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Intraocular Retinal Transplants

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Embryonic rat retinae transplanted into the anterior chamber of adult rat eyes of the same or different strain survive and grow. Light and electron microscopic studies show that the transplants undergo histogenetic differentiation, resulting in the development of mature inner and outer layer neurons and Müller glial cells. Vascular connections develop between the host iris and the retinal transplant. These initial observations indicate that retinal transplantation to a recipient eye is a procedure which offers ample opportunities for the study of problems related to neural development, retinal plasticity and repair. *Invest Ophthalmol Vis Sci* 26:1182-1185, 1985

Encouraged by recent successful attempts to ameliorate neural deficits by means of neural transplants,¹⁻³ we are performing in oculo retinal transplants in the rat^{4,5} to study the histogenetic evolution of the transplant, the implant-host interactions, and, eventually, the capability of the implants to repopulate damaged areas of the host retina. The initial results are described in this report.

Materials and Methods. The first experiments were conducted to examine the importance of donor age and recipient strain in graft development (Table 1). Long-Evans embryos, aged 13 to 16 postconceptual days (E 13-16), served as donors. The embryonic retina at this age is formed by a distinct layer of

pigment epithelium surrounding a central mass of mitotically active germinal cells (Fig. 1). No neurons exist in the prospective neuroretina except for a few ganglion cells in the central region of E16 retinae. The transplant consisted of both the neural retina and the pigment epithelium. Under general anesthesia, the lids are opened by passing lid sutures. A corneal

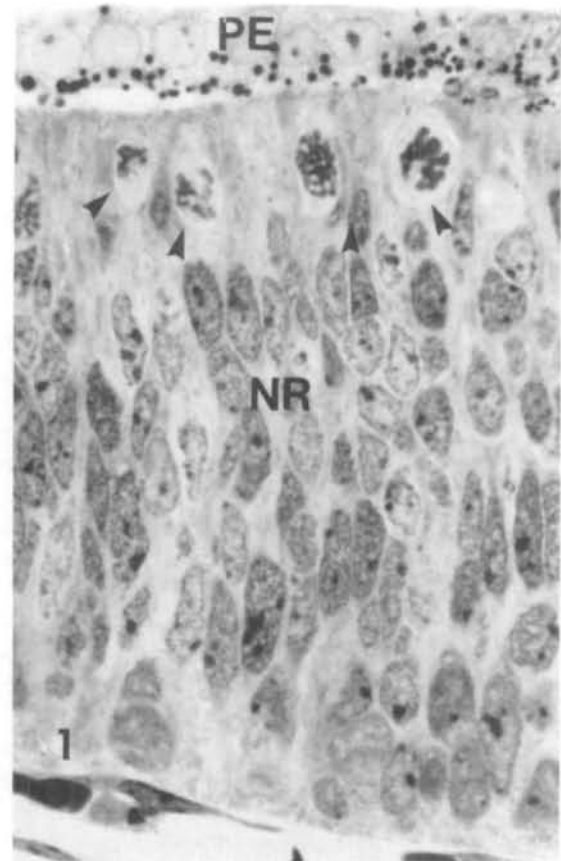


Fig. 1. The retina at E16 shows a well differentiated pigment epithelium layer (PE). The neural retina (NR) is formed by rows of germinal cells, many of which are in the process of mitosis (arrowheads). A few ganglion cells may be found in the innermost aspect of the central retina, outside this microscopic field ($\times 850$).

Table 1. Summary of experiments

Group	Donor strain	Host strain	Donor age	Survival days	Number of hosts
1	Long Evans	Long Evans	13 DPC*	14-30	2
			14 DPC	0-28	3
			16 DPC	0-91	16
2	Long Evans	Lewis	13 DPC	14-30	11
			14 DPC	0-18	3
3	Long Evans	Fischer	14 DPC	5-28	6

* DPC: days postconception.

incision was then made by using a microscalpel (the same type that is used for cataract extraction) and the wound was then extended for a total of about 2 mm using corneoscleral scissors. Following this, the embryonic retina was taken and introduced into the anterior chamber using a fine corneal forceps or Pasteur pipette. One entire retina, including pigment epithelium, is normally transplanted into the host anterior chamber. After securing the transplant in the anterior chamber, the limbal wound was closed using two to three interrupted 10-0 nylon sutures. Garamycin eye ointment was applied at the end of the procedure.

