Roller Organ Cultures of the Retina from Postnatal Rats

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Whole retinas of 2-14-day-old rats were cultured in a roller device for 2-14 days. Floating retinas of 7-14-day-old rats formed hole spheroid structures (spheroids) with the wall completely retaining the linear structure and layer-by-layer cellular and fibrous architecture, including the outer nuclear, outer plexiform, inner nuclear, inner plexiform layers, layers of ganglion cells and nerve fibers. The retina obtained at earlier terms of development often formed folds, with pyknotic nuclei of dead neurons in their deep compartments. In organ cultures of the retina isolated from rats at early postnatal periods, rosettes were formed in sites of local injury to the outer nuclear layer and pigmented epithelium. Roller organ cultures can be used for *in vitro* studies of the development and experimental diseases of the retina.

Key Words: *retina; roller organ culture; photoreceptors; ganglion cells; rosettes*

Organotypic culturing of animal retina sections, apart from multilayer cell cultures, is used for the studies of the development and experimental diseases of mammalian retina [1,4,5,10]. The first organ cultures of the whole retina embedded in a plasma clot were obtained in 1930s [9]. Now, long-living organ cultures of the retina from newborn and postnatal mice and rats are prepared by culturing on semipermeable membranes. Histological, ultrastructural, and immunocytochemical studies showed good preservation of the cytoarchitectonics (including the ganglion cell layer) and cell-cell interactions in these organ cultures [3,6,9,11].

We studied early postnatal rat retinal organ cultures obtained by roller culturing.

MATERIALS AND METHODS

All experiments were carried out in accordance with the Regulations for Handling Experimental Animals and requirements of ARVO (Association for Research in Vision and Ophthalmology) on the use of animals in ophthalmological studies. The eyes of 2-14-day-old rats were removed under deep ether narcosis, washed in 70% ethanol and 2 portions of sterile Dulbecco's phosphate buffered saline (DPBS; Sigma). Whole retina was isolated in cold DPBS with 0.8% glucose (Gl-DPBS). A circular incision along the interface between the retina and albugineous membrane was made and the cornea, lens, and vitreous body were removed. The retina was separated from the ora serrata and optic nerve head. The isolated retina was washed in Gl-DPBS and transferred into a Petri dish with 2 ml cold nutrient medium. Ten retinas were thus isolated one-by-one, transferred into 50-ml flasks with 10 ml nutrient medium containing 90% minimum Eagle medium (Sigma), 10% FCS (Sigma), 0.8% glucose, 2 mM glutamine, 0.2 U/ml insulin, 2 ml/100 ml B-27 additive (Gibco), 25 mM HEPES buffer, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Sealed flasks were cultured on a horizontal roller (36.5°C, 60 rpm) [2]. The majority of cultures were cultured for 2-14 days. The medium was replaced by $^1/_3$ every 3 days.

For histological study the retinas from each flask were washed in DPBS and fixed (5 min) in

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modified Tellesnitskii's fixative (70% 96° ethanol, 20% formalin, and 10% glacial acetic acid). After dehydration in ethanol (70%) and three portions of dehydrated (99.9%) isopropyl alcohol (Sigma) the retinas were placed into melted (58°C) paraffin (Sigma) for 3 h or overnight [1]. After 1-h incubation in a new portion of paraffin the retinas were embedded in blocks. Paraffin sections (8 μ) were mounted on gelatin-coated slides. Deparaffinized sections were stained with Gil's hematoxylin (Sigma), eosin, and post-stained with 0.1% Cresyl Violet strong on acetate buffer (pH 3.4).

RESULTS

Floating retinas change their configuration during roller culturing and are transformed into vesicular bodies (spheroids); the structure of spheroids formed by retinas isolated from 2-6 and 7-14-day-old rats is different.

Intact retina from 7-14-day-old rats usually retains the linear structure and forms a spheroid with a central cavity persisting over the entire period of culturing (Fig. 1, *a*). Histological studies of serial sections showed that the wall of the spheroid includes cellular and fibrous structures characteristic of mature retina: outer nuclear and retinal layers, inner nuclear and retinal layers, layers of ganglion cells and nerve fibers (Fig. 1, *b*). The pigmented epithelial layer was absent in roller cultures of the retina from 7-14-day-old rats. No degenerating neurons and rosettes were detected in the photoreceptor layer, inner nuclear layer, and ganglion cell layer at the initial stages of culturing. Solitary hyperchromatic neurons were detected at later terms of culturing (9 days and later).

