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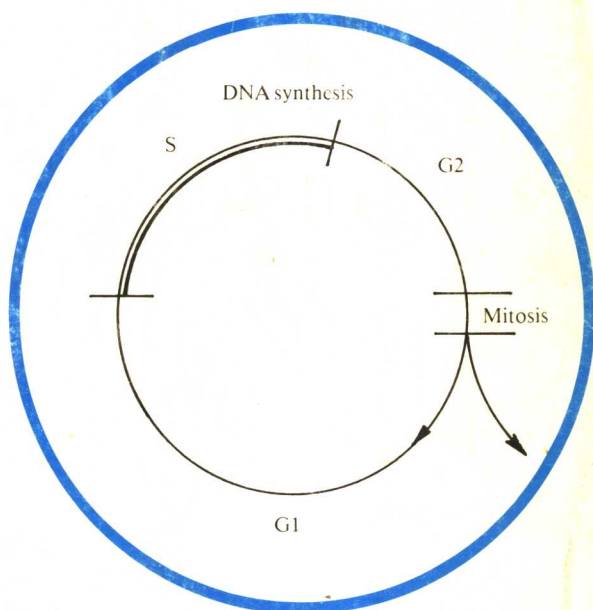
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cell culture for biochemists

R. L. P. ADAMS

2nd revised edition



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CELL CULTURE FOR BIOCHEMISTS

Second revised edition

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List of abbreviations

2,4-D	2,4-diphenoxyacetic acid
ACTH	adrenocorticotrophic hormone
Ad2	adeno 2 virus
AMP, ADP, ATP	adenosine monophosphate, diphosphate, triphosphate
ATCC	American Type Culture Collection
BHK cells	baby hamster kidney cells
BME	basal medium (Eagle)
BPL	β -propiolactone
BRL	Buffalo rat liver
BSA	bovine serum albumin
BSS	balanced or buffered salt solution
BUdr	bromodeoxyuridine
CHO cells	Chinese hamster ovary cells
CMF	calcium- and magnesium-free BSS
CMP, CDP, CTP	cytidine monophosphate, diphosphate, triphosphate
CMRL	Connaught Medical Research Laboratories
CMS	Calgon metasilicate
CPE	cytopathic effect
dAMP, dTMP	deoxyadenosine monophosphate, deoxythymidine monophosphate, etc.
DAPI	2-diamidino-phenyl indole
Decon	a detergent
DePex	mounting medium available from G.T. Gurr Ltd. (Appendix 3)
EC10	Eagle's MEM supplemented with 10% calf serum
EDTA	ethylenediaminetetraacetic acid (versene)

EFEC	Eagle's MEM supplemented with foetal calf serum
EGF	epidermal growth factor
EMS	ethyl methane sulphonate
ESG	Ewing sarcoma growth factor
ETC	Eagle's MEM supplemented with tryptose phosphate (10%) and calf serum (10%)
FBS	foetal bovine serum
FGF	fibroblast growth factor
FITC	fluorescein isothiocyanate
G0-phase	the resting stage of the cell cycle
G1-phase	the first gap in the cell cycle (between M and S)
G2-phase	the second gap in the cell cycle (between S and M)
GMP, GDP, GTP	guanosine monophosphate, diphosphate, triphosphate
GS-filter	0.22 μm membrane filter supplied by Millipore Corp
HAT medium	medium supplemented with hypoxanthine, aminopterin and thymidine
HAU	haemagglutinin unit
HECS	human endothelial cell supernatant
Hep cells	human epithelial cells
HPRT	hypoxanthine phosphoribosyl transferase
HSV	herpes simplex virus
HTC cells	hepatoma tissue culture cells
IAA	indole acetic acid
ITES	medium supplement containing insulin, transferrin, ethanolamine and selenium
M	mitosis
MCDB	media developed at the Department of Molecular, Cellular and Developmental Biology, University of Colorado
MEM	minimum essential medium
Methocel	carboxymethyl cellulose type MC (4000 centripoises) sold by Dow Chemical Co.
MNNG	<i>N</i> -methyl- <i>N</i> -nitro- <i>N</i> -nitrosoguanidine
m.o.i.	multiplicity of infection
MOPC	mineral oil-induced plasmacytoma

