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Journal

Investigative Ophthalmology & Visual Science, 39(11)

ISSN

0146-0404

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Publication Date

1998-10-01

Peer reviewed

Intact Sheets of Fetal Retina Transplanted to Restore Damaged Rat Retinas

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PURPOSE. The aim of this study was to establish a model for morphologic retinal reconstruction after destruction of photoreceptors.

METHODS. Rat embryos were prelabeled by injection of bromodeoxyuridine (BrdU) into timed pregnant rats on 2 to 6 consecutive days. Pieces of fetal retinas (embryonic day [E] 17 to E22) were embedded in growth factor-reduced matrigel for protection and stored in medium on ice. With the use of a custom-made implantation tool, trimmed embedded pieces were placed into the subretinal space of albino rats whose photoreceptors had been damaged by continuous exposure to blue light for 3 to 4 days.

RESULTS. Donor cells were unequivocally identified by the BrdU label. Approximately 25% of transplants in the subretinal space developed parallel layers, with photoreceptor outer segments facing the host pigment epithelium. Transplants developed rosettes if host pigment epithelium had been damaged, if trauma to the donor tissue occurred during preparation or transplantation, and if the donor tissue was misplaced into the choroid or into the epiretinal space on top of the host retina. If the surgery was performed more than 4 weeks after the light damage, continued degeneration of the host retina caused secondary pigment epithelium damage, and transplants did not develop parallel layers of photoreceptor outer segments.

CONCLUSIONS. After transplantation to the subretinal space of a degenerated retina, gel-protected fetal retina can develop parallel layers and photoreceptor outer segments in contact with host pigment epithelium. Transplants can develop good fusion with the inner retina of a photoreceptor-deficient recipient. (*Invest Ophthalmol Vis Sci.* 1998;39:2121-2131)

In many inherited retinal degenerations, blindness is often due to specific degeneration of photoreceptors and/or retinal pigment epithelium (RPE), even though the remaining neural retina that connects to the brain is still functioning.¹⁻³ If the destroyed photoreceptors and/or RPE can be replaced, and if the new cells can connect to the functional part of the retina, it is possible that retinal degeneration might be prevented or eyesight restored.

Retinal diseases that affect the photoreceptors (e.g., retinitis pigmentosa and age-related macular degeneration), are among the leading causes of blindness.^{3,4} Retinitis pigmentosa is a group of inherited diseases with mutations in photoreceptor (for a review see Berson⁵) and RPE proteins.⁶ Macular degeneration is thought to be primarily caused by RPE dystrophy.^{7,8} The progression of retinitis pigmentosa can be slowed with vitamin A treatment,⁹ but there is currently no treatment

that can reverse the degenerative processes once the photoreceptors are lost.

Retinal transplantation aims at replacing lost photoreceptors or dysfunctional RPE. Clinical trials of RPE transplantation^{10,11} and photoreceptor transplantation¹² have been reported.

In animal experiments, the donor tissue for transplantation is derived from various sources: fetal¹³⁻¹⁹ or mature,^{20,21} freshly harvested^{16,21-25} or cultured.^{10,17,26,27}

Most donor tissue has been transplanted as cell aggregates^{15,16,28-31} or cell suspensions.^{22,32} Few groups have transplanted sheets of donor tissue. Patch transplants of cultured RPE have been done.¹⁷ Intact sheets of 8-day postnatal or mature retina or photoreceptors, but not of fetal retina, have been transplanted to rat retinas with photoreceptor degeneration.^{20,21} Silverman reported opsin-stained outer segments in transplants derived from "postnatal" retina.³³ Transplantation of intact sheets of fetal retina was first reported in 1995³⁴ and again later.^{35,36}

Our laboratory has consistently used fresh fetal retinal donor tissue. Fetal or embryonic tissue has a high capacity to develop different cell types, to sprout neuronal processes, and to produce trophic factors. Embryonic cells in general are well tolerated immunologically by a host of the same species when placed into the central nervous system or the eye, because they as yet lack antigenic sites.³⁷⁻³⁹ Fetal transplants are not rejected if they are derived from the same species unless there is major surgical trauma; xenografts need immunosuppression to survive.^{29,40-42}

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Supported by National Institutes of Health, Bethesda, Maryland, Grant EY08519; by the Vitreoretinal Research Foundation, Louisville, Kentucky; by the Jewish Hospital Foundation, Louisville, Kentucky; by the Murray Foundation Inc., New York, New York; by an unrestricted grant from the Research to Prevent Blindness, New York, New York; by the German Humboldt Foundation, Bonn, Germany; and by funds from an anonymous sponsor.

Submitted for publication March 24, 1998; accepted June 4, 1998. Proprietary interest category: N.

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TABLE 1. Overview of Experiments

Donor Age	No. of Animals	Transplant Surgeries	Time after Transplantation (mo)	Trauma*†	No. of Surviving Transplants	Rejection‡	No Trace of Graft
Total (E15-E21)	67	109	0.4-10	15 (13.8)	75 (68.8)	14 (12.8)	20 (18.3)
E15-E17	6	10	1-6.4	0	9	1	—
E19	20	30	0.5-8.7	7	21	3	6
E20	22	38	0.5-10	4	24	6	8
E21	13	22	0.4-9	2	14	2	6

* Damage to optic nerve or excessive damage to host retina.

† Values in parentheses are percentages.

‡ Infiltration with macrophages.

With our previous method, retinal cell aggregates were transplanted to a retinal lesion site,²⁸ and fetal retinal cells were found to survive, form a transplant, and develop most retinal cell types, including photoreceptors, after transplanta-

tion.^{13,14,24,29} The cell layers of aggregate transplants are organized in rosettes (spherical structures with the photoreceptors around an outer limiting membrane, inner and outer segments toward the lumen, and the inner retinal layers on the outside of

