volve attachment of pigment epithelial processes to rod outer segment tips, outer segment invagination by these processes or detachment of the tips. Any of these events could alter ionic gradients involved in transduction, particularly in the vicinity of the tip, and lead to a reduction in sensitivity. The large desensitization 1.5 hr after light onset seen in dominant retinitis pigmentosa could reflect an alteration of a greater than normal fraction of the outer segment membrane associated with disc shedding, since these outer segments are thought to be already shortened. The prolongation of this desensitization up to 8 hr after light onset in the patients could derive from a delay in reestablishing baseline membrane conductance subsequent to shedding or a delay in the termination of shedding itself throughout the light period, as might occur with diseased photoreceptors. Prolonged recovery of psychophysical rod sensitivity following a bleach has been reported in some patients with retinitis pigmentosa,11,12 and, while the physiological bases of these delays are also presently unknown, they could be related to factors responsible for the delayed ERG recovery described in this study. Further investigations will be needed to establish more conclusively whether at least some patients with retinitis pigmentosa show a defect in outer segment renewal.

Key words: retinitis pigmentosa, electroretinogram, rod, outer segment, phagocytosis, diurnal rhythm

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Donor Age Influences on the Success of Retinal Grafts to Adult Rat Retina

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The rat retina can be successfully grafted within a long time period which extends into the first 2 weeks of postnatal life. Postnatal grafts taken 1–2 days (PN 1–2) after birth demonstrate no significant differences in their ability to form successful grafts. However, grafting success begins to diminish gradually starting between PN 2–4 and reaches a low point in organization and survival by PN 14. PN 21 grafts rapidly degenerate by 1 to 2 days after transplantation. Although early postnatal retinal tissue can be successfully grafted, E 15 embryonic retinas make better grafts for their ability to form consistent laminae and to integrate with host tissue in a fresh lesion paradigm. Invest Ophthalmol Vis Sci 29:498–503, 1988

The eye has been successfully used numerous times as a recipient of tissue grafts. However, almost all intracocular graftings have dealt with nonretinal tissue grafts placed into the anterior chamber where the iris offers a rich vascular substrate.1 With the exception of one earlier report,2 only recently has the fetal retina been grafted into the anterior chamber where it survived and continued to differentiate.3,4 Although retinal grafting has been demonstrated to be a simple and highly successful technique,5 no attempts in the past have been made to graft these cells into an adult retinal penetrating lesion site in order to initiate repair.
and possible return of function. Recently we have reported the development of a successful model for grafting embryonic rat retina into an adult rat retinal lesion site adjacent to the vitreal body. In the present paper we begin to define optimum conditions for successful retinal grafting by reporting on what effect the age of the donor tissue has on successful transplantation.

**Materials and Methods.** Fifty-one young adult male Sprague-Dawley rats weighing between 200–250 g were used as hosts in all experiments and received bilateral retinal grafts in a manner previously described. Graft tissue was taken from E 15 embryos after cesarean section of time-pregnant rats (day 0 = vaginal plug) or from 1, 2, 4, 6, 8, 10, 14 and 21 postnatal day (PN) pups. After survival times of 4–7 weeks, the animals were anesthetized and sacrificed with an overdose of sodium pentobarbital. The enucleated eyes were fixed in Bouin’s solution for 5 hr, placed in 80% ethanol overnight, and then changed to 95% and 100% ethanol. A piece of the whole eye containing the graft and optic nerve was trimmed out and subsequently processed through xylene and embedded in paraffin. Sections were cut at 10 μm and stained with hematoxylin and eosin.

Grafts were analyzed for successful transplantation according to four criteria of a modified Evaluation Index (E.I. score) established previously. The modified E.I. score used in these experiments analyzed the graft according to: (1) the filling of the lesion site by the graft; (2) how well host/graft tissues integrate; (3) the degree of lamination of the graft; and (4) the viability of the grafted tissue, ie, healthy and/or degenerated cells. Values were analyzed statistically according to the student t-test.

One set of experiments compared the differentiation of E 15 and PN 1 grafts in either a fresh or conditioned lesion site at 7 and 6 weeks respectively after transplantation. A second set of experiments was designed to evaluate the potential for postnatal transplants (ie, PN 1, 2, 4, 8, 10, 14 and 21) to form successful grafts at 4 weeks after transplantation.

Animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research and NIH policies on the subject.

**Results.** Comparison of E 15 and PN 1 Graft Development: E.I. score evaluation of E 15 grafts indicated that they achieved approximately 70% of the maximum value compared to 50% for PN 1 transplants (Fig. 1A). The major difference in the four categories measured was a significantly higher degree of lamination exhibited by the E 15 graft irrespective of whether they were located in a fresh or conditioned lesion site (Fig. 1E). E 15 grafts exhibited an increase in the number of laminae (ie, six to seven of the nine laminae present, not including the retinal pigment epithelium) compared to only three to five for PN 1 grafts. Light microscopic evaluation indicated the presence of ganglion cell (GCL), inner plexiform (IPL), inner nuclear (INL), outer plexiform (OPL), outer nuclear (ONL) and outer limiting membrane (OLM) layers (Fig. 2B, D). In contrast, PN 1 grafts possessed only a GCL, IPL, INL and ONL (Fig. 2A, C) under these conditions. It should be noted that the use of the term GCL does not indicate the presence of surviving retinal ganglion cells, which has yet to be established. Characteristically absent from both E 15 and PN 1 grafts was a continuous optic fiber layer and inner limiting membrane (Fig. 2A–D). In addition, E 15 grafts were found to be better able to integrate with the host retina in the lesion site under fresh lesion conditions than PN 1 grafts (Figs. 1D and 2E, F). However, no significant difference was found between E 15 and PN 1 integrative abilities in the con-
Fig. 2. Light micrographs of retinal transplants showing the degree of lamination and integration found in E 15 and PN 1 grafts. (A) PN1 graft (G) found in the lesion site (L) between and overlapping the cut edges (E) of the host retina (H) at 6 weeks after transplantation. Note that the graft laminar pattern consists only of a ganglion cell (GCL), inner plexiform (IPL), and mixed inner (INL) and outer nuclear (ONL) layers. Bar equals 100 μm. (B) E 15 graft (G) found in the lesion site (L) between and overlapping the cut edges (E) of the host retina (H) at 7 weeks after transplantation. Note that the graft laminar pattern consists of distinct and well defined GCL, IPL, INL, outer plexiform layer (OPL) and ONL. Bar equals 100 μm. (C) Higher magnification of (A) showing more clearly the diminished laminar organization when compared to (D) below. Bar equals 50 μm. (D) Higher magnification of (B) which reveals in more detail the presence of a more complete laminar pattern. Also note the appearance of an outer limiting membrane (arrowheads). Bar equals 50 μm. (E) A PN 1 graft (G) located in the lesion site (L) between and overlapping the cut edges (E) of the host retina (H). Note a small area of fusion at the cut edge of the host retina (E); however, in contrast to (F), a barrier has formed between host/graft tissue outside the lesion site (arrowheads). Bar equals 100 μm. (F) An E 15 graft (G) located in the lesion site (L) between and overlapping the cut edges (E) of the host retina (H). This graft not only fuses with the host tissue at the cut edges but also along one of its overlapping surfaces (arrowheads). Bar equals 100 μm. Arrows point in the direction of the optic disk (E, F).
tioned lesion sites, which was apparently due to increased connective tissue growth in the conditioned lesion area. There was no significant difference between E 15 and PN 1 grafts with respect to graft filling and viability under either fresh or conditioned lesion situations (Fig. 1C, F). However, due to the significant differences particularly in lamination and somewhat in integration the total E.I. score values for E 15 grafts were significantly increased over PN 1 values for both fresh and conditioned lesion paradigms (Fig. 1B).

**Postnatal Donor Age Influence on Grafting Success:** According to our E.I. evaluations, PN 1–21 grafts, observed 4 weeks after transplantation, revealed that successful grafting can occur within the first 2 postnatal days. In all categories there were no measured significant differences between PN 1 and PN 2 values (Fig. 3). The first critical period of differences in graft development occurred between PN 2–4. All E.I. score values continued to decline until by PN 14 the grafting success was at a minimal level (Fig. 3A–F). By PN 21 there was a rapid degeneration of the tissue within 2 days after transplantation, with no survival indicated. Light microscopic observations indicated a fairly good laminar representation in portions of some PN 1 grafts but by PN 8 very little organization was present (Fig. 4A–C). The majority of cells surviving at PN 8 appeared to be the dark staining photoreceptor cells (Fig. 4C). Also by PN 8 there appeared to be a wide gap between graft and host tissue if the transplant was placed in the middle of the die-back zone. Occasionally, if the graft overlapped onto portions of the intact host retina during transplantation a thick connective tissue-like sheet formed around the graft separating it from the host tissue (Fig. 4D). Surviving PN 14 tissue was almost completely devoid of recognizable retinal cells; however, even at this late date there appeared to be no rapid degeneration (Fig. 4E). In contrast, by only 2 days after PN 21 transplantation no viable grafted tissue could be found in the lesion site (Fig. 4F).

