EXPERIMENTAL BIOCHEMISTRY

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Dedicated to the Memory of
Robert L. Dryer
1921–1984

This book is a resultant of two separate volumes used together in our course for several years. The original theoretical component was written as a syllabus by Dr. Dryer shortly before his untimely death; the experimental section was in large part also assembled by him. Both portions have been revised during the preparation of this volume, but the genesis of the idea for this product must properly be attributed to Dr. Dryer.

ACKNOWLEDGMENTS

I would like to acknowledge the good-natured patience and efficiency of Brenda Kunkel and Cay Wieland in typing the manuscript, as well as the competence of Todd Erickson in drawing the illustrations.

Introduction

From its beginning, biochemistry has been a largely experimental science. Early biochemists were forced, by the crudity of their tools and methods, to work with whole plants or animals. As the field progressed, successive generations of biochemists were enabled to study tissue slices, tissue homogenates, isolated organelles, and even what are now popularly known as "molecular" systems. This search for ever simpler systems arose from the belief that the simpler the system the more precisely it could be defined and studied.

Recently, as part of the generalized fusion of the biological sciences, we have seen a return to more complex systems such as tissue culture, in which intact cells may be isolated, grown, harvested, and then marvelously manipulated. Even more complex systems, such as isolated pancreatic islets, are now considered to be effective biochemical tools for certain studies. Bacteria are being "engineered" into producing materials that are rare and costly when produced by more traditional methods. With all of this, we now have analytical methods capable of dealing with nanogram (and frequently with subnanogram) quantities of materials.

Unfortunately, however, baccalaureate students of today get an all too limited exposure to basic laboratory science. It is time-consuming and very expensive to mount a good experimental course using modern tools and techniques. Student time is at a premium, given the pressures of other courses and legitimate interests. In spite of these difficulties, we have a strong belief in the value of laboratory training in baccalaureate as well as in graduate programs. We hope to introduce young students to what is new and exciting, but at the same time we want to be sure they are reasonably grounded in what is not so new, perhaps not so exciting, but still very important. In this book and in the course from which it was derived, emphasis is placed not just on the operational mechanics of the experiments, but also on an understanding of the rationale behind each protocol and of the theoretical bases for the individual methods and procedures used. The book is divided into two parts. In section I are presented fundamental treatments of physical, chemical, and mathematical topics to serve as a background for the experiments in section II. At the beginning of each experiment, reference is made to those chapters in section I that

most directly pertain to the experiment; a student should study them together with any other assigned readings before coming to class.

The experiments in section II constitute a tested, one-semester course, which is required of our undergraduate majors usually in the first semester of their junior year. Most of the students have completed at least one semester of didactic biochemistry and are co-registered in another. This class involves one weekly lecture and two laboratory periods of four to eight hours each.

The experiments we have selected emphasize three major points: first, all systems that obey the laws of chemistry are "molecular"; second, it is necessary to be as quantitative as possible in all experimental work; third, basic principles and fundamental knowledge form the basis of all later professional work. Only a few of the later exercises deal with what might be called molecular biology; its full treatment requires an additional course dedicated to that purpose. We make no claim to originality in these experiments. Indeed, they all had their genesis in previously published research. Neither is this collection the work of any single individual. Since its inception, a number of our biochemistry faculty have made significant contributions. In particular, we want to recognize the efforts of Bryce Plapp, Peter Rubenstein, Earle Stellwagen, and Joseph Walder. Additionally, at least several dozens of graduate student assistants have helped us refine these protocols to their present state.

This program is not static; it will continue to evolve, reflecting technical developments in the tools of biochemistry as well as its expanding theoretical horizons.

Contents

Section I Theory

Chapter 11. Introductory Aspects of Molecular Biology

SECTION I

Theory