CULTURE OF REAGGREGATED BRAIN CELLS IN HIGH-SPEED MINIROLLERS

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Previously dissociated cells of embryonic brain tissue, cultured in a constantly mixed suspension, reaggregate and form complex tissue fragments or glioneuronal aggregates, in which processes of biochemical and morphological cell differentiation take place, leading to the formation of mature neurons and synaptic connections between them [4-6, 8, 9]. As a result of histogenetic processes such as migration and sorting of cells in glioneuronal aggregates, the neuron-architectonics of the brain structures taken for dissociation may be formed, for example, the cerebellum, hippocampus, neocortex, and so on [2, 3]. In all investigations to obtain and culture glioneuronal aggregates known to the present writers a constant-temperature rotating water shaker bath was used [7], into which were placed conical flasks with a volume of 10-25 cm^3, containing 3-5 ml of the cell suspension. The diameter of rotation of the flasks (1.3-1.9 cm) and the speed of rotation (70-90 rpm) led to the formation of a funnel and increased the likelihood of adhesion of the cells in suspension.

In the investigation described below methods of obtaining and culturing aggregates of dissociated embryonic brain cells in high-speed portable rollers, placed in a laboratory incubator of the usual type, were developed.

EXPERIMENTAL METHOD

The portable roller apparatus, designed by the writers (Fig. 1), is intended for work in an incubator with type 3Ts1125 MU-42 water jacket, which determines its size. The roller consists of a rectangular metal frame 1 in which are mounted parallel aluminum rollers (length of roller 50 cm, diameter 28 mm). The leading roller 2, connected by a rubber belt drive with the other rollers, is driven by the electric motor 3. We used a single phase reversing motor of type RD-09 (78 rpm). The flasks used were kept under antibiotics and

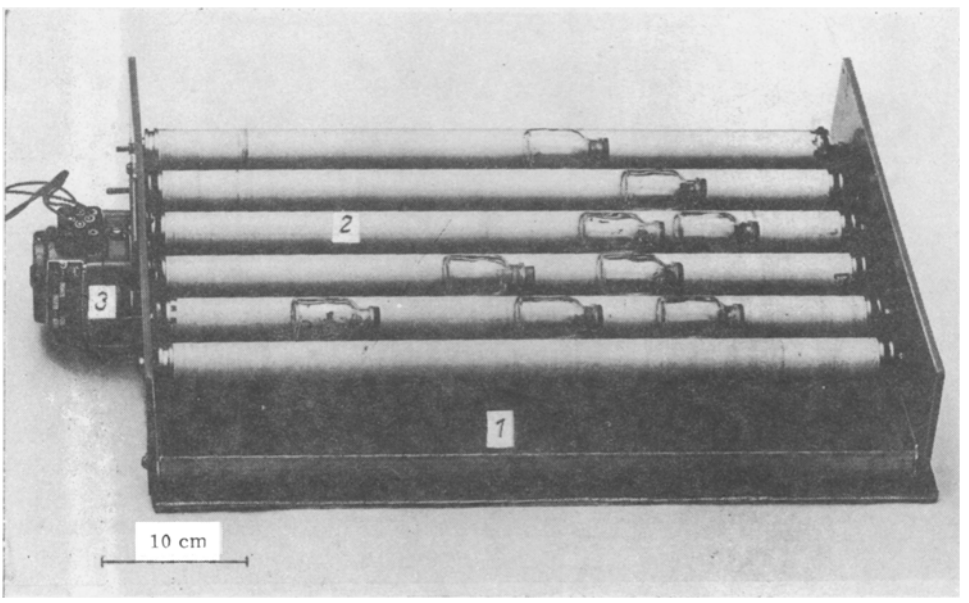

Fig. 1. Portable roller apparatus. Explanation in text.