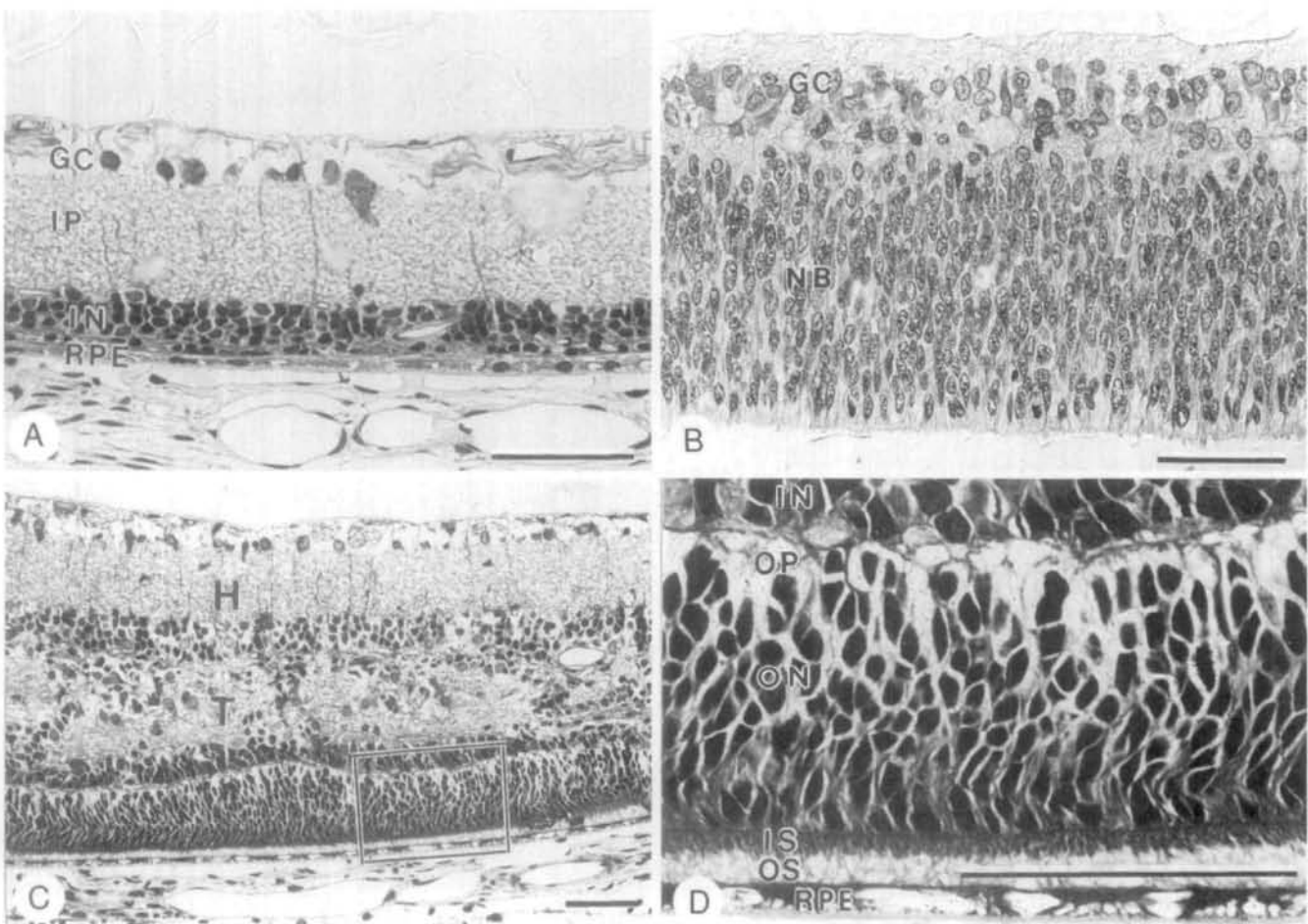


FIGURE 1. Light-damaged recipient retina, embedded donor tissue, and early development of transplants. (A) Dorsal midperiphery of light-damaged retina, 4 days of continuous exposure to blue light (peak 420 nm), 10 days after light damage. The outer nuclear layer is absent, and the inner nuclear layer is directly apposed to the RPE. (B) Example of embedded donor tissue. Rat E19 retina, showing developed ganglion cell layer and neuroblastic outer retina. (C) Rat E19 transplant 13 days after transplantation to light-damaged rat host retina. (D) Enlargement of box area in (C). The developing transplant photoreceptors adjacent to the host RPE are just beginning to form outer segments. All are 2- μ m histocryl sections. Scale bars, 50 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. GC, ganglion cell layer; H, host; IN, inner nuclear layer; IP, inner plexiform layer; IS, inner segments of photoreceptors; NB, outer neuroblastic layer; ON, outer nuclear layer; OP, outer plexiform layer; OS, outer segments of photoreceptors; RPE, retinal pigment epithelium; T, transplant.

TABLE 2. Transplant Placement (% of Surviving Transplants)

Epiretinal (in Vitreous)	Epiretinal and Subretinal	Subretinal	Choroid
18/75 (24.0)	11/75 (14.7)	41/75 (54.7)	5/75 (6.7)
Total	52/75 (69.3)		

Values in parentheses are percentages.

the rosette). The transplanted tissue can develop different degrees of integration with the host retina. Transplant cells can send out neuronal processes to form synapses in the host retina.⁴³ Aggregate transplants develop better organization in the subretinal than in the epiretinal space³⁰ and have provided a good model for study of the developmental capacity of fetal retinal cells after transplantation (for a review see Ref. 44).

However, they appear to have little potential for functional replacement of lost photoreceptors.

Our hypothesis is that fetal retinal transplants will have the greatest potential to improve light perception in damaged retina, if they can be organized in parallel layers so that transplant photoreceptors develop outer segments in contact with RPE. It would be especially important that transplant photoreceptors or interneurons establish functional synaptic contacts with such host retinal cells, as ganglion, bipolar, and horizontal cells. Many retinal diseases destroy photoreceptors and/or RPE, but, at least for some time, leave inner retinal layers and ganglion cells still functional.¹⁻³

Fetal cells have a high capacity to reestablish neuronal connections. Therefore, it was our goal to transplant fetal retina in intact sheets in the hope that such transplants would develop parallel layers resembling normal retina. Because of the extraordinary difficulty of handling fragile fetal tissue, it needs to be protected during transplantation. This can be done

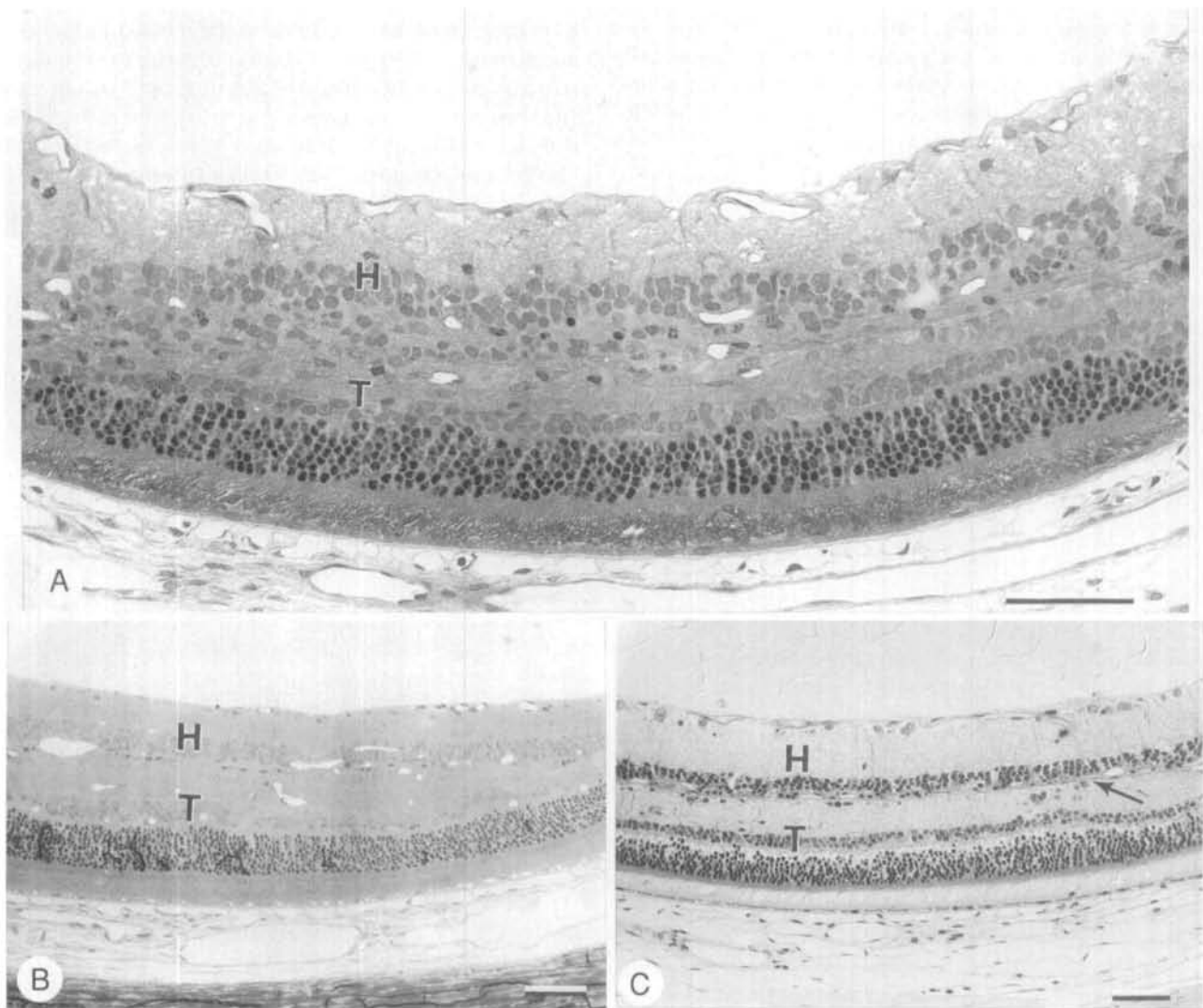


FIGURE 2. Reconstructed retina 7 weeks after transplantation surgery. (A) E21, (B) E20, and (C) E19 transplant. The transplants in (A) and (B) show large areas of integration between transplant and host, whereas the transplant in (C) has formed an inner limiting membrane (arrow) toward the host retina. (A, C) 1.5- μ m histocryl sections; (B) 0.5- μ m Epon section. Scale bars, 50 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. H, host; T, transplant.

