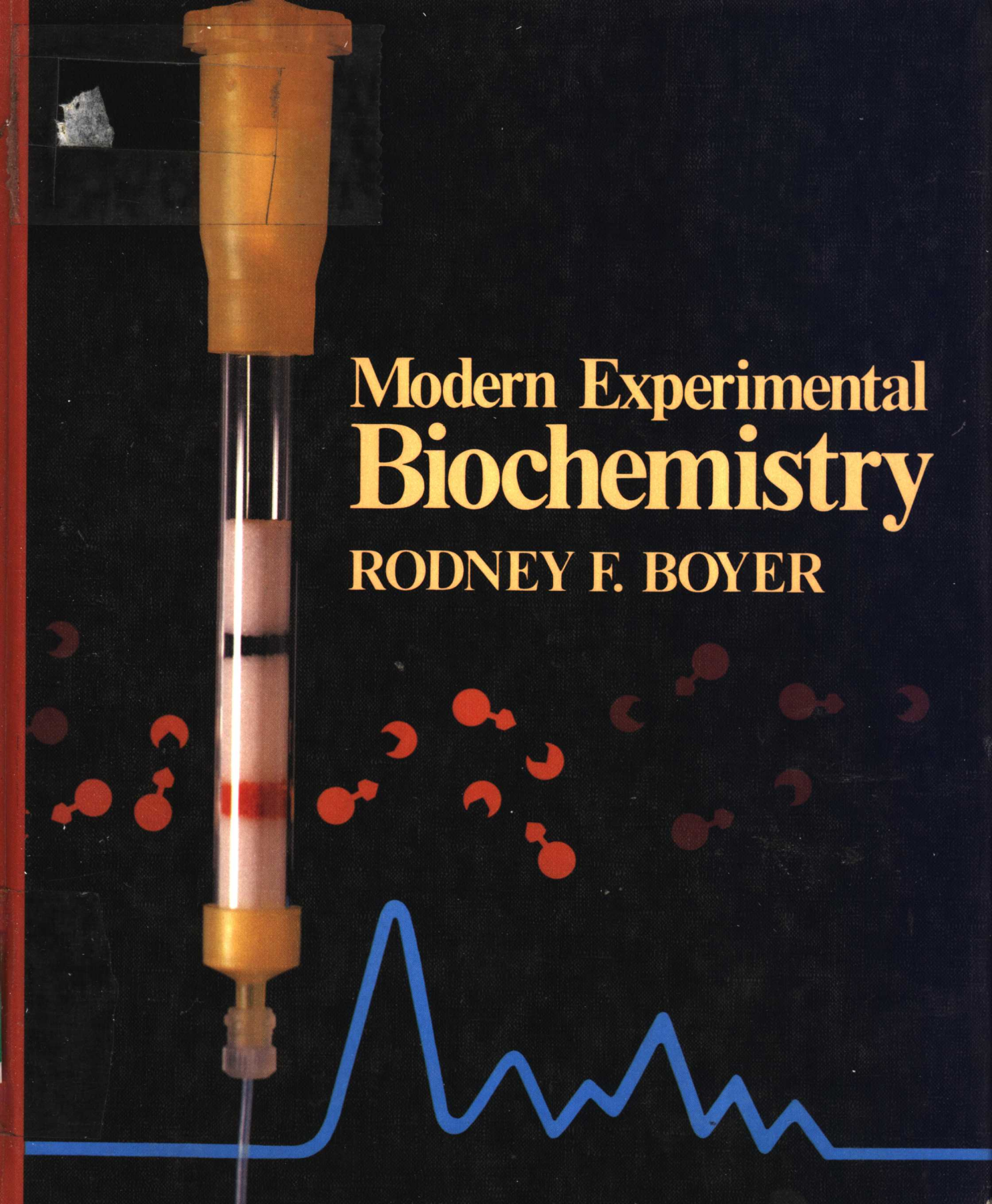
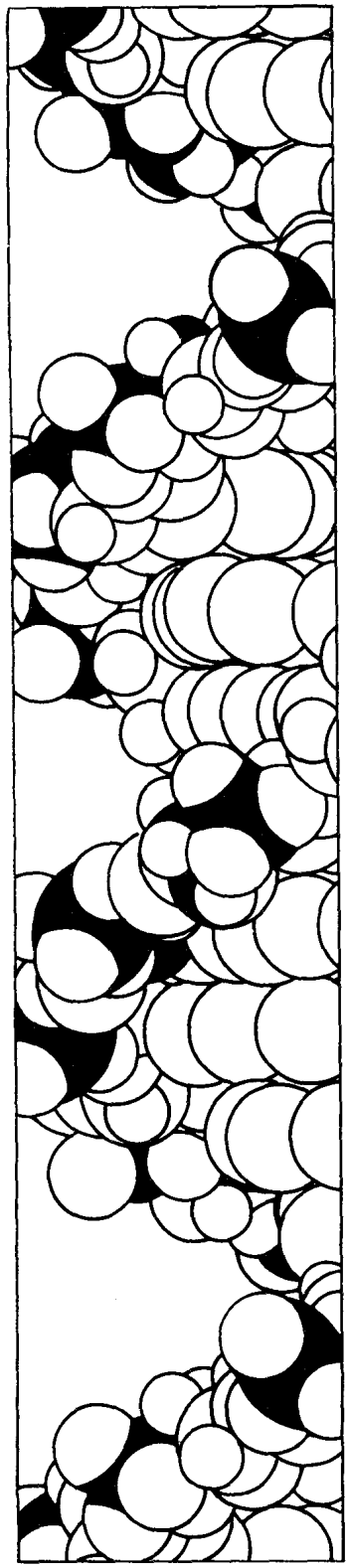