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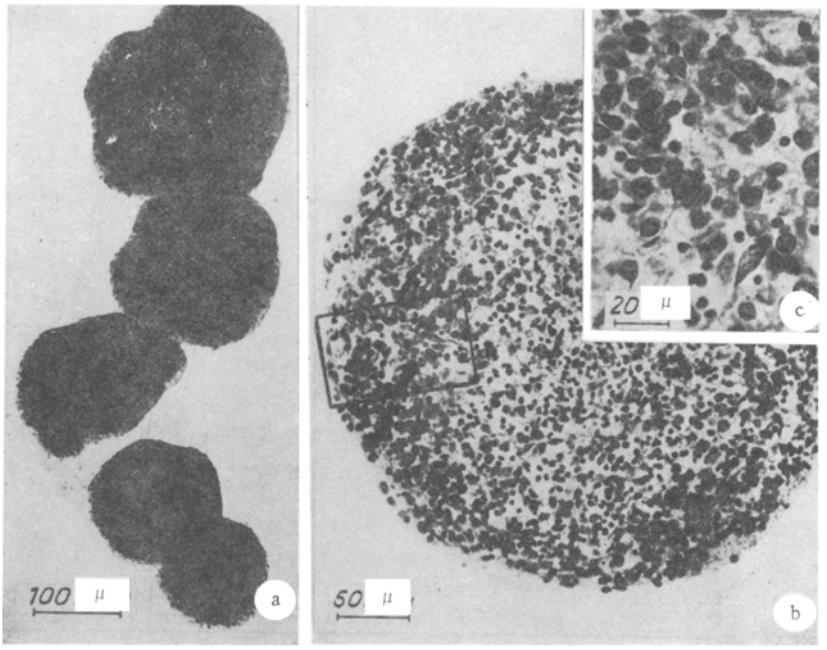

Fig. 2. Glioneuronal aggregates formed in suspension of dissociated cortical cells from brain of 17-18-day mouse embryos, cultured in high-speed miniroller. a) Group of glioneuronal aggregates (2nd day of culture); b) cellular structure of glioneuronal aggregate (5th day of culture); c) fragment of cortical zone of mature glioneuronal aggregate (5th day of culture).

contained 1-2 ml of the cell suspension. The diameter of the rollers of the miniroller and the diameter of the flasks were chosen so that the speed of rotation of the flasks was 60-80 rpm. Experiments showed that this rotation speed of the flasks ensured optimal conditions for cell aggregation. At slower rotation speeds adhesion of the cells and aggregates to the flask walls takes place. At high rotation speeds the aggregation of the cells is disturbed. To obtain the cell suspension, the usual methods of enzymic or mechanical dissociation of embryonic brain tissue were used [1, 7]. The number of cells in the suspension was $5 \cdot 10^6$-$5 \cdot 10^7$ cell/cm^3. The culture medium consisted of 30% serum, 50% Eagle's minimal medium, and 20% balanced salt solution and it contained 600 mg% of glucose, 0.2 U/ml of insulin, and 10^{-2} M of HEPES organic buffer. The use of airtight flasks and of medium containing HEPES buffer made it unnecessary to provide a special gaseous environment. The medium was changed twice a week.

EXPERIMENTAL RESULTS

During culture of dissociated embryonic brain cells by the suggested methods, good and complete reaggregation of the cells is obtained as early as at the end of the first day of the experiment. A large number of small round glioneuronal aggregates measuring 0.3-0.5 mm is formed initially (Fig. 2a). This is followed by an increase in size of the aggregates due to proliferation of their component glial cells and adhesion of smaller separate tissue fragments. The size and shape of the aggregates are largely determined by the age of the embryo and the anatomical features of the brain structures used for dissociation. In the present experiments mature (7-10 days) glioneuronal aggregates of neocortex from 17-18-day embryos attained a size of 1-1.5 mm and had the characteristic cellular architectonics (Fig. 2b). The surface regions of the aggregates were formed by a narrow zone containing thin nerve fibers (molecular layer). The subjacent wide layer, consisting of many neurons, is the analog of the cortical structure (Fig. 2c). In the central zones of the aggregates, corresponding to a subcortical white matter, mainly densely packed glial cells are present.

