

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

Third Edition

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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 prominently 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.

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1986

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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-(<i>p</i> -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)
P _i	inorganic phosphate
PP _i	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)

<i>Physical Quantity</i>	<i>Name of SI Unit</i>	<i>Symbol</i>
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.

<i>Physical Quantity</i>	<i>Name of Unit</i>	<i>Symbol</i>
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.

$$\begin{aligned}
 1 \text{ litre (l)} &= 1 \text{ dm}^3 = 10^{-3} \text{ m}^3 \\
 1 \text{ millilitre (ml)} &= 1 \text{ cm}^3 = 10^{-6} \text{ m}^3 \\
 1 \text{ microlitre } (\mu\text{l}) &= 1 \text{ mm}^3 = 10^{-9} \text{ m}^3
 \end{aligned}$$

Powers of Units – Prefixes

<i>Multiple</i>	<i>Prefix</i>	<i>Symbol</i>
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

<i>Unit</i>	<i>SI equivalent</i>
ångström (Å)	100 pm = 10^{-10} m
atmosphere (standard) (760 mmHg at s.t.p.)	101 325 Pa
calorie	4.186 J
centigrade ($^{\circ}\text{C}$)	($t^{\circ}\text{C} + 273$) K
Curie, Ci	$3.7 \times 10^{10} \text{ s}^{-1}$
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)	$8.314 \text{ J K}^{-1} \text{ mol}^{-1}$
Planck constant (h)	$6.63 \times 10^{-34} \text{ J s}$
Molar volume of ideal gas at s.t.p.	$22.41 \text{ dm}^3 \text{ mol}^{-1}$
Faraday constant (F)	$9.648 \times 10^4 \text{ C mol}^{-1}$
Speed of light in a vacuum (C)	$2.997 \times 10^8 \text{ m s}^{-1}$

Contents

1.	General principles of biochemical investigations by I. Simpkins	1
1.1	Introduction	1
1.2	pH and buffers	4
1.2.1	Effect of pH on biological processes	4
1.2.2	The pH-dependent ionisation of amino acids and proteins	5
1.2.3	Buffer solutions for biological investigations	9
1.3	Physiological solutions	10
1.3.1	Introduction	10
1.3.2	Microbial media	11
1.3.3	Higher plant media	11
1.3.4	Animal media	12
1.3.5	Media for organelle isolation and tissue homogenisation	12
1.4	Whole organism techniques	13
1.4.1	Animal studies	13
1.4.2	Plant studies	15
1.5	Organ and tissue slice techniques	15
1.5.1	Perfusion of isolated organs	15
1.5.2	Slice techniques	16
1.6	Cell and tissue culture	17
1.6.1	Microbial culture	17
1.6.2	Animal cell and tissue culture	18
1.6.3	Plant cell and tissue culture	21
1.6.4	Cell sorting	23
1.6.5	Cell counting	25
1.6.6	Cryopreservation	26
1.6.7	Culture collections	27
1.7	Mutants in biochemistry	27
1.7.1	Introduction	27
1.7.2	Mutant classification	28

viii Contents

1.7.3	Mutant selection	29
1.8	Cell fractionation	29
1.8.1	Introduction	29
1.8.2	Methods of disrupting tissues and cells	31
1.9	Microscopy	32
1.9.1	Introduction	32
1.9.2	Ion probe analysis	33
1.9.3	Preparation of specimens for microscopy	35
1.9.4	Cytochemistry	37
1.10	General approaches to metabolic investigations	39
1.11	Suggestions for further reading	42
2.	Centrifugation techniques by A. Griffiths	44
2.1	Introduction	44
2.2	Basic principles of sedimentation	45
2.3	Centrifuges and their use	49
2.3.1	Small bench centrifuges	50
2.3.2	Large capacity refrigerated centrifuges	50
2.3.3	High speed refrigerated centrifuges	50
2.3.4	Continuous flow centrifuges	51
2.3.5	Preparative ultracentrifuges	51
2.3.6	Analytical ultracentrifuges	52
2.4	Design and care of preparative rotors	54
2.4.1	Materials used in rotor construction	54
2.4.2	Fixed angle and swinging bucket rotors	55
2.4.3	Vertical tube rotors	55
2.4.4	Continuous flow rotors	56
2.4.5	Elutriator rotors	56
2.4.6	Zonal rotors	57
2.4.7	Care of rotors	60
2.5	Sample containers	61
2.6	Density gradient centrifugation	62
2.6.1	Formation and choice of density gradients	62
2.6.2	Sample application to the gradient	63
2.6.3	Recovery and monitoring of gradients from centrifuge tubes	64
2.6.4	Nature of gradient materials and their use	64
2.7	Preparative centrifugation	65
2.7.1	Differential centrifugation	65
2.7.2	Density gradient centrifugation	69
2.7.3	Centrifugal elutriation	71
2.8	Selection, efficiency and application of preparative rotors	71
2.9	Analysis of subcellular fractions	74
2.9.1	Assessment of homogeneity	74
2.9.2	Presentation of results	75
2.10	Some applications of the analytical ultracentrifuge	75