TABLE 3. Organization of Subretinal Transplants

Disorganized	Rosettes Only	Rosettes + Small Area with Parallel Layers and Outer Segments	Large Area with Parallel Layers and Outer Segments
9/52 (17.3)	23/52 (44.2)	7/52 (13.5)	13/52 (25.0)

Values in parentheses are percentages.

by embedding it in a gel. Results are presented here that show that this approach can produce well-organized retinal transplants integrated in a damaged retina. Part of this work has been previously reported in abstract form.^{34,35}

METHODS

Experimental Animals

Sixty-seven female albino Sprague-Dawley rats (weight, 200 to 250 g) were used as transplant recipients. Twenty-eight timed-pregnant Long-Evans rats were used as donors for fetal tissue. All animals were treated according to the regulations in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. An overview of the experiments is shown in Table 1.

Light Damage

Female albino Sprague-Dawley rats were exposed for 3 or 4 days to blue light. A custom-made acrylic plastic/wire cage was surrounded by 48-in daylight fluorescent light bulbs, which were covered with a blue plastic filter (Lee filter No. 197, transmission peak at 420 nm). The light intensity was 678 to 1291 lux (63 to 120 foot-candle [ft-c]). After the exposure to light, the rats were returned to normally lighted cages in the

animal facilities (12 hours light on/off; light intensity 8 to 320 lux, 0.5 to 30 ft-c). The light damage procedure was described in abstract form.⁴⁵ An example of a light-damaged retina is shown in Figure 1A.

Preparation of Donor Tissue

To prelabel the donor tissue, timed-pregnant rats were injected with 40 mg/kg bromodeoxyuridine (BrdU), a thymidine analogue, on 2 to 6 consecutive days.¹⁹ To harvest the donor tissue, rat embryos (embryonic days [E] 17 to E21) were removed by cesarean section and stored in ice-cold oxygenated hibernation medium (according to the method of Kawamoto and Barrett⁴⁶). The tissue was kept cold during dissection. Fetal retinas were carefully dissected free from surrounding tissues, and then embedded in growth factor-reduced matrigel (GRF-matrigel; Collaborative Biomedical Products, Bedford, MA). The gel was polymerized at 37°C in an incubator for 30 to 60 minutes. The tissue was then stored on ice in hibernation medium and used for transplantation within 1 to 5 hours after embedding. An example of embedded donor tissue is shown in Figure 1B.

The embedded tissue was cut into rectangular pieces (0.4- to 0.8-mm wide and 0.8 to 1.5 mm long) to fit our custom-made implantation tool (patent pending). This tool was designed to "place" the donor tissue, not to inject or push it.

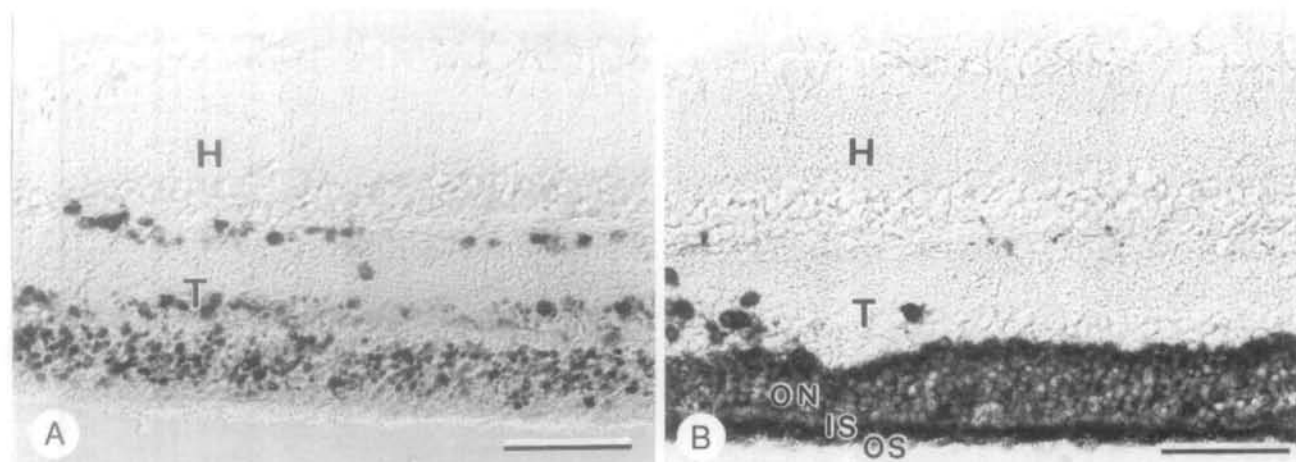


FIGURE 3. Labeling for (A) bromodeoxyuridine (BrdU) and (B) rhodopsin (E20 transplant, 12 weeks after surgery). (A) Many of the donor cells were labeled before transplantation. BrdU was injected into the donor mother on 6 different days. BrdU labels donor cells synthesizing DNA at the time of injection. Different retinal cell types in the transplant are labeled. There is no label in the host retina. (B) A section of the same transplant stained for rhodopsin. The transplant photoreceptors and their outer segments are immunoreactive. No rhodopsin staining is seen in host retina above the transplant. The transplant outer segments have broken apart from the host RPE during tissue processing. All are 8- μ m paraffin sections. Scale bars, 50 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. H, host; IS, inner segments of photoreceptors; ON, outer nuclear layer; OS, outer segments of photoreceptors.

