follow when handling and dissecting preserved animal specimens. Other suppliers use similar chemicals for their preserved animal specimens. You should study carefully the safety information supplied with any preserved specimens before you begin to handle or dissect them.

To achieve the necessary level of safety in the laboratory, each instructor should be familiar with all chemicals present and the necessary precautions to be taken in using them.

Carolina provides specimens preserved in alcohol, *Carosafe*™ (contains ethylene glycol), and formalin solutions. Information is provided in the catalog regarding which particular preservative is used with a certain type of specimen. Note that specimens are never provided in a formalin preservative unless this is specifically requested by the customer. Note also that the specimens that are preserved with embalming fluids and are never treated with *Carosafe*™, are provided with a specific Material Safety Data Sheet (MSDS) prepared for that specific embalming fluid. Regardless of the preservative that is used, we recommend you follow these safety tips whenever working with preserved specimens:

1. Wear appropriate protective eyewear at all times.
2. Wear appropriate protective equipment such as gloves and lab coats.
3. Work only in a well-ventilated area.
4. Prohibit eating, drinking, and smoking in the work area.
5. In the event of contact, wash skin with soap and water, flush eyes with water.
6. If overexposure to any chemical occurs, seek medical attention immediately.
7. Be careful with sharp objects such as pins, scalpels, and the spines and teeth of specimens.

Formalin preserved or embalmed specimens should always be used in a well-ventilated area to prevent irritation to eyes, skin, or respiratory tract. The use of goggles lessens eye irritation from formaldehyde vapors. If direct contact to eyes or skin occurs, wash thoroughly with water.

Isopropanol is very flammable, so avoid all sparks, open flames, and excessive heat.

Although it is unlikely to be ingested, ethylene glycol can be toxic if taken orally. Due to the low vapor pressure of ethylene glycol, it is very unlikely that any vapors would ever be encountered, but vapors may be a problem if the liquid is heated to excessive temperatures. We know of no reason that this should occur under normal conditions of use.

When working with preserved materials, be careful with sharp objects such as pins, scalpels, and the spines and teeth of specimens. When using a scalpel, we recommend cutting away from oneself and ensuring that fingers are kept out of the cutting path at all times.

Carolina preserved specimens are available in *Carosafe*™, an ethylene glycol based shipping and holding fluid. *Carosafe* is not a fixative; it is a preservative designed to prevent mold and tissue deterioration after the tissue has been properly fixed with formalin. *Carosafe* is an effective substitute for the standard formalin preservative and acts to hold the unpleasant odor of formaldehyde to an absolute minimum. Additionally, Carolina preserved animals may be ordered "damp-packed." Our tradename for this improved method of packaging is Caropak. Preserved animals shipped in Caropaks have been processed with Carosafe, and are as "odorless" as effective fixation and preservation techniques allow.

The following table contains further safety and health information regarding the three most common chemicals used by Carolina in the preservation process. This information is given in the form of a columnar table that contains all of the information required by OSHA to be present on a Material Safety Data Sheet (MSDS) under the Hazard Communication Standard (29 CFR 1910.1200).