2.10.1	Determination of molecular weight	75
2.10.2	Estimation of purity of macromolecules	78
2.10.3	Detection of conformational changes in macromolecules	78
2.11	Safety aspects in the use of centrifuges	79
2.12	Suggestions for further reading	79
3.	Enzyme techniques by K. Wilson	80
3.1	Introduction	80
3.2	Enzyme units and enzyme purification	82
3.2.1	Enzyme units	82
3.2.2	Protein estimation	83
3.2.3	Enzyme purification	84
3.3	Steady-state enzyme kinetics	87
3.3.1	Initial rates	87
3.3.2	Variation of initial rate with substrate concentration	89
3.3.3	Variation of initial rate with enzyme concentration	95
3.3.4	Variation of initial rate with temperature	95
3.3.5	Variation of initial rate with pH	95
3.3.6	Influence of inhibitors on initial rate	96
3.3.7	Cellular control of enzyme activity	99
3.4	Enzyme assay techniques	100
3.4.1	General considerations	100
3.4.2	Visible and ultraviolet spectrophotometric methods	100
3.4.3	Spectrofluorimetric methods	103
3.4.4	Luminescence methods	103
3.4.5	Radioisotope methods	104
3.4.6	Manometric methods	104
3.4.7	Ion-selective and oxygen electrode methods	105
3.4.8	Immunochemical methods	105
3.4.9	Microcalorimetric methods	105
3.4.10	Automated enzyme analysis	106
3.5	Substrate assay techniques	106
3.6	Pre-steady-state enzyme kinetics	107
3.6.1	Rapid mixing methods	107
3.6.2	Relaxation methods	109
3.6.3	Detection of intermediates	109
3.7	Protein-ligand binding studies	110
3.7.1	General principles	110
3.7.2	Equilibrium dialysis	112
3.7.3	Ultrafiltration	113
3.8	Immobilised enzymes	113
3.9	Suggestions for further reading	114

x Contents

4. Immunochemical techniques by D.H. Burrin	116
4.1 General principles	116
4.1.1 Introduction	116
4.1.2 Definitions	118
4.1.3 The precipitin reaction	120
4.1.4 Preparative uses of the antigen-antibody interaction	120
4.2 Production of antibodies	121
4.2.1 Production of antisera (polyclonal antibodies)	121
4.2.2 Production of monoclonal antibodies	123
4.3 The precipitin reaction in free solution	127
4.3.1 Principles	127
4.3.2 Qualitative analysis of antigen	127
4.3.3 Quantitative analysis of antigen	128
4.3.4 Hapten inhibition test	128
4.4 The precipitin reaction in gels: immunodiffusion(ID)	130
4.4.1 Principles	130
4.4.2 Single (simple) immunodiffusion	131
4.4.3 Double immunodiffusion	131
4.4.4 Immunoelectrophoresis (IE)	134
4.4.5 Visualisation and recording of precipitin lines in gels	137
4.5 Radioimmunoassay (RIA)	138
4.5.1 Principles	138
4.5.2 Practical aspects	139
4.5.3 Immunoradiometric assay (IRMA)	141
4.6 Enzyme immunoassay (ELISA)	141
4.6.1 Principles	141
4.6.2 Practical aspects	145
4.6.3 Applications	146
4.7 Fluorescence immunoassays (FIA)	146
4.7.1 Immunofluorescence (IF)	146
4.7.2 The homogeneous substrate-labelled fluorescent immunoassay (SLFIA)	147
4.7.3 Delayed enhanced lanthanide fluorescence immunoassay (DELFLIA)	148
4.7.4 Flow cytofluorimetry and fluorescence activated cell sorting (FACS)	149
4.8 Particle counting immunoassays (PACIA)	149
4.9 Complement fixation	150
4.9.1 Principles	150
4.9.2 Practical aspects	152
4.10 Suggestions for further reading	152
5. Molecular biology techniques by S. Boffey	153
5.1 Introduction	153
5.2 Structure of nucleic acids	153

