A CYTOLOGICAL STUDY OF A MONOLAYER CULTURE OF CHICK EMBRYO BRAIN TISSUE

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M. K. Karakuyumchyan and N. G. Fel'dman

Laboratory for Virus Cytopathology and Anti-Rabies Laboratory, Moscow Scientific Research Institute of Virus Preparations
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Prior to 1930, the possibility of growing nervous tissue outside the living organism was regarded as a doubtful proposition. The majority of authors held the view that nerve and glial tissue disappeared and only mesenchymatous derivatives—smooth muscle, fibroblasts, and histiocytes would grow in cultures [12].

Since 1931 many Soviet research workers [1,3-5, 7-9] have demonstrated the growth of nerve and glial elements in explanted fragments of nerve tissue. The results of investigations conducted during the last decade [6, 10, 11, 13, 15] have testified still further to the possibility of culturing nerve and glial tissue outside the living organism.

All research workers have removed small pieces of brain tissue (1 mm) to hanging drop cultures composed of blood plasma and embryo extract. Nerve and glial elements have grown out from the explanted fragments into the solid medium.

Attempts at obtaining cell cultures from transplants of brain tissue have only been successful on one occasion, when an Italian research worker [14] obtained monolayer cultures of cells from the cerebral cortex of an adult monkey and a rabbit. These cultures consisted of multipolar and fusiform cells.

EXPERIMENTAL METHOD

The material used consisted of brain tissue from chick embryos after 13-20 days incubation. The brain received the same treatment as any other organ being used as a source of material for monolayer culture: small fragments, up to 1-2 mm in size, were cut with the aid of sterile scissors and then treated with 0.3% trypsin solution, after which they were centrifuged at 1500 revs/min and resuspended in nutritive medium. The cellular suspension was poured into tubes with cover glasses or mica plates and the tubes were then kept in a glass, thermostatically controlled incubator at 37°. A microscopical examination of the growth of the culture in the glass tube was carried out daily. Over a period of 120 h, cover glasses or mica plates with cells growing on them were removed from the tubes and placed in various fixatives. The cells were subsequently stained with common histological stains or given special neurohistological treatment (subvital staining with methylene blue using F. M. Lazarenko's method, the impregnation technique of Compass or Nissl's method).

EXPERIMENTAL RESULTS

A drop of the brain suspension after trypsinization was fixed, stained and examined under the microscope. Morphologically it exhibited few differences from the general cellular mass [2], consisting, like the latter, of rounded cellular elements, between which distinct fragments of nerve fibers were rendered visible by impregnation techniques.

After 24 h cultivation a considerable number of cellular elements had settled on and become attached to the cover slips and mica plates (Fig. 1). These attached cells had undergone a change of form compared with those of the general cell mass, and were fusiform or stellate instead of rounded. Some of the cells were binucleate and had increased in size prior to division, whereas others had already divided. Certain of the processes radiating from the

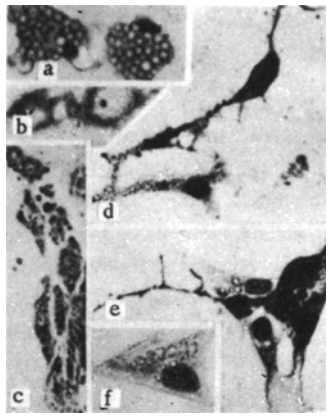

Fig. 1. Cell elements from brain tissue of chick embryo after 24 h cultivation. a) Granular spheres; b) neurons; c) binucleate cells; d,e) nerve cells; f) multipolar neuron. Supravital staining with methylene blue using Lazarenko's method. Obj. 40×, ocular 7×.

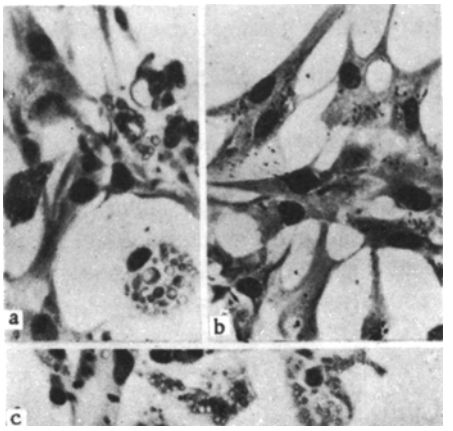

Fig. 2. Cell elements from brain tissue of chick embryo after 48 h cultivation. a, b, c) Different parts of culture, consisting of multipolar, anastomosing elements and granular spheres. Stained with Sudan III and hematoxylin. Obj. 40×, ocular 7×.