Comparative Safety of Preservatives

	Formaldehyde	Isopropanol	Carosafe™ (Ethylene Glycol)
Physical Data			
Hazardous Components (OSHA—1994)	Methanol (TWA 200 ppm) Formaldehyde (TWA 0.75 ppm)	Isopropanol (TA 400 ppm)	Ethylene Glycol (TWA = 50 ppm Ceiling concentration)
Flash Point	184° Fahrenheit (Combustible)	53° Fahrenheit (Flammable)	241° Fahrenheit
Lower Explosion Limits LEL	7%	2%	3.2%
Fire Extinguishing Media	Alcohol Foam, Water Fog, Carbon Dioxide, Dry Chemical	Alcohol Foam, Carbon Dioxide, Dry Chemical	Water Fog, Carbon Dioxide, Dry Chemical
Unusual Fire or Explosion	Vapor heavier than air, may travel along ground to distant ignition source and flash back.	No unusual fire hazards noted. Closed containers exposed to fire may explode.	None
Threshold Limit Value (TLV) ACGIH	200 ppm (TWA) Methanol 0.3 ppm Ceiling Formaldehyde	400 ppm (TWA)	50 ppm Ceiling
Effects of Overexposure			
Eyes	Vapor causes severe irritation, redness, tearing, blurred vision. Liquid may cause severe or permanent damage.	Direct contact may cause irritation.	Direct contact may cause irritation.
Skin (Contact)	Irritation, dermatitis, strong sensitizer.	Mild irritation possible.	Mild irritation possible.
Inhalation	Irritation of respiratory tract, dyspnea, headache, bronchitis, pulmonary edema, gastroenteritis.	Irritation of respiratory tract, headache, and at high concentrations, narcosis.	Reported irritant effects at extremely high (10000 mg/cubic meter) concentrations of vapor.
Ingestion	May be fatal or cause blindness if ingested. LD50 (oral-rat) = 500 mg/kg (RTECS, 1986)	May cause nausea, vomiting, headaches, dizziness, gastrointestinal irritation. LD50 (oral-rat) = 5045 mg/kg (RTECS, 1986)	May be harmful or fatal if ingested. Ethylene glycol has been reported as causing liver and kidney damage when ingested LD50 (oral-rat) = 4700 mg/kg (RTECS, 1986)
Chronic Effects	Listed by the National Toxicology Program (NTP) as reasonably anticipated to cause cancer in humans. Also listed by IARC and OSHA as possible human carcinogen.	Not listed as causing cancer by NTP, IARC, or OSHA. No other chronic effects noted.	Not listed as causing cancer by NTP, IARC, or OSHA. No other chronic effects noted.
Target Organs	If inhaled, eyes, nasal passages, throat.	None	Liver and kidneys (if ingested)
First Aid Measures	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If ingested, if conscious, immediately induce vomiting. If eye or skin contact, immediately flush with flooding amounts of water for at least 15 minutes. Seek medical attention for all instances of overexposure to this chemical.	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If ingested, if conscious, immediately induce vomiting. If eye or skin contact, immediately flush with flooding amounts of water for at least 15 minutes. Seek medical attention for all instances of overexposure to this chemical.	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If ingested, if conscious, immediately induce vomiting. If eye or skin contact, immediately flush with flooding amounts of water for at least 15 minutes. Seek medical attention for all instances of overexposure to this chemical.
Spill Control Measures	If a spill occurs, cleanup personnel should wear full protective clothing and NIOSH approved self-contained breathing apparatus. Eliminate sources of ignition. Keep non-essential personnel away. Absorb spilled material on vermiculite or other suitable absorbent. Containerize for disposal.	Eliminate sources of ignition. Cleanup personnel should wear proper protective clothing and equipment to avoid contact with liquid. Respiratory protection may be required. Absorb material on activated carbon or other suitable absorbent. Containerize for disposal. Flush area of spill with water.	Cleanup personnel should wear proper protective clothing and equipment to avoid contact with liquid. Absorb material on vermiculite or other suitable absorbent material. Containerize for disposal. Flush area of spill with water.
Disposal	Dispose in accordance with all applicable local, state, and federal regulations. Contact local or state waste agencies if disposal questions arise.	Dispose in accordance with all applicable local, state, and federal regulations. Contact local or state waste agencies if disposal questions arise.	Dispose in accordance with all applicable local, state, and federal regulations. Contact local or state waste agencies if disposal questions arise.
Personal protection	Wear gloves, lab coat, splash goggles and any other appropriate equipment suggested by the laboratory supervisor.	Wear gloves, lab coat, splash goggles and any other appropriate equipment suggested by the laboratory supervisor.	Wear gloves, lab coat, splash goggles and any other appropriate equipment suggested by the laboratory supervisor.
Storage Information	Store tightly closed in a location suitable for general chemical storage.	Store in a location suitable for flammable liquid storage.	Suitable for storage in a general chemical storage area.