Modern Experimental Biochemistry

RODNEY F. BOYER



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Hope College

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Preface

The introduction of modern instrumental methods has revolutionized experimental biochemistry. The training of future life scientists must include both a theoretical and practical exposure to current biochemical techniques and concepts. The purpose of this textbook is to give students a thorough experience in modern experimental biochemistry. The book is designed for a teaching laboratory, accompanying a lecture course for junior-senior students. Its primary use will be in the undergraduate curriculum for students who have had one year of organic chemistry with laboratory. These students are principally in chemistry, biochemistry, biology and preprofessional (premedical, predental, preveterinarian) programs. This type of laboratory is probably offered in every four-year college and university, but the differences among these courses are vast. Biochemical equipment, staff expertise, scheduling, and student backgrounds vary considerably; hence, present laboratory courses are tailored to match local realities. This laboratory textbook was designed to respond to the needs, desires, and equipment limitations present in most biochemical teaching laboratories. Every effort has been made to produce a book that not only trains students in modern biochemical techniques, but one that does so in a flexible and generally usable fashion.

The book is divided into two parts. Part I, Theory and Experimental Techniques, introduces students to background material that is usually not provided in lecture courses nor readily available in existing laboratory textbooks. Since some laboratory procedures are used in several experiments, all of this information is consolidated in Part I for easy reference. Basic theory and practical information are offered for each experimental technique. This section will also become a useful reference for students during future research work.

Twenty-six experiments representing all areas of biochemistry are included in Part II. This broad coverage should lead to a well-rounded experience for the student and also allow the instructor to choose experiments that are appropriate or adaptable under a variety of local conditions.

The general outline for each experiment is as follows.

INTRODUCTION, THEORY, AND OBJECTIVES

In this section it is assumed that a student has studied the general subject in lecture. Only a summary or review of significant aspects of background is included. The general discussion includes practical information

that is generally not available in biochemistry textbooks. The general thrust of the experiment is explained, and a flow chart of the experiment is presented, if appropriate. This outline of the experiment allows the student to recognize the importance of each part of the experiment to the achievement of the overall objective.

EXPERIMENTAL

Following a list of all materials and supplies is a carefully written procedure for the experiment. It is divided into logical parts for ease of completion and to facilitate the interruption of an experiment, if necessary.

ANALYSIS OF RESULTS

In this section the student is instructed in the proper collection and handling of data from the experiment. Each table or graph that is to be constructed is explained and sample calculations are outlined. Typical data for the experiment may be disclosed to the student but only to aid the student in interpretation of results.

QUESTIONS AND PROBLEMS

Several questions and problems are provided at the end of each experiment. Some questions will deal with various details of the experiment, and numerical problems are emphasized.

In the development of each experiment, primary consideration was given to the use of modern procedures and techniques. Students will learn procedures that are now used in actual laboratory settings, whether academic or industrial. Each experiment presents a challenging laboratory situation or problem to be solved by the student. In each experiment, a technique is introduced that allows students to obtain and evaluate some property of a biomolecule or biosystem. For each set of experiments dealing with a particular class of biomolecules, students proceed through three levels of investigation:

1. Isolation and purification of a macromolecule or cell fraction/organelle
2. Physical and chemical characterization of the macromolecule or cell fraction
3. Biological characterization or study of dynamic function

If students proceed through these three levels, they will gain an understanding and appreciation of the essence of biochemistry—the relationship between chemical structure and biological function.

