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Principles and Products

M Butler

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Preface and Acknowledgements

Over the past 5 to 10 years, biotechnology has been introduced as an integral component of most degree-level courses in the biological sciences. Biotechnology is a broad based subject and its component parts fit into a range of traditionally accepted biology units — biochemistry, microbiology and immunology.

Animal cell technology is one such component. It is a technology which has developed from years of cell culture study on a small laboratory scale (~ 100 ml). Such cell cultures have been used to investigate some of the fundamental areas of biology — cellular metabolism, growth and the action of hormones. Such small-scale cultures have been found increasingly useful in toxicological testing, and where possible being preferred to whole animal experiments.

More recent developments have allowed animal cells to be cultured at a larger scale (100–10000 l) for the production of an increasing range of biological compounds. They are high value compounds with medical and veterinary applications — for diagnosis, prophylaxis or therapy. They have a high specific activity *in vivo* and enable appreciable metabolic changes to result from the clinical administration of relatively low doses (~ ng/ml of blood plasma) which can be several orders of magnitude lower than those of bacterial therapeutics such as antibiotics. The commercial production of these animal cell biologicals normally requires cell culture at a scale of 100–1000 litres — animal cell cultures above this volume being quite exceptional.

Having identified an animal cell product of commercial value, there are a number of possible methods for production. The first to be considered may be biochemical extraction from bulk biological tissue. However, this method has a number of disadvantages — it is unreliable, suffers from batch variation and often insufficient tissue is available to satisfy the demand for the final purified product. Alternative approaches include chemical synthesis, animal cell culture or culture

of genetically engineered bacteria. For each product the preferred approach may be different. Chemical synthesis may suit relatively small molecules. Production from genetically engineered bacteria may be favoured for proteins which are normally unmodified by eucaryotic post-translational events, or where activity is unaffected by glycosylation. Although once considered difficult, animal cell cultures can now produce routinely acceptable yields of complex products. The most common in production are the viral vaccines, marking the beginning of many of the now established principles of animal cell technology.

The first part of this book describes the basic principles involved in the use of animal cell cultures, the possibilities for genetic modification and the problems to consider in scaling up such cultures. In the scond part of the book, several selected cell products are chosen as case studies for considering alternative strategies for large-scale production. The background of our understanding of these cell products is discussed and related to the requirements of large-scale production.

Clearly, each cell product will have an associated set of arguments favouring one or other process for its large-scale production. In many instances such production involves genetic engineering in bacteria, and no excuse is offered for introducing these techniques in a book on animal cell technology. Future developments may change the preferred production methods for some of these cell products. One particular prediction may be the increased use of genetically engineered animal cells, the large-scale culture of which may allow production processes which express the advantages (but few of the disadvantages) of using genetically engineered bacteria.

The products selected for discussion in this book are those that have become prominent in most people's minds when considering large-scale process production of animal cell products. Clearly, the full list of commercially valuable cell products is much longer than those described and many more novel products are expected in the near future. The development of production processes for these new compounds will undoubtedly be promoted by the on-going discussion concerning production of those more well established biologicals which are described in this book.

I am grateful to those many students who unwittingly became guinea pigs to the development of some of the thoughts and ideas in this book. Although the work is primarily aimed at the level of final year degree students, it may also be of benefit to post-graduate or other research workers when first entering the maze of animal cell technology. It assumes a basic knowledge of cell biology and biochemistry which most biological science students will study in the initial years of their course. Hopefully this book will help in solving some of the existing set of problems so as to continue the rapid advance of animal cell technology.

I would like to express my gratitude to my colleagues at Manchester Polytechnic, particularly Dr Maureen Dawson and Dr Peter Gowland for their valuable comments and criticism during the preparation of the manuscript. Also, my thanks go to Professor Bryan Griffiths for his thorough review and comments before publication and to my wife, Elisabeth for her patience during many hours of proof reading.

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Fig. 2.7 Kluft, C. *et al.* (1983) *Adv. Biotech. Processes* **2**, 98-109.
Fig. 2.9 Birch, J.R. *et al.* (1987) in 'Plants and Animal cells: Process Possibilities' (Webb, C. and Mavituna, F. Eds.) pp. 162-171.Chichester, Ellis Horwood.
Table 2.2 Katinger, H. and Scheirer, W. (1985) in 'Animal Cell Biotechnology' vol. 1 (Spier, R.E. and Griffiths, B. Eds.) pp. 167-193 London, Academic Press.
Table 2.3 Griffiths, B. (1986) *Trends in Biotech.* **4**, pp. 268-272.

Chapter 3
Table 3.2 Petricciani, J.C. (1985) in 'Large-scale Mammalian Cell Culture' (Feder, J.B. and Tolbert, W.R. Eds.) London, Academic Press.

Chapter 5
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Chapter 6
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SECTION I

Animal Cell Technology: Principles

Chapter 1

The Development of Cell Cultures

Historical Background

The first recorded attempts to maintain animal cells in culture can be attributed to Ross Harrison, who in 1907 devised the hanging drop technique. He suspended dissected nerve tissue from frog embryos in lymph fluid which was allowed to clot as a droplet on the underside of a microscope cover slip. Figure 1.1 shows how this was mounted on a hollow microscope slide and sealed with wax. By this method Harrison was able to observe growth of the embryonic nerve cells for several weeks.

