

Intraretinal xenografts of differentiated human retinoblastoma cells integrate with the host retina *

M. del Cerro ^a, M.F. Notter ^{a,**}, G. Seigel ^a, E. Lazar ^a, G. Chader ^b and C. del Cerro ^a

^a Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642 (USA)
and the ^b National Institutes of Health, Bethesda, MD (USA)

(Accepted 29 January 1992)

Key words: Retinoblastoma; Y79 cell; Transplant; Mitotic arrest; Xenograft; Differentiation

We report on the successful use of chemically modified Y79 human retinoblastoma cells for intraretinal xenografting into damaged adult mammalian eyes. Y79 cells were exposed *in vitro* to retinoic acid/butyrate to induce differentiation. Using a multisite transplantation method, the suspension was injected into the subretinal space of Fischer 344 rats. The survival, integration, and differentiation potential of these cells was studied, following their return to the intraocular milieu from which the progenitor cells originated. The grafted cells survived and differentiated into immature photoreceptor elements in the subretinal and intraretinal locations, as multiple clusters of rosette-forming cells intimately attached to the host neuroretina. The differentiation process included development of synaptic connectivity of the ribbon type with the surrounding neuropil. No signs of renewed cell division were found within grafts performed on 42 rat eyes, and there was no indication of cell-mediated host reaction against the transplants. This study indicates that tumorigenicity can be suppressed in mitotically arrested Y79 cells, and that these cells are capable of undergoing differentiation *in vivo*. This provides evidence of the remarkable differentiation properties of human retinoblastomas while indicating that Y79 cells may ultimately be able to substitute for fetal cells in experimental retinal transplantation.

INTRODUCTION

Retinoblastomas are the most common ophthalmologic malignancies of childhood. These tumors have been the subject of numerous studies at the cellular and molecular levels, and success has been achieved in obtaining continuous retinoblastoma cell lines. Y79 retinoblastoma cells represent a long-established cell line derived from a human intraocular retinoblastoma²². The histogenetic capabilities of the line have been the subject of repeated study during the last decade. Early histochemical observations showed that this line produces astroglial and neuronal cells in monolayer cultures¹², while more recent work suggests that the Y79 line derives from embryonic tumoral precursors capable solely of photosensory differentiation⁹. Electron microscopic observations have shown that these cells are indeed capable of undergoing photoreceptor differentiation^{17,27}. Chemically induced differentiation, using

a variety of agents, has been attempted to revert the tumoral nature of these cells. However, in most instances, only small percentages of treated Y79 cells actually attain the differentiated state. Markers such as the presence of neuron-specific enolase, S-antigen, and photoreceptor cell morphology have been employed to characterize morphological and biochemical conversion.

In a recent study, Y79 cells were treated in suspension with butyrate and/or corticosteroids and subsequently implanted subcutaneously into nude mice to establish an *in vivo* model of growth control and differentiation¹¹. However, in that study, Y79 cell growth *in vivo* remained unchecked and tumor formation was apparent. To date though, it appears that no study has involved grafting Y79 cells intraocularly into the retinal area from which the cells originated. Therefore, with this information as a background, we designed a series of experiments aimed at testing the potential of Y79

* Portions of this study have been presented in abstract form¹⁸.

** Deceased.

Correspondence: M. del Cerro, Department of Neurobiology and Anatomy, University of Rochester School of Medicine, PO 603, Rochester, NY 14642, USA. Fax: (1) (716) 442-8766.

cells, initially differentiated in vitro, to undergo further differentiation in oculo. In addition to giving important information as to tumor cell biology in vivo, we thought that this also would address the question of the suitability of Y79 cells as potential donor cells for intraretinal grafting.

Our experimental design required the subjugation of aggressively malignant Y79 retinoblastoma cells in order for them to serve as suitable graft material upon their return to the retina. To this end, Y79 cells were grown in the presence of butyrate and retinoic acid for 7 days as monolayer cultures. These conditions were chosen due to an observed enhancement of retinoic acid-induced growth inhibition by butyrate in vitro, without a concomitant loss in cell viability^{14,17}. Our results show that under these conditions, mitotically arrested Y79 retinoblastoma cells are capable of further morphological differentiation in vivo with no evidence of tumor formation. We propose that this model will prove to be useful in the field of retinal transplantation, as well as in the study of cell differentiation and suppression of malignancy.

MATERIALS AND METHODS

Preparation of donor cells

Y79 Human retinoblastoma cells were used as donor tissue. Cells were obtained from the American Type Culture Collection and grown under standard conditions. Stock cultures were grown in suspension in RPMI medium supplemented with 15% fetal calf serum (FCS) (heat inactivated, Hyclone) 0.02% glutamine and 50 µg/ml gentamycin at 37°C with humid 95% air, 5% CO₂. For transplantation studies, Y79 cells were transferred to coated, 100-mm tissue culture dishes for attachment and differentiation. Dishes were pretreated with 50 µg/ml poly-D-lysine for 30 min at room temperature to generate an adhesive surface as described¹⁵. Y79 cells (5 × 10⁶/dish) were cultured overnight in standard medium (MEM + 10% FCS) for adhering cells. After 48 h, 50 µM of retinoic acid and 0.5 mM butyrate were added simultaneously to inhibit mitosis and to differentiate Y79 cells as described¹⁷. For this, cells were cultured for 7 days in the presence of both agents and then processed for transplantation. Monolayers were rinsed in calcium magnesium-free phosphate-buffered saline with 0.2% EDTA and treated with 0.06% trypsin (Gibco) for 10 min at 37° to remove cells from the adherent surface. Cells were centrifuged at 900 rpm, and rinsed with calcium magnesium-free phosphate-buffered saline with 0.2% EDTA. After pelleting, the cells were suspended in cold human plasma for transplantation, at a final concentration of 4000 cells/µl. Since the cells showed a marked tendency to agglutinate, the dissociated state was maintained by aspirating the suspension through the tubing connected to a butterfly needle, (Abbott Hospitals, North Chicago, IL) and then releasing them through the same port. By varying the needle gauge and the number of aspiration–ejection cycles, it was possible to maintain fine control over the final degree of dissociation.

Hosts and anesthesia

Healthy male adult albino rats of the Fischer 344 strain served as hosts. A total of four identical groups of six rats each were used sequentially. The animals were 4–6 months old at the beginning of the study. The choice of the Fischer rat was dictated by the fact that this particular strain is known to be affected by an age-related retinal degeneration. Thus, the retinal periphery offers the grafted cells a background of depleted photoreceptor cell population. The animals

were anesthetized with a mixture of chloral hydrate and sodium pentobarbital (Chloropent, Henry Schem Inc. Port Washington, NY) at a dose of 3 ml/kg, given intramuscularly. In addition, eye drops of 1% Alcaine (propaine hydrochloride, Alcon, Fort Worth, TX) were also used as a topical anaesthetic. The eyes were dilated pre-operatively with one drop each of 1% neosynephrine and 1% mydriacyl (Alcon, Fort Worth, TX). All hosts received daily injections of Cyclosporine A at a dose of 10 mg/kg, starting the day of transplantation.

Delivery system and transplantation procedure

For this set of experiments, a 29-gauge needle tightly sheathed in plastic, with 1.2–1.4 mm of the needle tip left exposed was connected to a microliter syringe (Series 1700, Hamilton, Reno, NV), prior to the procedure. A plastic sheath placed on the needle served as an adjustable regulator. By setting it at the appropriate depth, depending on the animal model being used, it limited the depth of penetration and provided protection against over-penetration (Lazar and del Cerro, in preparation). The plastic sheath could be regulated so that only enough of the needle tip was exposed to reach the subretinal space without actually penetrating the retina. In this way, the formation of large retinal holes or tears were prevented.

