GLIO-NEURONAL AGGREGATES IN COLLAGEN GEL: A MODEL FOR STUDYING FACTORS AFFECTING BRAIN CELL DEVELOPMENT

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Suspension cultures of reaggregated embryonic brain cells [13] are widely used in neurobiological research in order to study processes of cytogenesis and histogenesis. Investigations conducted on deaggregated cultures have shown that disconnected brain cells form spherical tissue fragments, or glio-neuronal aggregates (GNA), during culture in a continuously mixed suspension, and these aggregates can reproduce the histotypical organization of the original brain structure, such as the neocortex, hippocampus, or cerebellum [5, 9-11].

Previous investigations showed that GNA, cultured on a solid collagen substrate, can be used to study the principles governing growth of axons and migration of glial cells [1].

The aim of this investigation was to study the development of GNA in a three-dimensional collagen gel containing nutrient media of different composition.

EXPERIMENTAL METHODS

GNA of neocortical cells of 16-18-day mouse embryos of the C57BL strain were obtained by the method described previously [2]. Formed aggregates were transferred on the 1st, 2nd, and 3rd days to the bottom of plastic dishes 35-40 mm in diameter, and mounted in collagen gel containing nutrient medium (CGNM). The method of preparation was as follows [6]: Collagen acetate, obtained from rat tail tendons [8], was dialyzed against acidified (0.1 M HCl) solution of Eagle's minimal medium in water (1:9, pH 4.0) for 24 h. The dialyzed collagen can be kept at 4°C for 2 weeks. The dialyzed collagen was mixed at 2°C with 0.15 M NaOH in salt solution or nutrient medium in tenfold concentrations (Table 1); 1.0-1.5 ml of CGNM was uniformly spread over the bottom of the dishes, into which the GNA had been introduced beforehand. Coagulation of the collagen took place after 2-3 min, and a three-dimensional CGNM was formed, in which the GNA was embedded. Covering each dish with CGNM should not take more than 1 min, for solidification of the collagen gel takes place at neutral pH as a result of elevation of its temperature.

The GNA, embedded in CGNM, were cultured at 36.0°C in an incubator (5% CO_2 + 95% air, relative humidity 98%). The cultures were examined and photographed on Biostar (Reichart, Austria) and MBI-13 (USSR) inverted microscopes.

RESULTS

The first signs of growth of GNA embedded in CGNM were observed 3-4 h after the beginning of culture, when axons with cones of growth appeared at the periphery of the aggregates.

As a result of an increase in the number of axons and in their length toward the end of the first day of culture a zone of growth consisting of radial fibers, diffusely arranged in the layer of collagen gel, formed around the aggregates (Fig. 1). On the 2nd-3rd day of culture there was a further increase in the number and length of the axons, which formed collaterals and thin terminal branches; some axons formed thin glio-axonal bundles (Fig. 2). In this period single glioblasts, characteristically drop-like in shape, migrated into the zone of growth (Fig. 3a). On the following days of culture there was a further increase in the area of the zone of growth of the aggregates. Some migrating glioblasts were transformed into cells giving off numerous processes and similar to fibrous astrocytes (Fig. 3b).

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TABLE 1. Composition of Media Used to Culture GNA in CGNM

CGNM	Eagle's minimal medium · 10 (ml)	Balanced saline · 10 (ml)	Sodium bi-carbonate (7.5%), ml	L-glutamine (200 mM) ml	Embryonic calf serum, ml	NaOH (0.15 M), ml	Distilled water, ml	Collagen solution, pH 4.0, ml	Total volume of CGNM, ml
Collagen gel + saline	—	0,74	—	—	—	0,05	0,76	5,9	7,45
Collagen gel + amino-acid medium	0,74	—	—	—	—	0,05	0,76	5,9	7,45
Collagen gel + complete nutrient medium	0,45	—	0,11	0,05	0,56	0,05	0,33	5,9	7,45

Note. Final volume of CGNM indicated above is sufficient to cover seven plastic Petri dishes 35 mm in diameter. Instead of the above volumes of water, test solutions of biologically active compounds in tenfold concentrations can be added as components of the CGNM.

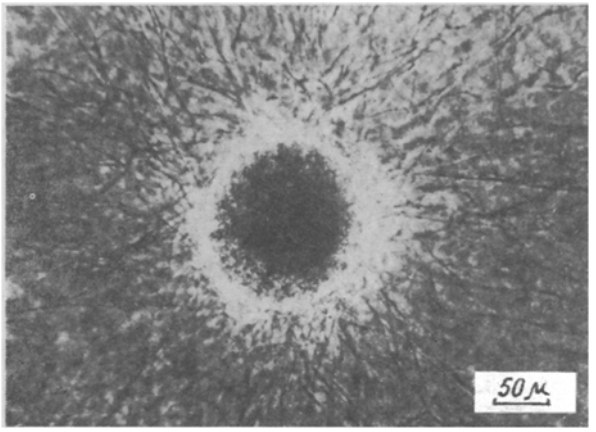

Fig. 1. GNA of neocortical cells of 18-day mouse embryo in collagen gel. First day of culture in CGNM after culture for 3 days in rotating flask. Phase contrast. Scale 50 μ.

