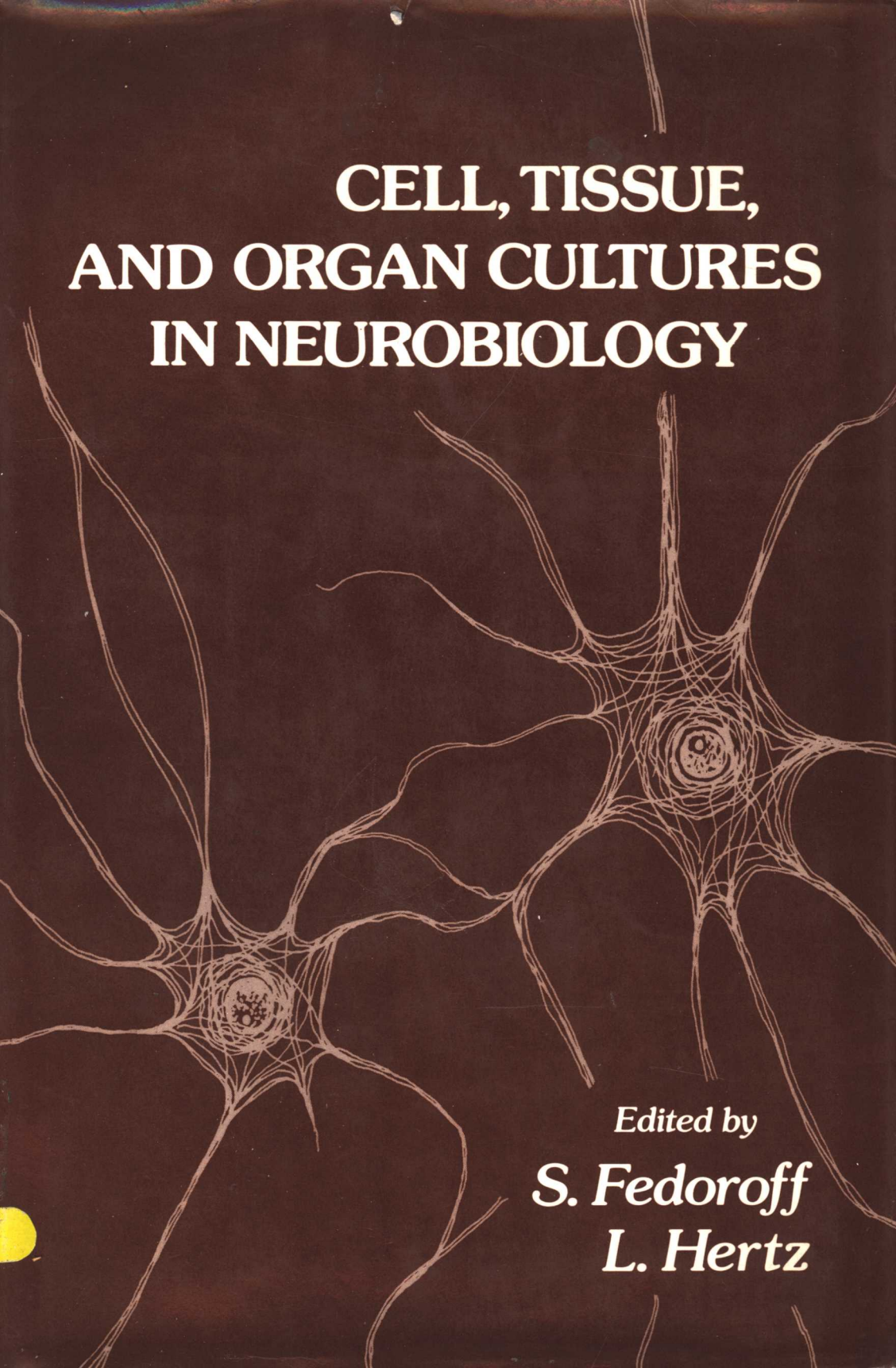
CELL, TISSUE, AND ORGAN CULTURES IN NEUROBIOLOGY



Edited by

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ACADEMIC PRESS
New York San Francisco London 1977
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data

Main entry under title:

Cell, tissue, and organ cultures in neurobiology.