The microliter syringe was preloaded with the suspension of retinoblastoma cells. After the animal was appropriately anesthetized, collibri forceps (Storz, St. Louis, MO) were used to firmly grasp the sclera at the limbus and rotate the globe anteriorly. Then, using a stereomicroscope for direct visualization, the needle was manually inserted through the sclera and gently rotated until the tip could be directly viewed through the retina. Then, the tip was advanced further so as to slightly elevate the retina while the plastic protective sheath prevented over-penetration of the needle and perforation of the neuroretina. At this point, with the bevel of the needle facing the globe, the 3- to 4-µl injection of cells was made into the subretinal space. Following the injection, the needle was quickly withdrawn and the procedure repeated at a point 180° opposite to the first injection site in the same eye. Typically, two microinjections were made into each eye, in the retinal periphery, anterior to the eye's equator. One was made superiorly at the 12 o'clock position and the other at the 6 o'clock position inferiorly. The needle was quickly withdrawn following each injection, and upon completion of the procedure, a topical lubricant was placed on the cornea to prevent drying.

In vivo exams

All surgery was performed using an Olympus SZH stereomicroscope fitted with a 35-mm photographic camera as well as videotaping apparatus. Indirect and direct ophthalmoscopy was routinely performed on all the transplant recipients. Using a stereomicroscope (SZH, Olympus Corp., Lake Success, NY), and incident illumination, the animals were examined at regular intervals. This allowed us to continuously monitor the growth and condition of the transplant as well as the host eye. Photographs were taken through the microscope using a Nikon 8008 camera in aperture priority mode. Fast color reversal film (Kodak Ektachrome 800/1600 ISO), was used to neutralize possible blur caused by eye movements in the living animal.

Histological procedures

Animals receiving cell injections were sacrificed as scheduled at predetermined times ranging from 30 to 60 posttransplantation days (PTD). The eyes were enucleated and the animals sacrificed under deep anesthesia using an intramuscular injection of ketamine, 100 mg/ml, at a dose of 90 mg/kg (Quad Pharmaceuticals, Indianapolis, IN) and intramuscular Rompun, 20 mg/ml, at a dose of 8 mg/kg (xylazine, Mobay Corp., Shawnee, KS).

The eyes were enucleated and fixed in 6% glutaraldehyde in cacodylate buffer for 24–48 h. They were then rinsed in buffer and split along a sagittal axis, extending from the cornea to the optic nerve. The hemisected eyes were examined and photographed under a stereomicroscope, and were then embedded in plastic (Eponate 12,

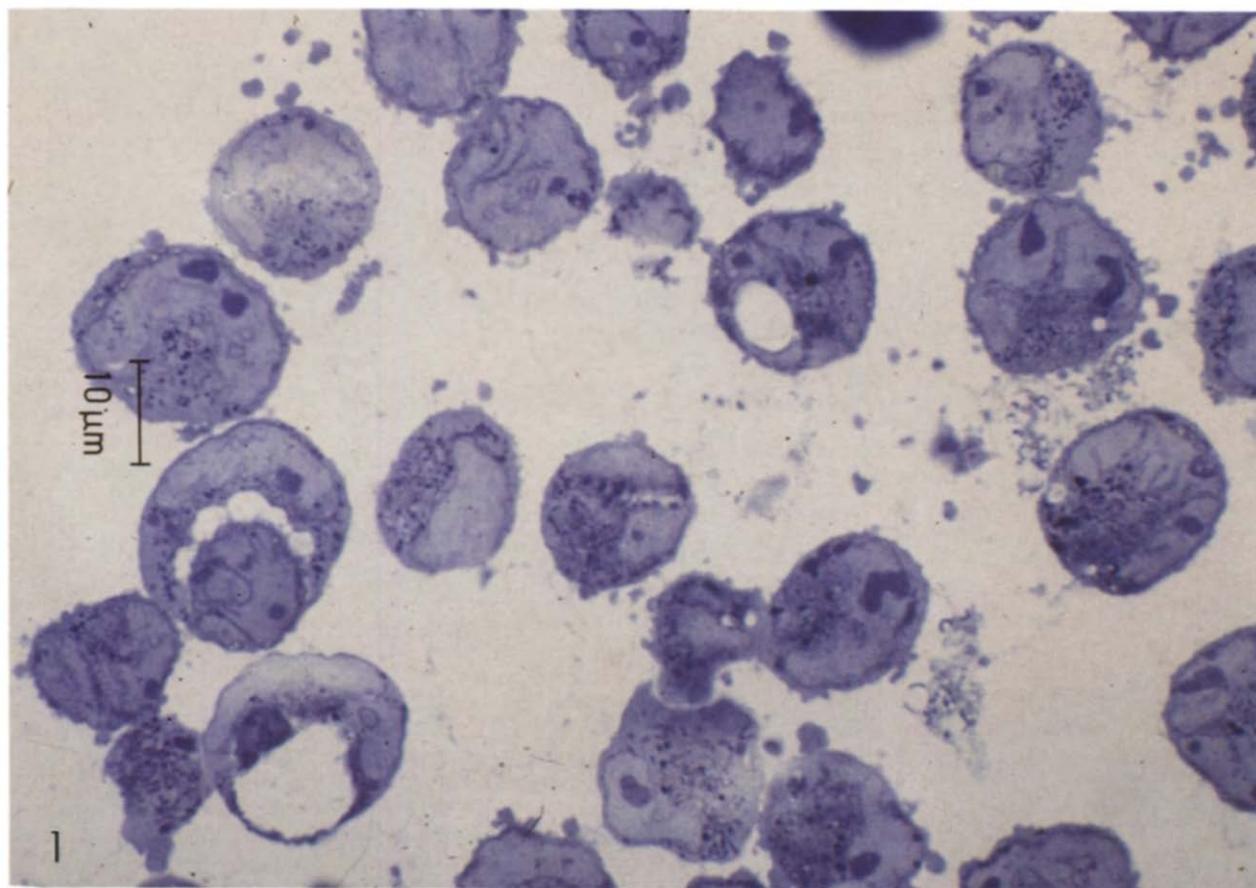


Fig. 1. A 1- μm -thick plastic section of a pellet of Y79 cells treated for 7 days with butyrate and retinoic acid as described in the text. The picture shows a population of large spherical cells, with a high nuclear-cytoplasmic ratio and prominent nucleoli. Some of the cells have clearly visible cytoplasmic vacuoles. Magnification = 1,300 \times .

Ted Pella, Redding, CA). One- μm -thick sections were cut and stained for light microscopic study. Ultrathin sections were cut with a diamond knife for electron microscopic studies. They were stained with lead acetate²⁸ and studied under a Zeiss 10 electron microscope operating at 80 kV.

RESULTS

Microscopically, the suspension of Y79 cells, freshly obtained after dissociation, consisted of rounded cells of variable size. One- μm -thick plastic sections of pellets formed by centrifugation of these cells allowed us to observe the large size attained by some of the cells; values higher than 20 μm in diameter were not uncommon (Fig. 1). Light microscopy also indicated the presence of a very high nuclear/cytoplasmic ratio, as well as large nucleoli, geographic nuclear profiles, and multinucleated cells. In spite of a general appearance resembling that of tumoral cells, no mitoses were observed in counts encompassing 500 cells counted in 5 different sections. This result was considered important as it underscored the efficacy of the mitotic arrest achieved by the retinoic acid-butyrate treatment, and it was a predictor of the non-tumoral behavior of the cells after transplantation.

