Cell Culture in the Neurosciences

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CURRENT TOPICS IN NEUROBIOLOGY

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Preface

A fundamental problem in neuroscience is the elucidation of the cellular and molecular mechanisms underlying the development and function of the nervous system. The complexity of organization, the heterogeneity of cell types and their interactions, and the difficulty of controlling experimental variables in intact organisms make this a formidable task. Because of the ability that it affords to analyze smaller components of the nervous system (even single cells in some cases) and to better control experimental variables, cell culture has become an increasingly valuable tool for neuroscientists. Many aspects of neural development, such as proliferation, differentiation, synaptogenesis, and myelination, occur in culture with time courses remarkably similar to those *in vivo*. Thus, *in vitro* methods often provide excellent model systems for investigating neurobiological questions.

Ross Harrison described the first culture of neural tissue in 1907 and used morphological methods to analyze the cultures. Since that time the technique has been progressively modified and used to address an ever widening range of developmental questions. In recent years a convergence of new or improved cell culture, biochemical, electrophysiological, and immunological methods has occurred and been brought to bear on neurobiological questions. This volume is intended not to be comprehensive but rather to highlight some of the latest findings, with a review of previous important work as well, in which combinations of these methods are used. The chapters cover a broad range of topics, but they can be divided into two major sections: (1) morphological and biochemical studies and (2) electrophysiological studies. All of the authors have made significant contributions in their respective areas of research.

The section on morphological and biochemical studies comprises nine chapters. The chapter by Bottenstein briefly describes the different

types of culture preparations, the major components of a culture system, and, in more detail, the study of the molecular requirements for growth of cultured neurons and glial cells using serum-free methods. The importance of soluble and extracellular matrix factors and the cell type-specificity of these requirements are described. Fields reviews the antigenic markers presently available that have proven so useful for unambiguously identifying the different neural cell types *in vitro*. She describes neural cell surface antigens, including a discussion of their use in perturbing cellular function and in cell separation. Both polyclonal and the newer monoclonal antibodies are described, some of which discriminate between subpopulations of neurons and glial cells. Perez-Polo describes the discovery of nerve growth factor and other more recent neuronotrophic factors and their importance in neuronal survival, differentiation, and possibly regeneration. He discusses the benefits of using clonal cell lines for studying the responses to neuronotrophic factors. The chapter by Saneto and de Vellis covers the area of hormonal regulation of the proliferation and differentiation of primary CNS glial cells. They emphasize the advantages of using isolated populations of cells for studying these phenomena. Landis describes the classic experiments which first demonstrated the plasticity of the neurotransmitter phenotype in superior cervical ganglion cells in response to epigenetic regulation. Analysis at the single-cell level provides evidence for a transition from noradrenergic to cholinergic function, with an intermediate dual-function stage, as a result of interaction with target cells. The ability to distinguish induction from selection for already committed cells is only possible using *in vitro* methods. She also correlates the *in vitro* results with *in vivo* data, including electron microscopic analysis. Sieber-Blum and Sieber discuss the influence of soluble factors and the extracellular matrix on the migration and differentiation of neural crest cells. Clonal cultures of neural crest cells indicate that differentiation into melanocytes and adrenergic neurons can occur in the absence of noncrest cells, contrary to a widely accepted belief. Honegger's biochemical studies of reaggregate cultures of fetal rat brain, containing a mixture of cell types, illustrate the influence of several growth factors and hormones on both neuronal and glial differentiation. The effects on cholinergic neurons are described in the greatest detail. Serum-free culture methods provide a significant improvement for analyzing these phenomena. Guroff compares the properties of the PC12 clonal cell line with adrenal chromaffin cells and sympathetic neurons. He concludes that PC12 cells are an excellent model system for studying the mechanism of action of nerve growth factor and some aspects of neuronal differentiation, e.g., neurite extension and synapse formation. The

chapter by Eddé and Darmon reviews the work of several groups investigating neuronal differentiation in embryonal carcinoma cells, which are multipotential stem cells able to give rise to derivatives of all three germ layers. When these cells differentiate, they are no longer tumorigenic and thus provide a unique model system. The authors describe several inducers of neuronal differentiation.

The section on electrophysiological studies comprises three chapters. The role of electrical activity in modulating neuronal survival and cholinergic differentiation in fetal mouse spinal cord neurons is discussed by Brenneman and Nelson. Blockade of electrical activity results in decreased survival and expression of cholinergic function. They also defined the critical period and suggested that electrical activity releases trophic factors that mediate the effects seen. Smith and Barker give a general introduction to classical electrophysiological techniques, i.e., intracellular stimulation and recording. They describe the underlying ionic mechanisms of neurotransmitter-mediated inhibitory and excitatory postsynaptic potentials in embryonic spinal cord and hippocampal neurons in culture. Quantitative analyses, including the use of voltage-clamping, can be made with cultured neurons that are difficult if not impossible to apply to CNS neurons *in vivo*. The more recent development of the patch-clamp method is discussed by Huang. Further biophysical analysis of single ion channels and their regulation, including aggregation of channels, is now possible using cultured neurons. Conventional neurotransmitter-activated and voltage-sensitive channels are described, as is the discovery of several new species of channels.

The authors have used a variety of preparations and asked many different questions. They have described the advantages and the limitations of their respective preparations and have given us a preview of future directions. Many of the experiments described here illustrate the individuality of growth and differentiation requirements of different neural cell types and their enormous plasticity during development in response to environmental influences. While cell culture is not the only or necessarily the best method to study a particular neurobiological question, this volume contains some of the best examples of when cell culture can be most profitably employed in neuroscience.

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Contents

I. Morphology and Biochemistry