TWA—Time Weighted Average; ACGIH—American Conference of Governmental Industrial Hygienists; IARC—International Agency for Research on Cancer; OSHA—Occupational Safety and Health Administration; PEL—Permissible Exposure Limit; NIOSH—National Institute for Occupational Safety and Health; RTECS—Registry of Toxic Effects of Chemical Substances. LD50—Lethal Dose for 50% of a population.

Source: Carolina Biological Supply Company, 2700 York Road, Burlington, North Carolina, 27215, 910-584-0381

Handling and Care of Animals in the Laboratory

The study of anatomy and physiology of animals is fundamental to the training of zoology students. Many students find that working with living and preserved animals is one of the most interesting and beneficial aspects of their education. Prospective employers in business and industry, and admissions committees of graduate programs, as well as medical, dental, and veterinary schools, have frequently emphasized the importance of such practical experience.

Research with laboratory animals has led to important scientific advances in physiology, genetics, behavior, nutrition, ecology, and other fields. Advances in human medicine that are direct results of experimentation involving animals include immunization against polio, diphtheria, measles, and other diseases; insulin production and therapy; blood transfusions; chemotherapy; electrocardiography, open-heart surgery, and artificial heart valves; organ transplantation; and kidney dialysis.

Major advances in veterinary medicine resulting from experimentation with animals include the development of vaccines for rabies, distemper, swine cholera, and brucellosis; medication for dog heartworms; artificial insemination, *in vitro* fertilization, and embryo transfer technology; methods for preserving endangered species; and surgical techniques for hip replacement. These veterinary advances have saved thousands of animal and human lives and have contributed greatly to the human food supply and to the quality of life of farm and companion animals.

Studies of animals from textbooks, photographs, charts, models, and computer simulations are good supplements, but they are not adequate substitutes for actual laboratory experience with living and preserved animals. Zoology students need to learn and practice proper methods to observe, handle, care for, experiment with, and dissect laboratory animals. Consider the dilemma of a neurosurgeon who has never observed, handled, or dissected an actual brain, but who is about to do his or her first operation on a member of your family with a brain tumor.

The handling and treatment of vertebrate animals is regulated by federal law under the Animal Welfare Act of 1966, amended subsequently in 1970, 1976, 1985, and 1990. Additional regulations governing the use and care of laboratory animals have been developed by the National Institutes of Health. Many individual states also have laws governing animal use. Invertebrate animals are generally not covered under these laws, but such animals should also be treated with care and respect as living creatures. Rare and endangered species are protected by special laws and may not be collected or used in laboratory studies except under special permits. All teachers and researchers must be familiar with these federal and state regulations and be responsible for using good judgment and for following appropriate procedures for handling and experimenting with all animals.

As a responsible citizen and a student of zoology, you should also handle living and preserved animals with care and respect. When working with both vertebrate and invertebrate animals, you should always take adequate precautions to avoid causing unnecessary stress or discomfort to the animals due to your handling or experimenting. Any animals kept in the laboratory must have a clean and appropriate environment, including adequate ventilation, food, water, and regular care. Be sure to follow the specific federal guidelines established for the care of animals kept in the laboratory for the duration of an experiment. At the end of the experiment, the animals must either be disposed of in an approved humane manner or returned to a permanent animal care facility as directed by your instructor.

Some people oppose the use of animals in the laboratory either for training or research because they believe it is unethical for humans to use animals in any way that might be harmful or detrimental to the animals for the benefit of humans or other animals. Appropriate usage of animals has been one of the most active controversies in the United States and elsewhere during the past several years.