Electron microscopic observations confirmed and further elaborated upon the light microscope observations. In particular, the irregular nuclear profiles and the presence of deep intranuclear cytoplasmic projections were made evident, as was the large size and heterogeneity of the nucleoli. A readily observed cytoplasmic feature was the presence of relatively high numbers of lipofuscin bodies and cytoplasmic vacuoles. Also notable was the scarcity of rough endoplasmic reticulum and the presence of innumerable free ribosomes in the cytoplasm (Fig. 2).

Observations of the grafted eyes in the living animals, by means of a stereomicroscope, or of an indirect ophthalmoscope, showed no signs of overgrowth of the transplanted cells into the vitreous cavity. Although the grafting site was readily identifiable in most cases as a minute defect on the choroidal and scleral surfaces, no retinal damage or inflammation was detected at these points.

All the graft-bearing animals ($n = 22$; 20 with binocular grafts, 2 with monocular grafts; grafting was not performed on two animals which showed corneal opacities at the time of grafting) survived to the scheduled dates of sacrifice in four consecutive experiments. His-

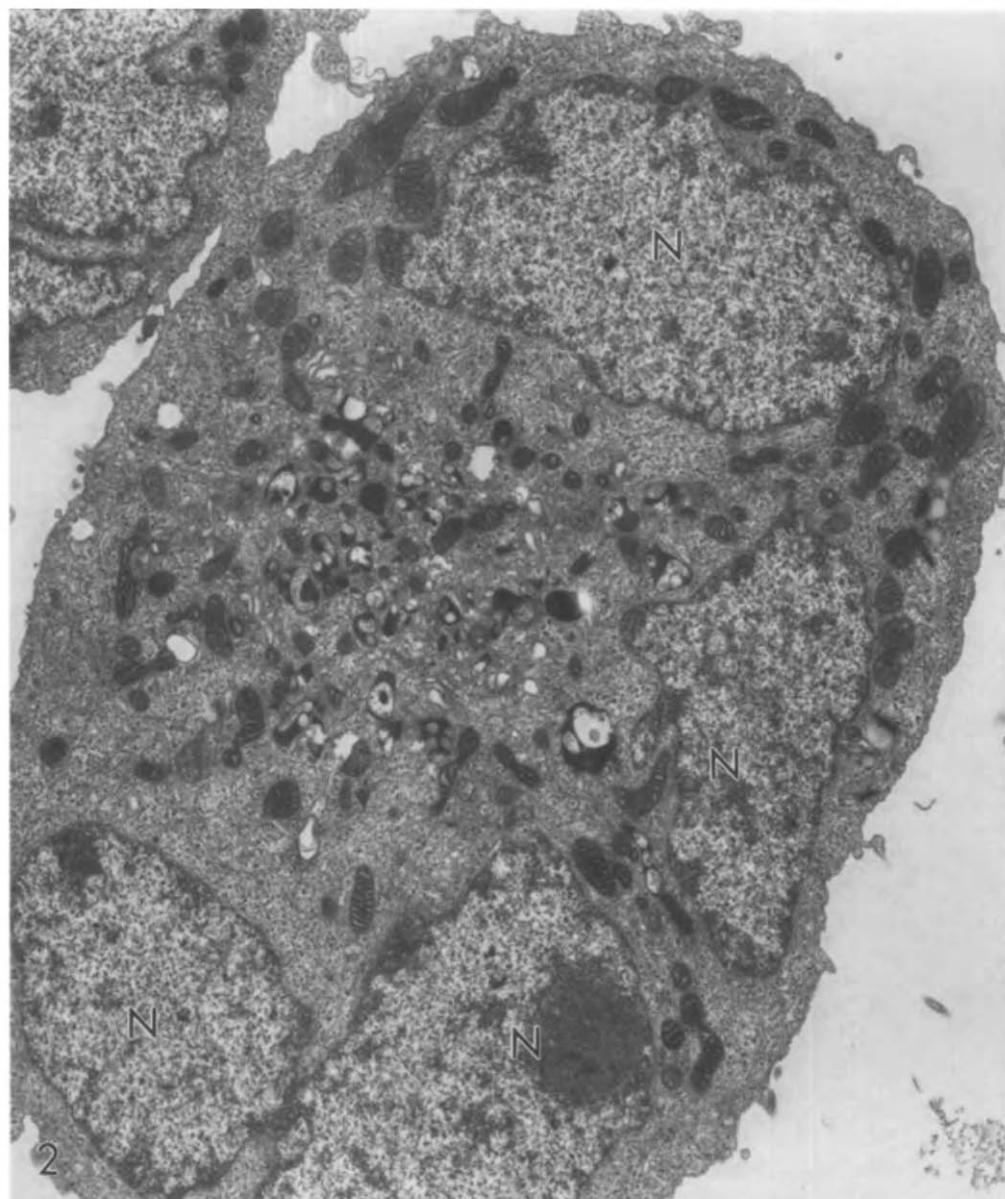


Fig. 2. Electron micrograph of a cell from the pellet shown in Fig. 1. The cell has an elliptical profile, with minute projections rising from its surface. The nucleus (N) is multi-lobed, while the cytoplasm contains evenly distributed dark mitochondria, small lysosomes and lipofuscin granules, and a multitude of free ribosomes. Magnification = 10,000 \times .

tological analysis showed that grafted cells survived in subretinal and, mainly, intraretinal locations. Multiple Flexner-Wintersteiner rosettes were observed in the transplants against a background of the photoreceptor-depleted host retina (Fig. 3A, C). The cells forming these rosettes were closely packed; they have large nuclei with finely dispersed chromatin and prominent and sometimes multiple nucleoli. The morphology of these cells was quite distinct from that of the rat photoreceptors (Fig. 3D). The cell shape, tendency to group in rosettes, and the presence of prominent or multiple nucleoli, clearly distinguished them from the neurons in the host inner nuclear layer (Fig. 3B and D). The differences were visible under the light microscope and were confirmed by electron microscopical

analysis (Fig. 4). Additionally, the electron microscope showed that the grafted cells developed a strong polarity. Their apical portions contacting the lumen of the rosettes, had cilia projecting into the lumen (Fig. 5) as well as a high mitochondrial concentration, which is typical of photoreceptor inner segments. Some club-shaped cytoplasmic enlargements were seen at the tip of these cilia. Laterally, the apical portion of the cells, were joined by an extensive system of intercellular junctions, closely reminiscent of the retinal outer limiting membrane (Fig. 5).