**Discussion.** It was determined that although PN 1 tissue could be successfully grafted, E 15 transplants were able to form more distinctive laminae at 7 weeks after transplantation. The retinal layers which the E 15 grafts expressed after 7 weeks but which were characteristically absent in most PN 1 grafts after 6 weeks of transplantation were the outer plexiform layer, outer limiting membrane and possibly the inner photoreceptor segments. The absence of the inner limiting membrane and of a definitive optic fiber layer has also been previously reported by us and in other studies involving retinal transplantation to the anterior eye chamber and superior colliculus. In the latter study optic fibers were found coursing randomly through the graft before exiting to enter the target tissue. We have also reported collections of fiber bundles leaving the GCL of PN1 grafts as demonstrated by SEM. These observations corroborate and expand upon those of del Cerro et al, where successful retinal grafting was reported in the anterior chamber of the adult rat eye using E 13 to PN 2 grafts.

E 15 grafts were also found to integrate better into the lesion site of host retinas in a fresh but not a conditioned lesion site. It was most interesting to observe that there was no significant difference between E 15 and PN 1 grafts in respect to their integration with the host retina in the conditioned lesion site. A recent report indicated that the areas of an adult CNS lesion not making immediate contact with grafted tissue appeared to develop a scar consisting of mesodermal elements and/or glial cells. These elements apparently formed as the lesion “aged” and because of their “barrier” characteristics decreased the likelihood of graft/host fusion. An absence or reduction in
Fig. 4. Light micrographs depicting the degree of survival of grafts transplanted at various postnatal ages (4 weeks survival with exception of F). (A) A PN 1 graft (G) showing a high degree of laminar organization. (B) A higher magnification of a section adjacent to (A) in the same graft showing that ganglion cell (GCL), inner plexiform (IPL), inner nuclear (INL), outer plexiform (OPL), outer nuclear (ONL) and outer limiting membrane (OLM) layers are present in portions of this PN 1 graft. (C) In contrast to (A, B) the PN 8 graft (G) shows no laminar organization. Also note that the graft appears much smaller than the PN1 transplant seen in (A) and is separated from the cut edges (E) of the host retina (H) in the lesion site (L) by a clear space. (D) A PN 10 graft (G) showing fewer surviving cells than in (C). Also note that a connective tissue-like capsule (arrowheads) separates the graft (G) from host (H) tissue. (E) A PN 14 graft (G) consisting mostly of fibrous connective-like tissue with very few recognizable retinal cells. (F) The transplantation site of a PN 21 graft only 2 days after grafting showing the cut edge of the host retina (E) at the transplantation site (T) which is filled with an influx of numerous cells (C). Note that no graft survives in the transplantation site. Bars in all photomicrographs equal 100 µm except (B) (25 µm).
the retinal glial response to lesion⁹ is a factor that should also be considered when interpreting these results.

In the present study we have attempted to determine which postnatal ages can be successfully grafted beyond PN 1. It was determined that successful postnatal grafting can be achieved through PN 2 without any appreciable reduction in the E.I. score. However, between PN 2 and 4 the capacity for retinal tissues to form good grafts begins to gradually diminish, reaching a low point in survival with PN 14 grafts. Numerous other reports have confirmed that postnatal tissues make less viable grafts as they reach a more mature state.¹⁰ The reported loss of the neuroepithelial layer by PN 4 in the developing rat retina may be correlated with the initial signs of graft deterioration seen between PN 2-4.¹¹ This phenomenon could certainly signal a reduced transplantation capacity or plasticity. PN 21 grafts rapidly degenerated 2 days after transplantation.

Key words: retinal grafts, donor age, eye

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