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Eleventh International Congress of Anatomy, Part A

GLIAL AND NEURONAL CELL BIOLOGY

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EDITOR **Sergey Fedoroff**

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Heinz von Mayersbach, Lawrence E. Scheving, and John E. Pauly, *Editors*

Preface

The papers in this volume are drawn from six symposia in the field of neurobiology held during the Eleventh International Congress of Anatomy in Mexico City in August 1980. Professor Enrique Acosta Vidrio, President of the Congress, had asked anatomists from many countries to suggest topics for the symposia, and those selected represent areas of rapid advance and wide interest.

Three symposia dealt with the topic Recent Advances in Structure and Function of Glial Cells, suggested by Dr. C. Leblond (Canada): one on Astrocytes was convened by Dr. S. Fedoroff (Canada), one on Oligodendrocytes, by Dr. R.P. Skoff (U.S.A.), and one on Microglia, by Dr. A. Privat (France).

A symposium on Morphological Aspects of Interneuronal Communication was convened by Dr. D.G. Jones (Australia), another, on Morphological Basis of Neurophysiology of the Cerebellum, by Dr. R. Llinas (U.S.A.), and one on Contribution of Metallic Impregnation to Neuroanatomy, by Dr. M. Marin-Padilla (U.S.A.).

Participants in the symposia were a broadly representative international group, coming from 12 countries: Australia, Belgium, Canada, France, Japan, Spain, Switzerland, The Netherlands, United Kingdom, U.S.A., Venezuela, and West Germany.

A unique feature of the Congress and hence of this volume was the organization of a separate symposium on each type of glial cell of the central nervous system, a sign of growing interest in the role of glial cells in the functioning of the nervous system.

I have arranged the papers for publication, including some special lectures presented at the Congress, in seven sections, and in so doing, have taken some liberties. Thus, the contents of each section do not correspond exactly to the program of each symposium. The final paper, on the topic Development of the Brain, was given as a special lecture.

I should also point out that it was the responsibility of each author to submit camera-ready copy; in the interest of speedy publication, some sacrifice of editorial consistency was made.

I would like to thank Dr. M.A. Galina, Coordinator of Scientific Sessions for the Congress, and Sra. Ma. Victoria de Acevèdo, Administrative Coordinator, who were extremely helpful in running the symposia and gathering manuscripts. I am grateful also to Dr. F. Oteruelo and Dr. M. Issa for assistance with the translation of some manuscripts, to E. Fedoroff for assistance with the editing, and to I. Karaloff for preparation of manuscripts.

Sergey Fedoroff

STRUCTURE AND FUNCTION OF ASTROCYTES

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PROPERTIES OF PUTATIVE ASTROCYTES IN COLONY CULTURES OF MOUSE NEOPALLIUM

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Nervous tissue has its embryonic origin in primitive proliferating cells, the ventricular cells. These cells diverge into a number of cell lineages each of which undergoes differentiation into specialized cell types. Tissue culture provides nearly ideal conditions for the study of cell lineages. Cells in primary cultures are direct descendants of cells in vivo and provide continuation of the in situ cell lineage (Fedoroff, 1977b). By carefully selecting the stage of development of the embryo and the culturing conditions it is now possible to obtain specific neural cell types including neurons (Wood and Bunge, 1975; Wood, 1976; Varon, 1977), astrocytes (Booher and Sensenbrenner, 1972; Fedoroff, 1977a, 1978; McCarthy and de Vellis, 1980), oligodendrocytes (McCarthy and de Vellis, 1980; Szuchet and Stefenson, 1980), Schwann cells (Wood and Bunge, 1975; Wood, 1976), or neural cells at a specific stage of their lineage (Fedoroff, 1978, 1980).

Colony culture method. In our laboratory we have developed a colony culture method and have used it to study the astrocyte cell lineage (Fedoroff, 1977a, 1978, 1980). We have used embryos from DBA/1J, C_3H/HeJ and Swiss mice as well as chick embryos to isolate the neopallium aseptically after carefully removing the meninges. The hemispheres were then freed of the basal ganglia, olfactory lobes and hippocampus and divided by means of microscalpels into small fragments which were then dissociated by gently forcing the tissue through sterile "Nitex" ® mesh (pore size 75 μm). The cells were then suspended in a growth medium consisting of Eagle's Minimum Essential Medium (MEM) containing a fourfold concentration of vitamins, a double concentration of

amino acids (except glutamine, which was kept at the 2 mM level), 7.5 mM glucose and 5% horse serum (v/v). Cell viability was determined by the Nigrosine dye exclusion technique (Kaltenback et al., 1958). The cells, in various dilutions in a total volume of 2.5 ml growth medium, were plated in 60 mm Falcon petri dishes. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. After three days of incubation, round, refractile cells could be seen attached to the plastic dish surface and many single cells, cell clumps and cell debris were floating in the medium. At this stage the cell debris and non-attached cells were removed and fresh medium was added. On subsequent culturing the dissociated cells began to proliferate and to form discrete colonies which varied in size, density and overall morphology (Fig. 1). The number of colonies formed depends on the number of viable cells initially plated as well as on the age of the embryo (Fedoroff, 1977a, 1978, 1980).

