PRO EXPERIMENTIS

Differentiation of Dissociated Neurons from Chick Embryo Cerebral Hemispheres Cultivated in ROSE Chambers With and Without Cellophane Membrane

The use of the ROSE multipurpose chamber[1] for long-term cultures of the nervous system has two basic advantages compared to the Maximow chamber. First, the possibility of changing the nutrient medium rapidly makes the chamber ideal for the study of the effects of various chemical factors on neuronal morphology and growth. Second, it allows optimal phase-contrast neurocytological and cinematographic studies[2,3].

The technique, which at first was used for cultivating tissue explants, makes use of a cellophane strip as an anchoring agent for the explants. The cellophane membrane, presumably by flattening the explant and thereby establishing a better contact between the cells and the substrate promotes growth and differentiation[4,5]. This was also described by RAIBORN and MASSEY[6], who used the cellophane strip in roller tube cultures for explants of chick embryo spinal cord.

The technique of the ROSE chamber with cellophane was later used for cultivating dissociated spinal ganglia cells[7] and dissociated cerebral hemisphere cells[8].

The use of the cellophane strip, however, can present difficulties in the cultivation of dissociated neuronal cells obtained from the cerebral hemispheres. The question arose, therefore, as to whether there was a real need for the cellophane in the dissociated cell cultures. In these cultures, in contrast to the tissue explants, the cells gravitate

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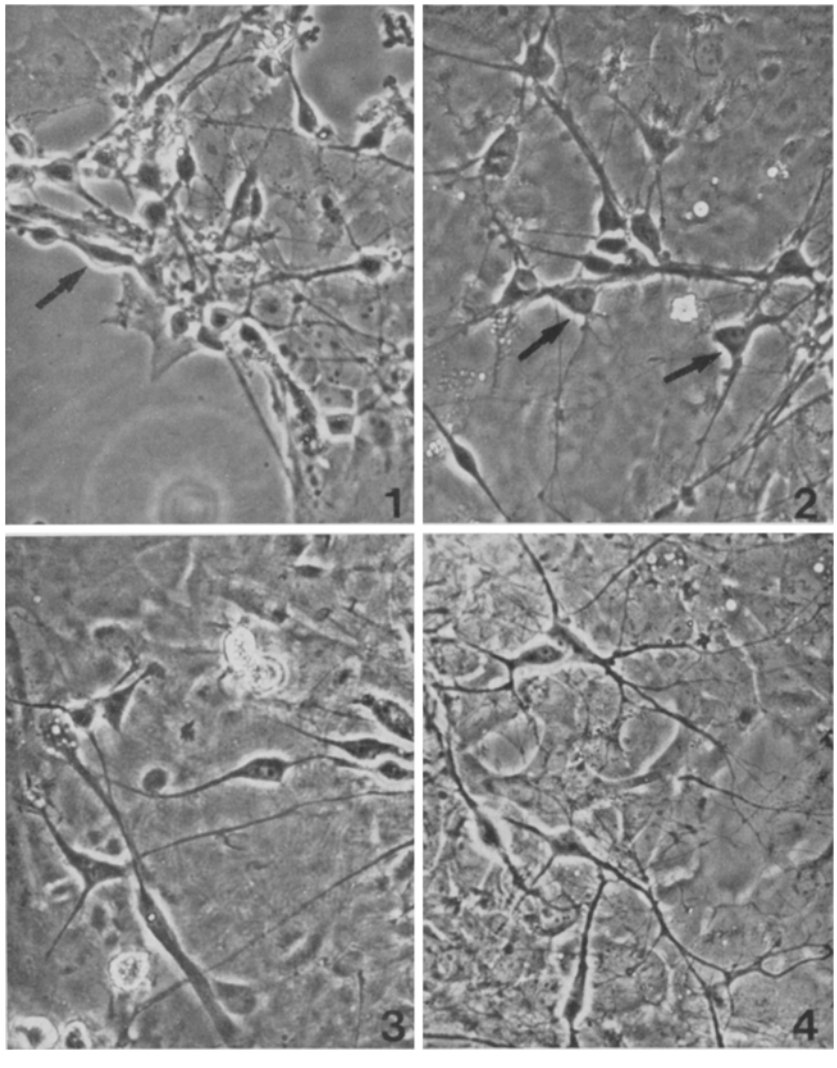

Fig. 1–4. Phase-contrast micrographs of dissociated cerebral hemisphere cells from 7-day-old chick embryos, cultivated in the ROSE chamber. × 250. 1. 6 days in culture, with cellophane. Fusiforme cell (arrow). 2. 6 days in culture, without cellophane. Multipolar neurons with clear nuclei and distinct nucleoli (arrows). 3. 21 days in culture with cellophane. 4. 21 days in culture without cellophane.