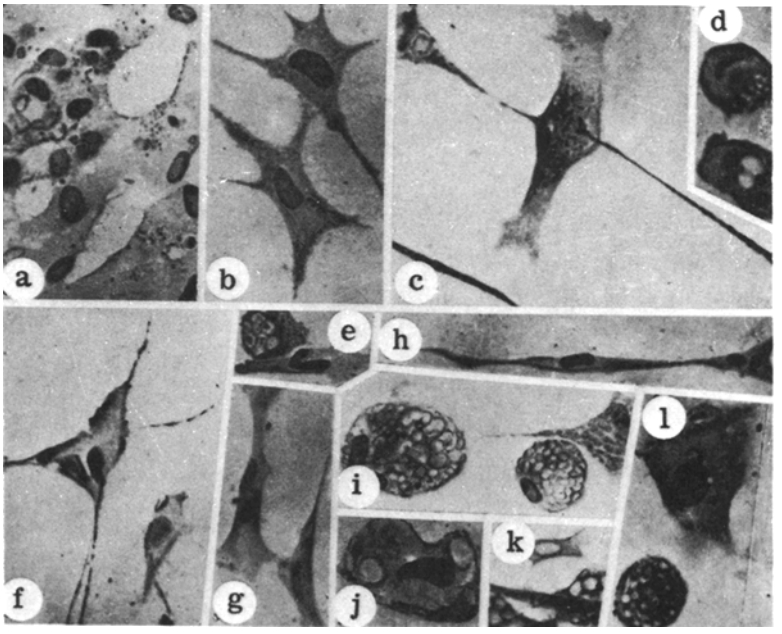

Fig. 3. Cell elements from brain tissue of chick embryo after 72 h cultivation. a) Epithelioid layer; b) multipolar neurons; c) bipolar neuron, in contact with 2 others; d, e) various stages in cell division; f) trinucleate neurons; g) 2 bipolar neurons with 2 nuclei; h) nerve cell with 3 nuclei; i) macroglial granular spheres; 1 with 2 nuclei; j) cell with pyriform nucleus; k) small neuron, fusiform glial element with 2 granular spheres; l) multipolar neurons, in the protoplasm of which are several microglial elements. Fixed in Bouin's fluid. Stained hematoxylin-eosin. Obj. 40×, ocular 7×.

cells had small varicose swellings along their length. Sometimes the cells were grouped so as to connect with each other. In terms of their size, and constancy with which their protoplasm, processes, and nuclei stained with methylene blue, the cells obviously belonged to two different types—nerve elements and glial elements.

After 48 h, the explants on the mica plates and cover glasses were larger than after 24 h (Fig. 2). Moreover, the form of the cells had become more diverse. Multipolar cells were encountered in the explants; some of the former were large, others small, and they all possessed light colored nuclei. In some of the cells the processes were of a pseudopodial type. Occasionally, the external boundary of the cells were scalloped. Granules were present in the protoplasm of these cells and such granules stained readily with Sudan III. The nucleus of the cell generally occupied a peripheral position. These multipolar cells, as described above, may be regarded as belonging to the macroglial type of granular sphere. We have not been able to observe typical mitoses, although our preparations contained binucleate cells and cells which had just divided. At the same time, a considerable increase in the number of cellular elements was noticed. All this appears to indicate that during the first 48 h of cultivation, cell division takes place amitotically.

After 72 h cultivation, the cellular forms became even more diverse. Their volume also increased. In certain parts of the culture epithelioid layers were visible (Fig. 3a). These epithelioid layers were not continuous, but were frequently interrupted by circular or oval cavities. In addition, there were many multipolar cells, with numerous processes; some of these cells possessed 2 and even 3 nuclei (Fig. 3b, f, h). Large vacoules (Fig. 3d, j) were observed in many of the cells. Some bipolar cells were present also (Fig. 3c). On the basis of cell shape, presence of a nucleus with 1 or 2 nucleoli, and the method of contact between one cell and another, we identified these elements as neurons. Many granular spheres—large, medium, and small (Fig. 3e, i, k, l) were observed in the cultures. In preparations which had undergone passage through alcohols, the protoplasm of these elements had a honeycomb appearance, composed of cells of various sizes. These elements represent macro- and microglial cells. After 120 h, fusiform and multipolar cells were found to have been formed as a result of intensive multiplication in certain parts of the culture; these cells were in direct contact with each other. Sometimes, 2 closely situated nuclei were found in a single cell body.

The data which we have obtained indicates the possibility of growing monolayer cultures of cells transplanted from the cerebral tissue of 13-20 day old chick embryos. There is an increase in the number of actual and dividing cells with each successive day of growth and development of the culture. A comparison between cells of the monolayer culture and the neurons and glial elements found growing in chick embryo brain explants in hanging drop cultures [4, 5] reveals a high degree of morphological similarity between them.

Thus, the results of cytological examination of cells from monolayer cultures and a comparison between such cells and elements growing in explants of brain tissue suggests that these particular monolayer cultures which have grown from trypsinized brain fragments of chick embryos, consist of macro- and microglial elements together with bipolar and multipolar cells which are apparently neurons.

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