Roller Organotypic Cultures of Postnatal Rat Retina

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Floating retinal sections from 7-12-day-old rats form ball-shaped retinal bodies during roller culturing. Histological studies of serial sections of retinal bodies showed that their outer surface is formed by the retina completely retaining organotypic cytoarchitectonics. Some retinal bodies have laminar structure consisting of several layers of the retina. At the initial stages of culturing some retinal bodies contain a cavity, which later is completely obliterated due to the growth of axons of ganglion cells and migration of glial cells and fibroblasts. This study demonstrated the possibility of long-term survival, differentiation, and *in vitro* axonal regeneration of ganglion cells, the main retinal efferent neurons, which can provide the basis for investigation of pathology and drug correction of injuries and stimulation of regeneration of these cells in experimental glaucoma models.

Key Words: *retina; organotypic cultures; postnatal animals; ganglion cells*

Cell and tissue cultures are widely used as experimental models of retinal development and pathologies starting from the 1960s [6]. Dissociated and reaggregated cell cultures [5,7,8,12] and organotypic tissue cultures [2,4,9-11] occupy the main place in *in vitro* studies of mammalian and avian embryonal, perinatal, and postnatal retina. There are only few studies of organotypic retinal cultures from late postnatal and adult mammals, including humans [2,14].

Organotypic culturing of the retina now employs methods previously developed for postnatal animal brain sections [3,13]. The advantage of postnatal retina culturing consists in the use of structure attaining higher phenotypical and neurochemical differentiation of its cells. Histological, immunocytochemical, ultrastructural, and electrophysiological studies showed that the retina developing in tissue cultures is characterized by high degree of differentiation of photoreceptors, neurons, and glial cells, and retains organotypic cytoarchitectonics, interneuronal and neuroglial relationships corresponding in general to the analogous structural parameters of mature retina *in vivo* [2,4,9-11]. On the other hand, many studies showed that axotomized ganglion cells in retinal organotypic cultures from perinatal and postnatal mammals undergo retrograde degeneration and die completely or partially within the first week of culturing [2,9,11].

Our aim was to develop a method for long-term organotypic culturing of postnatal rat retina maximally preserving ganglion cells.

MATERIALS AND METHODS

All experiments were carried out in accordance with the "Regulations for Handling Experimental Animals" and requirements of Association for Research in Vision and Ophthalmology on the use of animals in ophthalmological studies.

The retina of 7-12-day-old rats was cultured, because at this stage of development the retina attains structural differentiation comparable to that in adult animals.

The rats were decapitated under deep narcosis. Animal heads were treated with 70% ethanol. Enucleation was carried out under sterile conditions. The retina was isolated after circular dissection of the cornea, removal of the lens and vitreous, and washed twice in

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Ca^{2+}/Mg^{2+}-free Dulbecco phosphate buffer (DPB, Gibco) with 0.8% glucose. The retina (in DPB) was cut with a scalpel into rectangular fragments (1-2 mm^2); the fragments were transferred into nutrient medium consisting of 90% minimum Eagle medium (Gibco), 5% fetal calf serum (Gibco), 5% human placental serum, 0.8% glucose, 2 mM glutamine, 0.2 U/ml insulin, 25 mM HEPES buffer, 100 U penicillin, and 100 μg/ml streptomycin. Floating retinal sections were cultured in 25-ml flasks in 10 ml medium of the above composition. Sections of 2-4 retinas were placed into a flask with 10 ml nutrient medium and cultured for 6-12 days in a thermostat (36.5°C) on a horizontal roller with constant rotation (60 rpm) [1,15]. One-third of the medium was replaced twice a week or earlier if the medium was acidified.

For histological studies the retinal cultures from each flask were washed in DPB and fixed in modified Tellesnitskii fixative (70% of 96% ethanol, 20% of 39% formalin, 10% of glacial acetic acid, and 0.1% of eosin Y) during 10-15 min. After fixation the cultures were washed in 70% ethanol, dehydrated in 3 portions of isopropyl alcohol (isopropanol 99.9), 30 min in each portion, transferred into melted (58°C) paraffin (Paraplast Plus, Sigma), and left for 2-3 h or overnight; then the material was embedded into a new portion of paraffin. The sections (8 μ) were mounted on gelatin-treated slides, deparaffinated, and stained by the method of Nissl with 0.1% Cresyl Violet dissolved in 0.1 M acetate buffer (pH 3.4) or Jill Hematoxylin (Sigma) and 1% Eosin Y (Sigma). For detection of nerve fibers, the sections of retinal bodies (RB) were impregnated with silver by method of Bil'shovskii.