5.2.1	Components and primary structure of nucleic acids	153
5.2.2	Secondary structure of nucleic acids	155
5.3	Functions of nucleic acids	160
5.3.1	Classes of RNA	160
5.3.2	DNA replication	160
5.3.3	Transcription	161
5.3.4	Translation	162
5.4	Isolation of nucleic acids	163
5.4.1	DNA	163
5.4.2	RNA	165
5.5	Analysis of DNA	165
5.5.1	Electrophoresis	165
5.5.2	Sequencing of DNA	168
5.5.3	Protein sequencing	170
5.5.4	Renaturation kinetics	173
5.6	Outline of genetic manipulation	174
5.7	Enzymes used in genetic manipulation	175
5.7.1	Restriction endonucleases	175
5.7.2	Ligases	177
5.8	Cloning vectors	180
5.8.1	Plasmids	180
5.8.2	Viral DNA	184
5.8.3	Cosmids	185
5.8.4	Vectors used in eukaryotes	187
5.9	Isolation of specific nucleic acid sequences	188
5.9.1	Complementary DNA	188
5.9.2	Gene libraries	191
5.9.3	Colony hybridisation	191
5.9.4	Nick translation	192
5.9.5	Oligonucleotide probes	193
5.10	Expression of genes	194
5.11	Safety of cloning procedures	195
5.12	Applications of molecular biology	195
5.13	Suggestions for further reading	196
6.	Chromatographic techniques by K. Wilson	198
6.1	General principles and techniques	198
6.1.1	General principles	198
6.1.2	Column chromatography	200
6.1.3	Thin layer chromatography (TLC)	208
6.1.4	Paper chromatography	210
6.2	Adsorption chromatography	212
6.2.1	Principle	212
6.2.2	Materials and applications	212
6.3	Partition chromatography	213
6.3.1	Liquid-liquid chromatography	213

6.3.2	Countercurrent chromatography (CCC)	214
6.4	Gas-liquid chromatography (GLC)	215
6.4.1	Apparatus and materials	215
6.4.2	Applications	219
6.5	Ion-exchange chromatography	219
6.5.1	Principle	219
6.5.2	Materials	220
6.5.3	Practical procedure and applications	222
6.6	Exclusion (permeation) chromatography	225
6.6.1	Principle	225
6.6.2	Materials	227
6.6.3	Applications	229
6.7	Affinity chromatography	230
6.7.1	Principle	230
6.7.2	Materials	231
6.7.3	Applications	233
6.8	High performance (pressure) liquid chromatography (HPLC)	235
6.8.1	Principle	235
6.8.2	Apparatus and materials	236
6.8.3	Applications	241
6.9	Selection of a chromatographic system	242
6.10	Suggestions for further reading	243
7.	Electrophoretic techniques by M.G. Davis	245
7.1	General principles	245
7.2	Factors affecting electrophoresis	246
7.2.1	The electric field	246
7.2.2	The sample	248
7.2.3	The buffer	248
7.2.4	The supporting medium	250
7.3	Low voltage thin sheet electrophoresis	250
7.3.1	Materials	250
7.3.2	Apparatus and methods	252
7.4	High voltage electrophoresis (HVE)	253
7.5	Gel electrophoresis	254
7.5.1	Materials	254
7.5.2	Apparatus and methods	256
7.6	Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis	258
7.6.1	Principle	258
7.6.2	Apparatus and methods	259
7.7	Isoelectric focusing (IEF)	260
7.7.1	Principle	260
7.7.2	Apparatus and methods	261
7.8	Isotachophoresis	263
7.8.1	Principle	263