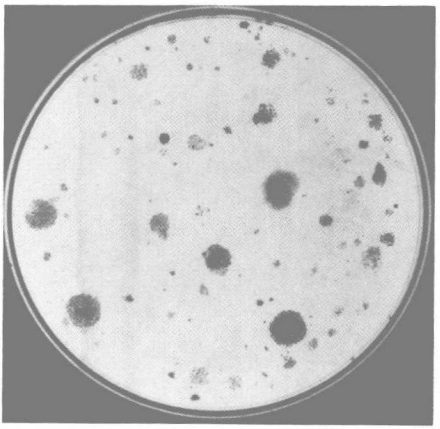

Fig. 1. Culture from dissociated 15-day mouse embryo cerebral hemispheres grown for 14 days in 60 mm Falcon petri dish. Cells form discrete colonies varying in size, density and morphology.

Colony types. Based on morphological criteria six distinct types of colonies have been identified and designated A to F (Fedoroff, 1977a). The frequency of occurrence of the various colony types varies according to the age of the embryo or postnatal mouse, the source of cells in the CNS, duration of culturing and the culture medium used (Fedoroff, 1977a, 1978, 1980; Fedoroff and Hall, 1979; Juurlink et al., 1980). In this paper however, only the relationship between type A colonies and type C colonies will be discussed. Cultures initiated from young embryos formed type A colonies in high frequency and type C colonies in low frequency; whereas cultures initiated from newborn animals formed type C colonies in high frequency and type A colonies in low frequency. Up to

seven days after plating incidence of the two colony types was about the same but after seven days the ratio between the two began to change. Also, at seven days, the size of the colonies was such that the various types could be identified easily. For these reasons, 7-day cultures were used to determine the frequency of occurrence of proliferating cell types in neural tissue. We believe that the 7-day colonies still reflect fairly closely the composition of the proliferating cell population removed from the CNS and can, therefore, be used to assay the cell types in this population at any given stage of CNS development. Shifts in the incidence of colony types on further culturing were used to trace cell differentiation or progression along the lineage (Fedoroff, 1979, 1980). For example, 7-day cultures prepared from the neopallium of 14 day old mouse embryos (E14) had a high frequency of type A and a much lower frequency of type C colonies. After 14 days in culture the frequency of type A colonies decreased and that of type C colonies increased (Fedoroff, 1978). These observations were interpreted to mean that type A colonies in cultures give rise to type C colonies (Fedoroff, 1978). This was subsequently confirmed through direct observation of the colonies by time lapse cinemicrography (Fedoroff, 1978). It should be noted that the change in the cell population of the CNS in embryos of increasing age, as assayed by colony cultures, was the same as the change observed when type A colonies were cultured for 3 or 4 weeks (Fedoroff, 1978, 1980). We are therefore convinced that the changes observed in cultures correspond to those occurring in vivo.

Cells of type A colonies. The type A colonies are composed of epithelial-like cells (Fig. 2) and as mentioned, occur in a very high frequency in cultures from younger embryos (Fedoroff, 1978). It does not seem likely that type A colonies originate from fibroblasts, endothelial cells, blood cells or smooth muscle cells, because each of these cell types has a quite different morphological appearance and growth pattern in culture. The most likely source of the cells in type A colonies seems to be the neuroectoderm. The only other derivatives of neuroectoderm with an epithelial-like appearance which might have been present in our cultures are cells of the pia mater. However, when we cultured cells from pia mater, type A colonies did not form (see also Sensenbrenner and Mandel, 1974; Moonen, 1980). We concluded, therefore that cells comprising the type A colonies are not descendants of pia mater, but originated directly from neuroectoderm. Ultrastructural examination of

type A colony cells indicated that the cells are indeed very immature. The cells have large oval nuclei with evenly distributed chromatin within which only a few patches of condensed chromatin were observed. The cytoplasm is rich in free ribosomes arranged in polysomes but contains relatively few mitochondria and cisternae of rough endoplasmic reticulum. Inclusion bodies, Golgi complexes, 10 nm filaments and microtubules were also observed in the cytoplasm (Fedoroff, 1980; Juurlink et al., 1980).

The fine structure of type A colony cells thus closely resembles that of the "pale" cells in the subventricular zone of newborn (PO) mice described by Smart (1961), Fisher (1967), Lewis (1968), Blakemore (1969), Blakemore and Jolly (1972), Privat and Fulcrand (1977), Imamoto et al., (1978) and Sturrock and Smart (1980). To confirm that the cells of type A colonies originated from the subventricular zone, the zone was carefully dissected from the rest of the neopallium. The number of colonies and the frequency of the type A colony-forming cells obtained from the subventricular zone and from the rest of the neopallium were then compared by the colony culture assay method (Juurlink et al., 1980). It was found that the subventricular zone of PO mice contributed 73% of all the colonies and 70% of all type A colony forming cells in the neopallium. These observations, together with the fact that the ultrastructure of the "pale" cells of the subventricular zone resembles that of the cells forming type A colonies, strongly suggest that the cells which form type A colonies originate from the subventricular zone of the neopallium.

Cells of type C colonies and formation of putative astrocytes. By observing type A colonies in cultures continuously for four weeks with phase contrast microscopy and time-lapse cinemicrography we noticed that type A colonies transformed into type C colonies. The epithelial-like cells of these colonies were no longer closely apposed to adjacent cells but made contact with them by means of many short slender, interdigitating processes (Fig. 3) (Fedoroff, 1977a, 1978, 1980).

The cells of the "mature" type C colonies (present in cultures of two weeks or more), grown in the presence of dBcAMP, form star-shaped cells which resemble astrocytes (Figs. 4 and 5). If the growth of marked individual type A colonies is followed for a period of four weeks during which dBcAMP is added to the medium for the last two weeks type A colonies are observed to gradually develop into type C colonies and eventually into colonies composed of star-shaped