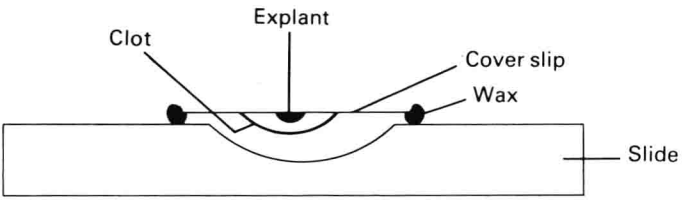

Fig. 1.1 Harrison's hanging drop technique (1907).

This work was extended by Burrows, who in collaboration with Alexis Carrel established techniques for the culture of a wide range of mammalian cells. They showed that strict aseptic control could enable the prolonged sub-culture of these cells for several years without infection. Good survival and growth of the cells was attained by the use of chick embryo extracts which were mixed with the plasma. The nutrients and growth factors were provided by the embryo extracts and the

substratum for cell attachment was provided by the matrix of the fibrin formed from the plasma. Cultures were maintained in Carrel flasks which were small flat-bottomed containers devised for ease of aseptic manipulation.

The use of trypsin, introduced by Rous for the dispersion of cells from tissue explants, was a major innovation in obtaining cell suspensions. This allowed cultures to be established from single cell types and distinguished the technique of 'cell culture' from 'tissue culture'. Although the terms are often (but incorrectly) interchanged, tissue culture involves the growth of cells *in vitro* in a tissue matrix which may involve several cell types, whereas cell culture involves the growth of cells as independent micro-organisms.

The use of trypsin became particularly valuable in establishing procedures for sub-culturing cells. However, before the 1940s the widespread use of culture techniques was still limited by the stringent sterility controls necessary. The perception of these difficulties was also intensified by Carrel's fastidious nature in insisting on full surgical dress akin to those used in hospital operating theatres. It was not until the late 1940s, with the advent of antibiotics, that further developments of cell culture techniques took place. The addition of antibiotics (such as penicillin and streptomycin) to culture media eased the handling of cultures by reducing the chances of bacterial infections — a particular problem with the chemically undefined biological fluids and extracts used at the time.

The use of an antibiotic-supplemented culture media which was less prone to infection established cell culture as a routine technique which could be developed further. During this period human carcinoma cells such as the well known HeLa cell line were isolated and found to proliferate vigorously in culture. Large-scale animal cell cultures were first considered after Enders' discovery in the late 1940s that viruses could be propagated in cell cultures and used as vaccines. The consequent development of cell culture scale-up for polio vaccine production in the 1950s heralded the beginning of animal cell culture as a developing technology.

In the early 1950s, Earle and Eagle made a significant contribution to this technology by their analysis of the nutritional requirements of cultured cells. In 1955 the media formulation known as Eagle's minimum essential medium (EMEM — Table 1.1) defined the growth requirements of HeLa cells and mouse *L* cells in terms of optimal concentrations of chemically defined nutrients. This formulation was based on the compounds required to replace the growth promoting biological fluids previously used for cell growth. This chemically defined media had the advantages of consistency between batches, ease of sterilization and lowered chance of contamination. EMEM and its subsequent various modifications have been used extensively for cell culture. However, these formulations do require an additional supplement (usually 10%) of chemically undefined blood serum to provide unidentified growth factors and hormones.

Since these developments in the 1950s the range of available cell lines has increased considerably. Many of the cells have undergone modifications from their original *in vivo* state. This has involved the transformation to continuous cell lines capable of infinite growth capacity, or in some cases their genetic manipulation to produce selected products. In 1975 Kohler and Milstein exploited the ability to

Table 1.1 Eagle's Minimum Essential Medium (MEM)

L-Amino acids	*mM*	*Vitamins/Co-factors*	*μM*
Arginine	0.6	Choline	8.3
Cystine	0.1	Folic acid	2.3
Glutamine	2.0	Inositol	11.0
Histidine	0.2	Nicotinamide	8.2
Isoleucine	0.4	Pantothenate	4.6
Leucine	0.4	Pyridoxal	6.0
Lysine	0.4	Riboflavin	0.27
Methionine	0.1	Thiamine	3.0
Phenylalanine	0.2		
Threonine	0.4	*Inorganic ions*	mM
Tryptophan	0.05	NaCl	116
Tyrosine	0.2	KCl	5.4
Valine	0.4	$CaCl_2$	1.8
		$MgCl_2.6H_2O$	1.0
Glucose	5.5	$NaH_2PO_4.2H_2O$	1.1
		$NaHCO_3$	23.8
Supplemented Serum	10%		
		Phenol red	5 mgl^{-1}

fuse cells of different types to produce genetically stable hybridomas capable of continuous secretion of pre-determined monoclonal antibodies. The range and potential of these cell hybrids is still being explored.

The era of recombinant DNA technology developed in the 1970s with the ability to express mammalian genes in bacteria, and this allowed the production of a range of useful mammalian proteins in bacterial cultures. These proteins included insulin, somatotrophin (growth hormone), interferon and many others which are discussed in later chapters. Although it was once considered that the use of such recombinant bacteria might replace the need for animal cell cultures, it is now clear that both systems have their own merits and demerits which should be considered for the commercial production of any biological compound. Now, a range of recombinant mammalian cells have been developed with capabilities of high specific production of selected compounds.

The range of commercially useful biologicals produced from animal cell cultures is now expanding. Most are proteins or glycoproteins and are of use as pharmaceutical, medical or veterinary products. There are available typically as extracellularly-released products of an expanding variety of cell lines which have been induced into culture. However, in order to supply would-be markets they are needed in relatively large quantities, and this requires a careful study and optimization of the production processes involved. Such a study should start with