Proceedings of the international workshop on cell, tissue, and organ cultures in neurobiology held at the University of Saskatchewan, Saskatoon, Canada, March 20–22, 1977.

1. Nerve tissue—Cultures and culture media—Congresses. 2. Cell culture—Congresses. 3. Tissue culture—Congresses. 4. Organs, Culture of—Congresses. I. Fedoroff, S. II. Hertz, L., Date [DNLM: 1. Tissue culture—Congresses. 2. Neurology—Congresses. QS530 C393 1977]
QP363.C44 599'.01'88 77-21066
ISBN 0-12-250450-X

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

In recent years the application of tissue culture in studies of the nervous system has widely increased. The uses of cell, tissue, and organ cultures in neurobiological research to the present time are reviewed in this fundamental reference book. We thus bring together an interdisciplinary perspective from morphology, biochemistry, pharmacology, endocrinology, embryology, and genetics. Several chapters deal with specific aspects of the nervous system *in vivo*. In order that cultured neural cells and tissues can be assessed as models of their known *in vivo* counterparts, the relevance of various tissue culture preparations for neurobiological investigations are evaluated.

The book will be of interest to people in a wide range of disciplines. It is directed toward tissue culturists concerned with the nervous system, as well as all neurobiologists, cell biologists, and embryologists interested in learning how neural cells and tissues behave in cultures and what has been learned about the nervous system using tissue culture methods, including the applicability of tissue cultures to the study of cell differentiation.

This volume is one outcome of an international workshop held at the University of Saskatchewan in March 1977. Many of the papers presented have been expanded, some considerably, to form chapters in the book; some points made during the discussions have been elaborated on and are included as chapters; the discussion sessions have been summarized and extensively edited to reflect the trends of thought and the interactions between the scientists of various disciplines present at the workshop. The ultimate aim of the volume is to provide a sound basis for the use of nervous tissue culture in the solution of neurobiological problems.

The publication of this volume had the generous support of the College of Medicine and Graduate Studies and the Department of Anatomy, University of Saskatchewan; the Pasadena Foundation for Medical Research; the National Institute on Aging, NIH, U.S. Public Health Service; the Muttart Foundation; the Multiple Sclerosis Society (Canada); and Gibco, Canada.

We are particularly grateful to D. G. Murphy and W. A. Opel for continuing interest in the workshop and to A. Boulton, I. Munkacsi, F. Oteruelo, P. Sulakhe, B. Wenger, K. Seemann, and the staff of the Department of Anatomy, University of Saskatchewan, for help in organizing the workshop.

Elaine Fedoroff, Elna Hertz, B. H. J. Juurlink, Anna Leslie, and Jean MacGregor gave invaluable assistance in the preparation of material for this volume;

Jean MacGregor created the cover design; the staff of the Modern Press in Saskatoon worked diligently in the preparation of the camera ready copy; the staff of Academic Press was patient and helpful in the final stages of publication.

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INTRODUCTION

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Nerve tissue culture was born just 70 years ago, when R. G. Harrison, cognizant perhaps of attempts then being made by others to maintain somatic cells *in vitro*, devised a simple and elegant procedure to investigate the genesis of the nerve-fiber. Being an experimental embryologist in the year 1907, he was well acquainted with the principle of "explantation"; but he carried it a step further by transferring a section of neural tube from the frog embryo to an indifferent, non-cellular substrate which consisted of clotted frog-lymph, enclosed in a transparent glass depression-slide. The naked outgrowing nerve fibers with their pseudopodal tips preceded the later-emerging supportive cells by a substantial time — and space — margin, thus strongly indicating that the fibers were not generated as a syncytium or nerve-net by cells along their course. The success of this experiment was spectacular enough to impress on others that the method was worth pursuing, and they took out after it from all directions in this country and abroad.