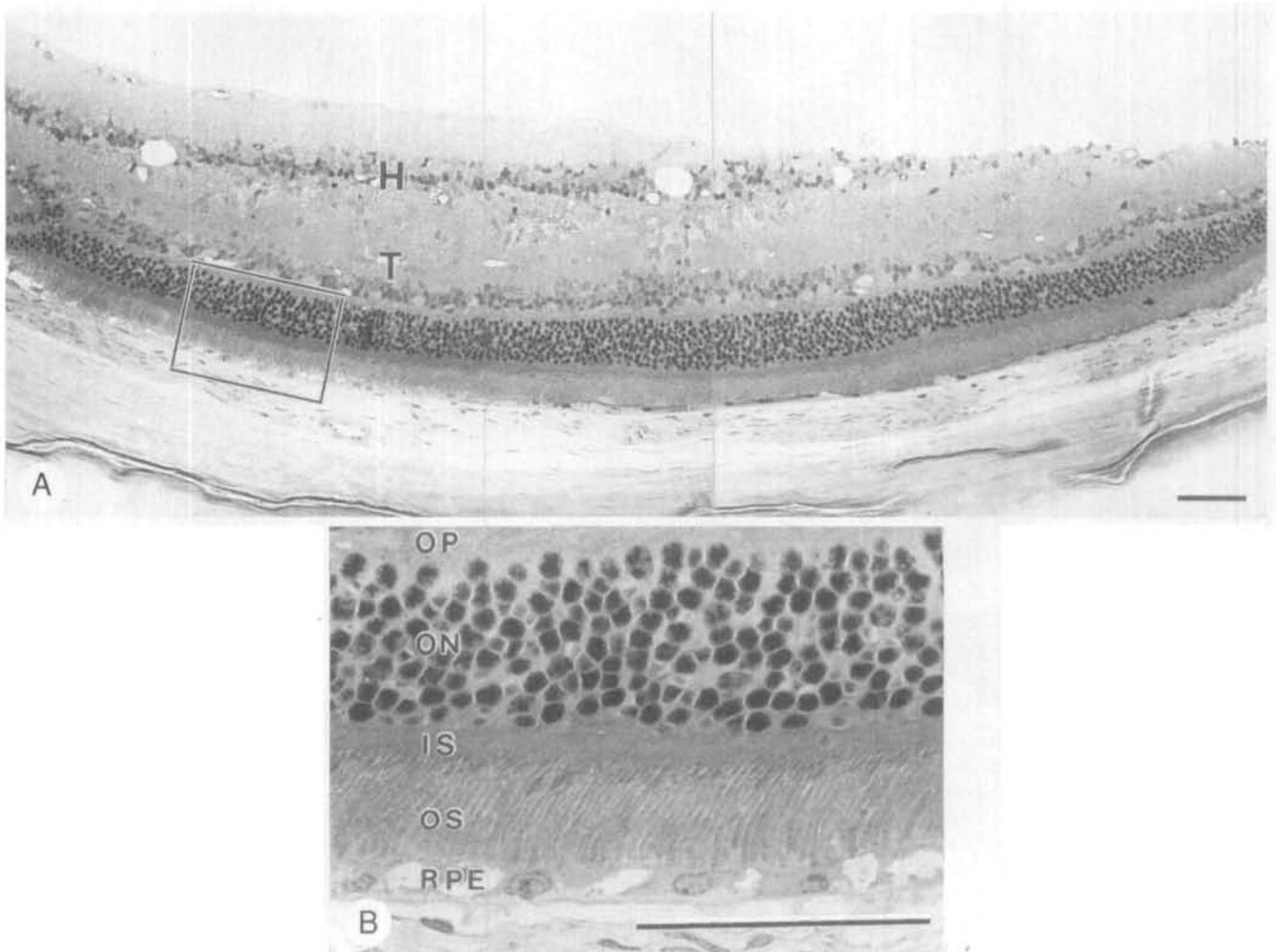


FIGURE 4. Transplant 6.5 months old. (A) Overview. Block only partly cut on the right. Note the good integration between transplant and host. The transplant photoreceptors have formed a long even outer nuclear layer with inner and outer segments in contact with the host retinal pigment epithelium (RPE), except for a rosette at the left edge of the transplant. The transplant continues well outside the photograph on the right. (B) Enlargement of box in (A). Epon section, 0.5 μm . Scale bars, 50 μm . All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. H, host; IS, inner segments of photoreceptors; ON, outer nuclear layer; OP, outer plexiform layer; OS, outer segments of photoreceptors; T, transplant.

Transplantation Procedure

Light-damaged rats were anesthetized by intraperitoneal injections of sodium pentobarbital (38–40 mg/kg) and atropine (0.4 mg/kg), and 10 minutes later by injection of xylazine (3–7 mg/kg). A small incision (0.5–1 mm) was cut in the pars plana or just behind, parallel to the limbus. Most transplants were placed in the superior quadrant of the rat eye. The implantation tool inserted the tissue into the subretinal space. However, as discussed below, the surgeon could not see where the tissue was placed. Although extreme caution was used to prevent damage to the host RPE, this frequently could not be avoided. After transplantation, the incision was closed with 10–0 sutures, and the eyes were treated with gentamycin eyedrops and artificial tears. After surgery, the rats were placed in an incubator to recover from the anesthesia.

Tissue Processing

The experimental animals were euthanized with an overdose of sodium pentobarbital (300 mg/kg). Most animals were per-

fusion-fixed with 4% paraformaldehyde with 0.18% picric acid in 0.1 N sodium phosphate buffer. The eyecups were dissected out, postfixed in cold Bouin's fixative (Sigma, St. Louis, MO) for periods from 14 hours up to several days, and embedded in low-melting paraffin or in histocryl plastic (Electron Microscopy Sciences, Fort Washington, PA). Eight-micrometer paraffin or 2- μm plastic sections were cut and stained with hematoxylin and eosin. Selected sections were processed for immunocytochemistry (see below).

Electron Microscopy

Selected rats were perfused with saline at 37°C, followed by 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 N phosphate buffer (PB). The eyecups were dissected out and immersion-fixed in the same fixative for at least 12 hours at 4°C, and, after several washes with PB, were postfixed in 2% OsO₄ in PB. Small pieces containing the transplant were dissected out. Usually, the transplants were cut into two pieces at the center of the graft. Tissue pieces oriented with the transplant center to face the knife edge

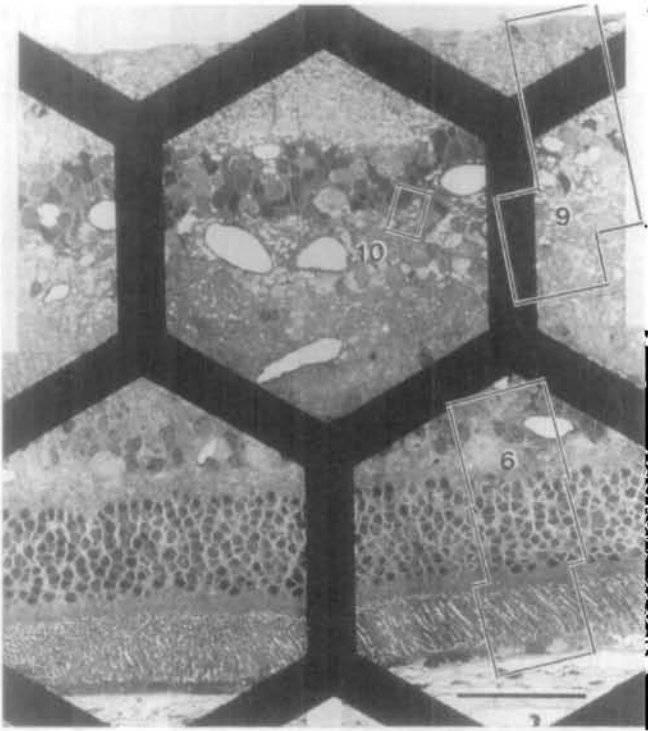


FIGURE 5. Overview montage of 6.5-month-old transplant (same as Fig. 4) in electron microscope. There are approximately 8 to 9 rows of photoreceptor nuclei in the transplant outer nuclear layer. The host retina contains no photoreceptors. Boxes indicate areas that are shown enlarged in Figures 6, 9, and 10. Scale bar, 50 μm . All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph.

were dehydrated through graded ethanols and embedded in Epon. Semithin sections (0.5 μm) were stained with toluidine blue. Ultrathin sections (60–80 nm), stained with uranyl acetate and lead citrate, were viewed in an electron microscope (SM20; Philips, Eindhoven, The Netherlands).

