A Biologist's Guide to Principles and Techniques of Practical Biochemistry

Third Edition

Edited by
Keith Wilson

and
Kenneth H. Goulding

Preface to the third edition

The decade that has passed since the first edition of this book was published has seen a quantum leap in our understanding of gene structure, gene expression and gene manipulation and has coincided with the emergence of biotechnology. The impact of the new technology associated with these developments has been to create great hopes for the diagnosis and control of numerous human genetic disorders, and for the introduction of commercially attractive characteristics into animal, plant and microbial cells. The developments have been made possible by the isolation and purification of numerous enzymes associated with nucleic acid metabolism, by the refinement and application of existing analytical techniques and the development of new ones. One of the greatest impacts has been made by the development of relatively simple procedures for the production of monoclonal antibodies which can be used in the detection and assay of specific proteins.

All of the principles and techniques associated with these new methodologies have quickly found their way into undergraduate curricula. Practical exercises based on them form an increasing component of courses in biochemistry, microbiology, genetics, plant physiology and immunology. We have attempted to respond to these developments by updating all chapters and by including appropriate new ones in this third edition. Chapter 1 has been expanded to cover a consideration of the rationale and methodology involved in *in vitro* and *in vivo* biochemical experimentation including cell and tissue culture, cryopreservation and the approaches to metabolic investigations. The chapter also considers the importance of mutants in biochemical studies and the applications of light and electron microscopy. Chapter 3 is a new chapter on Enzyme Techniques which covers the basic principles of enzymology, enzyme and substrate assays and ligand binding techniques. Chapter 4 on Immunochemical Techniques was first introduced in the second edition, but has been expanded to give appropriately greater consideration to monoclonal antibodies. Chapter 5 on Techniques in Molecular Biology is the second totally new chapter and considers the principles behind the recent developments in nucleic acid isolation, analysis and structure determination and of genetic manipulation including the isolation of specific genes, the production of gene libraries and gene cloning.

The new additions to the book have been made at the expense of the chapter on Manometric Techniques, the essential outlines of which are now

included in Chapter 1, and as a result of an increase in the total size of the book. The dilemma faced by all authors of undergraduate texts of balancing comprehensive cover against a reasonable text length and viable retail price is a difficult one to solve, but we hope that the moderate increase in the size of this new edition will be compensated by its wider appeal.

The general approach to the preparation of all the chapters remains unchanged from that of previous editions. Our aim was not to produce a comprehensive text for the specialist, but a general and, where necessary, simplified account for those students who have recourse to use some of the techniques during their undergraduate or postgraduate careers. Greatest attention has been given to those techniques which generally feature prominantly in undergraduate practical classes and less detailed coverage to other techniques which would be referred to in lectures and tutorials but which students are less likely to encounter in the laboratory. The main principles of the techniques and their associated instrumentation are discussed and reference given to their main applications and limitations. The book is intended for students on degree courses and Higher National Certificate and Higher National Diploma courses of BTEC in the biological, medical, paramedical and veterinary sciences in which biochemistry is an important component. It may also be of value to students on M.Sc. or other post-graduate courses who will be encountering biochemical techniques for the first time.

The third edition has been produced without the involvement of Bryan Williams who made such an invaluable contribution to previous editions. We are pleased to welcome Stephen Boffey as a first time contributor to the new edition. We would like to thank our two colleagues Dr Donald Bailey and Dr Michael Trevan for permission to reproduce their electron micrographs in Fig. 1.1. We are again indebted to the staff of Edward Arnold (Publishers) Limited, and particularly Nancy Loffler, for their continued enthusiastic support and helpful advice. We also gratefully acknowledge the unique scientific and linguistic skills of those people who have been responsible for the translation of previous editions into German, Italian, Russian and Spanish. We continue to welcome constructive comments and criticisms from all those who use the book as part of their studies.