Sequences of experiments that illustrate this process are:

I. Structure and Function of α-Lactalbumin

EXPERIMENT 4: Isolation and Purification of α-Lactalbumin, a Milk Protein

EXPERIMENT 5: Characterization of α-Lactalbumin

EXPERIMENT 8: Evaluation of a Regulatory Enzyme System: Lactose Synthetase

II. Lipids

EXPERIMENT 9: Isolation, Purification and Partial Characterization of a Lipid Extract from Nutmeg

EXPERIMENT 10: Characterization and Positional Distribution of Fatty Acids in Triacylglycerols

EXPERIMENT 12: Characterization of Erythrocyte Membrane Using Lipolytic Enzymes

III. DNA

EXPERIMENT 15: DNA Extraction from Bacterial Cells

EXPERIMENT 16: Characterization of DNA by Ultraviolet Absorption Spectrophotometry

EXPERIMENT 21: Ethidium Fluorescence Assay of Nucleic Acids: Binding of Polyamines to DNA

IV. DNA Recombination Techniques

EXPERIMENT 17: Growth of Bacteria and Amplification of ColE1 Plasmids

EXPERIMENT 18: Preparative-Scale Isolation and Purification of ColE1 Plasmids

EXPERIMENT 19: The Action of Restriction Enzymes on a Bacterial Plasmid or Viral DNA

EXPERIMENT 20: Rapid, Microscale Isolation and Electrophoretic Analysis of Plasmid DNA

Following Parts I and II is an appendix containing useful information including physical properties of biomolecules and solutions to selected questions. An instructor's manual describing the preparation of all reagents is available from Addison-Wesley.

Holland, Michigan *R. F. B.*

Acknowledgments

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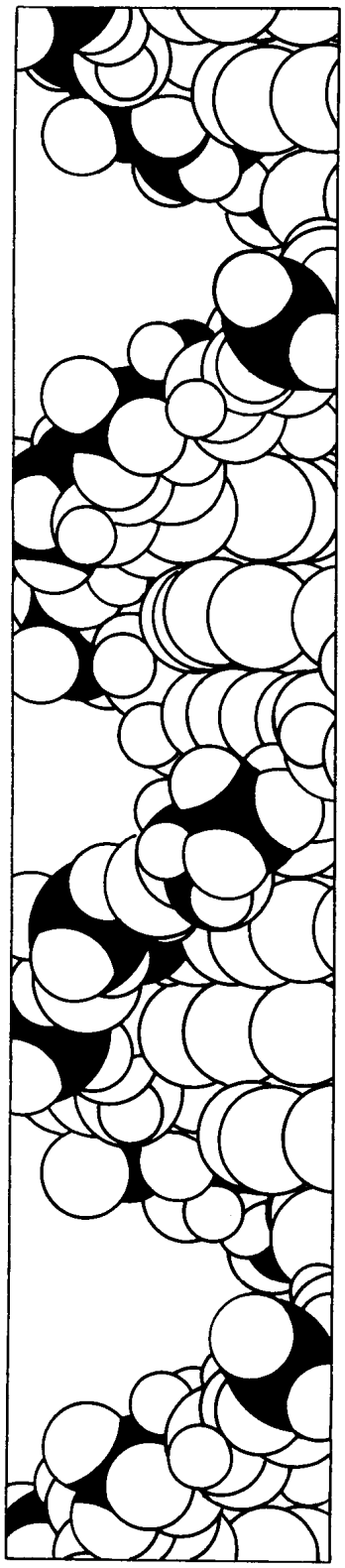

I

Theory and Experimental Techniques

Chapter **1**

Introduction to the Biochemistry Laboratory

Welcome to biochemistry laboratory! This is not the first chemistry laboratory course for most of you, but I believe you will find it to be among the most exciting and dynamic of those in which you have enrolled. Most of the experimental techniques and skills that you have acquired over the years will be of great value in this laboratory. However, you will be introduced to several new procedures. Your success in the biochemistry laboratory will depend, to a great extent, on your mastery of these specialized techniques and on your understanding of chemical-biochemical principles.

As you proceed through the schedule of experiments for this term, you will, no doubt, compare your work with previous laboratory experiences. In biochemistry laboratory you will seldom run reactions and isolate products on a gram-size scale as you did in the organic laboratory. Rather, you will work with milligram or even microgram quantities, and in most cases the biomolecules will be dissolved in solution so you never really "see" the materials under study. But, you will observe the dynamic chemical and biological changes brought about by biomolecules. The techniques and procedures introduced in the laboratory will be your "eyes" and will monitor the biochemical events occurring.

This chapter is an introduction to procedures that are of utmost importance for the safe and successful completion of a biochemical project. It is recommended that you become familiar with Sections A–F before you begin laboratory work.

A. SAFETY IN THE LABORATORY

The concern for laboratory safety can never be overemphasized. Most students have progressed through at least two years of laboratory work without even a minor accident. This record is, indeed, something to be proud of; however, it should not lead to overconfidence. You must always be aware that chemicals used in the laboratory are potentially toxic, irritating, or flammable. Such chemicals are a hazard, however, only when they are mishandled or improperly disposed. It is the author's experience that accidents happen least often to those students who come to each laboratory session mentally prepared and with a complete understanding of the experimental procedures to be followed. Since dangerous situations can develop unexpectedly, though, you must be familiar with general safety practices, facilities, and emergency action. Students must have a special concern for the safety of classmates. Carelessness on the part of one student can often cause injury to other students.