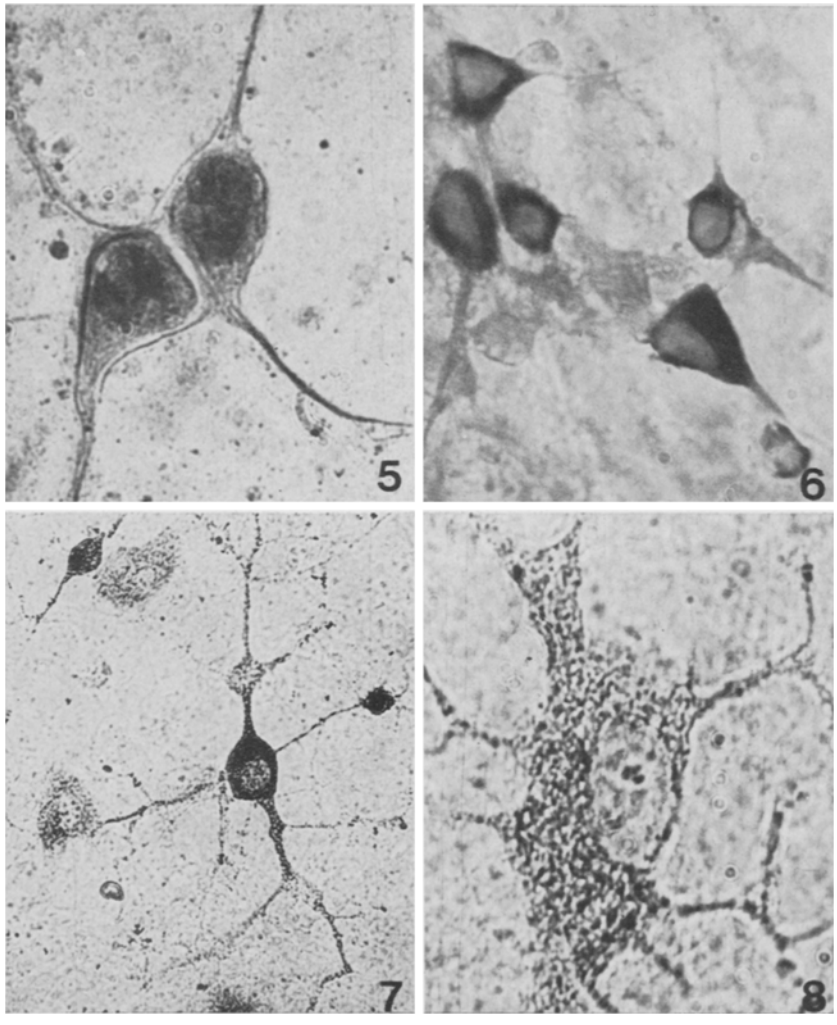

Fig. 5–8. Histochemical studies of dissociated cerebral hemisphere cells from 7-day-old chick embryos, cultivated in the ROSE chamber without cellophane. 5. 7 days in culture, Holmes impregnation. ×1000. 6. 14 days in culture, thionine staining. ×1000. 7. 1 month in culture, acetylcholinesterase activity. × 250. 8. 1 month in culture, acetylcholinesterase activity. ×1000.

to the coverslip and thus establish good contact with the substrate.

Material and methods. Cerebral hemispheres from 5-, 7- and 10-day-old chick embryos were dissociated and the cells cultivated on a layer of collagen in the ROSE chamber by the method described previously[8]. The nutrient medium used consisted of Eagle's basal medium fortified with 20% fetal calf serum. In half of the cultures, the cellophane strip was omitted from the ROSE chamber assembly.

The cultures were observed by phase-contrast microscopy. Some preparations were stained by the modified Holmes impregnation method[9], some with thionine[10] to show Nissl formation, while others were used for acetylcholinesterase reactions[11].

Results and discussion. In both types of cultures, with and without cellophane membrane, the growth of the nerve fibres started after about 12 h in culture. There was no great difference observed in survival between cells cultivated by the two methods. In culture without cellophane, there were a larger number of long fibres and of growth cones. There was also an appreciable difference between the general appearance of the cultures in those without cellophane. The cells were spread out evenly throughout the culture, whereas in the cultures with cellophane, cell fibre growth seemed to be confined to areas within the preparation.

After 6 days in culture, differentiation of neuronal elements was in general more advanced in the cultures without cellophane. Multipolar neurons appeared with large, well-defined nuclei and distinct nucleoli (Figure 2). In the culture with cellophane most of the cells remained fusiformed at this stage (Figure 1).

By the 2nd week in culture, the neurons attained a well differentiated stage in both culture conditions. In the cultures without cellophane, however, there were many more large neurons and more fibres, many of which formed fascicles and networks. In the cultures with cellophane a great number of macrophages were observed.