The clusters formed by the grafted cells were intimately integrated into the host neuroretinal tissue (Fig. 6). Here, a narrow band of neuropil often separated the apical portion of the Y79 cells which formed the

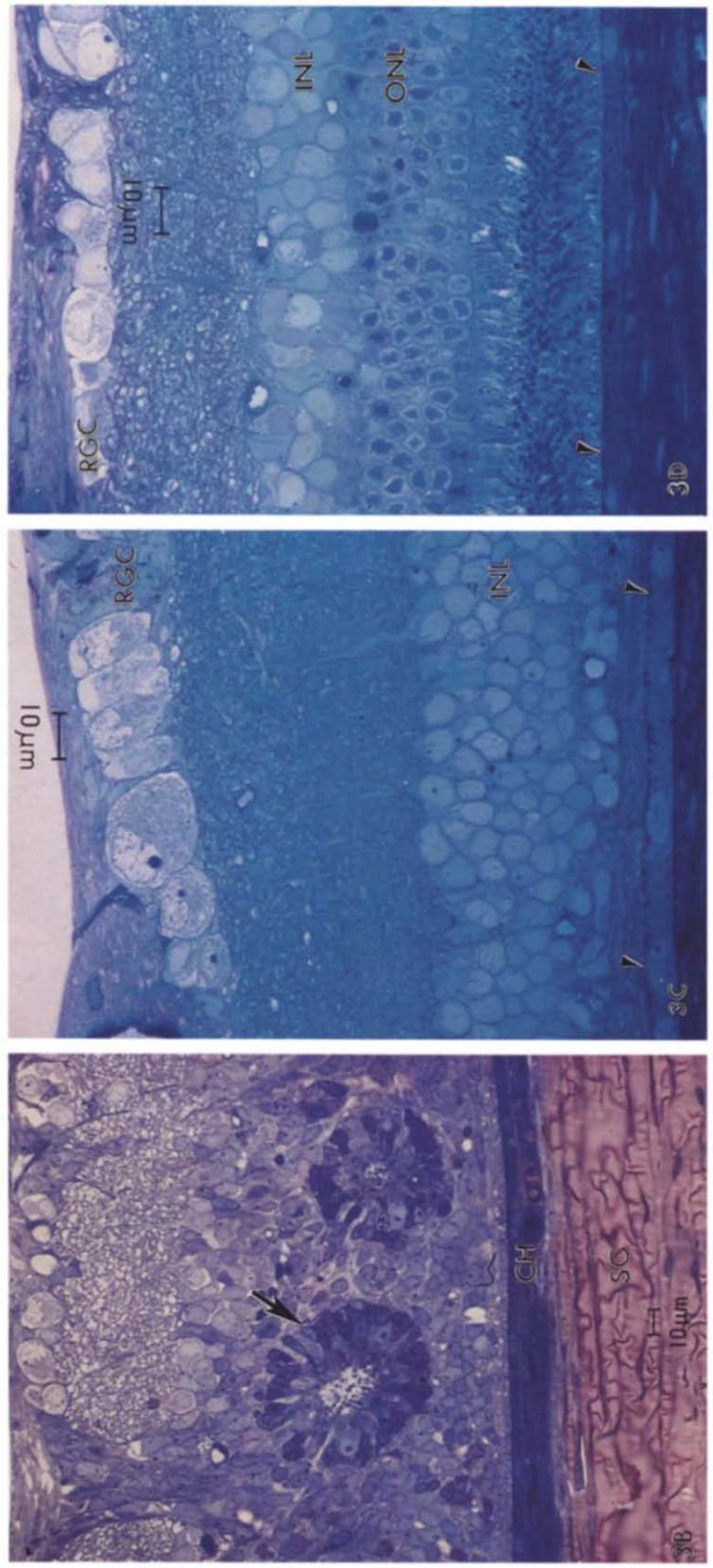
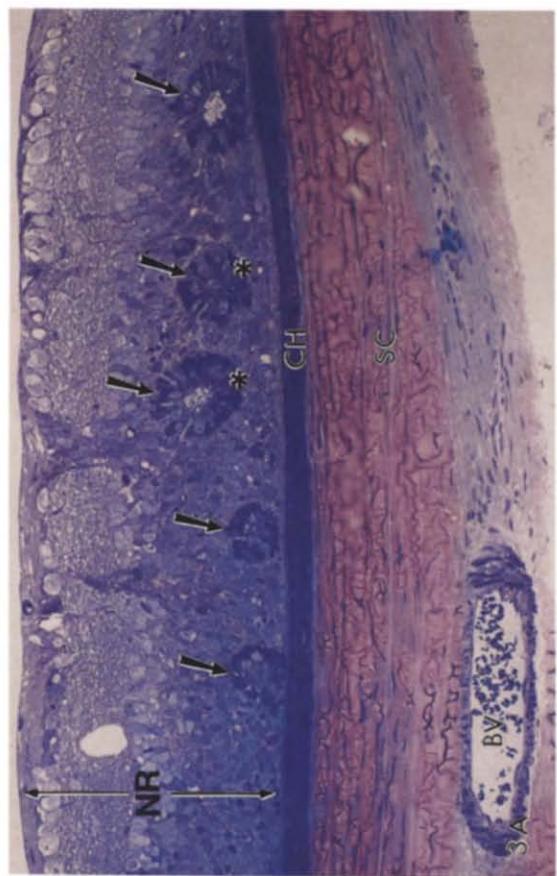


Fig. 3. A: 1- μm -thick plastic section from the eye of a Fischer 344 rat intraretinally grafted with differentiated Y79 cells. At posttransplantation day (PTD) 57, the grafted cells form rosettes (arrows) within the outermost region of the host neuroretina (NR), which is affected by the age-related retinal degeneration characteristic of this rat strain. The lack of visible trauma attests to the benign nature of the grafting procedure, while the lack of inflammatory reaction suggests immunological tolerance. Details of the rosettes indicated by asterisks are shown in 3B. CH, choroid; SC, sclera; BV, blood vessel. Magnification = 162 \times . B: Details of the two rosettes indicated in A. The presence of cytoplasmic projections into the space can be appreciated, as well as the intimate integration of the grafted cells with the host retina. The virtual absence of host photoreceptor cells in this field is characteristic of areas affected by age-related retinal degeneration in the Fischer 344 rat strain. The arrow points to the rosette shown in the electron micrographs. CH, choroid; SC, sclera. Magnification = 325 \times . C: 1- μm -thick plastic section from the eye of a Fischer 344 rat showing the age-related degeneration characteristic of the strain. The outer nuclear layer and photoreceptor layers are absent. The inner nuclear layer (INL) confronts the pigment epithelium (arrowheads). Non-grafted eye, contralateral to that shown in A and B. RGC, retinal ganglion cell layer. Magnification = 585 \times . D: 1- μm -thick plastic section from the eye of a normal Fischer 344 rat retina which has not received transplants. The picture illustrates the morphological features of normal rat neuroretinal cells. Comparison of A and B documents fundamental differences between the appearance of the host neurons and those of the differentiated Y79 cells. RGC, retinal ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Arrowheads, retinal pigment epithelium. Magnification = 585 \times .

←

rosettes from the host retina. Electron microscopically, it was observed that the basal cytoplasmic regions of the grafted cells projected short expansions into this neuropil. We unexpectedly found synaptic contacts between the basal expansions of the Y79 cells and the fibers forming the neuropil. These synapses, which were frequently observed, were of the ribbon type (Fig. 7), characteristic of the synapses formed at the basal portion of normal photoreceptor cells.

No signs of continued cell division were found within the transplants in any of the four experimental series run for this study. Therefore, within the time frame of these observations, the chemically induced mitotic arrest was both uniform and irreversible. There was no indication that a cell-mediated host reaction had occurred against the grafted material (Fig. 3A and B), presumably an indication that the immunosuppressive regimen was appropriate to prevent rejection of the xenograft.