RESULTS

Initially flat transverse sections of the retina no larger than 1-2 mm^2 change their configuration and form round or ellipsoid RB for the entire period of culturing. The wall of round RB is formed by one layer of the retina retaining its cytoarchitectonics, while some oval RB have a complex laminar structure including several retinal layers (Fig. 1, *a*, *b*). Solitary rosettes can form in multilaminar RB in retinal cultures derived from 7-day-old rats. These rosettes are formed by a photoreceptor layer (Fig. 1, *b*).

At the initial stages of culturing some RB contain an internal cavity (Fig. 1, *a*), which is later completely obliterated due to the growth of axons of ganglion cells and migration of glial cells and fibroblasts.

Histological studies of serial sections of RB showed that their outer surface is formed by the retina completely retaining its organotypic cytoarchitectonics (Fig. 2, *b*) similar to cell structure of the retina taken from rats at the same term of postnatal development (Fig. 2, *a*). It is noteworthy that nerve fiber layer containing axons of ganglion cells is poorly developed in the native retina of 7-day-old rats (Fig. 2, *a*), while after 8-day culturing of cells derived from the retina of rats of the same age this layer appreciably increased in size due to migration of glial cells and growth of regenerating axons of ganglion cells (Fig. 2, *b*, *c*).

Poorly developed layer of external and internal segments of photoreceptors, external nuclear layer containing nucleus-containing bodies of photoreceptors, external retinal (plexiform) layer, internal nuclear layer containing amacrine, horizontal, bipolar neurons and Muller cells (identification of these cells in preparations stained with basophil stains is difficult), internal retinal (plexiform) layer, ganglion layer; and nerve fiber layer are detected in histological preparations of RB stained with basophil stains by the method of Nissl and impregnated with silver (Fig. 2, *b*, *c*; 3, *a-c*).

Hence, RB forming in roller cultures of floating sections of postnatal rat retina are tissue fragments

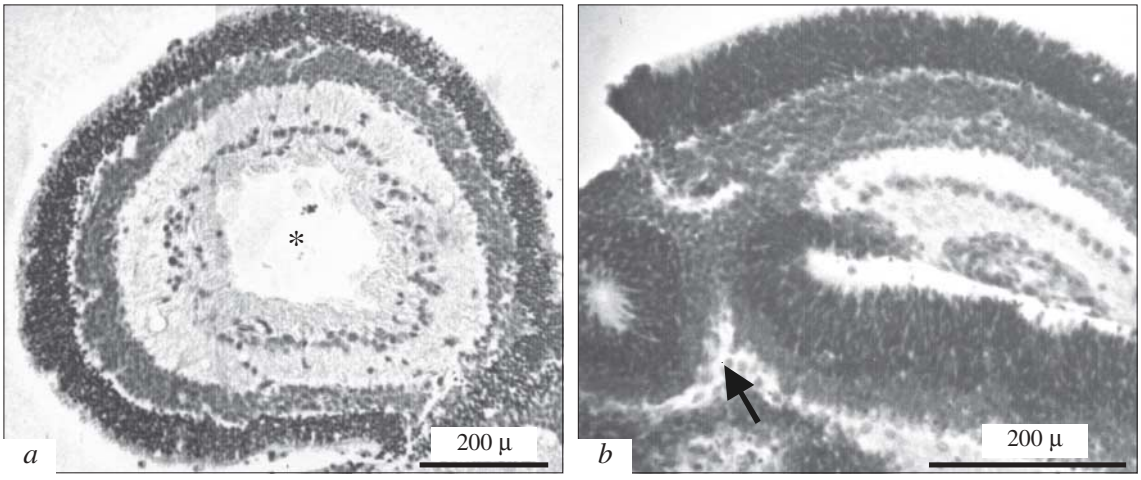

Fig. 1. Retinal body. Cresyl Violet staining by Nissl method. *a*) transverse section, the asterisk shows the inner cavity; *b*) fragment of section showing several retinal layers and a newly formed rosette (arrow).