For these pioneers, practically every observation yielded pay-dirt, and though nearly everyone at some time had a try at growing nerve-tissue or made incidental observations on it, those reports were almost buried in the general accumulation of tissular riches during the ensuing score of years. They were easy to bury because as Harrison's successors developed culture techniques to accommodate other, proliferative, histological types, nervous tissues, especially CNS, for a variety of reasons were found to be refractory. The definitive neurons did not proliferate nor enjoy a long life in culture; they remained viable no more than a week or two, as a rule. Harrison had luck as well as genius going for him in his choice of problem and material: A question that could be answered in short-term (a few days) culture of embryonic amphibian cells which carried with them their own native nutrients in the form of yolk.

Nevertheless, even in its primitive state the culture method could be utilized effectively by others for nerve study. In 1913 Ingebrigtsen, who as a neurosurgeon was interested in the possibility of regeneration, addressed himself with some success to one of the contemporary problems: he cultured

cerebellum from the chick and a number of mammals in plasma clots and established that severed CNS axis cylinders of higher vertebrates were able to regenerate — growing out as they did from his explants in typical beaded form, though soon suffering the granular degeneration that (as described by Nauta in 1950) is now well-known to pathologists. The significance of these observations went generally unrecognized and unacclaimed. Then as now, it took unusual showmanship or a unique train of circumstances to breach the habitual thought patterns of medical savants unacquainted with the living cell. Neurobiologists were not far behind them; in 1944 Nonidez felt obliged to publish a review on the status of the Neuronfrage, in a final effort to lay at rest the tenaciously held nerve-net theory. Let us admit that a worker with a large investment in technique or hypothesis does not readily give it up on the publication of an adversary. And in justice it should be said that in the light of more modern technical practices culture conditions then were far from ideal, and results could therefore be suspect. Nevertheless, the principle cannot be enunciated too often: that anything a cell is seen to do in culture must be counted among its potentialities.

In an introduction it would not be possible or desirable to cover even summarily the development of nerve tissue culture in method and substance over the three-score years and ten that have preceded the present sessions designed to illuminate the status quo. Moreover, substantial reviews are now available and these have approached the subject from different angles as the field has become diversified (Murray, 1965, 1971; Sato, 1973; Bunge, 1975; Nelson, 1975; Crain, 1976). The last decade particularly has seen great expansion and multiplication of technical procedures concomitant with the setting of new goal-horizons. Ancillary methods such as light and electron microscopy with their recent advances, also continuing biochemical, neurochemical and pharmacological specializations, have been combined and recombined with enhanced culture procedures in the pursuit of more precisely defined objectives; and since in addition, each experimental run put out by a collaborative group tends to be published as a separate paper with a different senior author, the resultant literature expansion has become unmanageable. As regards the body of nerve tissue culture, the right hand no longer knows what the left hand is doing.

The symposial gathering held in Saskatoon in March 1977 was designed to resolve this dilemma by bringing together practitioners of varied persuasions and goals in order that they might learn from each other and set up a system of cross-fertilization. The group was small but participants were selected on an ecumenical basis and their communications grouped in topical rationale. Provision was made each day at the end of the practical sessions for discussions in depth that might deal with problems of general significance suggested by the reports. No holds were barred, and these lively interchanges provide invaluable information as well as

perspective. Now that tissue culture has become established as an adjunct to nearly every discipline, the most urgent general question that faces the research worker today may be the validity of his material as model: its degree of applicability to situations as they occur spontaneously *in situ*. To what extent can principles deduced from observations upon nervous tissues isolated in culture (of whatever type) be presumed to hold in the intact organism? The separation of parts from the whole and of individual factors from a complex is basic to the experimental method; this is accomplished par excellence in tissue culture. Nevertheless, other considerations must also be taken into account in exploring the "Model" problem and these may be of greater or less importance depending on specific circumstances, — as these discussions make abundantly clear.