DISCUSSION

This study revealed that chemically differentiated Y79 retinoblastoma cells have a truly remarkable capacity to remain mitotically arrested, and to differentiate into neural cells *in vivo*. Upon return to a retinal milieu, even as xenografts, these cells undergo an extraordinary transformation. At the time of grafting, these cells retain morphological characteristics denoting their tumoral origin, such as a large nuclear-cytoplasmic ratio, large nucleoli, and undifferentiated cytoplasm rich in free ribosomes. Thirty days later, these cells formed rosettes within the host retina. Furthermore, they acquired a definite apical-basal polarity. Their apical regions showed the mitochondrial accumulation associated with photoreceptor inner segments. The presence of a single cilium, which ends in an enlarged tip, indicates an abortive effort to form a photoreceptor outer segment. The presence of lateral

intercellular junctions which form an equivalent of the retinal outer limiting membrane, indicates that domains within the cell membrane also partake in the differentiation effort of the cell. However, the most important and unexpected differentiation trait expressed by the grafted cells was their ability to form synaptic connections of a specific type, i.e. ribbon synapses that bear a strong resemblance to those normally seen in photoreceptor neurons. An unexplained observation was the tendency of these cells to penetrate the host retina when injected into the subretinal space. No similar behavior has been observed in experiments where human fetal retinal cells were grafted into the subretinal space⁴.

These observations are striking, considering the malignant, proliferative nature of untreated Y79 cells derived from a human intraocular retinoblastoma. In a parallel series of studies we have found that Fischer rats of the same age, injected under identical conditions with undifferentiated Y79 cells, and kept under cyclosporine treatment, develop fast-growing intraocular tumors, which aggressively invade the host retina, vitreous, and optic nerve¹. However, morphological indications of Y79 differentiation *in vitro* have been shown in response to such agents as dibutyryl cAMP¹⁴, serum-free conditions²⁷, retinal pigmented epithelium-derived growth factor²⁶, as well as sodium butyrate^{2,17}. Neuronal markers of differentiation have been found *in vitro*, such as S-antigen induction by gamma-interferon¹⁰, increased neuron-specific enolase⁹ and interphotoreceptor retinoid-binding protein (IRBP) upon butyrate treatment¹⁶. Of particular importance to the present study is that the use of butyrate to halt cell division also induces IRPB, a specific marker for photoreceptor-like differentiation²³. In addition to these neuronal markers, detectable glial markers such as glial fibrillary acidic protein (GFAP)^{3,12} and the S-100 antigen²⁵ *in vitro* suggest the possibility of a varied differentiation potential within Y79 cell populations.

In recent studies, 48-h butyrate/hydrocortisone treatment in vitro proved ineffective for differentiation or tumor growth inhibition in vivo¹¹. However, in our

studies, the particular combination of 7 day sodium butyrate/retinoic acid treatment in vitro, coupled with possible restorative effects of the in vivo retinal envi-

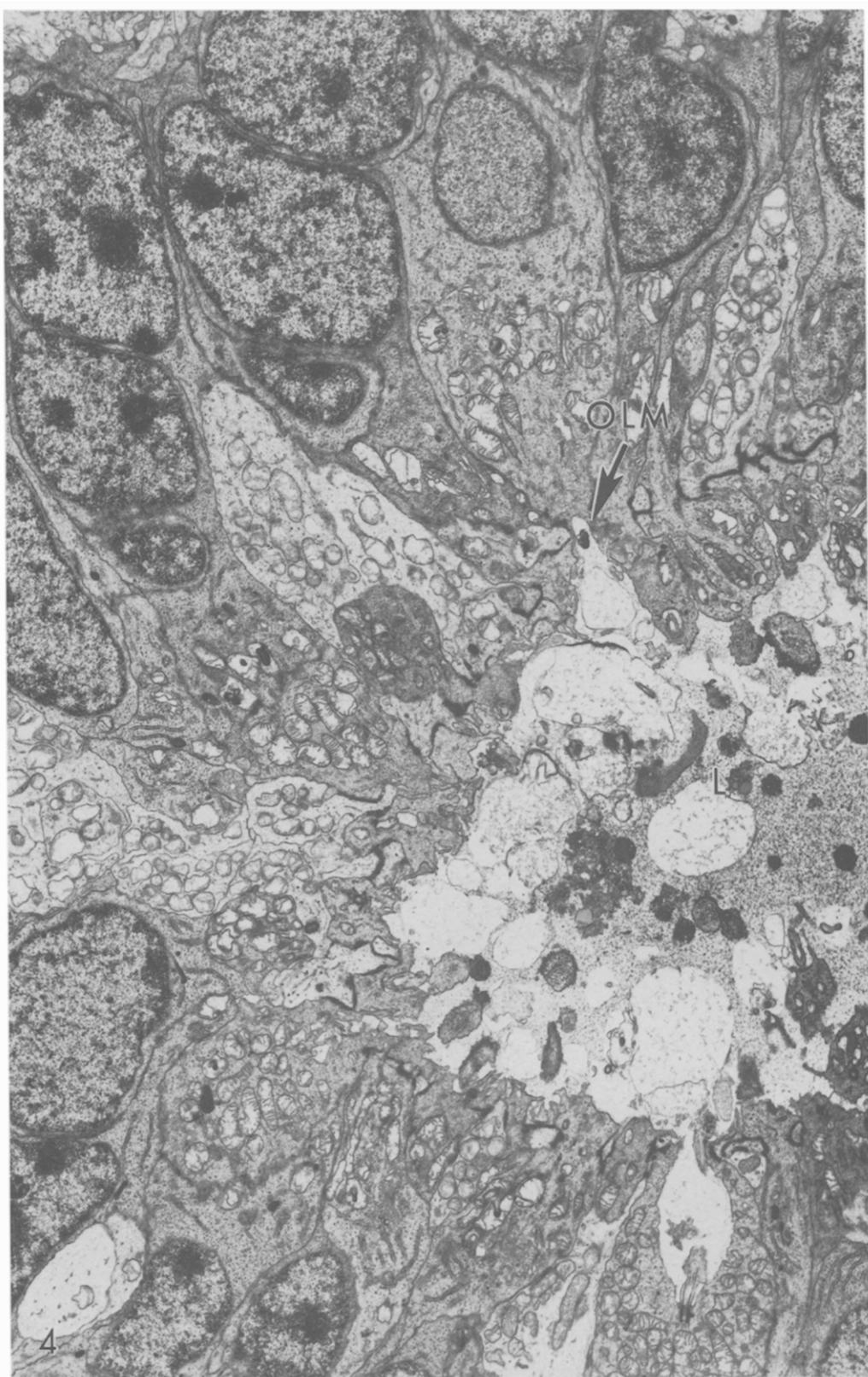


Fig. 4. Electron micrograph of the lumen of the rosette indicated in Fig. 3B. The cells forming the rosette have developed a well defined apical-basal polarity. Their apical portions contain most of the mitochondria present in the respective cells and have developed an extensive system of intercellular junctions reminiscent of the normal outer limiting membrane (OLM). Details of the cells projecting into the lumen (L) are seen in Fig. 5. Magnification = 5,000 \times .

ronment appeared to have profound effects on Y79 cell growth and differentiation, as thus far, no tumors have been detected under these conditions in any of our relatively large number of grafts ($n = 42$). Logisti-

cal considerations required that the population used in this study were divided into four groups of six animals each. The fact that in each separate instance the differentiated Y79 cells gave no indication of a return to the

