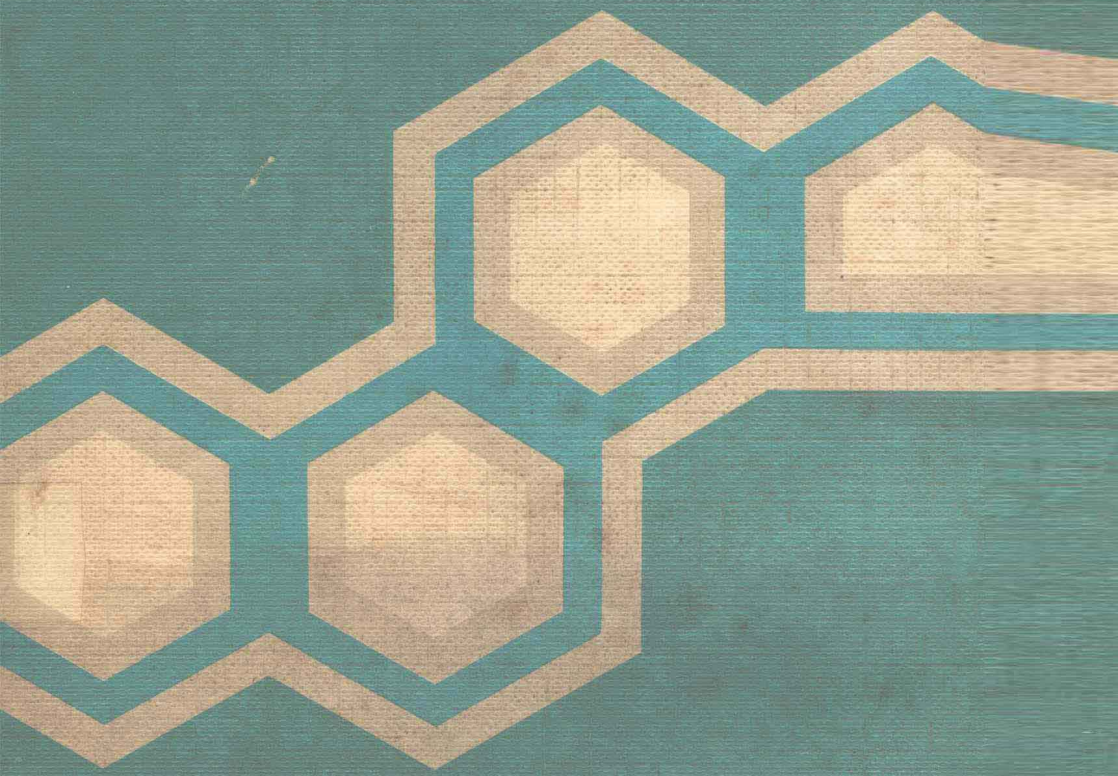
An Introduction to Practical Biochemistry

Second Edition

David T. Plummer



An introduction to practical biochemistry

Second edition

David T Plummer
Senior Lecturer in Biochemistry
Chelsea College, University of London

McGRAW-HILL Book Company (UK) Limited

London . New York . St Louis . San Francisco . Auckland . Beirut . Bogotá
Düsseldorf . Johannesburg . Lisbon . Lucerne . Madrid . Mexico . Montreal
New Delhi . Panama . Paris . San Juan . São Paulo . Singapore . Sydney
Tokyo . Toronto

Published by
McGRAW-HILL Book Company (UK) Limited
MAIDENHEAD . BERKSHIRE . ENGLAND

British Library Cataloguing in Publication Data

Plummer, David Thomas
An introduction to practical biochemistry.
2nd ed.
1. Biological chemistry — Laboratory manuals
I. Title
574.1'92'028 QP519.7 77-30195
ISBN 0-07-084074-1

2345 WC&S 80798

PRINTED AND BOUND IN GREAT BRITAIN

To Ruth, Malcolm, Jonathan and Martyn

Psalm lll v 2 (R.S.V.) Great are the works of the Lord, studied by all who have pleasure in them.

Preface to the Second Edition

An Introduction to Practical Biochemistry was first published at the end of 1971, and the time is now ripe for it to be revised and updated. The straight addition of new material and experiments would produce a larger and probably less useful and more costly book, yet there is still a need for a comprehensive practical book with a wide range of experiments to satisfy the various courses taught in the subject. The case for the inclusion of new subject matter was strong and the dilemma became what to eliminate from the original work. The author has attempted to solve this problem by removing some of the old or superfluous experiments and most of those needing specialized equipment. The new edition has thus been updated and much of it rewritten, yet it still contains a comprehensive range of basic experiments, it will therefore be suitable for teaching the fundamental concepts of practical biochemistry to large classes with the minimum of specialized apparatus. Some of the experiments are more suited to smaller groups, but can still be used in a large class by operating several of these experiments at the same time and moving groups of students from one set to another on a weekly basis.