Immunocytochemistry

Sections were deparaffinized, washed with phosphate-buffered saline (PBS), and incubated in 20% blocking serum (horse serum for mouse antibodies, goat serum for rabbit antibodies). For BrdU staining, sections were pretreated with 1% pepsin in 2 N HCl for 30 minutes at 37°C (a modification of the procedure of Ref. 19) and washed several times with PBS before being treated with blocking serum. The following primary antibodies were used for staining, usually overnight incubation at 4°C: mouse monoclonal anti-BrdU (DAKO, Carpinteria, CA) 1:50; mouse monoclonal anti-rhodopsin rho1D4 and rho4D2⁴⁷ 1:50; rabbit anti-rhodopsin 1:100,000.⁴⁸ After several washes with PBS, the binding of the primary antibody was detected using the Vector Elite ABC kit for mouse or rabbit antibodies (Vector Laboratories, Burlingame, CA).

RESULTS

Transplant Survival and Placement

An overview of the surgical outcome is shown in Table 1. Of the 109 surgeries performed, 75 (ca. 69%) resulted in surviving

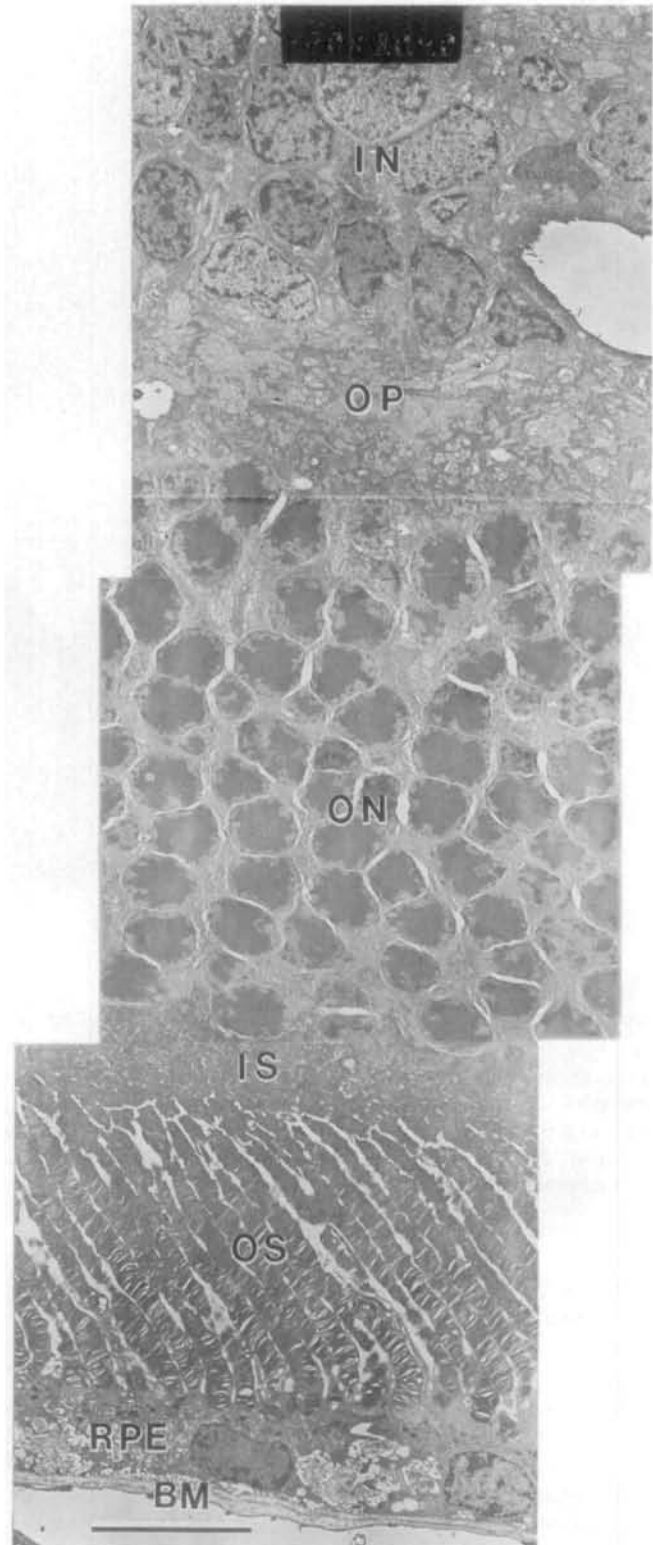


FIGURE 6. Montage of transplant layers (same transplant as in Figs. 4 and 5). See box in Figure 5. Scale bar, 10 μm . All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. BM, Bruch's membrane; IN, inner nuclear layer; IS, inner segments of photoreceptors; ON, outer nuclear layer; OP, outer plexiform layer; OS, outer segments of photoreceptors; RPE, retinal pigment epithelium.

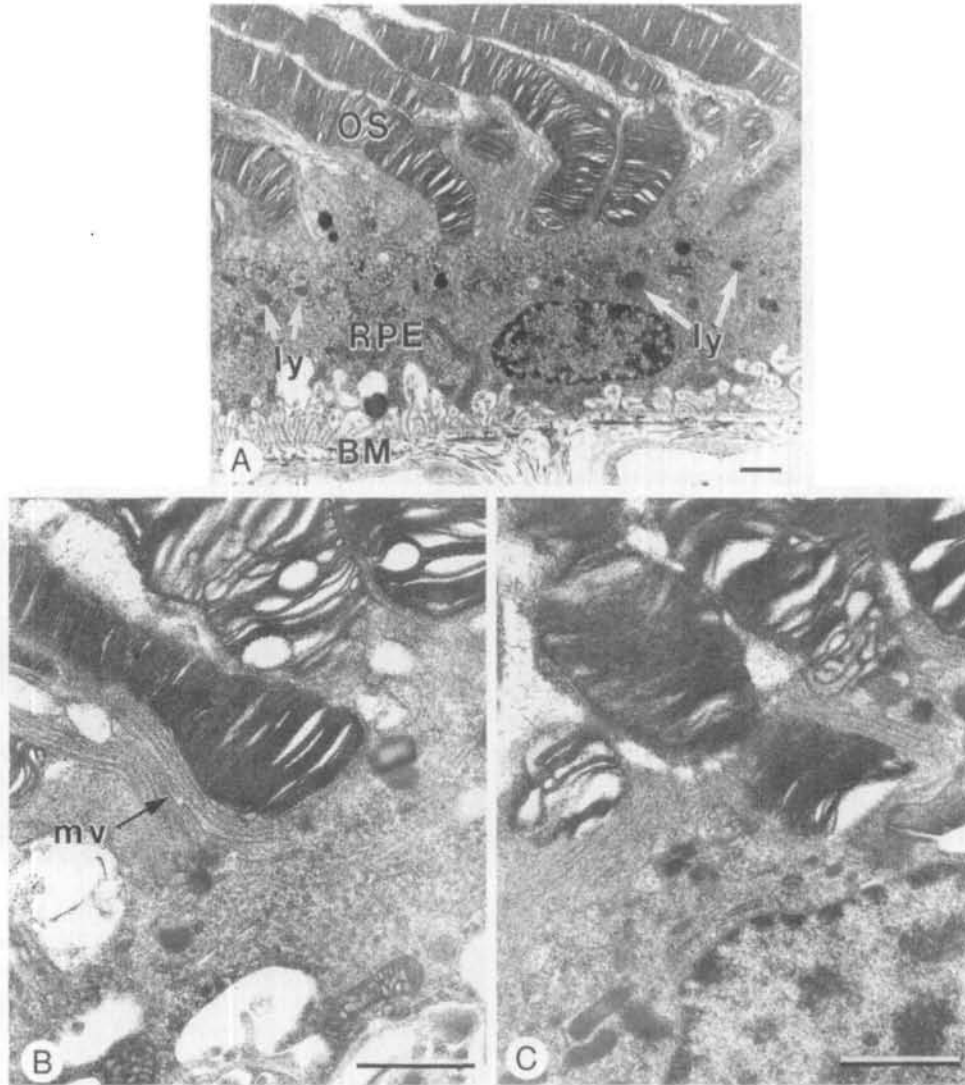


FIGURE 7. Transplant outer segments and host retinal pigment epithelium (RPE). **(A)** Same transplant as in Figure 2B (E20, 7 weeks after surgery). The transplant outer segments are contacting a host RPE cell that contains several lysosomes. The RPE cell looks normal, with many basal in-foldings. **(B, C)** Same transplant as in Figures 4, 5, and 6. **(B)** Host RPE microvilli interdigitate with transplant outer segments. **(C)** A piece of an outer segment has broken off and bound to the RPE cell (nucleus in lower right corner). Scale bars, 1 μm . All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. BM, Bruch's membrane; ly, lysosomes; mv, microvilli; OS, outer segments of photoreceptors.

transplants. Surgical complications, such as optic nerve damage or extensive damage to the host retina, were seen in 15 eyes (ca. 14%). Table 2 shows the distribution of transplant placements. Approximately 24% of these transplants had been misplaced in the epiretinal space (on top of the retina facing the vitreous). Most surviving transplants (ca. 69%) were found in the subretinal space between host retina and RPE. Approximately 7% of the transplants had been incorrectly placed in the choroid.