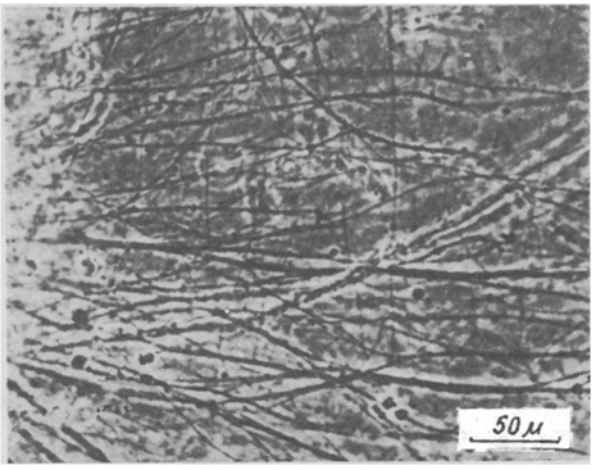

Fig. 2. Zone of growth of GNA. Besides thin isolated axons, more massive glio-axial bundles are visible on 3rd day of culture in CGNM after 3 days of culture in rotating flask. Phase contrast. Scale 50 μ.

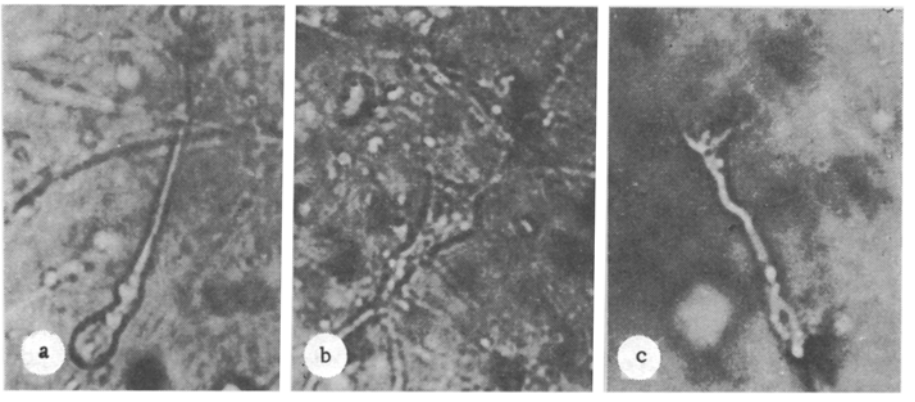

Fig. 3. Glial cells in zone of growth of GNA. Light field. Blurring of picture with this magnification is due to the fact that the object was surrounded by a layer of collagen gel. Scale 10 μ [as in Russian original]. a) Migrating glioblast (1st day of culture in CGNM); b) differentiated astrocyte (4 days of culture in CGNM). GNA transferred into collagen gel on 3rd day of culture in rotating flasks. [Fig. 3c not explained in Russian original].

Observations on development of the aggregates in collagen gel containing salt solution, Eagle's medium, or complete nutrient medium (Table 1) showed that on the first 2 days of culture no significant differences were found between them in the character of growth of the axons or migration of the glioblasts. Definite differences in development of the aggregates, due to the composition of the nutrient medium included in the collagen gel could be observed after the 3rd-4th day of culture, when degeneration and cloudy swelling of the axons began in CGNM containing salt solution. In collagen gel with Eagle's amino-acid medium, this took place on the 4th-6th day, and in gel with complete nutrient medium the axons lasted until the 8th-10th day (the longest time of culture). In all cases isolated axons degenerated first, whereas glio-axonal bundles still remained visibly unchanged.

The method of culture in CGNM, first used in the present investigation for short-term cultures of GNA of embryonic brain cells, was used for organ cultures of spinal, sympathetic, and parasympathetic ganglia [6, 7]. This collagen gel, composed of thin fibrils, has a loose, dispersed fibrous structure, and forms a three-dimensional substrate, surrounding the tissue in culture. An undoubted advantage of this substrate is that nutrient media of definite chemical composition and biologically active substances can be incorporated into it in order to study their effect on the development of nerve tissue in culture.