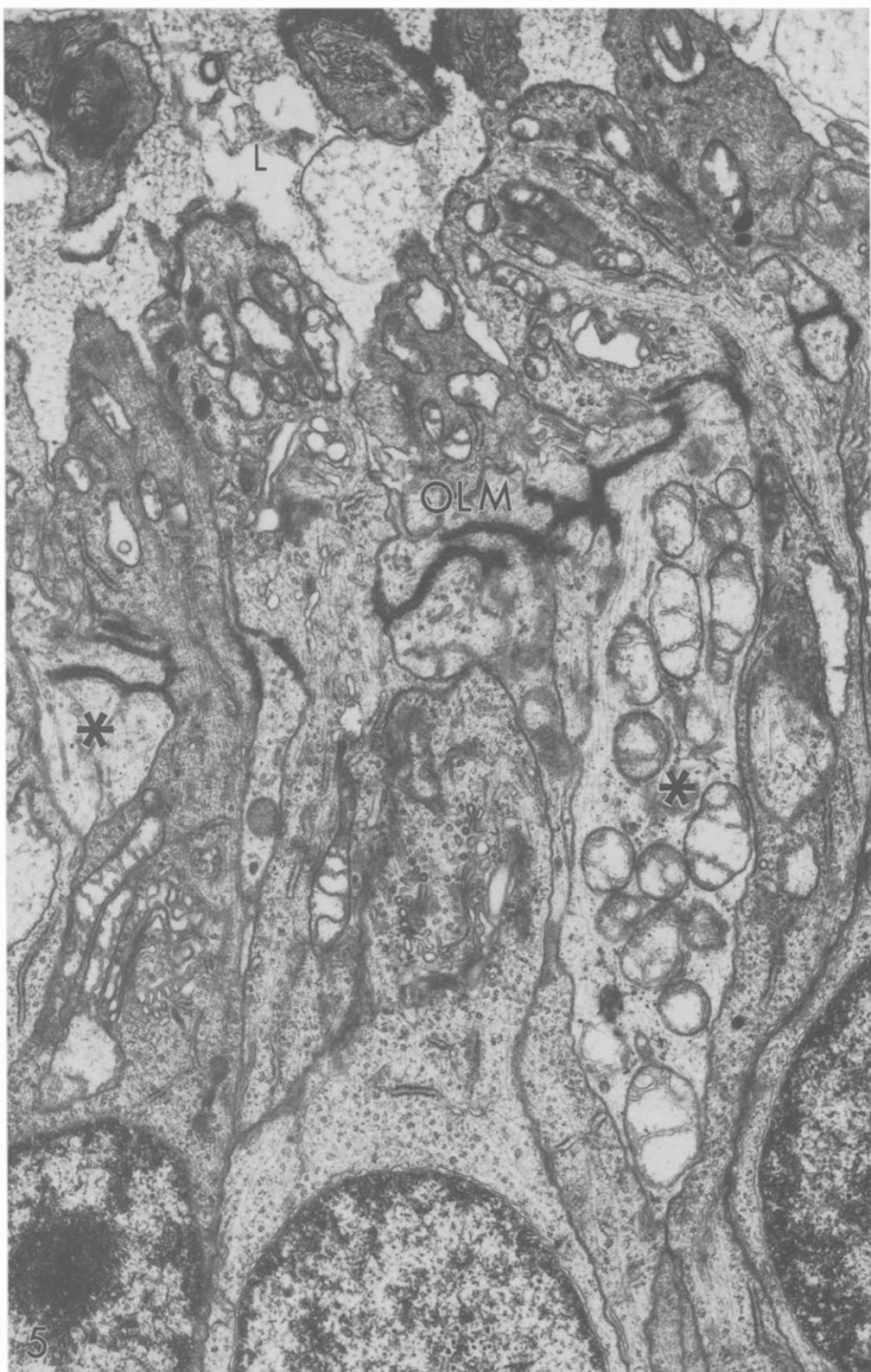


Fig. 5. The polarization of mitochondria into the most apical regions of the cells can be further visualized at this magnification. A few cells containing "light", i.e. low electron-density, cytoplasm (asterisks) are intermingled with the denser cells forming the wall of the rosette. L, lumen; OLM, outer limiting membrane. Magnification = 16,000 \times .

tumoral condition, attests to the reliability of the differentiation treatment. Our results indicate an ability to overcome the malignant phenotype consistent with Rb gene deletions inherent in Y79 cells^{19,20}. Suppression of the Y79 malignant phenotype has been demonstrated in vitro by Y79- NIH 3T3 cell fusion and Rb genetic complementation²⁴. As early as 1984, Kyritsis et al., speculated that the in vitro differentiation effects of butyrate and retinoic acid on Y79 cells could be useful for in vivo studies. Our studies, based on graft-

ing in vitro differentiated Y79 cells into host eyes affected by an age-related retinopathy, represent the first confirmation of such a hypothesis.

In a related field, a number of studies have been performed with human and rodent neuroblastoma cell lines in which these cells have been proposed as an alternate donor source for CNS transplantation. Results using neuroblastomas grafted into the CNS have been variable: some lines do not survive, others form tumors^{6,7}, and still others induce functional recovery in