7.8.2	Apparatus and methods	263
7.9	Preparative electrophoresis	264
7.10	Detection, recovery and estimation	266
7.11	Suggestions for further reading	268
8.	Spectroscopic techniques by D.H. Burrin	270
8.1	General principles	270
8.1.1	Radiation, energy and atomic structure	270
8.1.2	Types of spectra and their biochemical usefulness	272
8.1.3	Basic laws of light absorption	273
8.2	Visible and ultraviolet (UV) spectrophotometry	274
8.2.1	Principles	274
8.2.2	Instrumentation	275
8.2.3	Applications	278
8.3	Infra-red (IR) spectrophotometry	283
8.4	Circular dichroism (CD) spectroscopy	284
8.4.1	Principles	284
8.4.2	Instrumentation	285
8.4.3	Applications	285
8.5	Spectrofluorimetry	287
8.5.1	Principles	287
8.5.2	Instrumentation	288
8.5.3	Applications	290
8.6	Luminometry	294
8.6.1	Principles	294
8.6.2	Instrumentation	295
8.6.3	Applications	295
8.7	Atomic/flame spectrophotometry	296
8.7.1	Principles	296
8.7.2	Instrumentation for atomic emission spectrophotometry	298
8.7.3	Instrumentation for atomic absorption spectrophotometry	299
8.7.4	Flameless atomic absorption spectrophotometry	299
8.7.5	Applications of flame spectrophotometry	299
8.7.6	Atomic fluorescence spectrophotometry	300
8.8	Electron spin resonance (ESR) spectrometry	300
8.8.1	Principles	300
8.8.2	Instrumentation	302
8.8.3	Applications	303
8.9	Nuclear magnetic resonance (NMR) spectrometry	304
8.9.1	Principles	304
8.9.2	Instrumentation	306
8.9.3	Applications	306
8.10	Mass spectrometry	307

8.10.1	Principles	307
8.10.2	Instrumentation	308
8.10.3	Applications	310
8.11	Tabular summary of spectroscopic techniques	311
8.12	Suggestions for further reading	312
9.	Radioisotope techniques by K.H. Goulding	314
9.1	The nature of radioactivity	314
9.1.1	Atomic structure	314
9.1.2	Atomic stability and radiation	315
9.1.3	Types of radioactive decay	315
9.1.4	Radioactive decay energy	316
9.1.5	Rate of radioactive decay	316
9.1.6	Units of radioactivity	318
9.1.7	Interactions of radioactivity with matter	318
9.2	Detection and measurement of radioactivity	319
9.2.1	Absolute and relative counting	319
9.2.2	Methods based upon gas ionisation	320
9.2.3	Methods based upon excitation	323
9.2.4	Methods based upon exposure of photographic emulsions	334
9.3	Other practical aspects of counting radioactivity and analysis of data	337
9.3.1	Counter characteristics	337
9.3.2	Sample and isotope characteristics	338
9.3.3	Supply, storage and purity of radiolabelled compounds	339
9.4	Inherent advantages and restrictions of radiotracer experiments	339
9.5	Applications of radioisotopes in the biological sciences	340
9.5.1	Investigating aspects of metabolism	340
9.5.2	Analytical applications	341
9.5.3	Other applications	342
9.6	Safety aspects of the use of radioisotopes	343
9.7	Suggestions for further reading	344
10.	Electrochemical Techniques by M.R. Jenkins	345
10.1	Introduction	345
10.1.1	The range of electrochemical techniques	345
10.1.2	Electron transport processes	346
10.1.3	Principles of electrochemical techniques	351
10.2	Measurement of pH by glass electrodes	354
10.2.1	Principles of operation	354
10.2.2	The pH-stat	356
10.2.3	Measurement of Δ pH and $\Delta\psi$	357
10.3	Ion-selective electrodes (ISE) and gas sensors	359
10.3.1	Introduction	359

	10.3.2 Uses of ion-selective electrodes	361
10.4	Oxidation-reduction (redox) potentials	362
	10.4.1 Principles	362
	10.4.2 Potentiometric titrations of oxidation-reduction reactions	363
	10.4.3 Oxidation-reduction indicators (redox-dyes) and their uses	363
10.5	The oxygen electrode	366
	10.5.1 Principles	366
	10.5.2 Types of oxygen electrode	366
	10.5.3 Operation of a Clark electrode (Rank electrode)	367
	10.5.4 Applications of oxygen electrodes	369
10.6	Biosensors	372
	10.6.1 Introduction and principles	372
	10.6.2 Enzyme electrodes	373
	10.6.3 Bacterial electrodes (cell-based biosensors)	375
	10.6.4 Enzyme-immunosensors	377
10.7	Electrochemical detectors	377
10.8	Suggestions for further reading	380

Index		381
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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 micro-organisms, 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

2 General principles of biochemical investigations

energy source. Organisms which rely on inorganic electron donors are said to be *lithotrophic* while 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.