Both types of cultures developed well during the 3rd week in culture. After the 3rd week, apart from the presence of many macrophages in the cultures with cellophane, there was no striking difference between the two types of cultures (Figures 3 and 4).

Histochemical studies of some of the cultures have demonstrated that in the neurons differentiated without cellophane there was a normal development of neurofibrillar material (Figure 5), of Nissl bodies (Figure 6) and of acetylcholinesterase activity (Figures 7 and 8).

The results of this study show that there was no hindering of differentiation of dissociated cerebral hemisphere nerve cells from young chick embryos when the cellophane

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membrane was omitted. On the contrary, in many cases there was a definite improvement of differentiation. Our findings indicate that the cellophane membrane can be omitted, when dissociated cerebral cortical cells from young chick embryos are cultivated, thus simplifying the technique of culture in ROSE chambers.

The ROSE chamber culture system affords advantages for testing the effect of growth factors. It is therefore necessary to be able to cultivate the nerve cells without cellophane membrane when some factors are unable to dialyze through this membrane.

The system of cultivating dissociated nerve cells offers new possibilities for electro-physiological studies. These studies, as previously performed by CRAIN, PETERSON and BORNSTEIN[12] on explants, were done in Maximow assembly. ROSE chambers with cellophane were used for this purpose with some difficulties. Stripping out the cellophane, what is necessary in these experiments, could evoke a 'mechanical shock' of the cells and influence their membrane potential and functional abilities (LODIN, personal communication)[13].

Zusammenfassung. Dissoziierte Nervenzellen der Gehirnhemisphären von Hühnerembryonen wurden in ROSE-Kammern mit und ohne Cellophanmembran kultiviert. Die Differenzierung der Neurone wurde mit Phasenkontrast und histochemischen Methoden untersucht.

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A Simple Method for the Determination of the Growth Rate in Human Fibroblast Cell Cultures

A variety of methods are available for measuring the rate of multiplication of cells in culture, the most common among them being based on cell counts after trypsinization and resuspension, or protein determinations at regular intervals[1,2]. Each of these methods has its advantages and disadvantages, but we found that neither was suitable for comparative growth studies of a large number of human fibroblast cell lines with chromosome anomalies[3].

The method finally chosen was based on the technique originally used by PUCK, MARCUS, and CIECURA[4] to measure the clonal growth of HeLa cells. They deposited single cell suspensions in nutrient medium in Petri dishes and counted the number of cells in some of the resulting clones at various time intervals thereafter. However, unlike heteroploid cells such as HeLa cells which are epithelial in nature and which usually remain together after cell division to form a discrete colony, human fibroblasts have a pronounced tendency to migrate, and after the two- or three-celled stage it is impossible to recognize individual colonies at the concentrations needed for optimal growth. Therefore, instead of counting the cells in a single colony, we counted the number of cells in the same delimited area daily, thus measuring the number of cells in colonies initiated from single cells less those cells which migrated out of the delimited area. We made the

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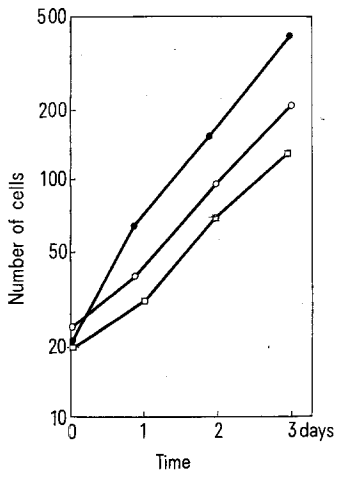

Fig. 1. Representative growth curves, L 205 cells. Open circles: 9th passage, 20,000 cell inoculum. Closed circles: 17th passage, 10,000 cell inoculum. Open squares: 13th passage, 15,000 cell inoculum.

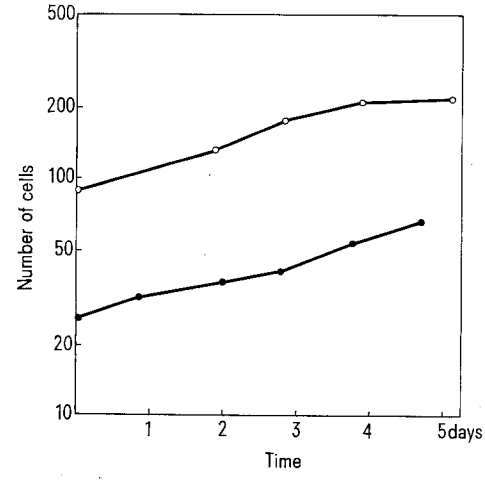

Fig. 2. Growth of L1116 cells. Closed circles: 13th passage, 10,000 cell inoculum. Open circles, 14th passage, 20,000 cell inoculum.