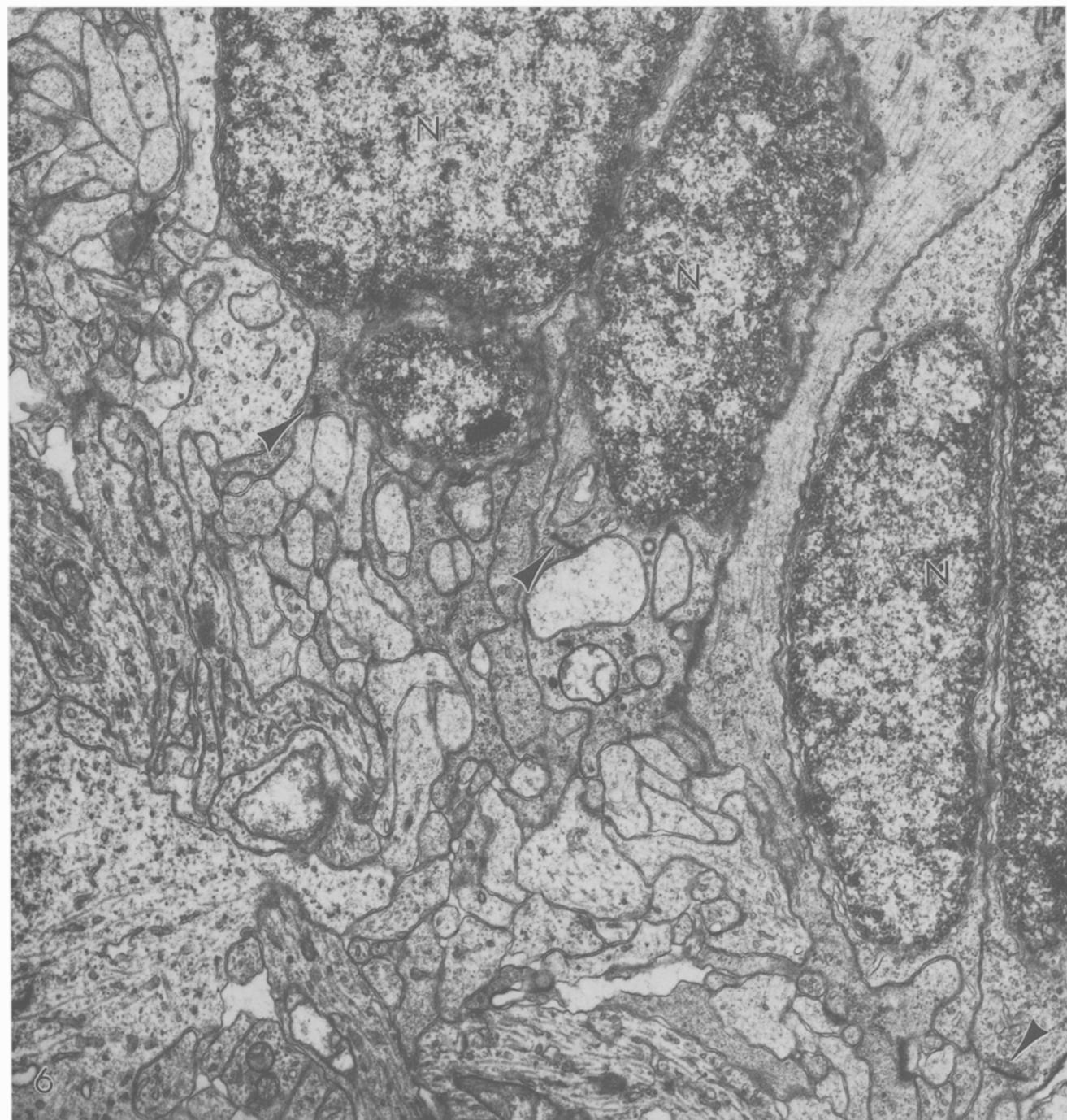


Fig. 6. Electron micrograph depicting the basal regions from the cells in the same rosette seen in the preceding figures. Even at this level of magnification, a total integration between the rosette and the surrounding neuropil can be observed. Several ribbon synapses (arrowheads) are present in the field. Details of the basal cytoplasm from the two cells in the lower right part of the illustration are seen in Fig. 7. N indicates cell nuclei. Magnification = 16,000 \times .

animals exhibiting a neurotransmitter-deficient, neurological disorder^{13,21}. In regard to the latter transplant studies, neither full integration of donor cells within the host, nor appropriate neuritogenesis and synaptogenesis have been seen. This makes the findings of the present study all the more significant in that Y79 retinoblastoma cells *in vivo* readily develop synaptic contacts. Whether those contacts are with the host retinal cells or with other graft-derived neuroretinal elements, a bona fide attempt to establish circuitry is evident. Planned behavioral tests on blinded rats grafted with differentiated Y79 cells may provide answers to the question of functional integration with the host. The possibility is also open, as suggested by a reviewer of this manuscript, to use fluorescence-activated cell sorting (FACS) to select those subpopulations of Y79 cells which have the greatest tendency to differentiate as photoreceptors. Work on obtaining fur-

ther differentiation of retinoblastoma cells is underway.

Some workers have claimed that certain cell lines, such as Y79, which have been propagated *in vitro* for extended periods, cannot be considered phenotypically representative of retinoblastoma cells⁸. Should this be the case, it may help to explain why, under appropriate treatment, and within the experimental circumstances of our experiments, the Y79 cells permanently lost their tumorigenic properties and differentiated as photoreceptor cells to a considerable degree. However, it should be pointed out that a parallel study in our laboratory using untreated Y79 cells for injection into the subretinal space of Fischer 344 rats, resulted in the development of fast-growing, highly invasive ocular tumors¹ (also del Cerro et al., in preparation). This result indicates that without the prolonged butyrate/retinoic acid treatment, Y79 cells retain a strong tu-

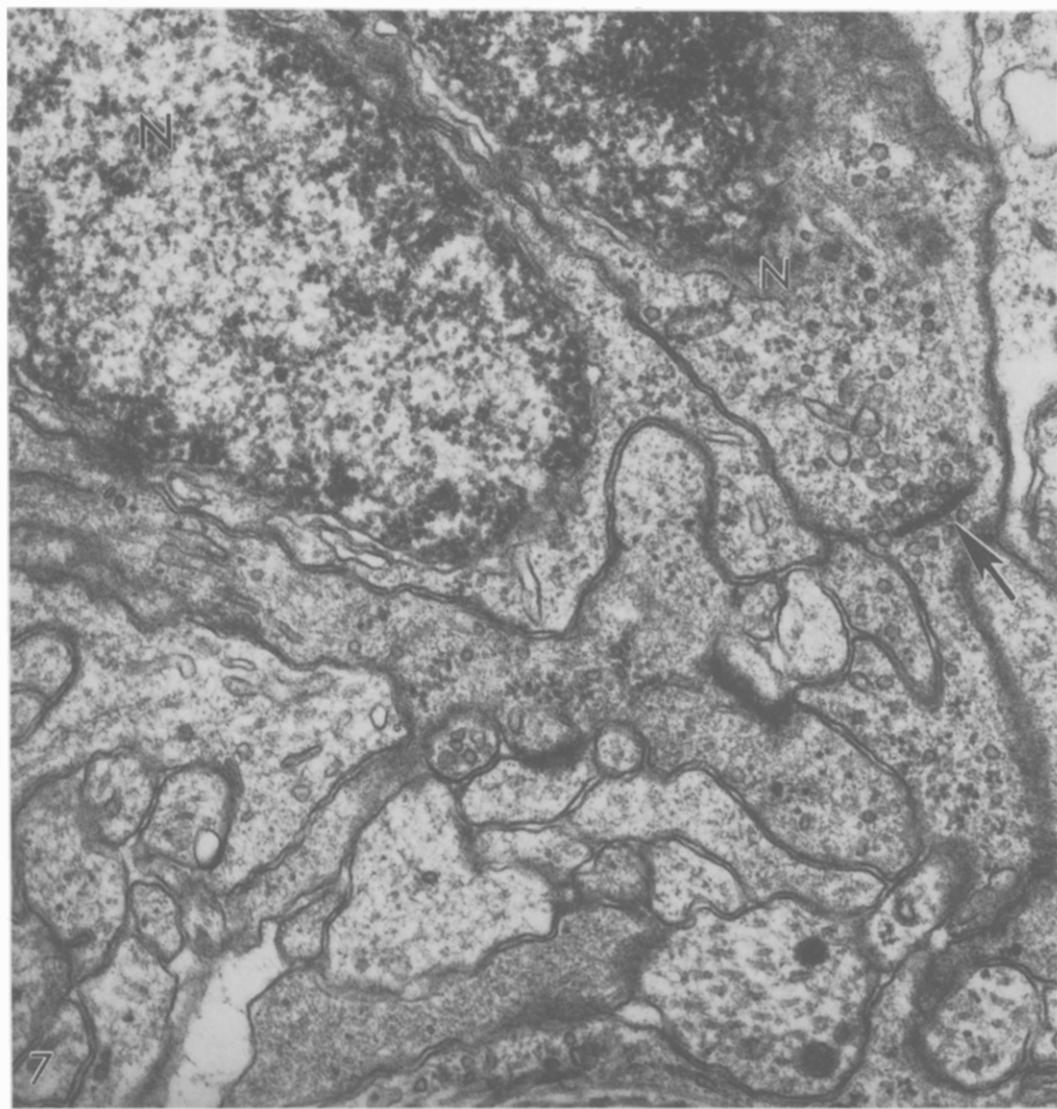


Fig. 7. High-magnification view of an area seen at the lower right corner in Fig. 6. One of the cells shows an accumulation of vesicles clustered around a ribbon (arrow), with the typical arrangement seen in normal photoreceptor cells. N indicates cell nuclei. Magnification = 28,500 \times .

moral character in vivo. Furthermore, it makes clear the fact that xenografting and/or cyclosporine treatment, are unable to induce mitotic arrest in these cells.

Another noteworthy aspect of the present study was the lack of any apparent reaction in the host retina directed towards the grafted cells. It is known that cells from the Y79 and WERI Rb 1 retinoblastoma lines express significant levels of HLA antigens⁵. Thus, it is particularly fortunate that immunosuppression with moderate doses of a single immunomodulator (Cyclosporine A) was sufficient to assure graft acceptance.