Such opponents of animal use seek to reduce or eliminate the use of animals in teaching and research based on their convictions. They often cite alternatives to the use of animals in research and testing, such as computer simulations, models, films or videos, tissue culture, and *in vitro* chemical tests, as effective substitutes. While many scientists agree that alternatives to the use of animals are effective in some cases, no adequate alternatives are available in many other cases. Most scientists agree that the rational use of animals for teaching and research continues to be essential for the progress of human health and welfare. This position has been endorsed by several prestigious scientific bodies, including the American Society of Zoology, the American Association for the Advancement of Science, the Society of Sigma Xi, the National Science Teachers Association, the National Association of Biology Teachers, and several state academies of science.

The continuing controversy over the use of animals for teaching and research, as well as the escalating costs of obtaining and caring for laboratory animals, has already resulted in substantial reductions in the number of animals used for study and in research and improvements in the care and handling of animals in the laboratory. Concerns over the use of animals have also led to numerous governmental regulations on the use and handling of animals in the laboratory. Therefore, in addition to learning about the animals themselves, zoology students must also learn the rules and methods for the proper care and handling of the animals.

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Part I

Animal Cells, Tissues, and Development

Chapter One

Microscopy

Objectives

After completing the laboratory work in this chapter, you should be able to perform the following tasks:

1. Identify the main parts of a compound microscope and explain their function.
2. Define and explain focus, working distance, resolving power, and magnification.
3. Describe the proper use and care of both compound and stereoscopic microscopes.
4. Explain the difference between compound and stereoscopic microscopes and give examples of appropriate uses of each type.
5. Use both compound and stereoscopic light microscopes in the correct way.
6. Explain the operating principles of a phase contrast microscope and give examples of its use.
7. Describe the two main types of electron microscopes and give examples of their use.
8. Explain the importance of microscopes in biological studies.

The Compound Microscope

The **compound microscope** is one of the most important and useful tools of the zoologist. It is used to study cells and cell parts, the organization of tissues, the structure of bone, and the structure of developing embryos, among many other important applications. Since many of the exercises in this course will require the use of the compound microscope, it is important to review some aspects of its construction, use, and care.

A modern compound microscope is illustrated in figure 1.1. Since there are numerous makes and models of compound microscopes in use, the microscope assigned for your use may differ slightly from the one illustrated. The operating principles and procedures, however, will be similar to those outlined later. Your laboratory instructor will point out any important differences between your microscope and the one illustrated.

A microscope is an expensive precision instrument and must be handled with care. Always carry your microscope **with both hands.** Grasp the arm of the microscope firmly with one hand and support the base with the other hand. Place the microscope carefully on your table with the arm facing you. Do not "clunk" it on the table.

Materials List

Living specimens
- *Artemia* (brine shrimp) larvae

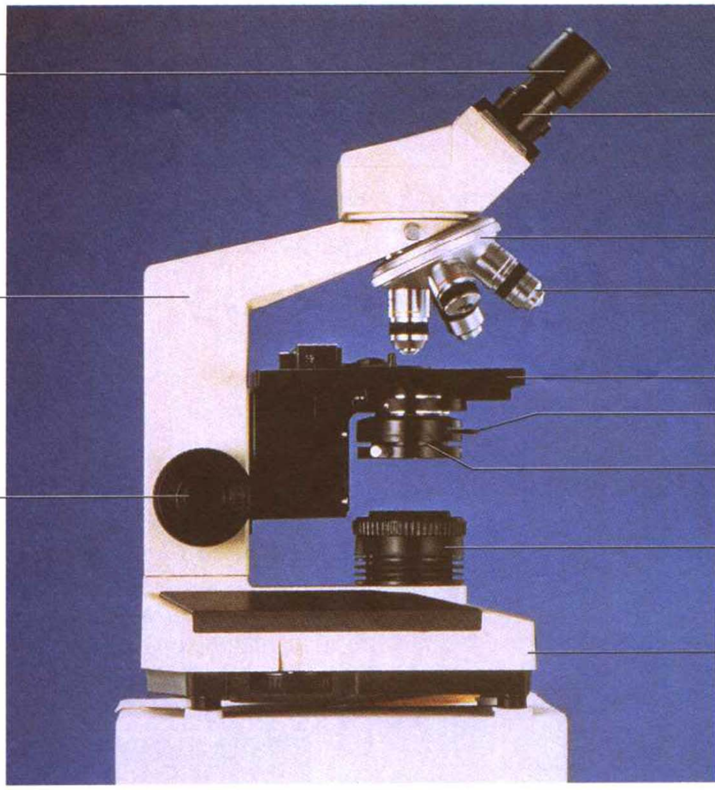
Prepared microscope slides
- Letter "e"
- Frog blood