MSE	Medical and Scientific Equipment Ltd.
MVM	minute virus of mouse
NCTC	National Cancer Tissue Culture
NDV	Newcastle disease virus
PBS	phosphate buffered saline
PCA	perchloric acid
p.f.u.	plaque forming unit
PGE	prostaglandin E
PHA	phytohaemagglutinin
PPLO	pleuropneumonia-like organism
PPO	diphenyloxazole
p.s.i.	pounds per square inch (14.7 p.s.i. = 1 atmosphere = 10^5 Pascal)
PyY cells	polyoma transformed BHK cells
RPMI	Roswell Park Memorial Institute
RSV	Rous sarcoma virus
SDS	sodium dodecyl sulphate
S-phase	DNA synthetic phase of cell cycle
SSC	saline sodium citrate
SV40	simian virus 40
T3	triiodothyronine
T-antigen	transplantation antigen
TC	tissue culture
TCA	trichloroacetic acid
<i>t</i> G1, <i>t</i> G2, <i>t</i> S and <i>t</i> M	the time for the cell phases G1, G2, S and M, respectively
TK	thymidine kinase
TRITC	tetramethylrhodamine isothiocyanate
ts	temperature sensitive
UMP, UDP, UTP	uridine monophosphate, diphosphate, triphosphate
VSV	vesicular stomatitis virus
7X	a detergent

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Contents

<i>List of abbreviations</i>	<i>v</i>
<i>Acknowledgements</i>	<i>ix</i>
<i>Chapter 1. Introduction</i>	<i>1</i>
1.1. Background	1
1.2. Some advantages	2
1.3. Applications	4
1.3.1. Differentiation	4
1.3.2. Genetics	4
1.3.3. Immunology	5
1.3.4. Hormones and growth factors	6
1.3.5. Virology and cell transformation	6
1.3.6. Cytotoxicity testing	7
1.4. Animal cell biotechnology	8
<i>Chapter 2. Characteristics of cultured cells</i>	<i>11</i>
2.1. Types of cells	11
2.2. Primary cells and transformation	13
2.3. Growth control	16
2.3.1. Nutritional requirements	16
2.3.2. Cell cycle and growth cycle	18
2.4. Attachment and spreading	19
2.4.1. Anchorage dependence and growth in suspension	19
2.4.2. Density dependent regulation (contact inhibition)	20
2.4.3. Fibronectin	21
2.5. Growth factors	23
2.5.1. The serum component	23
2.5.2. Growth factors for haemopoietic cells	26
2.5.3. Mechanism of action of peptide growth factors	27
2.5.4. Oncogenes	31
2.5.5. Steroid hormones	32
2.6. Differentiated functions in cell cultures	32
<i>Chapter 3. Culture vessels</i>	<i>35</i>
3.1. Design of culture vessels	35
3.1.1. Gaseous exchange	35