Transplant Organization

The organization of subretinal transplants is shown in Table 3. Transplants placed in the epiretinal space developed mostly rosettes (data not shown). Of the transplants placed in the

subretinal space, 25% showed large areas with parallel layers and fully developed photoreceptors with outer segments in contact with the host RPE. Thirteen percent of the subretinal transplants contained small areas with transplant outer segments contacting host RPE and the remainder as rosettes, and the rest contained only rosettes (ca. 44%) or were disorganized (ca. 17%).

Recipient Retina, Donor Tissue

The recipient retinas had lost most of their photoreceptors at the time of grafting, but the RPE still appeared intact (Fig. 1A). The donor retinas (E17-E19) contained a ganglion cell layer and a mostly neuroblastic outer retina (e.g., Fig. 1B).

Light-Microscopic and Electron-Microscopic Features of Organized Transplants

Two weeks after transplantation, the still-developing transplant could be recognized by the presence of spindle-shaped photoreceptor nuclei. An example of such a transplant is shown in Figures 1C and 1D. The transplant photoreceptors were just beginning to develop outer segments at this stage.

Several examples of transplants at 7 to 8 weeks after grafting are shown in Figure 2. At this stage, the transplants contained a complete outer nuclear layer with approximately 6 to 10 rows of photoreceptor nuclei, with inner and outer segments facing the host RPE. The inner layers of the transplants appeared to be less well developed than the outer layers. Many transplants had partly integrated with the light-damaged host retina (Figs. 2A, 2B), but others appeared to be separated from the host retina by an inner limiting membrane (Fig. 2C). Organized transplants with parallel layers of photoreceptor outer segments were found up to 10 months after grafting.

The donor tissue could be unequivocally identified by immunohistochemistry for BrdU (Fig. 3A). The outer segments and the cell bodies of transplant photoreceptors stained for rhodopsin (Fig. 3B).

Transplants 7 weeks (Fig. 2B) to 6.5 months (Fig. 4) after transplantation were processed for EM (Figs. 5, 6, 7, 8, 9, and 10). The transplant photoreceptors had developed well-organized outer segments (Fig. 6) that were in intimate contact with the host RPE (Fig. 7). Photoreceptor terminals in the outer plexiform layer of the transplant formed ribbon synapses with horizontal and bipolar cells (Fig. 8). The transplant was well integrated with the host retina (Figs. 4, 9, and 10), making it often impossible to determine where the transplant ended and the host retina began.

DISCUSSION

The results of this study show that pieces of fetal retina transplanted in intact sheets to a damaged rat retina devoid of photoreceptors can develop parallel layers similar to those of a normal retina, with fully developed photoreceptor inner and outer segments in contact with the host RPE. The success rate of these difficult experiments is relatively low for several reasons, mostly related to tissue handling and surgical technique.

The dissection and the handling of the donor tissue during and after embedding have to be performed very carefully. Any break or disturbance of the tissue will lead to rosette formation.

The implantation into the subretinal space of the rat is very demanding because the surgeon cannot see where the transplant is placed after entering the subretinal space. If Bruch's membrane is disrupted or too much trauma or bleeding occurs during surgery, the transplant is exposed to the peripheral immune system and can apparently be easily rejected. Transplants can survive well after transplantation into the choroid; however, because they do not have contact with host RPE, they will form rosettes. Likewise, damage to host RPE before surgery (due to the light damage) or during surgery (by the implantation instrument) will hinder the development of photoreceptor outer segments and lead to rosette formation. If the retinal piece is placed on the retinal surface, facing the vitreous, it will fold up and form rosettes.



FIGURE 8. Transplant outer plexiform layer. Several rod terminals with ribbon synapses can be seen. Photoreceptor cell nucleus in bottom of micrograph. Same transplant as in Figures 4, 5, and 6. Scale bar, 1 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph.

Probably because of the internal pressure of the eye, retinal transplants tend to be displaced from the initial placement area when the insertion tool is withdrawn. Another reason that they tend to be displaced is that part of the donor tissue may stick to the tool. This may even result in the tissue coming out through the lesion site.

The time of surgery appears to be important for transplant success. Transplants implanted more than 4 weeks after the end of light damage usually did not develop very well. This might be because the degeneration process continues after light damage, and the RPE cells start to degenerate after all photoreceptors are lost (see Ref. 45 and authors' unpublished data, in preparation). Normal RPE appear to be necessary for the full development of transplant photoreceptors.

Transplant outer segments in contact with host RPE have been reported by Gouras et al.^{16,49} and Silverman et al.^{20,21,33} Silverman used much older donor tissue (youngest donor age postnatal day 8). Photoreceptor transplants from mature tissue only maintained their outer segments when part of the inner retina was included with the transplant.

It was shown previously that fetal retinal aggregate transplants can grow neuronal fibers into the recipient's retina and make synaptic connections with host retinal cells.⁴³ It was encouraging to see the degree of integration between organized transplants and recipient retina in many of these experiments, but it is not known why this happens. Do the transplants integrate better when the host retina remains mostly untouched during surgery or, in contrast, when it is injured by the insertion tool? It is known that cytokines are produced after retinal injury⁵⁰ and brain injury,^{51,52} and they may promote integration.