Audiovisual materials
- Wall charts showing parts of compound and stereoscopic microscopes

Parts of the Microscope

Identify the principal parts and controls of the microscope with the aid of figure 1.1. At the top of the microscope is the **eyepiece,** or ocular lens, which is inserted in an inclined **body tube.** Microscopes with one eyepiece and body tube are monocular microscopes; those equipped with two eyepieces and body tubes for simultaneous viewing with both eyes are binocular microscopes. Below the eyepiece is the **arm** attached to the **base.** Also attached to the arm is the movable **stage,** which holds a microscope slide or other object for viewing. **Stage (slide) clips** aid in holding the slide in position on the stage. Above the stage is the **revolving nosepiece** with two or more **objective lenses.** Within the stage is another lens system, the in-stage **condenser,** which serves to concentrate light rays from the built-in **illuminator.** On the base near the illuminator is the **light switch.** Instead of a built-in illuminator, some microscopes have a **substage mirror** to reflect light from an auxiliary light source.

Figure 1.1 Compound microscope.
Courtesy of the Olympus Corporation.



Beneath the condenser, locate the **disc aperture diaphragm.** Rotating the disc diaphragm increases or decreases the amount of light on the specimen. More expensive microscopes often have an **iris diaphragm** with movable elements instead of a disc diaphragm. Raising and lowering the condenser also regulates the illumination of the specimen, although in most of your work in this course you will obtain satisfactory results by adjusting the condenser to the position that gives maximum illumination (usually near its uppermost position) and then making any further needed reductions in illumination with the disc diaphragm. This simplified method of controlling light does not produce the precise illumination required for advanced microscopy, but it produces results satisfactory for most routine purposes.

Accurate observation of a specimen requires positioning the objective lens at a specific distance from the specimen; this distance is determined by the specific construction of each objective lens and is called the **working distance** of the lens. When the objective lens is located at the proper working distance, the specimen will be in **focus.** The working distance of the lens varies **inversely** with the magnification of the objective; low-power objectives have longer working distances and high-power objectives have shorter working distances.

Focusing the lens system is accomplished by mechanically changing the distance between the specimen and the objective lens. Coarse and fine **adjustment knobs** for this purpose are provided on the side of the arm near the base. On some modern microscopes both coarse and fine adjustments are controlled by a single knob with dual function, but most models have separate controls. How many focusing control knobs are there on your microscope?

When you observe a specimen through a compound microscope, several images are formed by the optical system of the microscope. Three of these images are of special importance—the **real image,** the **virtual image,** and the **retinal image.**

The **real image** is formed at a specific distance above the objective lens. What you actually see when you look through the objective lens is the **virtual image,** which appears both larger and farther away than the specimen on the stage. The **retinal image** is formed by the rays of light striking the retina of your eye. The virtual image can be helpful to you in estimating the size of an object viewed through a microscope as explained later.

Magnification

The principal purpose of a microscope is to magnify the image of an object. The **magnification** of an object is determined by the construction of the ocular and objective lenses of the microscope, and the total magnification is the product of the separate magnification of these two lenses.