3.1.2. Sealed vessels	36
3.1.3. Perfusion techniques	38
3.2. Small scale cultures	38
3.3. Intermediate scale cultures	41
3.4. Large scale cultures	41
3.4.1. Roller vessels	41
3.4.2. Cell factories	44
3.4.3. Perfusion vessels	45
3.4.4. Capillary beds	46
3.5. Suspension cultures	47
3.6. Microcarriers	50
3.6.1. Positively charged microcarriers	51
3.6.2. Negatively charged microcarriers	52
3.6.3. Collagen or gelatin (denatured collagen) beads	54
3.6.4. Cell entrapment	55
3.7. Air lift systems	55
3.8. Bioreactors	56
<i>Chapter 4. Subculturing</i>	<i>59</i>
4.1. Dissociation techniques	59
4.1.1. Trypsin	59
4.1.2. Pronase	60
4.1.3. Collagenase	60
4.1.4. Dispase	61
4.1.5. EDTA (Versene)	61
4.1.6. Mechanical means	62
4.2. Subculture of a cell monolayer	63
4.2.1. Viable cell count	64
4.2.2. Bacterial check	64
4.3. Subculture of cells growing in suspension	64
4.4. Protocol for setting up microcarrier cultures	65
4.4.1. Preparation of the microcarrier	65
4.4.2. Preparation of the culture vessel	65
4.4.3. Initiating a culture	66
4.4.4. Non-stirred microcarriers	66
4.5. Subculture of cells growing on microcarriers	67
4.6. The growth cycle	68
<i>Chapter 5. Cell culture media</i>	<i>71</i>
5.1. Introduction	71
5.2. Balanced salt solutions	72
5.2.1. Zwitterionic buffers	75
5.3. Eagle's medium	75
5.3.1. Powdered media	77
5.4. More complicated media	78
5.5. Simple media with unspecified additives	79
5.6. Antibiotics	79
5.7. Serum	80

5.7.1. Removal of small molecules from serum	82
5.8. Serum-free media	84
5.8.1. Low serum media	87
5.8.2. Defined media	88
5.8.3. Media for isolation of secreted products	89
5.8.4. Commercial media	91
5.8.5. Isolation of factors from culture supernatants	92
5.9. Media for culture of insect cells	93
5.10. Media for culture of plant cells	94
<i>Chapter 6. Primary cells</i>	<i>97</i>
6.1. Introduction	97
6.2. Lymphocytes	97
6.2.1. Isolation of leukocytes and autologous plasma	98
6.2.2. Purification of lymphocytes	98
6.2.3. Glass-bead column method	98
6.2.4. Gradient centrifugation method	99
6.2.5. Cultured lymphocytes	100
6.3. Human skin biopsies	101
6.4. Mouse or rat embryo cultures	103
6.5. Chick embryo cells	105
6.6. Chick embryo liver cells	106
6.7. Rat hepatocytes	107
6.8. Primary kidney cells	108
6.9. Endothelial cells	111
6.10. Mammary epithelial cell cultures	111
6.11. Colonic epithelial cells	112
6.12. Rat or chick skeletal muscle cells	112
6.13. Mouse macrophage cultures	112
6.14. Ascites cells	113
6.15. Dipteran cell culture	114
<i>Chapter 7. Techniques</i>	<i>117</i>
7.1. Cell cloning and plating efficiency	117
7.1.1. Measurement of plating efficiency	118
7.1.2. Four simple cloning methods	119
7.1.3. Cloning under agar	120
7.1.4. Cloning with a feeder layer	121
7.2. Cell counting procedures	122
7.2.1. Haemocytometer	122
7.2.2. Electronic cell counter	123
7.2.3. Comparison of the methods	126
7.3. Storage of cells	127
7.3.1. Freezing procedure	129
7.3.2. Storage procedure	132
7.3.3. Recovery of cells from liquid nitrogen	134
7.3.4. Organisation of stocks of frozen cells	135
7.3.5. Cell banks and transport of cells	135