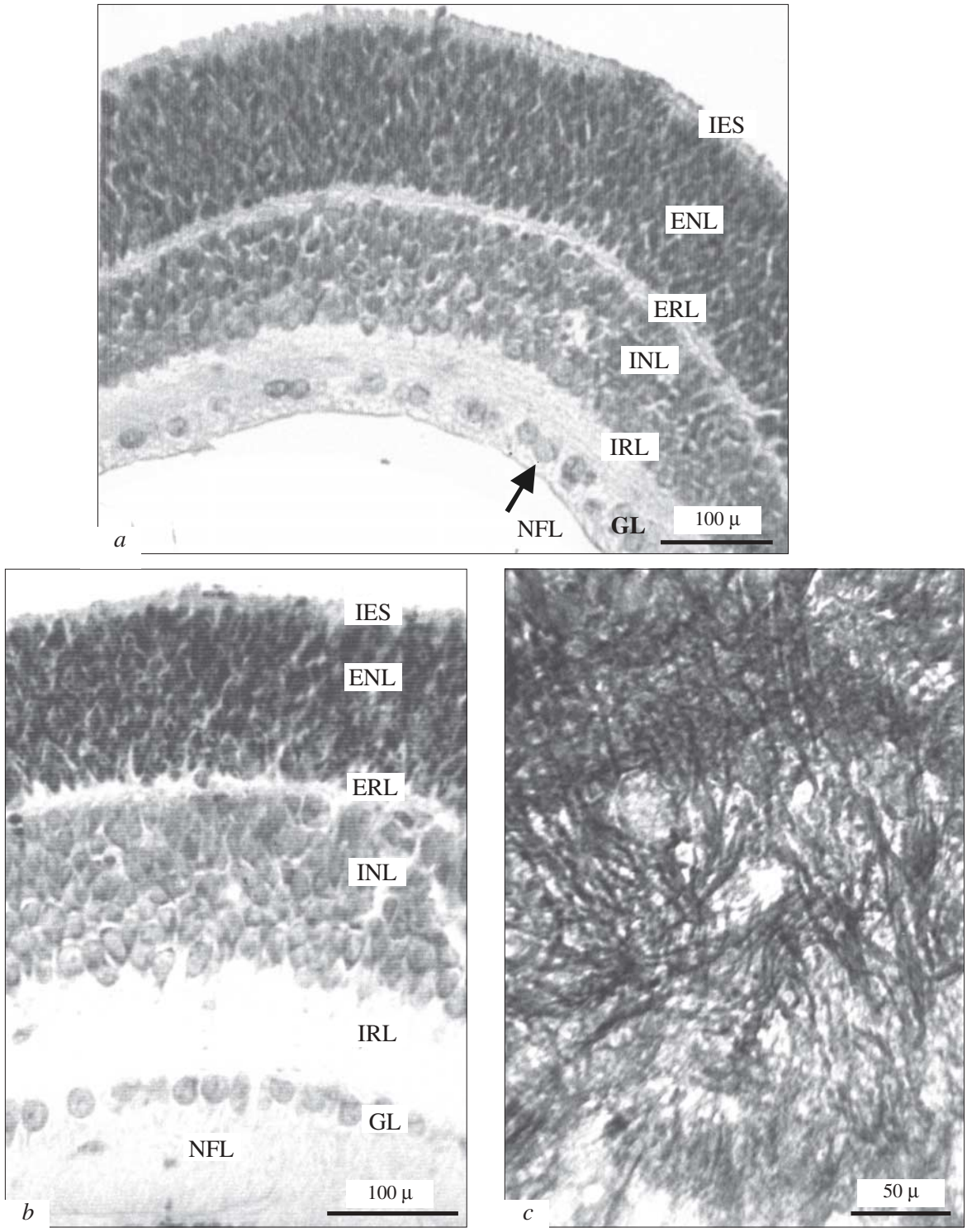

Fig. 2. Histology of serial sections of retinal bodies. *a*) section of the native retina from a 7-day-old rat before culturing; *b*) retinal body wall from a 7-day-old rat after 8-day culturing; *c*) ganglion cell axons in retinal nerve fiber layer, 7-day-old rat, 8 days in culture. IES: internal and external segments of photoreceptor cells; ENL: external nuclear layer; ERL: external retinal layer; INL: internal nuclear layer; IRL: internal retinal layer; GL: ganglion layer; NFL: nerve fiber layer. *a*, *b*: Cresyl Violet staining after Nissl; *c*) Bil'shovskii impregnation with silver.

completely retaining cell organotypic structure and cell-cell relationships characteristic of mature retina, which on our opinion is crucial for its preservation and development in long-term organotypic culturing.

