

EXPERIMENTS AND METHODS IN BIOCHEMISTRY

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Preface

Because biochemistry is an experimental science, it is essential that students have a thorough knowledge of basic biochemical techniques and procedures. The purpose of this manual is to acquaint advanced undergraduate students and beginning graduate students with some of these techniques and their application to the solution of biochemical problems by experimental means. Rather than covering all of biochemistry in a superficial, qualitative manner, this manual attempts to deal with selected topics in a detailed, quantitative fashion. Nevertheless, these topics cover a broad range of compounds of biological interest and a variety of sources of biological materials. The student will be introduced to many of the more important biochemical techniques, including colorimetry, chromatography, gas exchange, isotope assay, equivalence measurement, enzyme purification, and cell and tissue disruption techniques.

Proper execution of an experiment and the interpretation of the data obtained require a knowledge of the theory behind the experiment. Sections of theory and of pertinent references to the literature are therefore given for each experiment. Furthermore, a description of the principles of operation for commonly used instruments and the pitfalls involved in their use is presented, in the hope that the data obtained from these instruments will be more meaningful.

Another important facet of laboratory work is the treatment of data and calculation of results. To familiarize the student with biochemical calculations, problem sets relevant to the experiments are given. In addition, sections on reporting and calculating of results are included as a guide to the student.

Most of the experiments in this manual are based on a biochemistry laboratory course taught at Cornell by Dr. Walter Nelson and ourselves over the past four years. These experiments have been performed by many graduate and undergraduate students. The majority of these students have obtained excellent data for the experiments without difficulty.

The manual deliberately contains more experiments than can reasonably be accomplished in a one-semester laboratory course. Also, some of the experiments are divided into many detailed sections. This was done to give the manual flexibility and allow the instructor to select experiments which are most suited to the needs and interests of his students. The experiments are arranged in order of increasing complexity. The first two experiments ("Equivalence Measurements" and "Characterization of Glycogen and Amylopectin") may be used to acquaint the student with the laboratory and basic quantitative techniques prior to his exposure to the more difficult experiments.

Most of the experiments can be performed with rather modest equipment. Thus reasonably large numbers of students can be trained without an undue strain on the budget. Even so, we have found that it is practical to run several different experiments simultaneously to prevent an overload on the equipment.

Finally, it is hoped that this course will not only increase the students' laboratory expertise, but also impart some enthusiasm for research in biochemistry.

We wish to extend our sincere thanks to our colleagues who contributed many ideas and helpful criticisms to the content of this text, particularly Drs. Walter L. Nelson, Ray Wu, Michael Cusanovich, Donald B. Zilversmit, and Barry Hughes. Details of experiments were clarified greatly by the help of several teaching assistants, especially Mr. Mark Anderson who also read numerous portions of the manuscript. We owe a debt of gratitude to those students who acted as guinea pigs for the experiments. This manuscript would never have appeared without the generous cooperation of our typist, Mrs. Joyce Broadhead. We are thankful to Mrs. Vickie Shaff for typing many of the rough drafts. Lastly, but not least, we appreciate the patience of our wives and families during the writing of this text.

D. C. W.
R. E. M.

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PART I

Introduction to Experimental Biochemistry