Spheroids with preserved fragments of pigmented epithelium formed in retinal cultures derived from 2-6-day old rats. The retina forming the spheroid wall lacked the outer plexiform layer. In some cultures the retina of 2-6-day-old rats formed deep folds with pyknotic nuclei of dead neurons in the inner nuclear layer and ganglion cell layer of these folds, which seemed to be caused by hypoxia in these compartments of the retina (Fig. 2, *b*). The formation of folds of the outer nuclear layer is described for organ cultures of fetal and early postnatal retina grown on semipermeable membranes [7,9]. Folds can also form in organ cultures of 7-14-day-old rats if the retina was damaged during isolation.

Rosettes formed by radially oriented photoreceptor cells were detected in the outer nuclear layer of roller cultures of the retina from 4-7-day-old rats (Fig. 3, *a*, *d*). Some rosettes formed in sites of local

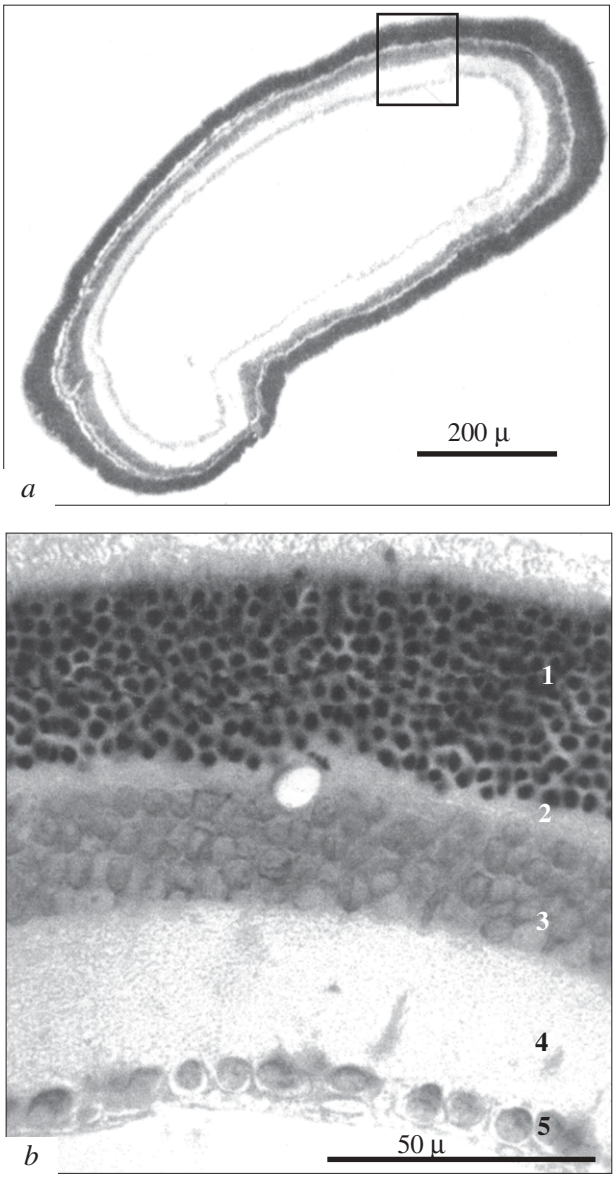

Fig. 1. Retinal spheroid derived from a 9-day-old rat, 8 days of culturing. *a*) transverse section; *b*) fragment of the wall shown by the frame in Fig. *a*. *1*) outer nuclear layer; *2*) outer plexiform layer; *3*) inner nuclear layer; *4*) inner plexiform layer; *5*) layers of ganglion cells and nerve fibers. Here and in Figs. 2, 3: staining with hematoxylin and eosin, cresyl echt violet.

injury to the outer nuclear layer or pigmented epithelium; at first a pit emerged on the retinal surface, which grew deeper, its edges fused, and a circular rosette was thus formed (Fig. 3, *b-d*).