Following World War II the barriers to neuron longevity in culture were resolved by modifications of medium, substrate and culture-chambers, so as to allow the production of "organotypic" microcosms (Murray, 1965) of differentiating cell communities which with meticulous handling could survive months of isolation in essentially normal form and function. Their LM verisimilitude was amply reinforced by fine-structural and bio-electrical findings. The first region of the nervous system to be brought under control was the sensory ganglion, followed by the cerebellum, the cerebrum, the hypothalamus and spinal cord, and finally the sympathetic ganglia. Time-lapse moving-pictures, preeminently in the hands of Charles Pomerat, greatly accelerated and amplified the general medical understanding of living neurocytology and cellular movements (Hydén, 1967). For qualitative observations of development, specific cytopathology and nerve-cell physiology, the organotypic culture method, competently handled, is unmatched. But the amount of labor and skill involved tends to disqualify it for types of investigation that require more than micro-quantitative procedures, e.g., most problems of analytical neurochemistry.

It is not surprising, therefore, that the possibility of working with established strains of neoplastic neuroblasts and astroblasts attracted the enthusiasm of biochemists and their students, or that a truly enormous literature has burgeoned about these in the last decade, sparked by a high order of ingenuity on the part of their exploiters (Sato, 1973). Here was a source of material that could be propagated in bulk with relatively simple culture manipulations. However, the variability of these lines — even within clones, and a sober appraisal of their neoplastic character led neurobiologists to revert to dissociation methods (long practised by Moscona and his school) for the study of developing normal nervous tissues. By this means, starting with whole brains or regions, one could grow dispersed cells en masse for a substantial period, allow them to reaggregate (Moscona, 1965; Garber and Moscona, 1972) or examine them in monolayers, for whatever purpose. Though the ratio of viable neurons to supporting cells may be

small and tends to diminish with age *in vitro*, primitive contacts are formed between them which can be demonstrated by EM and by evoked action potentials. It now appears that with critical timing and treatment (e.g., with anti-mitotics) the relative amounts of various cell types to be cultured can be modified substantially, so as to produce "highly purified" populations of neural or non-neural cells. It should not be overlooked in the study of the *Neuroblastoma* that this tumor is not derived from the CNS, but from sympathetic ganglia or adrenal medulla, as any pathologist will confirm. The spontaneous neuroblastoma is a highly infiltrative tumor which often spreads massively to the spinal cord and quickly metastasizes further. Goldstein (1964), in working with the human type, sometimes finds differentiation in the direction of sympathetic ganglion cells, never of cord or brain neurons.

As these developments were taking place, the promoters of organotypic culture returned to the charge by confronting explants with dissociated cells from other selected regions and thus pinpointing the individual cell contacts on both morphological and physiological and macromolecular grounds. (Bunge *et al.*, 1974). Thus these methods, originally poles apart, have been combined to mutual advantage.

It is from this congeries of approach, method and emphasis that topics were assigned and representative participants were selected for the Saskatoon meeting. Along the way, histologists and embryologists were pressed into service to set forth some basic morphological criteria, electrophysiologists and chemists likewise in their fields, for mutual orientation among this group of sub-specialists.

The participants' communications in all these areas ably speak for themselves. This writer need only direct attention to the architectonics of the compilation. We are dealing here with living nervous tissues removed from their anatomic and histological surroundings *in situ* to an indifferent substrate and unaccustomed medium *in vitro* where questions of varying complexity are asked of them. It is of paramount importance to identify the cell-types which present themselves in a primary culture or its descendents — to recognize neurons, glial cells, Schwann cells and contaminants such as endothelial cells visually as far as possible, though conceding that reliance on other means may be necessary as well as confirmatory. To this end a session was devoted to the morphology, physiology and biochemistry of differentiated nervous tissues, particularly as the major types diverge in development. In the present state of our knowledge, the most generally or overall successful organotypic cultures are explanted in early life from fetal or newborn animals, at a time when morphogenetic movements have ceased and the region under study therefore has approximately its full quota of precursor cells which are sufficiently potent, and at the same time flexible or malleable enough to accommodate to the new environment and continue