The suggested method of suspension culture of reaggregated embryonic brain cells is simple and economical, it yields stable and reproducible results, and it provides great opportunities for the study of biologically active factors affecting the cytogenesis and histogenesis of nerve tissue.

LITERATURE CITED

1. I. V. Viktorov and T. L. Kryukoff, Byull. Eksp. Biol. Med., No. 9, 353 (1980).
2. R. G. De Long, Develop. Biol., 22, 563 (1970).
3. B. B. Garber, P. R. Huttenlocher, and L. H. M. Larramendi, Brain Res., 201, 255 (1980).
4. B. B. Garder and A. A. Moscona, Develop. Biol., 27, 217 (1972).
5. Z. Lodin, J. Faltin, V. Fleischmanova, et al., in: Ontogenesis of the Brain, ed. S. Trojan and F. Stastny, Vol. 3, Prague (1980), p. 137.
6. Z. Lodin, V. Fleischmanova, B. Hajkova, et al., Z. mikr.-anat. Forsch., 95, 701 (1981).
7. A. A. Moscona, in: Cells and Tissues in Culture, ed. E. N. Willmer, Vol. 1, New York (1965), p. 489.
8. N. W. Seeds, Proc. Nat. Acad. Sci. USA, 68, 1858 (1971).
9. N. W. Seeds and A. E. Valter, Proc. Nat. Acad. Sci. USA, 68, 3219 (1971).

ISOLATION OF BRAIN MONOAMINES BY HIGH-PERFORMANCE REVERSED PHASE LIQUID CHROMATOGRAPHY

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KEY WORDS: catecholamines; ion-pair chromatography; choice of eluant; column packing.

The development of high-performance liquid chromatography (HPLC), together with the introduction of electrochemical detectors (ED), has made the procedure of determination of monoamines and their precursors and metabolites in brain samples weighing only a few milligrams universally available. Early studies [8, 9] were based on cation-exchange chromatography with glass or metal columns 300-1000 mm long. The effectiveness of these columns for catecholamines was shown to be relatively low. Columns packed with a reversed phase, in combination with a mobile phase containing an ion-pair agent, were found to be much more suitable [2, 10].

To analyze monoamines the writers have used a microcolumn version of HPLC which has several advantages, especially when tissue samples of small size are used [1]. The internal diameter of the stainless steel columns was 2 mm and their length 60 or 120 mm. The eluant was supplied in some experiments by a "Du Pont 8800" pump (USA), in others by means of a pump of syringe type with electromechanical drive from an MSFP-3 microspectrophotometer (made by Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR). The sample was introduced either through a "Rheodyne 7124" injector (USA) or through the sample introducing unit of the "Milikhrom" chromatograph.

Concentrations of monoamines in the peaks were measured by means of an ED with glass-carbon working surface of the cell. The cell was designed and made on the basis of drafts and from materials described in the literature [4, 5, 10]. The electronic circuit of the instrument is similar to that given by Keller et al.[3], with minor modifications. The glass-carbon plate was cut out of chemical crucible glass of Soviet manufacture.

In this paper we examine the questions of packing the columns with sorbent, choice of stationary and mobile phases, preparation of specimens and standards, and reduction of the noise level during chromatography of monoamines in brain samples weighing a few milligrams.

Stationary Phase

We compared the properties of four types of sorbents: Lichrosorb RP_{18} (from Merck, West Germany), Silasorb C_{18} and C_8 (from Lachema, Czechoslovakia), and Nucleosil C_8 (Macherey, Nagel, West Germany). All sorbents had a particle diameter of 5 μ. Analysis showed that for catecholamines Lichrosorb RP_{18} and Silasorb C_{18} have approximately identical properties for use with catecholamines. Under selected conditions of

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