The formation of rosettes in organ cultures was observed in fetal and postnatal retinal cultures [7,9]. According to pathohistological data [8], the rosettes form as a result of developmental disorders of the eye in the ontogenesis, in retinal detachment, and pigmented degeneration, which indicates a relationship between the rosette formation and injury to the pigmented epithelium. Our data on the formation

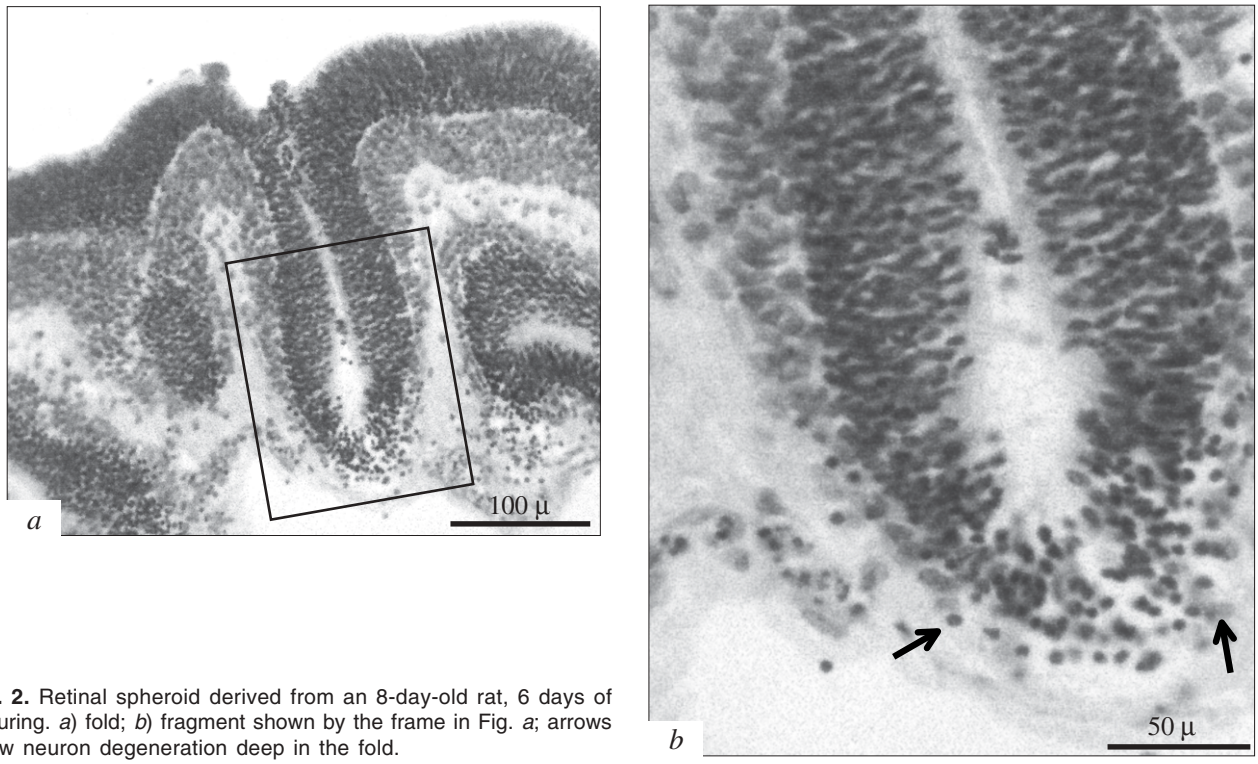

Fig. 2. Retinal spheroid derived from an 8-day-old rat, 6 days of culturing. *a*) fold; *b*) fragment shown by the frame in Fig. *a*; arrows show neuron degeneration deep in the fold.

