ADVANCES IN BIOTECHNOLOGICAL PROCESSES

Volume 5

Editors

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Preface

The great diversity in the field of biotechnology is reflected by the variety of subjects examined in this volume, the fifth in the series, **Advances in Biotechnological Processes.**

In addition to a chapter devoted to mushroom fermentation and its applications in industry, as well as in propagation of exotic species, there are three chapters that deal with aspects of biological degradation processes: one describes new trends in lignin biodegradation and possible applications to waste treatment, biotransformation processes, and the breakdown of cellulosic materials. Two other chapters analyze the role of biodegradation in treating water—effluents and wastewater—and how the breakdown of some chemicals can result in positive transformation of toxic wastes, or even energy production from heretofore toxic materials.

Other chapters serve to contrast old problems and procedures with new trends, focusing on production of poultry vaccines in embryonated eggs and on the biological manufacture of plasminogen activators. The role of culture media is further described in a chapter devoted to the propagation of mammalian cell cultures, viruses, and other biologicals.

New approaches in two other areas of interest are also part of this volume of **Advances in Biotechnological Processes:** plant tissue culture technology in crop improvement and the new horizon defined by protein modeling using computer graphics. Both chapters outline modern approaches to the study and application of biotechnology, and both also open new channels of scientific exploration through application of biologicals.

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Antonius L. van Wezel

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Culture Media for Propagation of Mammalian Cells, Viruses, and Other Biologicals

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I. INTRODUCTION

Cell, tissue, and organ culture were developed to isolate and analyze functions not discernible in whole animals. While whole-animal studies continue to provide useful information, extraneous factors such as biological variability, complex pharmacokinetics, and cellular heterogeneity, as well as the high cost of maintaining an animal center, have encouraged the utilization of mammalian cell culture techniques for a variety of applications. The primary objective of cell culture is to provide for the cells an exogenous medium that mimics the natural cellular environment by providing the appropriate nutrients, hormones, growth factors, and metabolites in a salt-buffered osmoticum, so that normal cellular growth and biological production may be sustained in vitro [1–3].

Initial attempts at cell culture placed cells directly into the humoral fluids from which they were derived [4]. However, as the cellular requirements were defined biochemically, customized formulations of defined media were designed that provided nutritionally required metabolites and vitamins in a buffered salt solution [5–7]. Mammalian serum and similar products have generally been considered as necessary additives to supplement the growth medium with necessary hormones and protein growth factors. Recent efforts to address the finite supply of fetal bovine serum and emerging requirements for more defined growth medium with reduced protein bulk have resulted in the formulation of more complex growth media that may be supplemented with purified growth factors to permit growth of certain specific cell types at reduced levels or in the absence of mammalian serum [8–9].

This chapter will provide a brief overview of the historical development of mammalian cell culture media and a discussion of the function of required components. The effects of serum on cellular growth and metabolism are discussed with observations regarding serum standardization and substitution by growth factors. The final section addresses selected applications of mammalian cell culture for large-scale production of biological materials and for clinical diagnosis.

II. MAMMALIAN CELL CULTURE MEDIA

A. Historical Development

The techniques of growing mammalian cells in vitro have been practiced for nearly a century. In many ways, however, tissue culture is still making the transition from an art form to a science.

In 1878, Bernard hypothesized that cells and tissues produce in vivo metabolic products that interact with other tissues to regulate their function

[10]. He advised that to discriminate independent tissue functions from organismic influences would require cell isolation within an artificial environment. In 1885, Roux performed the first "explantation" experiment with living tissue [11,12]. Roux isolated the medullary plate of a chick embryo in a warm saline solution and thus demonstrated that closure of the medullary tube was primarily a function of the constituent cells.

The ingenious experiments of Harrison further illustrated the suitability of tissue culture for investigating the structural elements of higher organisms [13]. The collaboration of Harrison and Carrel and colleagues [4,11,14–16] resulted in continued refinements to the salt and amino acid composition of growth medium and initiated the use of supplemental plasma and clotted lymph as growth-promoting additives. Numerous current applications of cell culture technology were initially addressed by this group, including inotropic effects in myocardial cells, comparative growth of normal and malignant cells, and the establishment of bioreactors for the propagation of viruses and other biological products.

