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cell culture for biochemists

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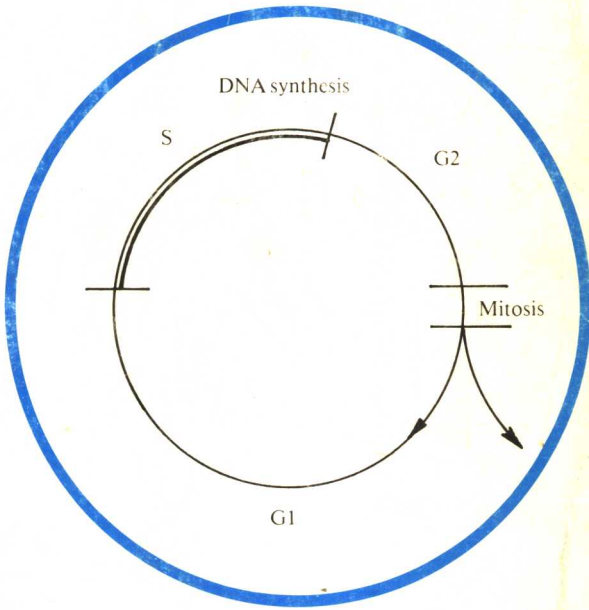

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CELL CULTURE FOR BIOCHEMISTS

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)

EFC	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	fluoroscein 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	*N*-methyl-*N*-nitro-*N*-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	trichloracetic acid
*t*G1, *t*G2, *t*S and *t*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

CHAPTER 1

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