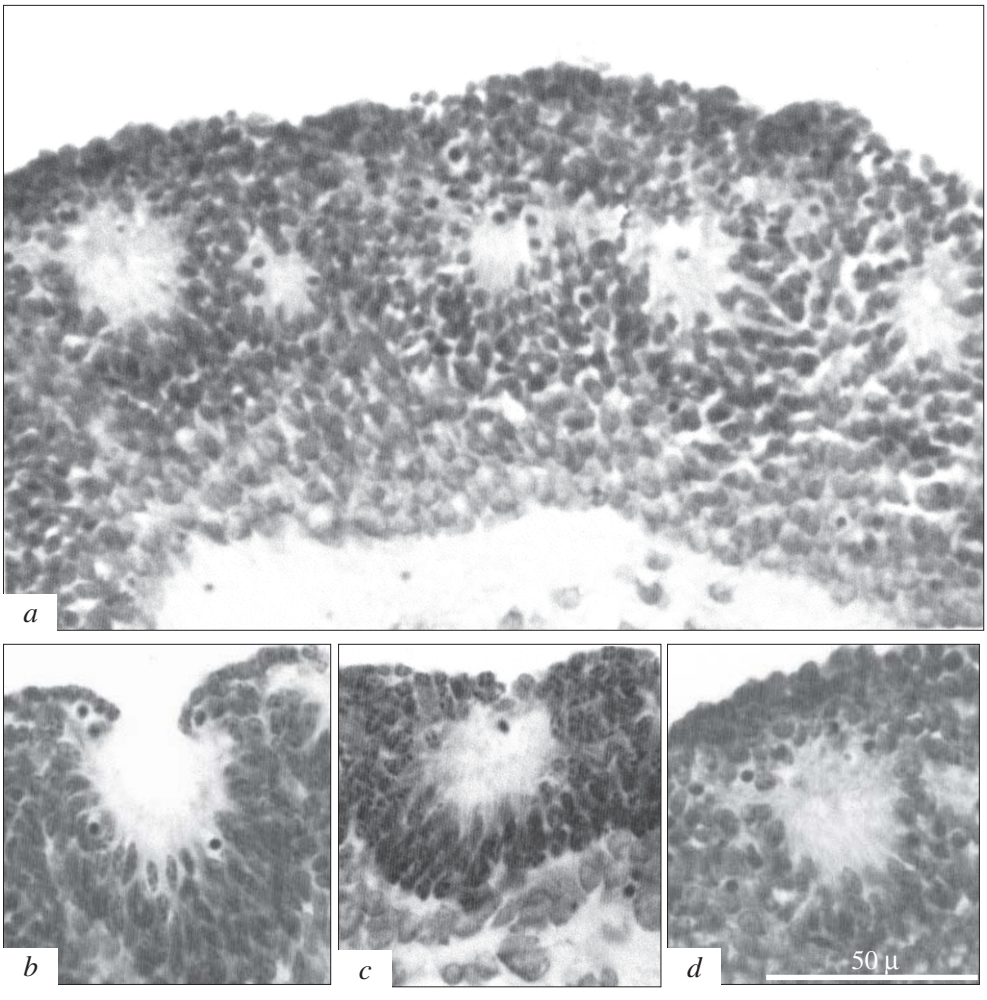

Fig. 3. Outer nuclear layer of retinal spheroid from a 6-day-old rat, 8 days in culture. *a*) rosettes in outer nuclear layer; no inner plexiform layer; *b*) initial stage of rosette formation in local injury to pigmented epithelium and outer nuclear layer; *c*) fusion of surface layer of cells; *d*) formed rosette.

of rosettes in retinal organotypic cultures in local injury to the pigmented epithelium and outer nuclear layer confirm this assumption.

Our experiments showed that 1-2-week-old rats are optimal for obtaining well-differentiated retinal organ cultures in roller culturing. More than 50% photoreceptor cells, neurons of the inner nuclear and ganglion layers die on days 3-5 of culturing in roller cultures derived from older rats (up to 1.5 months). Cell differentiation processes and formation of layer-by-layer architectonics are not over in the retina of rats of younger postnatal age, which is seen from the absence of the inner plexiform layer and formation of plicated spheroids and rosettes in the outer nuclear layer of the retina in roller organ cultures.

The main distinction of the roller organ culture method from stationary methods [3,6,9,11] is the possibility of culturing whole floating retinas of postnatal rats with completely retained cell structure. The advantage of this method is simultaneous culturing of numerous retinas under the same conditions, due to which some retinas from the same experiment can serve as controls in studies of experimental diseases of this structure.

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