Animal Cell Biotechnology

Volume 1

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Preface

"Animal Cell Biotechnology" is directed towards two groups of people. Those entering the field for the first time will find many details about how to set up and operate cell cultures in a variety of ways and at scales ranging between 0.001 and 10,000 litres. Others who are already engaged in the area will find that the comprehensive and detailed coverage of the selected topics written by experts in the various subjects will provide them with a well-referenced and well-indexed state-of-the-art report. This will enable them to expand their horizons further and to appreciate more fully those aspects of the subject with which they have not yet become deeply involved. It will also provide team leaders with the overview necessary to better coordinate and direct the various unit operations of manufacturing a product from animal cells in culture.

The contributing authors have been asked to assume that the reader will have been exposed at university or polytechnic level to two or more basic science subjects (mathematics, physics, chemistry and biology), although he/she may have qualified in the specialist disciplines of microbiology, chemical engineering, biochemistry, genetics, immunology or even biotechnology. In addition the writers have stressed, where appropriate, the underlying principles of the subject area and have provided many illustrations, diagrams, graphs and tables to present information which would otherwise take up much text and make for laborious reading. A balanced, thoughtful and fair assessment of alternative methods to achieve particular ends has been requested, yet in this burgeoning field the reader will have to contend with the clear enthusiasm of one or another of the various contributors. As editors, we are confident that there is a sufficient wealth of views for readers to have little difficulty in developing an appreciation of the strengths and weaknesses of alternative technologies and thereby be fortified in the choice of system for their specific application.

These volumes survey a new and as yet uncharted facet of biotechnology. They are designed both as an introduction and as an in-depth survey of the

present situation. In view of the exciting scientific achievements but disappointing practical results derived from trying to express mammalian genes in prokaryotic organisms and the new capabilities in the area of genetic engineering of animal cells, we can anticipate that animal cell biotechnology is likely to retain its place at the cutting edge of biotechnology for some time to come. We trust that these volumes will help with the realisation of that future.

R. E. Spier
J. B. Griffiths

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PART I

AN INTRODUCTION

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Introduction

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1. THE AIMS AND SCOPE OF THIS BOOK

A technological revolution in the way in which bacteria were used for generating useful products occurred over a period of some 30 years. This time period began with Weizman's pioneering acetone–butanol fermentation in 1914 and culminated in the development of unit processes based on stirred tank reactors of 20,000 litres to generate penicillin in the mid-to-late 1940s. A similar revolution is in progress with regard to the way in which animal cells in culture are grown and exploited for the generation of products. Its beginnings may be discerned in the late 1940s with Enders' work on the production of poliomyelitis virus from human embryonic tissues. This gave a direct impetus for the large-scale production, in tissue culture, of sizeable

quantities of immunogenic material to make polio vaccines. The transition from bench-scale cultures to full-scale industrial apparatus whose maximum development at present stands at 8000-litre cultures for cells which do not require a supporting solid substratum (suspension cells) and 1000-litre cultures for cells which require a substratum is a process which is still in its dynamic phase.

It is the purpose of this book to enable the reader to become knowledgeable about the current status of this rapidly advancing technology and to appreciate that much exciting work yet remains to be done both in the engineering of animal cell lines to meet specific industrial and academic requirements and in the innovation, design and development of new ways to cultivate animal cells on the large scale. This infers collaboration between experts in many diverse fields, including molecular biologists, biochemists, immunologists, cell biologists and engineers. An additional aim of this book is to enable scientists of these different disciplines to understand each other's aims and problems, and to be able to communicate with each other in order to gain a better collaboration. It has also necessitated the multiauthor approach that has been used so that the extensive scope of animal cell biotechnology can be expertly presented.

We have presented the material in such a way that the practical aspects of the subject have been emphasised. This has been coupled with a requirement of the contributors to provide well-referenced texts so that the reader may become acquainted with the finer details by such a comprehensive introduction to the growing body of published work. The level at which we have pitched the contributions is such that a person who is being, or has been, educated at a university/polytechnic in one of the basic areas of the biosciences would be able to grasp the principles and practices of this new area of activity. Also, the text has been so organised as to serve as a broad and extensive survey of the present state of the art for those who are currently engaged in advancing knowledge, understanding and capabilities in the area of the biotechnology of cultured animal cells.

The flow of subject matter follows naturally from the kinds of activities which proceed when generating products from animal cells. The early chapters describe the cells, the media they grow in and the unit operations which enable the cultured cells to be grown in equipment free of viable contaminants. This is followed by a section which deals, in some detail, with the different kinds of technologies which are currently in use and under development for the production of the two basically different sorts of animal cells: the suspension cells and the substratum-adherent cells, often referred to as "monolayer" or "anchorage-dependent" cells. Having grown the cells, attention is transferred to the kinds of products which are currently made from such biological substrates or raw materials. In this section emphasis is given to the two areas which dominate this field, these being the viruses for use in

vaccines and the antibodies, whose uses are presently expanding rapidly but already include applications for purifying immunoactive materials, diagnostic systems and detailed epidemiology and molecular analysis of disease-causing organisms. To have produced a material which forms the major and critical ingredient of a product is insufficient in itself. Such materials have then to be rendered in a suitable form to generate the maximum effect in the safest configuration. This is achieved by the series of downstream processing operations which result in more concentrated, less contaminated and safe to use materials. These are then formulated to potentiate their effects, prolong their shelf life and make them easy to use in the field. The bioproducts which result from these processes have to meet those requirements of safety and efficacy as determined by regulatory authorities whose function is to protect society at large against the dangers inherent in the applications of any prophylactic, therapeutic or other product which has been manufactured for direct bodily application. As such requirements are stringent, the many tests involved in the process of product acceptance have been given a separate section. It is important to realise that such tests may account for two-thirds of the total manufacturing costs (before packaging, storage overheads, sales, profits etc.). The agreement by a regulating agency to a particular testing regime (and the expected or acceptable results) also constitutes a major portion of the development costs of a product (typically in the range of £5–15 million) so that "regulatory agency requirements" impinge markedly on the design and development of the upstream equipment, processes and biocomponents.