Morgan et al. [17] developed a chemically defined medium capable of supporting the growth of chick embryo cells for an average of 4–5 weeks. This formulation is currently known as Medium 199 and is still widely used for maintaining a variety of primary cell cultures. Shortly thereafter, another chemically defined medium was developed capable of supporting growth of L cell mouse fibroblasts for 5 months in continuous culture [18].

The research of Eagle and co-workers elucidated the nutritional requirements of cultured mammalian cells [19–21]. Eagle was unable to obtain reproducible growth of L cells without the addition of small amounts of serum, so he initiated a study to identify the missing nutrients. The specific amino acid and vitamin requirements determined by Eagle for this particular mammalian cell line have served as a basis for the majority of the strain-specific formulations currently employed in cell culture (e.g., the minimal essential media (MEM), Dulbecco's MEM, Iscove's modified DMEM, etc.—see also Table I).

A major landmark in the development of cell culture media was provided by Swim and Parker when, in 1958, they described the preparation of chemically defined media in powdered form [22]. Swim and Parker initiated their studies on the basic premise that tissue culture media in powdered form would not only be uniform but also be more economical and exhibit superior shelf life relative to liquid medium formulations.

The findings of Swim and Parker were extended by Hayflick et al. [23], who demonstrated the usefulness of dry powdered media for the successful passage of fastidious cell lines for research and vaccine production. Young et

TABLE I. Comparison of the Nutrient Composition of Selected Mammalian Cell Culture Media

	BME[a]	MEM	α MEM	RPMI 1640	Ham's F-12	DMEM	IMDM
Inorganic salts							
$CaCl_2$ (anhydrous)	200.00	200.00	200.00	100.00[b]	44.00[b]	200.00	165.00
KCl	400.00	400.00	400.00	400.00	223.60	400.00	330.00
$MgSO_4 \cdot 7H_2O$	200.00	200.00	200.00	100.00	122.00[c]	200.00	97.64[c]
NaCl	6,800.00	6,800.00	6,800.00	6,000.00	7,599.00	6,400.00	4,505.00
$NaHCO_3$	2,200.00	2,200.00	2,200.00	2,000.00	1,176.00	3,700.00	3,024.00
$NaH_2PO_4 \cdot H_2O$	140.00	140.00	140.00	1,512.00[d]	268.00[d]	125.00	125.00
$CuSO_4 \cdot 5H_2O$	—	—	—	—	0.00249	—	—
$FeSO_4 \cdot 7H_2O$	—	—	—	—	0.834	—	—
$ZnSO_4 \cdot 7H_2O$	—	—	—	—	0.863	—	—
$Fe(NO_3)_3 \cdot 9H_2O$	—	—	—	—	—	0.10	—
KNO_3	—	—	—	—	—	—	0.076
$Na_2SeO_3 \cdot 5H_2O$	—	—	—	—	—	—	0.0173
Amino acids							
L-alanine	—	—	25.00	—	8.90	—	25.00
L-arginine	17.40	126.00[e]	105.00	200.00	211.00[e]	84.00	84.00
L-asparagine	—	—	50.00	50.00	15.01	—	28.04
L-aspartic acid	—	—	30.00	20.00	13.30	—	30.00
L-cystine	12.00	24.00	24.00	50.00	—	48.00	91.24[f]
L-cysteine·HCl·H_2O	—	—	100.00	—	35.12	—	—
L-glutamic acid	—	—	75.00	20.00	14.70	—	75.00
L-glutamine	292.00	292.00	292.00	300.00	146.00	584.00	584.00
glycine	—	—	50.00	10.00	7.50	30.00	30.00
L-histidine	8.00	42.00[g]	31.00	15.00	20.96[g]	42.00[g]	42.00[g]
L-isoleucine	26.00	52.00	52.50	50.00	3.94	105.00	105.00
L-leucine	26.00	52.00	52.40	50.00	13.10	105.00	105.00