Previous investigations showed that GNA, formed in suspension cultures of dissociated embryonic brain cells, have a relatively constant cellular composition and an organ-specific cellular architechtonics, so that they can be regarded as multiple, mutually similar microcopies of the brain structures originally taken for dissociation [2, 5, 9-11]. As the results showed, the development of GNA, incorporated into CGNM, is characterized in the initial stages of culture by active growth of regenerating axons and migration of undifferentiated glial cells, during which the special features of the cullular organization of the aggregates determine synchronized growth of axons over the whole area of the zone of growth. Experiments with CGNM containing balanced salt solution, Eagle's medium, or complete nutrient medium demonstrated the synchronized development of DNA in CGNM of different composition in the initial period of culture, and showed that the duration of survival of the cultures is directly dependent on the composition of the nutrient medium included in CGNM. The results of these experiments indicate that simple salt solutions and serum-free media can be used in conjunction with various biologically active compounds to culture GNA of embryonic brain cells in CGNM in order to study the effect of these compounds on development of neurons and glia and on growth and regeneration of axons.

The main experimental model for studying the biological activity of neuronotrophic and gliotrophic factors and factors stimulating growth of axons at the present time are tissue and cell cultures of peripheral ganglia, consisting of relatively homogeneous neuron populations [7, 12]. In connection with the intensive development of research into regeneration of the CNS, there is an evident need for the creation of experimental test systems to study specific factors stimulating growth of the neurons and glial cells of the brain. In recently published investigations, explants of the retina [4] or spinal cord of early chick embryos [15] were used in test neuonotropic and growth-stimualting factors of the CNS. The use of tissue explants from other parts of the brain for these purposes seems less realistic because of differences in their cell composition and histotypical organization. Variability of the zones of growth of the explants arising as a result of this makes it difficult to make comparative quantitative estimates of the effect of these factors on axon regeneration. Cultures of dissociated embryonic brain cells are another experimental model for testing biologically active compounds [3, 14], but glio-neuronal interactions characteristic of nerve tissue and, perhaps determining the effect of neurotrophic factors, are disturbed in such cultures.

The practical importants of these methods of obtaining GNA and their subsequent culture in CGNM, which the present writers have developed, is that they enable organ-specific tissue fragmentsof various brain structures of similar size and cell composition to be obtained, suitable for use as test systems for studying the effect of biologically active compounds, introduced into collagen gel together with a nutrient medium of definite chemical composition, on the development of brain neurons and glial cells and on axonal growth and regeneration. The essential features distinguishing GNA cultured in CGNM are the synchronization of their development and the structural uniformity of the zones of growth formed, which means that parameters such as the number, length, and growth rate of the axons, the intensity of migration and differentiation of the glial cells, the area of the zones of growth, and the length of survival of the GNA can be used as criteria for the evaluation of the trophic and growth-stimulating activity of substances requiring testing.

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CIRCADIAN RHYTHMS OF THE ACID-BASE BALANCE AND BLOOD GAS COMPOSITION

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Any biological system, according to the law of structural-functional temporal discreteness, embodies time [3], and knowledge of the temporal characteristics of biological processes is an essential condition for the establishment of criteria of the physiological norm [6].

This paper describes a study of circadian fluctuations in the acid-base balance system and in the blood gas composition, and the use of cosinor analysis to investigate the parameters of the cirdadian rhythms in normal man.

EXPERIMENTAL METHODS

Twenty healthy volunteers aged 20-26 years were investigated at 3 and 9 a.m. and 3 and 9 p.m., corresponding to the four different parts of the 24-h period (night, morning, afternoon, evening), in the spring (March). All subjects confirmed to a standard program of daily activity, a standard diet, and a natural alternation of day and night. They slept from 11 p.m. until 7 a.m. Samples of capillary blood were taken from the finger to measure the following parameters with the aid of appropriate electrodes by the direct method: true pH (pH_t), partial pressure of carbon dioxide (pCO_2), and partial pressure of oxygen (pO_2). The measurements were made on the OP-210/3 biological microanalyzer (Radelkis, Hungary). The metabolic pH (pH_{met}), the actual bicarbonate (AB), standard bicarbonate (SB), buffer bases (BB), base excess (BE), and degree of oxygenation of hemoglobin (HbO_2) were determined by Severinghaus' method, based on the Siggaard–Andersen linearized nomogram [7]. The blood hemoglobin concentration was measured by a photocolorimetric method [1]. The numerical results were subjected to statistical analysis by computer by the Student–Fisher method and the cosinor program [8], which revealed the parameters of the circadian rhythms at a 95% level of significance: mesors, mean levels for the 24-h period; calculated acrophases, peaks of the maximum relative to the beginning of the 24-h period (midnight); and amplitudes. To

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