MICROCOPY RESOLUTION TEST CHART
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ON
NEURONAL CELL CULTURES
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Held at Vermont Academy
Saxton's River, Vermont
On July 19 - July 23, 1982
This conference was the first to provide an opportunity to survey the entire scope of the contributions of nerve tissue culture to neurobiology. The conference was designed to include all areas where substantive progress had been made by the study of nerve tissue in culture. In 1960 no more than six scientists would have qualified to present at the meeting; in 1982 it was difficult to reduce the total number of speakers for the nine sessions to 27. Of the 27 main speakers, three were from Europe, and the remainder from the U.S.A.

The conference was organized into nine sessions, with eight separate themes. Details of each of the sessions are provided in the enclosed program. The first session provided a survey of the contributions of nerve tissue culture to understanding mechanisms of nerve fiber growth. Dr. Dennis Bray of London, England reviewed the mechanisms by which the nerve fiber growth cone utilizes amoeboid activity to cause the nerve fiber to be elongated. Dr. Paul Letourneau of Minneapolis summarized recent immunocytochemical studies which allow identification of the proteins (particularly actin) present in the growing tip which provide the basis for its constant movement. Dr. Lloyd Greene of New York reviewed recent findings on the cell line designated PC12, which has the remarkable ability to extend nerve fibers in the presence of the well characterized trophic factor, nerve growth factor. This session provided a broad synopsis of our understanding of the mechanisms by which the nerve fiber is elongated during growth and during regeneration.

In the second session Drs. Varon, Thoenen and Barrett provided an overview of trophic factors able to influence neuronal survival and the initiation and promotion of neurite growth in tissue culture. Dr. Silvio Varon defined the types of influences which determine whether a specific nerve cell will attach to its substrate, will survive in culture, and will generate a neurite. He described several systems under study in his laboratory in which the separate aspects of nerve growth are being studied and the specific agents involved in neuronal survival and growth are being defined. Dr. Hans Thoenen described studies on the action of nerve growth factor on cultured dorsal root ganglion neurons and pointed out that these cells are maximally responsive to nerve growth factor for only a limited period of their embryonic development. Their survival then comes to depend on a second agent which Dr. Thoenen and his colleagues have very recently isolated from brain. The general concept evolving in this work is that each neuronal type may have unique factor requirements for its survival and for promotion of its growth; it may therefore be necessary to isolate specific components for each neuron under study.

In the third session tissue culture studies which have elucidated the influences on development of neurons of the autonomic nervous system were presented. Dr. Paul Patterson reviewed the extensive work by himself and his colleagues on the factors which cause adrenergic neurons placed under certain culture conditions to assume cholinergic properties. Dr. Mary Johnson presented evidence (using similar culture systems) that the expression of cholinergic properties is added to, and does not substitute for the basic adrenergic properties of the neuron; both characteristics are expressed in autonomic neurons under many culture conditions. The general concept evolving from this work is that the phenotype of at least certain neurons is not fixed
at the time of last neuronal mitosis but may be altered after the neuron has completed division and transmitter use of the neuron may subsequently be substantially changed. Dr. Darwin Berg reviewed work with parasympathetic neurons, including efforts to define the trophic factors which allow the long term culture of this neuron, and which are clearly not related to nerve growth factor.

In the fourth session the degree of sophistication in electrophysiological studies allowed by studies of cultures of dissociated nervous tissue was reviewed, by Drs. Philip Nelson, Gerald Fischbach and Edwin Furshpan. These workers demonstrated the degree of precise quantitation that one may obtain by the use of intracellular recordings from cultured neurons. This sophistication includes an effort to count the actual number of boutons present on specific cultured neurons and by studying the electrophysiological properties of the release of transmitter from these boutons to make calculations regarding the number of vesicles that are released per bouton for each electrical impulse. Dr. Nelson reached the rather surprising conclusion that approximately one vesicle of transmitter is released per electrical impulse. Dr. Fischbach reviewed the substantial data available regarding the formation of the neuromuscular junction in tissue culture, by the use of cocultures of neurons and muscle cells. These studies have led to the concept that the contact of nerve fibers on muscle influences the distribution of acetylcholine receptors on the surface of that muscle. Dr. Furshpan described in detail his studies on multifunctional neurons in culture, studies which taken together with immunochemical data, clearly show that the concept that neurons deal with only one transmitter is incorrect. Dr. Furshpan discussed neurons with the capability of releasing norepinephrine, acetylcholine and ATP.

In session five, Drs. Stanley Crain, John MacDonald and Monroe Cohen discussed various aspects of spinal cord physiology which have been elucidated by the use of nerve tissue cultures prepared from spinal cord itself and from spinal cord in combination with its input, that is the sensory ganglion and its output, the somatic motor neuron. In session six observations on myelin forming cells in both the central and peripheral nervous system made in tissue culture were thoroughly reviewed. The central nervous system was reviewed by Dr. Fred Seil of Portland, Oregon and the development of the Schwann cell as studied in culture was reviewed by Dr. Richard Bunge of St. Louis, Missouri. Dr. Martin Raff reviewed the extensive work of himself and his colleagues on the use of immunochemical markers to characterize both oligodendrocytes and Schwann cells. Taken together these results clearly indicated major differences in the cellular physiology of the central myelinating cell, the oligodendrocyte, and the peripheral myelinating cell, the cell of Schwann.

The seventh session dealt with the use of nerve tissue culture for the study of invertebrate nervous system. Dr. Schacher of New York discussed studies done with snail neurons and Dr. Leslie Henderson of Stanford, California discussed studies done with neurons from the leech. Dr. Stan Kater of the University of Iowa discussed elegant studies correlating findings in vitro and in vivo from the study of a freshwater snail. These studies clearly indicated that invertebrate neurons (as do their vertebrate counterparts) express their fundamental properties extremely well in culture. The system presented by Dr. Kater illustrated that certain properties observed to occur when neurons are damaged in the animal can be seen to be expressed by neurons placed in culture and damaged in a similar way. Dr. Kater presented the interesting concept that certain types of plasticity in the invertebrate nervous system can only be observed when both the presynaptic and postsynaptic members of a neuronal pair are damaged.
In sessions eight and nine the culture of specific parts of the central nervous system was reviewed. In particular, we heard presentations on the culture of neurons from the hippocampus, the brainstem, the cerebellum, the hypothalamus, the retina and the cerebral cortex. Of particular interest in this session was the demonstration by Dr. Toran-Allerand of New York that specific regions of the hypothalamus can be shown to respond to testosterone (and to estrogen) by the facilitation of substantial neurite growth and that this effect is limited to a certain period of embryonic development and is only expressed in very specific areas of the hypothalamus. This confirmation of the sensitivity of certain hypothalamic neurons to the sex steroids helps to elucidate the origins of the sexual dimorphism that has been clearly shown to be present in the mammalian hypothalamus.

The conference also included the presentation of approximately 12 poster sessions by the conference attendees other than the main speakers. Throughout the conference there was substantial and vigorous discussion of the topics under study both in the formal sessions and during the afternoon free time. The facilities, staff and meals were generally agreed to be excellent. At the end of the conference it was agreed by those present that the conference had been extremely useful and that it would seem reasonable to consider holding a conference of this type to review the use of nerve tissue culture at approximately two year intervals. Dr. Phillip Nelson was the designated organizer for the second conference.