Lancashire Polytechnic
The Hatfield Polytechnic
1986

Kenneth H. Goulding
Keith Wilson

Abbreviations and SI units

The following abbreviations have been used throughout this book without definition:

AMP	Adenosine 5′-monophosphate
ADP	Adenosine 5′-diphosphate
ATP	Adenosine 5′-triphosphate
DDT	2,2-bis-(*p*-chlorophenyl)-1,1,1-trichlorethane
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetate
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
mol. wt.	Molecular weight
NAD^+	Nicotinamide adenine dinucleotide (oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
Pi	inorganic phosphate
PPi	inorganic pyrophosphate
RNA	Ribonucleic acid
s.t.p.	Standard temperature and pressure
Tris	2-amino-2-hydroxymethyl propane-1,3-diol
e.m.f.	Electromotive force
e^-	Electron

Basic SI Units (Système International D'Unités)

Physical Quantity	*Name of SI Unit*	*Symbol*
Length	metre	m
Mass	kilogramme	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Amount of substance	mole	mol

Derived Units

Defined in terms of basic SI Units or other derived units.

Physical Quantity	*Name of Unit*	*Symbol*
Energy	joule	J
Force	newton	N
Pressure	pascal	Pa
Power	watt	W
Electric charge	coulomb	C
Electric potential difference	volt	V
Electric resistance	ohm	Ω
Frequency	hertz	Hz
Magnetic flux density	tesla	T
Area	square metre	m^2
Volume	cubic metre	m^3
Velocity	metre per second	$m\ s^{-1}$
Acceleration	metre per second squared	$m\ s^{-2}$
Density	kilogramme per cubic metre	$kg\ m^{-3}$
Electric field strength	volt per metre	$V\ m^{-1}$
Concentration	mole per cubic metre	$mol\ m^{-3}$
Magnetic field strength	ampere per metre	$A\ m^{-1}$
Dipole moment	coulomb metre	C m
Entropy	joule per kelvin	$J\ K^{-1}$

Volume

The SI unit of volume is the cubic metre, m^3. The litre has been redefined as being exactly equal to the cubic decimetre. Although the term litre still remains in common usage, it is recommended that both the litre and fractions of it (e.g. millilitre) are abandoned in exact scientific work.

1 litre (l) = 1 dm^3 = 10^{-3} m^3
1 millilitre (ml) = 1 cm^3 = 10^{-6} m^3
1 microlitre (μl) = 1 mm^3 = 10^{-9} m^3

Powers of Units – Prefixes

Multiple	*Prefix*	*Symbol*
10^9	giga	G
10^6	mega	M
10^3	kilo	k
10^2	hecto	h
10	deca	da

10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f

Conversion Table for Common Units to SI Equivalents

Unit	*SI equivalent*
ångström (Å)	100 pm = 10^{-10} m
atmosphere (standard) (760 mmHg at s.t.p.)	101 325 Pa
calorie	4.186 J
centigrade (°C)	(t°C + 273) K
Curie, Ci	3.7×10^{10} s^{-1}
erg	10^{-7} J
gauss (G)	10^{-4} T
micron, μ	1 μm
millimetre mercury (mmHg)	133.322 Pa
pound-force/sq in (lb f in^{-2}) (p.s.i.)	6894.76 Pa
ln x	2.303 $\log_{10} x$

Values of some physical constants in SI Units

Gas constant (R)	8.314 J K^{-1} mol^{-1}
Planck constant (h)	6.63×10^{-34} J s
Molar volume of ideal gas at s.t.p.	22.41 dm^3 mol^{-1}
Faraday constant (F)	9.648×10^4 C mol^{-1}
Speed of light in a vacuum (C)	2.997×10^8 m s^{-1}

Contents

1

General principles of biochemical investigations

1.1 Introduction

Biochemistry is concerned with understanding and exploiting the chemical unity and diversity of living organisms and seeks to correlate chemical structure with biological reactivity at the molecular, subcellular, cellular and organism level.