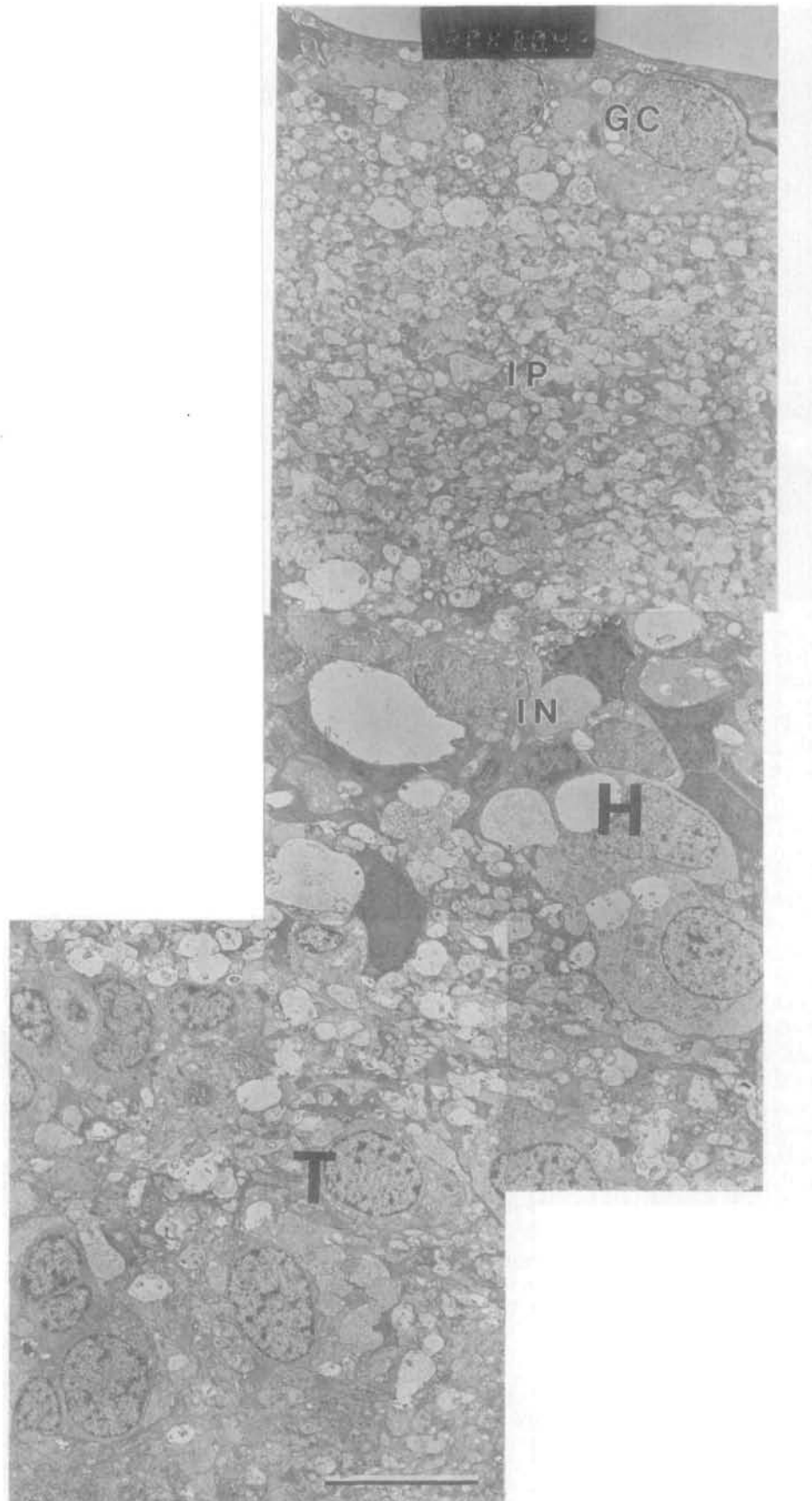


FIGURE 9. Montage of transplant-host interface. Same transplant as in Figures 4, 5, and 6 (see *box* in Fig. 5). Note the absence of glial barriers. It is difficult to tell where the transplant ends and the host retina begins. Scale bar, 10 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. GC, ganglion cell layer; IP, inner plexiform layer; H, host; IN, inner nuclear layer; T, transplant.

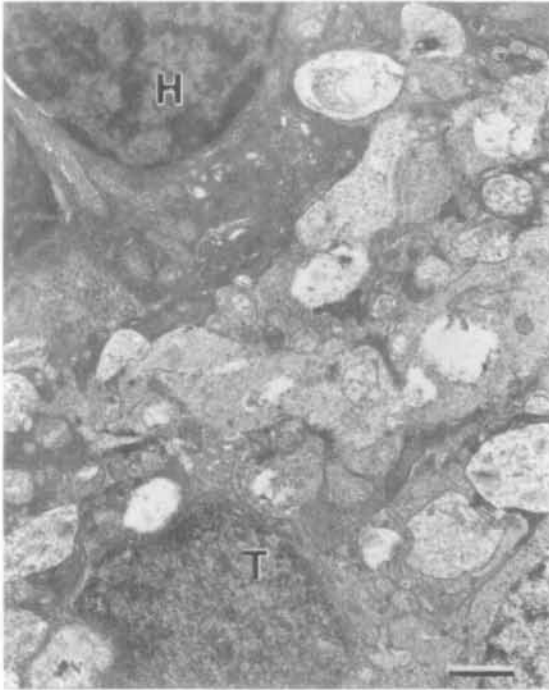


FIGURE 10. Host-transplant interface. Same transplant as in Figures 4, 5, and 6 (see box in Fig. 5). The nuclei in the left upper corner appear to belong to the host retina, the nuclei in the right lower corner appear to belong to transplant cells. It is unclear which neuronal fibers belong to the host and which to the graft. Scale bar, 1 μ m. All figures are oriented with the photoreceptor layer toward the bottom and the ganglion cell layer toward the top of the micrograph. H, host; T, transplant.

It is unknown which cell types, if any, in the transplants make synaptic connections with the host retina. The inner retinal layers of the transplants were usually less developed than the organized transplant photoreceptor layer.

It might be that the chances for meaningful synaptic connections between transplant and host retina could be increased by removing donor ganglion cells or by adding growth factors. Isolation of the outer part of the fetal retina was not attempted because any disturbance of the fragile fetal tissue would lead to rosette formation.

The fact that transplanted fetal retina can develop to resemble a normal retina has encouraged us to test the function of these transplants in light-damaged recipients. In summary, the photoreceptor organization in intact-sheet fetal retinal transplants makes it plausible that such transplants might restore light perception in recipients that have retinal degeneration but still have intact RPE and inner retinal layers.

Acknowledgments

The authors thank Carla Porter, Becky Perez, Lina Wang, Kelly Volk, and Barbara Kalinowska for their technical assistance; Lucinda Schultz of the University of Louisville and Mary Gail Engel of the University of Kentucky (Lexington) for cutting ultrathin sections; Eberhart Zrenner, Klaus-Dieter Miliczek, and Uwe Kretschmann, of the University of Tübingen, Germany, and Michael Sandberg, and Basil Pawlyk, of Berne-Gund Laboratory, Harvard University, Boston, MA, for their helpful discussions; Norman D. Radtke, of Audubon Hospital, Louisville,

KY, for his expert advice and support; and Paul O'Brien, and James B. Longley, for critically reviewing the manuscript.