Each chapter contains a summary of the relevant biochemistry and, in addition, every experiment has a section on the biochemical principles involved in that investigation. The book is meant to be more than just a 'cookery book' for use in the laboratory; it should provide a useful theoretical background to some of the more practical aspects of biochemistry and thus complement the standard theoretical text used by the student.

The experiments are suitable for the first two years of a British

BSc in biochemistry, for an MSc course which is largely a conversion course from another discipline, and for technical courses such as those leading to HNC, HND, and M.I. Biol. The book should also be useful to undergraduates and technicians studying biochemistry as a subsidiary or ancillary subject to their main course. It may also serve as a useful reference work for research workers in other fields whose investigations carry them into the unfamiliar area of practical biochemistry.

I must, of course, thank all my colleagues and associates who have made this book possible; many of them have kindly agreed to the inclusion of their experiments in this book for which I am most grateful. In particular, I would like to thank Dr Mike Perry and Dr John Wrigglesworth who have worked with me for several years in teaching the final year of our BSc. I am also indebted to Professor Tony Linnane and his colleagues of Monash University, Melbourne, Australia, with whom I spent a most useful and happy year of study leave. My thanks are also due to my colleagues Dr Malcolm Banner, Dr Peter Butterworth, Dr Derek Evered, and Dr Geoffrey Hall for helpful discussion and experiments and to Professor Harold Baum for his continued interest and encouragement. Finally I must thank my research assistants Dr Mitchell Fry, Dr Edwin Ngaha, Dr Charles Reavill, Dr Cecilio Vidal-Moreno, Mrs Claire Delpech, Miss Margaret Chai, and Miss Susan Seager, who have read the manuscript and proofs, and my technician Mr Paul Borella for carrying out some of the experiments. Last of all I must thank my wife Ruth for her love and encouragement and my family for all their patience during the preparation of the manuscript.

David Plummer

Technical notes

Solutions

A list of solutions required to carry out an investigation is given for each experiment together with the quantities needed. The figure 10 or 100 printed on the same line as the heading 'materials' means that the weights and volumes have been calculated assuming 10 or 100 students working in pairs. The amount required for a given class can, therefore, be readily calculated from this information. In arriving at the quantities needed, it has been assumed that estimations will be carried out in duplicate. In addition, an extra allowance over and above the bare minimum has been added and the solutions made up to the next most convenient volume. For example, if 300 ml is required for an experiment, then the recommended volume is given as 500 ml, but if 400 ml is needed then the volume suggested is 1 l.

Experiments with 100 marked by the 'materials' can be carried out by a class of that size but where 10 is marked there is probably some limitation on the availability of equipment or expensive reagents.

Finally, these quantities assume that the reagents are divided equally among the class with a small quantity in reserve. If reagents are left on a bench and people help themselves then much bigger quantities will need to be made up.

Laboratory Safety

In this book any obvious dangers in a particular experiment are pointed out, but some of the following general precautions are

worth noting. Always wear a laboratory coat and preferably safety spectacles when doing practical work.

Poisons

Don't be careless with poisons, always mark the danger on the solution of the toxic compound. It is recommended that any dangers inherent in the handling of a particular material be written plainly on the board in front of a class together with the common access routes to the body (i.e. mouth, lungs, skin, etc.).

All eating and drinking in the laboratory is, of course, strictly forbidden.

Never pipette poisons by mouth, use a safety bulb or dispense from a burette, preferably at one central point under academic or technical supervision. Safety pipettes should also be provided for handling corrosive materials such as strong acids or alkalis or highly reactive compounds, e.g., oxidizing and reducing agents.

Fire

The risks of fire are perhaps obvious when handling certain organic solvents, but vigilance is ever needed against this hazard. Inflammable solvents should not be evaporated over a naked flame and always check that a bunsen is not being used on an adjacent bench when handling solvents such as ether. Sparks from electrical equipment are a more subtle danger and, for this reason, solutions containing ether must not be centrifuged or left in a refrigerator. After use, organic solvents should be disposed of in special containers provided by the technical staff and never poured down the sink.

Biological hazards

The hazards involved in handling biological material are perhaps not quite so obvious as those mentioned above and it is the responsibility of the member of staff in charge of a class to point out any particular dangers. The booklet published by Imperial College (University of London) on precautions against biological hazards is very comprehensive and is recommended.

All *microorganisms* should be treated as potentially dangerous, but probably one of the greatest biological dangers is the risk of *serum hepatitis* (Australia antigen). Infection is effective primarily

through the blood but other body fluids can also transmit the virus. The mortality rate for this hepatitis can be as high as 30% of those infected and there is no known cure for the disease. Furthermore the disease can be carried by apparently healthy people so all apparatus in contact with human blood or urine should be sterilized immediately after use. The carrier rate may be as low as 0.1% (British population) or as high as 20% (some African populations). All human fluids should therefore be used with the greatest care and strict precautions taken.

Contents