Biochemical studies have substantiated the cell as the fundamental unit of life, since it alone possesses all the characteristics for independent energy transformation and replication. A unifying feature of all cells is that they contain many common chemical constituents, common metabolic pathways, and common mechanisms of cellular regulation. For example, only twenty different amino acids are found in proteins, and the membranes obtained from different organelles or even species are similar in phospholipid composition. There is overall similarity also in the chemical structure and function of enzymes and in the various metabolic pathways associated with the synthesis and degradation of carbohydrate, lipid, protein and nucleic acids. These unifying principles have assisted in the development of theories on biochemical evolution and phylogenetic interrelationships between organisms (*comparative biochemistry*) and they form the basis for a mode of biochemical deduction, based on extrapolation of results obtained in one species (usually of lower phylogenetic order) to another. Thus microorganisms, animal tissue cultures or laboratory animals are frequently used for monitoring the biochemical, physiological, pharmacological or toxicological responses to foreign exogenous compounds (xenobiotics) as a prelude to their use in humans. However, this approach must be treated with caution since biological variation between cell types or species is possible and there may be gross physiological differences, particularly between unicellular and multicellular species.

Metabolism depends on the enzymatically coupled turnover of a relatively few energy-rich *group transfer* molecules (such as certain acyl phosphates, nucleoside diphosphates and triphosphates, and enoyl phosphates) and strongly reducing substances, generated in catabolism, (such as reduced pyridine nucleotides and flavin nucleotides) being used to overcome thermodynamic barriers in biosynthesis. Nutritional classification is based on both the external source of electrons for reduction purposes and the

energy source. Organisms which rely on inorganic electron donors are said to be *lithotrophic* whilst those which rely on organic sources are said to be *organotrophic*. To each of these classes may be added the prefix *photo* if energy is provided by light within the visible and far-red region of the spectrum or *chemo* if energy is provided by oxidation of either organic or inorganic compounds.

Eukaryotes exhibit a much narrower range of nutritional types than prokaryotes but display great heterogeneity in differentiation of cells, tissues and organs which perform especial physiological functions. Each different cell type in a multicellular organism must reflect accompanying biochemical and physiological differences operating within these cells and invoke mutual cooperation of cells in physiological processes. A large part of developmental biochemistry is concerned with elucidating, at the molecular level, the mechanisms of selective gene expression leading to differentiation.

Essentially two types of biochemical investigation are possible. The *in vivo* technique uses intact, whole organisms (plants or animals) or, alternatively, parts of animals subjected to perfusion techniques, to maintain as far as possible the integrity of tissues. The advantage of the *in vivo* method is that artefacts are reduced but often it does not permit precise analysis to be undertaken because of permeability barriers, the complexities of metabolism associated with the multicellular state and mutual cell interference. The *in vitro* method involves the incubation of biologically-derived material in artificial physical and chemical environments. The term is equally applicable to enzyme preparations, to isolated organelles, to intact microorganisms and to excised parts of animals or plants. Conditions may be chosen to promote a limited degree of growth, differentiation and development as for instance in *cell, tissue and organ culture* of animals and plants. The specific advantage of cell and tissue culture methods is that they reduce the physiological and biochemical constraints imposed by contiguous cells. The approach has found widespread application in the biosciences. In its most fundamental sense, cell culture facilitates the investigation of the developmental potential (*totipotency*) of cells, i.e. the capacity, within the limits of its genetic constitution, of one cell to form any other type of cell, given an appropriate artificial chemical and physical environment. A general criticism of experimentation *in vitro* is that extrapolation to the situation *in vivo* may be unjustified, i.e. that the methodology *in vitro* is the study of artefacts.

Biochemical investigations frequently require the purification of a particular compound from a complex mixture. In *analytical separations*, the objective is to identify and estimate small amounts of the compounds and frequently it is not necessary to recover the compound after the separation process. In *preparative separations* the main aim is to isolate and recover as large an amount as possible of the compound in a high degree of purity, in order, subsequently, to study its chemistry and/or its biological properties. Whether an analytical or preparative approach is being adopted may well dictate the choice of separation and purification techniques, mainly because a preparative approach requires much larger amounts of starting material and need not employ techniques which give a high percentage recovery.