This study demonstrated the possibility of long-term survival, differentiation, and axonal regeneration of ganglion cells, the main efferent neurons of the retina *in vitro*, which can be used in studies of retinal

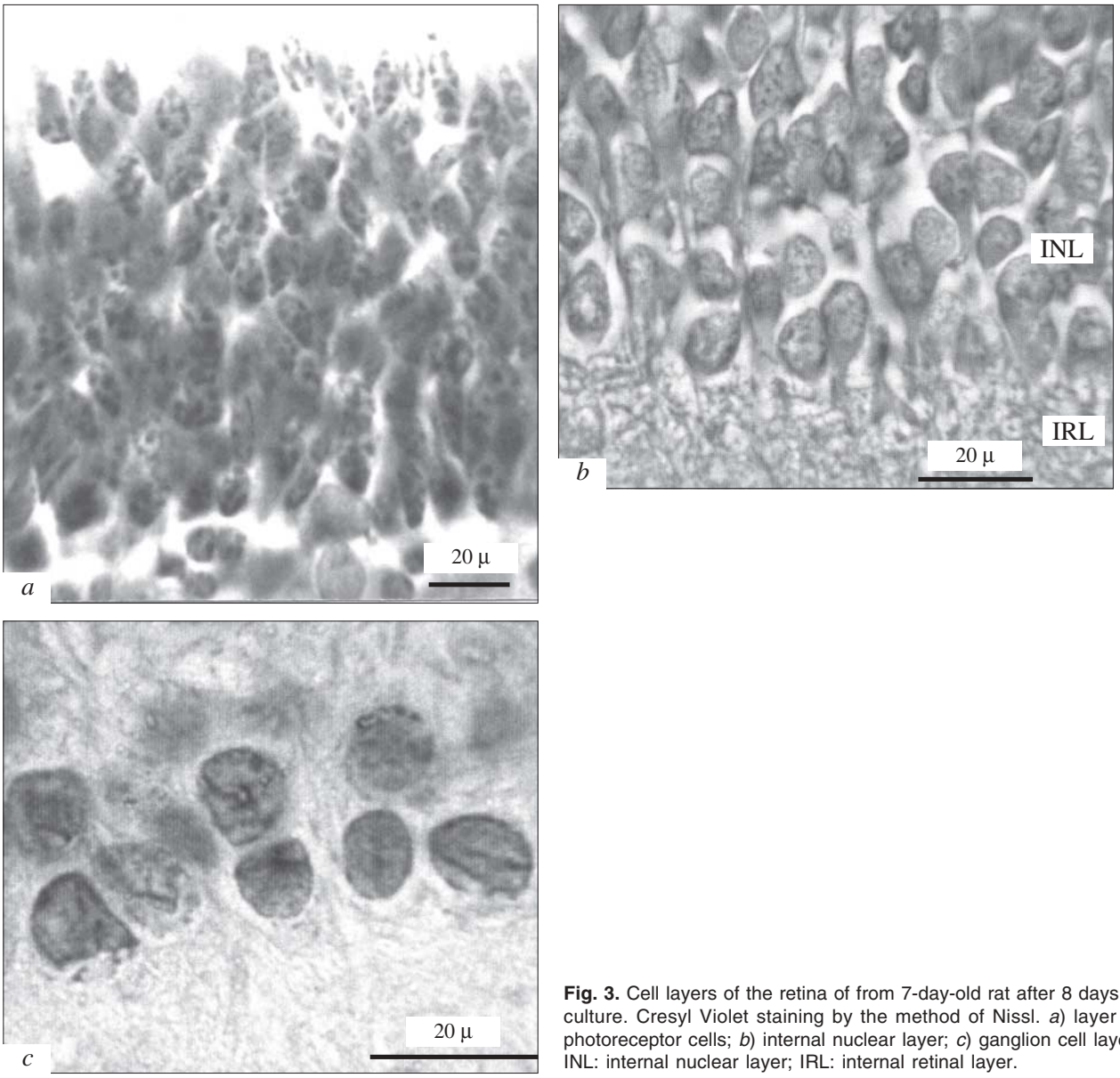

Fig. 3. Cell layers of the retina of from 7-day-old rat after 8 days in culture. Cresyl Violet staining by the method of Nissl. *a*) layer of photoreceptor cells; *b*) internal nuclear layer; *c*) ganglion cell layer. INL: internal nuclear layer; IRL: internal retinal layer.

diseases and subsequent drug correction of neuronal injuries on experimental glaucoma models.

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