7.4. Karyotyping	136
7.4.1. Chromosome preparation	137
7.4.2. Karyotyping	138
7.4.3. Q-banding	140
7.4.4. G-banding	140
7.4.5. Chromosome sorting	141
7.5. Cell transfection	142
7.5.1. Calcium phosphate method	142
7.5.2. Liposomes	144
7.5.3. Electroporation	144
7.6. Cell visualisation	146
7.6.1. Phase contrast microscopy	147
7.6.2. Fluorescence microscopy	148
7.7. Sub-cellular fractionation	148
<i>Chapter 8. Glassware preparation and sterilisation techniques</i>	<i>151</i>
8.1. General	151
8.1.1. Washing procedure	152
8.1.2. Bottles and pipettes	152
8.1.3. Rubber stoppers	153
8.1.4. Glass washing machines	153
8.2. Sterilisation by heat	153
8.2.1. Hot air	154
8.2.2. Autoclaving	154
8.2.3. Control of sterilisation	155
8.3. Sterilisation by filtration	156
8.3.1. Self-assembled filter apparatus	156
8.3.2. Disposable filter apparatus	159
<i>Chapter 9. Contamination</i>	<i>165</i>
9.1. Bacterial contamination	165
9.1.1. Glass and plasticware	165
9.1.2. Cells	165
9.1.3. Media	166
9.2. Sterility checks	166
9.3. Analysis of bacterial contamination	168
9.4. Airborne contamination	168
9.4.1. Aseptic technique	169
9.4.2. Laminar flow systems	172
9.5. Antibiotics	173
9.6. Disposal of contaminated material	174
9.7. Mycoplasmas	174
9.7.1. Effect on cell cultures	176
9.7.2. Culture of mycoplasmas	176
9.8. Testing for mycoplasma	177
9.8.1. Orcein stain	178
9.8.2. Autoradiography	178
9.8.3. Fluorescence staining	179

9.8.4. Cell growth test for mycoplasmas	182
9.8.5. DNA probe for mycoplasma	183
9.9. Elimination of mycoplasmas	183
9.10. Viral contamination	184
<i>Chapter 10. The cell cycle</i>	<i>187</i>
10.1. Description	187
10.2. Mitosis	188
10.3. S-phase	190
10.4. Control of the cell cycle	191
10.4.1. The G ₀ -phase and commitment to cycle	191
10.4.2. P34 and cyclins	195
10.5. Distribution of cells around the cycle	196
10.6. Growth fraction	199
10.7. Cell cycle analysis	199
10.7.1. Tritiated thymidine pulse method	200
10.7.2. Continuous labelling method	201
10.7.3. Accumulation functions	203
10.7.4. Graphical analysis	206
10.7.5. Flow microfluorometry (FACS)	207
<i>Chapter 11. Cell synchronisation</i>	<i>211</i>
11.1. Introduction	211
11.2. Selection of mitotic cells	212
11.2.1. Shaking	212
11.2.2. Trypsinisation	213
11.2.3. Selection from microcarriers	214
11.3. Selective killing of cells in particular phases	215
11.4. Selection of cells by size	215
11.4.1. Electronic cell sorting	215
11.4.2. Zone sedimentation	216
11.4.3. Centrifugal elutriation	219
11.5. Synchronisation by subculture	222
11.6. Serum deprivation	224
11.7. Isoleucine starvation	226
11.8. Blockade of S-phase	228
11.8.1. Action of aminopterin and amethopterin (methotrexate)	229
11.8.2. Action of 5-fluorodeoxyuridine	232
11.8.3. Action of high concentrations of thymidine	232
11.8.4. Action of hydroxyurea	235
11.9. Procedure for inducing synchrony at the G ₁ /S interphase	235
11.9.1. Isoleucine starvation and hydroxyurea	235
11.9.2. Stationary phase cells and aminopterin	236
11.9.3. Double thymidine block	236
11.9.4. Comparison of the methods	236
11.10. Synchronisation in G ₂	237
11.11. Synchronisation in M	238