The experiments in this book are designed with an emphasis on safety. However, no amount of planning or pretesting of experiments substitutes for awareness and common sense on the part of the student. All chemicals used in the experiments outlined here must be handled with care and respect. Some chemicals and procedures will require special attention. When this is the case, a precautionary note will appear at the beginning of the experimental procedure. Here, the dangerous properties of the chemicals will be described along with recommendations for safe handling, first aid, and proper disposal.

So that all students are equally aware of potential laboratory hazards, it is imperative that a set of rules be developed for each laboratory situation. The following can serve as guidelines.[1]

1. Some form of eye protection is required at all times. Safety glasses with wide side shields are recommended, but normal eyeglasses with safety lenses may be permitted. *Contact lenses should never be worn in the laboratory.* Naturally, there is controversy surrounding the use of contacts (see Kingston, 1981). The major problem with contact lenses is that they reduce the rate of self-cleansing of the eye. If a chemical splatters into the eye of a contact lens wearer, he or she will possibly have greater injury because of less effective irrigation of the eye. Furthermore, the wearer may be reluctant to use an eye wash for fear of losing the contact lenses.

[1] Adapted from *Safety in Academic Chemistry Laboratories*, 3rd Edition, August, 1979. A publication of the American Chemical Society Committee on Chemical Safety.

2. Never work alone in the laboratory.
3. Be familiar with the physical properties of all chemicals used in the laboratory. This includes their flammability, reactivity, toxicity, and proper disposal.
4. Eating, drinking, and smoking in the laboratory are strictly prohibited.
5. Unauthorized experiments are not allowed.
6. Mouth suction should not be used to fill pipets or to start siphons.
7. Become familiar with the location and use of standard safety features in your laboratory. All chemistry laboratories should be equipped with fire extinguishers, eye washes, safety showers, fume hoods, chemical spill kits, first-aid supplies, and containers for chemical disposal. Any questions regarding the use of these features should be addressed to your instructor or teaching assistant.

Rules of laboratory safety are not designed to impede productivity nor should they instill a fear of chemicals or laboratory procedures. Rather, their purpose is to create a healthy awareness of potential laboratory hazards and to improve the efficiency of each student worker. The list of references at the end of this chapter includes books and manuals describing proper and detailed safety procedures.

B. THE LABORATORY NOTEBOOK

The biochemistry laboratory experience is not finished when you complete the actual experimental procedure and leave the laboratory. All scientists have the obligation to prepare written reports of the results of experimental work. Since this record may be studied by many individuals, it must be completed in a clear, concise, and accurate manner. This means that procedural details, observations, and results must be recorded in a laboratory notebook *while* the experiment is being performed. The notebook should be hardbound with quadrille ruled (gridded) pages, and used only for biochemistry laboratory. This provides a durable, permanent record and the potential for construction of graphs, charts, etc. It is recommended that the first one or two pages of the notebook be used for a constantly updated Table of Contents. Although your instructor may have his or her own rules for preparation of the notebook, the most readable notebooks are those in which only the right-hand pages are used for record-keeping. The left-hand pages may be used for your own notes, reminders and calculations. The following outline may be used for each experimental write-up. The basic outline follows that required by most biochemical research journals. Note that Parts I–IIb are labeled as Prelab and should be completed before you enter the laboratory.

Brief Outline of Experimental Write-up

Prelab

I. Introduction
   (a) Objective or purpose
   (b) Theory
   (c) References

II. Experimental
   (a) Table of materials and reagents
   (b) List of equipment
   (c) Record of procedure

III. Data and Calculations
   (a) Record of all raw data
   (b) Method of calculation with statistical analysis
   (c) Present data in tables, graphs, or figures when appropriate

IV. Results and Discussion
   (a) Conclusions
   (b) Compare results with known values
   (c) Discuss the significance of the data
   (d) Was the original objective achieved?

Details of Experimental Write-up

I. Introduction

This section begins with a three- or four-sentence statement of the objective or purpose of the experiment. For preparing this statement, ask yourself, "What are the goals of this experiment?" This statement is followed by a brief discussion of the theory behind the experiment. If a new technique or instrumental method is introduced, give a brief description of the method. Include chemical or biochemical reactions when appropriate. Any references (textbooks, journals, etc.) used to prepare this section should be listed at the end.

II. Experimental

Begin this section with a list of all reagents and materials used in the experiment. The sources of all chemicals and the concentrations of solutions should be listed. Instrumentation is listed with reference to company name and model number.

The write-up to this point is to be completed as a Prelab. The experimental procedure followed is then recorded in your notebook as you proceed through the experiment. The detail should be sufficient so that a