In summary, our work not only yields information on the cell biology of retinoblastomas, but also indicates that suppression of tumorigenicity may allow differentiated, mitotically arrested Y79 cells to become a viable alternative to human fetal tissue for retinal transplantation.

Acknowledgements. This paper is dedicated to the memory of Mary F.D. Notter whose dedication and inspiration made this work possible. Supported by NEI Grants 05262 and 052314, the Rochester Eye Bank, T32AG00107 (G.S.), and generous private donations. The authors wish to thank D. Hodari Brooks and David DiLoreto Jr. for their assistance with some aspects of this work and to Ann Paxhia for her technical assistance. Helpful suggestions by two anonymous reviewers are sincerely appreciated.

REFERENCES

- 1 Brooks, H., Notter, M., Seigel, G., Lazar, E., del Cerro, C. and del Cerro, M., Intraretinal grafts of Y79 cells into rats. An in vitro model of human retinoblastoma, *Soc. Neurosci. Abstr.*, 17 (1991) 1137.
- 2 Campbell, M., Karras, P. and Chader, G., Y-79 Retinoblastoma cells. Isolation and characterization of clonal lineages, *Exp. Eye Res.*, 48 (1989) 77-85.
- 3 Detrick, B., Evans, C., Chader, G., Percopo, C. and Hooks, J., Cytokine-induced modulation of cell proteins in retinoblastoma, *Invest. Ophthalmol. Vis. Sci.*, 32 (1991) 1714-1722.
- 4 del Cerro, M., Lazar, E., Grover, D., Gallagher, M., Sladek, C., Chu, J. and del Cerro, C., Intraocular transplantation and culture of human embryonic retinal cells, *Invest. Ophthalmol. Vis. Sci.*, 31 (1990) 593.
- 5 Fournier, G.A., Sang, D.N., Albert, D.M. and Craft, J.L., Electron microscopy and HLA expression of a new line of retinoblastoma, *Invest. Ophthalmol. Vis. Sci.*, 28 (1987) 690-699.
- 6 Freed, W., Patel Vaidya, U. and Geller, H., Properties of PC12 pheochromocytoma cells transplanted to the adult rat brain, *Exp. Brain Res.*, 63 (1986) 557-666.
- 7 Geller, H., Adinolfi, A., Laskin, J. and Freed, W., Implantation of catecholamine-secreting cell lines into the rat and mouse brain, *Progr. Brain Res.*, 78 (1988) 643-646.
- 8 Greig, S., Heise, K., Kindler-Röhrlborn, A. and Rajewsky M.F., In vitro differentiation of human retinoblastoma cells into neuronal phenotypes, *Differentiation*, 45 (1990) 250-257.
- 9 Herman, M., Perentes, E., Katsetos, D., Darcel, F., Frankfurter, A., Collins, V.P., Donoso, L.A., Eng, L.F., Marangos, P.J., Wiechmann, A.F., May E.E., Thomas, Ch.B. and Rubinstein, L.J., Neuroblastic differentiation potential of the human retinoblastoma cell lines Y-79 and WERI-Rb 1 maintained in an organ culture system, *Am. J. Pathol.*, 134 (1989) 115-132.
- 10 Hooks, J., Chader, G., Evans, C. and Detrick, B., Interferon-gamma enhances the expression of retinal S-antigen, a specific neuronal cell marker, *J. Neuroimmunol.*, 26 (1990) 245-250.
- 11 Howard, M., Wardell, S. and Albert, D., Effect of butyrate and corticosteroids on retinoblastoma *in vitro* and *in vivo*, *Invest. Ophthalmol. Vis. Sci.*, 32 (1991) 1711-1713.
- 12 Jiang, Q., Lim, R. and Blodi, F., Dual properties of cultured retinoblastoma cells: immunohistochemical characterization of neuronal and glial markers, *Exp. Eye Res.*, 39 (1984) 207-215.
- 13 Kordower, J., Notter, M.F. and Gash, D., Neuroblastoma cells in neural transplants: a neuroanatomical and behavioral analysis, *Brain Res.*, 417 (1987) 85-98.
- 14 Kyritsis, A., Joseph, G. and Chader, G., Effects of butyrate, retinol and retinoic acid on human Y79 retinoblastoma cells growing in monolayer cultures, *J.N. Cl.*, 73 (1984) 649-654.
- 15 Kyritsis, A., Tsokos, M. and Chader, G., Attachment culture of human retinoblastoma cells: long-term culture conditions and effects of dibutyryl cyclic AMP, *Exp. Eye Res.*, 38 (1985) 411-421.
- 16 Kyritsis, A., Wiggert, B., Lee, L. and Chader, G., Butyrate enhances the synthesis of interphotoreceptor retinoid-binding protein (IRBP) by Y79 human retinoblastoma cells, *J. Cell. Physiol.*, 124 (1985) 233-239.
- 17 Kyritsis, A., Tsokos, M. and Chader G., Control of retinoblastoma cell growth by differentiating agents: current work and future directions, *Anticancer Res.*, 6 (1986) 465-474.
- 18 Lazar, E.S., del Cerro, M., Notter, M.F., Seigel, G. and del Cerro, C., Intraretinal xenografts of mitotically arrested human retinoblastoma, *Soc. Neurosci. Abstr.*, 17 (1991) 1137.
- 19 Lee, W., Bookstein, R., Hong, F., Young, L., Shew, J. and Lee, E., Human retinoblastoma susceptibility gene: cloning, identification and sequence, *Science*, 235 (1987) 1394-1399.
- 20 Levine, A. and Momand, J., Tumor suppressor genes: the P53 and retinoblastoma sensitivity genes and gene products, *Biochim. Biophys. Acta*, 1032 (1990) 119-136.
- 21 Mena, M., de Yebenes, J., Dwork, A., Fahn, S., Latov, S., Herbert, J., Flaster, E. and Slonim, E., Biochemical properties of monoamine-rich human neuroblastoma cells, *Brain Res.*, 486 (1989) 286-290.
- 22 Reid, T., Albert, D., Rabson, A., Russell, P., Craft, J., Chu, E., Tralka, T. and Wilcox, J., Characteristics of an established cell line of retinoblastoma, *J.N. Cl.*, 53 (1974) 347-360.
- 23 Rodrigues, M., Wiggert, B., Shields, J., Donoso, L., Bardenstein, D., Katz, N., Friendly, D. and Chader, G., Retinoblastoma. Immunohistochemistry and cell differentiation, *Ophthalmology*, 94 (1987) 378.
- 24 Sasabe, T. and Inana, G. Mechanisms of suppression of malignancy in hybrids between Y-79 retinoblastoma and NIH 3T3 cells, *Invest. Ophthalmol. Vis. Sci.*, 32 (1991) 2011-2019.
- 25 Terenghi, T., Polak, J., Ballesta, J., Cocchia, D., Michetti, F., Dahl, D., Marangos, P. and Garner, A., Immunocytochemistry of neuronal and glial markers in retinoblastoma, *Virchows Arch.*, 404 (1984) 61-73.
- 26 Tombran-Tink, J. and Johnson, L., Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells, *Invest. Ophthalmol. Vis. Sci.*, 30 (1989) 1700-1707.
- 27 Tsokos, M., Kyritsis, A., Chader, G. and Triche, T., Differentiation of human retinoblastoma in vitro into cell types with characteristics observed in embryonal or mature retina, *Am. J. Pathol.*, 123 (1986) 542-552.
- 28 Venable, J.H. and Cogeshall, R.A., Simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.*, 25 (1965) 407-408.