<i>Chapter 12. Use of radioactive isotopes in cell culture</i>	239
12.1. Estimation of rates of DNA synthesis	239
12.1.1. Flooding the pool	243
12.1.2. Blocking the endogenous pathway	245
12.1.3. Allowing for endogenous dTTP	246
12.1.4. Comparison of the methods	247
12.1.5. Application to suspension cultures	248
12.2. Estimation of rates of RNA and protein synthesis	249
12.3. Autoradiography	250
12.3.1. Fixation and staining	251
12.3.2. Emulsions	251
12.3.3. Stripping film	252
12.3.4. Liquid emulsion	253
12.3.5. Autoradiography in dishes	255
12.3.6. The value of grain counting	255
12.3.7. Background grains	256
12.3.8. Autoradiography of water-soluble cell components	257
12.3.8.1. Cell fixation	257
12.3.8.2. Covering with emulsion	258
12.4. Labelling with bromodeoxyuridine	259
12.5. DNA repair	259
12.4.1. Ultraviolet radiation	260
12.4.2. Estimation of repair synthesis	261
<i>Chapter 13. Cell mutants and cell hybrids</i>	263
13.1. Auxotrophic mutants	263
13.2. Selection of mutants	264
13.2.1. Procedure for isolation of TK ⁻ mutants	266
13.3. Temperature sensitive mutants	267
13.3.1. Selection of G1 mutants and S mutants	267
13.3.2. Selection of G2 mutants and M mutants	268
13.4. Replica plating of animal cells	268
13.5. Somatic cell hybridisation	269
13.6. Myeloma culture and monoclonal antibody production	271
13.6.1. Isolation of spleen cells	272
13.6.2. Harvesting of myeloma cells	273
13.7. Methods of fusion	273
13.7.1. Cell fusion with lysolecithin	273
13.7.2. Cell fusion using polyethyleneglycol	273
13.7.3. Electrofusion	274
13.8. Cell communication	275
13.8.1. Grain counting and cell communication	276
<i>Chapter 14. Viruses</i>	279
14.1. Introduction	279
14.1.1. Animal virus classification	280
14.1.2. Precautions to be taken when using virus infected cells	280

14.2.	Virus production	283
14.2.1.	Procedure for production of herpes simplex, pseudorabies or EMC virus	283
14.2.2.	Procedure for production of SV40 virus	284
14.2.3.	One-step growth curve of SV40	286
14.2.4.	Sendai virus – production and inactivation	287
14.2.4.1.	Production	287
14.2.4.2.	Inactivation by UV	287
14.2.4.3.	Inactivation by β -propiolactone	287
14.3.	Virus detection	288
14.3.1.	Plaque assay	288
14.3.1.1.	Viral dilution	289
14.3.1.2.	Suspension assay	289
14.3.1.3.	Monolayer assay	289
14.3.1.4.	Agar overlay assay	290
14.3.2.	Fluorescent antibody techniques	292
14.3.2.1.	Preparation of antisera	293
14.3.2.2.	Preparation of globulin fraction	293
14.3.2.3.	Conjugation of antisera with fluorescein or rhodamine	293
14.3.2.4.	Staining techniques	294
14.3.3.	Haemadsorption and haemagglutination	295
14.4.	Production and testing of viral vaccines	296
14.5.	Viral transformation of cells	297
14.5.1.	Methods of transformation	298
<i>Chapter 15. Differentiation in cell cultures</i>		<i>301</i>
15.1.	Erythroid differentiation of Friend cells	301
15.1.1.	Induction of globin synthesis in Friend cells	302
15.2.	Skin and keratinocytes	303
15.3.	Teratocarcinoma cells	305
15.4.	Differentiation of muscle cells	307
15.5.	Differentiation of adipose cells	308
15.6.	Differentiated hepatocytes	308
<i>Chapter 16. Appendices</i>		<i>311</i>
1.	Media formulations	311
2.	Stains and fixatives	325
3.	Suppliers	327
4.	Sterility checks	331
5.	Assays	333
<i>References</i>		<i>335</i>
<i>Subject index</i>		<i>355</i>

Introduction

1.1. Background

Although a number of books are available giving a great deal of information about various aspects of cell culture this book is designed rather for the biochemist or molecular biologist, whose interest in cell culture extends only as far as this technique provides him with material with which he may perform biochemical experiments.

This is the second edition of this book and, although some sections have been extensively revised, others are little changed. For instance the details of glassware preparation have not changed over the last 10 years but what has changed is the use of glassware which has been superseded almost entirely with presterilised plastic ware.

The aspects which have changed most over the last 10 years are those which result from a greater understanding of the controls over cell division, e.g. the role of growth factors and the use of serum-free medium.