In setting the scene for the depiction of the present state of animal cell biotechnology it was mentioned that this area is presently in the throes of its revolution. The final section, therefore, attempts to indicate the direction in which future changes in product profiles and technological developments can be expected. This area does not yet constitute an open and closed package in the history of technology. It is the view of the authors that we are but at the beginning of a journey of exploration and exploitation of the biochemical potential inherent in the animal cell in culture. Where we shall be in the decades ahead may well depend on you, the reader. We, as editors and authors, will be well satisfied if we have helped in some way to extend the scope of the applications and increase the rate of progress in this rapidly moving area of endeavour.

2. THE HISTORY OF THE USE OF LARGE-SCALE CULTURES OF ANIMAL CELLS

Acceptance of the idea that a cell belonging to a higher animal could be removed from that animal and thence made to grow and replicate in an *in*

vitro situation did not occur until the early decades of the 20th century. Once it became apparent that such procedures were possible a second phase of activity began with the demonstration that the filterable infectious agents, viruses, could be grown and replicated in the extra-corporeal environment in such cells. A third phase may be delineated from the time of the demonstration of the production of useful amounts of virus for vaccine applications up to the time when (1) it became possible to insert and express particular exogenously derived genes in animal cells and (2) it was shown that it is possible to grow in culture a population of cells derived from a single cell. When such populations are derived from an antibody-excreting cell, the antibody molecules of the culture supernatant are all alike. The implications and consequences of these latter two capabilities are currently under intensive investigation. This marks the beginning of a fourth phase of activity and brings this historical survey up to the present. These phases will be considered in more detail below.

2.1. Growing the Cells of Higher Animals in Culture (*29*, *34*, *35*)

The demonstration of the capability of animal cells to grow and divide in culture required that a number of techniques were available. These may be summarised as

1. A means of obtaining some animal cells free of exogenous prokaryotes and fungi.
2. The development of a medium in which such excised cells could thrive.
3. A methodology to view such cells and observe their developments.
4. A means of continuously propagating such *in vitro* cultures and of keeping them free of other biological agents.

The intellectual background to these developments was provided by the development of the concept of a cell as the fundamental unit of living organisms of both the animal and plant kingdoms. This idea arose from Hooke's 1665 observation that the vesicles and cavities observable in cork were like the "cells" which form part of a monastery. Such observations were extended by Malpighi (1674) and Greur (1682), who observed plant cells filled with fluid and bounded by cell walls. Later (1806) Treviranus noted, as did Hugo von Mohl in 1830, that meristem cells elongated and divided. It was left to Schleiden to formulate a definitive theory of the cellularity of plants (1838), in which he included an important role for the nucleus, previously discovered by Brown in 1831. While comparisons between animal and plant tissues had been made by such as Muller (1835) and Henle and Purkinge (1837), it was Theodor Schwann, having met Schleiden in 1837 and dis-

cussed the existence of nuclei in both animal and plant cells, who published in 1839 a series of papers with the title "Mikroskopische Untersuchungen uber die Ubereinstimmung in der Structur und dem Wachstum der Tiere und Pflanzen" and thereby established the universality of the cellular basis for all the then known forms of animal and plant life. For these reasons our modern concept of the cell is said to have arisen from these two pioneers—Schleiden and Schwann (*31*).

A second idea, that of Claude Bernard (1813–1878), held that it is a characteristic of living things to preserve the status of their internal conditions despite changes in their external environment. His concept related to the organism as a whole, yet it can be equally well applied to a cell growing in culture outside the body. It followed from this approach that a cell outside the normal environment of an animal would seek to maintain its internal conditions and that it would be most likely to grow and divide when the difference between its internal environment and its external environment was minimal. Such thinking led to the development of fluids capable of sustaining and promoting cellular life outside the body.

At about the same time a number of investigators were showing that living tissue could be maintained outside the body. W. Roux (1885) retained the viability of a medullary plate of a chick embryo in warm saline and became an active publicist of this *in vitro* embryology. In 1887, Arnold demonstrated that the cells which had colonised a piece of alder pith when inserted into frogs could be shown to leave their new home when it was transplanted into an *in vitro* situation. Loeb (1897) demonstrated the survival of the cells of the blood and connective tissues in tubes of serum and plasma, and Ljunggren (1898) showed that he could keep an explant of human skin sufficiently viable in ascitic fluid for its successful reimplantation.

Additional experiments were performed by Jolly (1903), who observed cell division in hanging drops containing salamander leucocytes, and Beebe and Ewing (1906), who observed the same phenomena in an explanted canine lymphosarcoma.

Following Roux's activities, Ross Harrison became interested in such work and developed the "hanging drop" technique further (*15*), using small pieces of excised frog medullary tube embedded in a clot of frog lymph and held in this structure on the underside of a coverslip perched over a hollow in a glass slide. In 1907, by using such chambers, he was able to observe the growth of nerve cells over periods of several weeks. He quotes growth rates for such fibres as up to 20 μm in 25 min.

While the Harrison experiments were organised to answer questions relating to the physiology of frog nerve cells, the techniques which he used were applied to other cells from the tissues of warm-blooded animals by Burrows, who used a fowl plasma clot in place of a lymph clot (1910). In the