References

- Eisenfeld AJ, LaVail MM, LaVail JH. Assessment of possible transneuronal changes in the retina of rats with inherited retinal dystrophy: cell size, number, synapses, and axonal transport by retinal ganglion cells. *J Comp Neurol*. 1984;223:22-34.
- Papermaster DS, Windle J. Death at an early age: apoptosis in inherited retinal degenerations. *Invest Ophthalmol Vis Sci*. 1995;36:977-983.
- Voaden MJ. Retinitis pigmentosa and its models. *Prog Retinal Res*. 1990;10:293-331.
- Bird AC. Retinal photoreceptor dystrophies. LI. Edward Jackson Memorial Lecture. *Am J Ophthalmol*. 1995;119:543-562.
- Berson E. Retinitis pigmentosa. The Friedenwald Lecture. *Invest Ophthalmol Vis Sci*. 1993;34:1659-1676.
- Maw M, Kennedy B, Knight A, et al. Mutation of the gene encoding cellular retinaldehyde-binding protein in autosomal recessive retinitis pigmentosa. *Nat Genet*. 1997;17:198-200.
- Oshinskic IJ. Age-related macular degeneration. *Optom Clin*. 1996;5:25-53.
- Segato T, Midena E, Blarzino MC. Age-related macular degeneration. *Aging (Milano)*. 1993;5:165-76.
- Berson E, Rosner B, Sandberg M, et al. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol*. 1993;111:761-772.
- Algere PV, Berglin L, Gouras P, Sheng Y. Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. *Graefes Arch Clin Exp Ophthalmol*. 1994;32:707-16.
- Algere PV, Berglin L, Gouras P, Sheng Y, Kopp ED. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. *Graefes Arch Clin Exp Ophthalmol*. 1997;35:149-58.
- Kaplan HJ, Tezel TH, Berger AS, Wolf ML, Del Priore LV. Human photoreceptor transplantation in retinitis pigmentosa: a safety study. *Arch Ophthalmol*. 1997;115:1168-1172.
- Aramant R, Seiler M, Ehinger B, Bergström A, Adolph AR, Turner JE. Neuronal markers in rat retinal grafts. *Dev Brain Res*. 1990;53:47-61.
- Aramant RB, Seiler MJ. Human embryonic retinal cell transplants in athymic immunodeficient rat hosts. *Cell Transplant*. 1994;3:461-474.
- Del Cerro M, Ison JR, Bowen GP, Lazar E, Del Cerro C. Intraretinal grafting restores function in light-blinded rats. *Neuroreport*. 1991;2:529-532.
- Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest Ophthalmol Vis Sci*. 1994;35:3145-3153.
- Sheng Y, Gouras P, Cao H, et al. Patch transplants of human fetal retinal pigment epithelium in rabbit and monkey retina. *Invest Ophthalmol Vis Sci*. 1995;36:381-390.
- Seiler M, Turner JE. The activities of host and graft glial cells following retinal transplantation into the lesioned adult rat eye: developmental expression of glial markers. *Dev Brain Res*. 1988;43:111-122.
- Seiler M, Aramant R. Transplantation of embryonic retinal donor cells labelled with BrdU or carrying a genetic marker to adult retina. *Exp Brain Res*. 1995;105:59-66.
- Silverman MS, Hughes SE. Transplantation of photoreceptors to light-damaged retina. *Invest Ophthalmol Vis Sci*. 1989;30:1684-1690.
- Silverman MS, Hughes SE, Valentino T, Liu Y. Photoreceptor transplantation: anatomic, electrophysiologic, and behavioral evidence for the functional reconstruction of retinas lacking photoreceptors. *Exp Neurol*. 1992;115:87-94.
- Li L, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp Eye Res*. 1988;47:911-917.

23. Li L, Sheedlo HJ, Turner JE. Long-term rescue of photoreceptor cells in the retinas of dystrophic rats by RPE transplants. *Prog Brain Res.* 1990;82:179-185.
24. Seiler MJ, Aramant RB. Photoreceptor and glial markers in human embryonic retina and in human embryonic retinal transplants to rat retina. *Dev Brain Res.* 1994;80:81-95.
25. Sharma RK, Ehinger B. Cell proliferation in retinal transplants. *Cell Transplant.* 1997;6:141-148.
26. Sheedlo HJ, Li L, Turner JE. Effects of RPE age and culture conditions on support of photoreceptor cell survival in transplanted RCS dystrophic rats. *Exp Eye Res.* 1993;57:753-761.
27. Lopez R, Gouras P, Kjeldbye H, et al. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest Ophthalmol Vis Sci.* 1989;30:586-588.
28. Aramant R, Seiler M, Turner JE. Donor age influences on the success of retinal transplants to adult rat retina. *Invest Ophthalmol Vis Sci.* 1988;29:498-503.
29. Aramant R, Seiler M, Ehinger B, et al. Transplantation of human embryonic retina to adult rat retina. *Restor Neurol Neurosci.* 1990;2:9-22.
30. Aramant R, Seiler M. Cryopreservation and transplantation of rat embryonic retina into adult rat retina. *Dev Brain Res.* 1991;61:151-159.
31. Seiler MJ, Aramant RB, Bergström A. Co-transplantation of embryonic retina and retinal pigment epithelial cells to rabbit retina. *Curr Eye Res.* 1995;14:199-207.
32. Juliusson B, Bergström A, van Veen T, Ehinger B. Cellular organization in retinal transplants using cell suspensions or fragments of embryonic retinal tissue. *Cell Transplant.* 1993;2:411-418.
33. Silverman MS, Hughes SE, Valentino TL, Liu Y. Photoreceptor transplantation to dystrophic retina. In: Anderson RE, Hollyfield JG, LaVail MM, eds. *Retinal Degenerations*. Boca Raton, FL: CRC Press; 1991:321-335.
34. Aramant RB, Seiler MJ. Organized embryonic retinal transplants to normal or light-damaged rats. *Soc Neurosci Abstr.* 1995;21:1308.
35. Aramant RB, Seiler MJ. Transplants to restore damaged rat retinas [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):S332. Abstract nr 1548.
36. Ghosh F, Arnér K, Ehinger B. Transplant of full-thickness embryonic rabbit retina using pars plana vitrectomy. *Retina.* 1998;18:136-142.
37. Widner H, Brundin P. Immunological aspects of grafting in the mammalian central nervous system: a review and speculative synthesis. *Brain Res Rev.* 1988;13:287-324.
38. Dunnett SB. Neural transplantation in animals models of dementia. *Eur J Neurosci.* 1990;2:567-587.
39. Kupsch A, Oertel WH, Earl CD, Sautter J. Neuronal transplantation and neurotrophic factors in the treatment of Parkinson's disease-update February 1995. *J Neural Transm Suppl.* 1995;46:193-207.
40. Aramant R, Turner JE. Cross species grafting of embryonic mouse and grafting of older postnatal rat retinas into the lesioned adult rat eye: the importance of Cyclosporin A for survival. *Dev Brain Res.* 1988;41:303-307.
41. Duan WM, Widner H, Brundin P. Temporal pattern of host responses against intrastriatal grafts of syngeneic, allogeneic or xenogeneic embryonic neuronal tissue in rats. *Exp Brain Res.* 1995;104:227-242.
42. Little CW, Castillo B, DiLoreto DA, et al. Transplantation of human fetal retinal pigment epithelium rescues photoreceptor cells from degeneration in the Royal College of Surgeons rat retina. *Invest Ophthalmol Vis Sci.* 1996;37:204-211.
43. Aramant RB, Seiler MJ. Fiber and synaptic connections between embryonic retinal transplants and host retina. *Exp Neurol.* 1995;133:244-255.
44. Aramant R, Seiler M. Retinal cell transplantation. In: Lanza RP, Chick WL, eds. *1996/1997 Yearbook of Cell and Tissue Transplantation*. Dordrecht, Netherlands: Kluwer Academic Publishers; 1996:193-201.
45. Seiler MJ, Aramant RB, Petry HM, Callahan TL, Sandberg MA, Pawlyk BS. A new effective light-damage model selective for photoreceptors in albino rats-anatomical, electrophysiological and behavioral results [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):S1027. Abstract nr 4795.
46. Kawamoto JC, Barrett JN. Cryopreservation of primary neurons for tissue culture. *Brain Res.* 1986;384:84-93.
47. Molday RS. Monoclonal antibodies to rhodopsin and other proteins of rod outer segments. *Prog Retinal Res.* 1989;8:173-209.
48. Plantner JJ, Hara S, Kean EL. Improved, rapid immunoassay for rhodopsin. *Exp Eye Res.* 1982;35:543-545.
49. Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Reconstruction of degenerate rd mouse retina by transplantation of transgenic mouse photoreceptors. *Invest Ophthalmol Vis Sci.* 1992;33:2579-2586.
50. Wen R, Song Y, Matthes MT, Yasumura D, LaVail MM, Steinberg RH. Injury-induced upregulation of bFGF and CNTF mRNAs in the rat retina. *J Neurosci.* 1995;15:7377-7385.
51. Ip NY, Wiegand SJ, Morse J, Rudge JS. Injury-induced regulation of ciliary neurotrophic factor mRNA in the adult rat brain. *Eur J Neurosci.* 1993;5:25-33.
52. Frautschy SA, Walicke PA, Baird A. Localization of basic fibroblast growth factor and its mRNA after CNS injury. *Brain Res.* 1991;553:291-299.