Before a biochemist will apply himself to using the technique of cell culture he must be assured that it offers him significant advantages which outweigh any disadvantages. Furthermore, he must not imagine that the methods are too laborious for routine use or that some degree of black magic is required before success can be achieved. To some extent such fears are based on the experience of workers in the field up to about 1960. In the preceding 30 years nearly all major cell types had been cultivated for varying periods and much descriptive information obtained but this was only as a result of constant dedicated effort.

Since 1960 many of the obstacles have been removed from the path of the biochemist. Perhaps the most important is that commercial companies now supply media, sera, cells and culture vessels

which enable cells to be cultured occasionally or routinely on a scale varying from a growth surface of less than one square centimetre up to several square metres. This service is available only as a result of the description of simple media in which the cells grow well and the development of simple methods for isolation of primary cells, selection of clones and storage of cell lines.

The other major fear is one of cost. On a weight for weight basis cultured cells are several orders of magnitude more expensive than, say, rat liver. Thus a rat costs under £5 and yields about 10 g of liver. 10^6 cells obtained from a commercial supplier cost about £2 and so 10 g of cells (2×10^{10} cells) could cost £40,000 although significant price reductions are obtained when buying in quantity. One can produce the same number of cells in one's own laboratory for less (the cost of medium may be as little as £25), but this hides the cost of overheads. Nevertheless, the use of cultured cells must offer marked advantages before it is worthwhile to embark on large scale production, and there is seldom any justification for using cultured cells as a source of material for an enzyme purification when sources like rat liver or rabbit kidney would do as well. At the other extreme, however, many experiments may be performed with 10^2 – 10^6 cultured cells at a cost equivalent to the alternatives.

1.2. Some advantages

One major advantage offered by cultured cells which cell biologists make full use of but which is often ignored by biochemists is that the living cells may be watched under the microscope. It is essential that healthy cells are used in an experiment and that they remain alive throughout the experiment. That this is the case may be monitored regularly and moreover quantitative estimates of the proportion of viable cells are readily obtained. It is often impossible to know the state of an animal's kidney until the end of the experiment and then usually only in a qualitative manner.

Cells in culture offer a homogeneous population of cells of virtually identical genetic make-up, growing in a constant environment. Moreover, the environment may be changed, within limits, at

the whim of the experimenter who may thereby investigate the effect of pH, temperature, amino acid and vitamin concentration etc. on the growth of the cells. Growth may be measured over a short time period either by measuring an increase in cell number or size, or by following the incorporation of a radioactive tracer into DNA. These are real advantages over a whole animal system, placing cultured cells on a par with microorganisms as an experimental system. Using cultured cells, the growth requirements of human cells were analysed in a few weeks thus confirming decades of work with people of different genetic background living in different environments (Eagle, 195a,b; see § 2.3.1).

Moreover, significant results may be obtained with very few cells. An experiment which may require 100 rats or 1000 humans in order to clarify some point may be statistically equally valid if 100 coverslip cultures or one microwell plate are used. If each cell is regarded as an independent experiment then one coverslip culture may yield more reliable results than a hospital full of people. This is obviously a major advantage as far as man is concerned but also overcomes the ethical problems which often arise when large numbers of animals are used for experimental purposes. However, in the final analysis, many experiments must be performed on whole animals, but this is no justification for not using cultured cells for the preliminary work.

Because cells in culture are easily available for manipulation by the biochemist, radioactive tracers, drugs or hormones etc. may be applied in a known concentration and for a known time period. The amounts of such compounds required may be an order of magnitude less than with comparable experiments on whole animals. There is no fear that the drug whose effect is to be investigated is being metabolised by the liver stored in the muscles and excreted by the kidney. It is usually a simple matter to establish that a substance added to a cell culture remains in contact with the cells in unchanged form at a known concentration for a given time. This enables experiments to yield realistic figures for the rates of incorporation or metabolism of compounds. Such experiments are not without hazards in cultured cells (see Chapter 12) but are very difficult to interpret in whole animals. However, when the aim of the