Three kinds of host have been used: (1) young adult Long-Evans outbred rats; (2) Lewis strain inbred rats; and (3) Fischer strain inbred rats. Survival times ranged from 0 to 90 days post-transplantation. The animals received repeated ophthalmologic examinations during the survival time; they were deeply anesthetized, and the eyes were removed and prepared for light and electron microscopic study using procedures previously described.⁶ Briefly stated, the eyes are removed, hemisected, and fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde, in a 0.1 M cacodylate buffer at a pH of 7.2-7.4. After postfixation in osmium tetroxide and plastic embedding, 1- μ m thick sections of the tissue are cut for light microscopy and ultrathin sections for electron microscopy.

Animal handling was performed in full compliance with the spirit and letter of the ARVO Resolution on the Care of Animals in Research and NIH policies on the subject.

Results. Survival and growth of the graft can be followed in vivo through the transparent cornea of the recipient eye. By a week to 10 days post-transplant (PTD), an irregular, whitish or pigmented mass can be seen growing in the anterior chamber. The mass continues to grow at a roughly linear rate until the end of the first post-transplantation month. At that time, growth ceases and the transplant size and appearance remain unchanged. All the transplants develop a translucent whitish mass, which microscopic examination shows to be neural portion of the retina. The pigment epithelium produces a heavily pigmented outgrowth, often adjacent to but not intermingled with the neural retina. Separate growth of the neural and pigmented components occurs in some animals producing clearly visible patches of either whitish or pigmented tissue. So far, the development of outer segments has only been observed when pigment epithelium was present near the rod cells, although not necessarily in contact with them. The transplants always appeared anchored inside the chamber as no free moving masses have been observed.

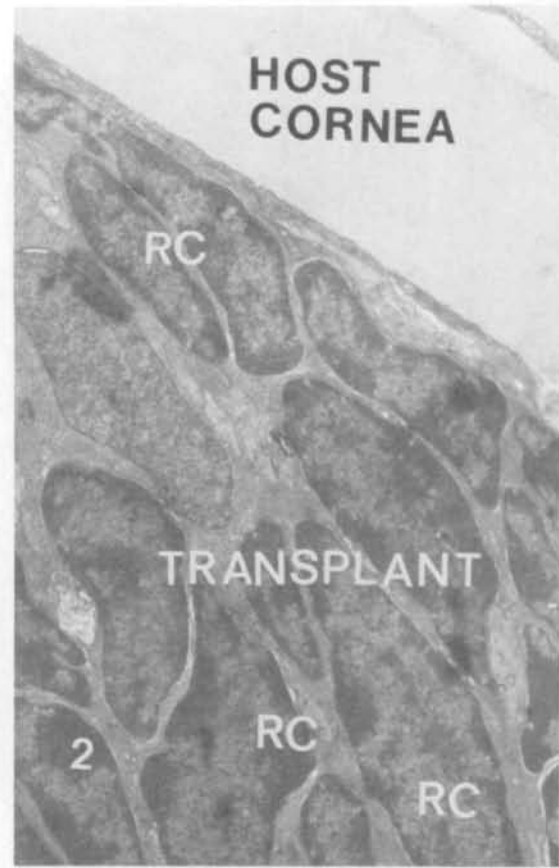


Fig. 2. Transplanted retina growing underneath the host cornea shows the closely packed rod cell somas (RC) ($\times 6,250$).

Microscopically, the transplant is often found in intimate contact with the host cornea and iris. At the point of contact, the cornea often loses its endothelial cells but remains undisturbed (Fig. 2). Although the exact mechanism by which the corneal thickness is maintained in the presence of retinal transplant in the anterior chamber has not been elucidated, several postulations can be made. Segmental endothelial cell loss may not always induce corneal edema since the remaining intact endothelium may maintain the functional integrity of the cornea. Another possibility is that the mechanical apposition of the transplant to the intact Descemet's membrane may prevent free passage of fluid from the anterior chamber into the stroma, thereby maintaining normal corneal thickness.

Iridal contacts can be extensive, with occasional invasion of transplanted retinal cells into the iris stroma or, more frequently, migration of pigmented cells from the iris to the periphery of the transplant. Vascular connection with the host is established via iridal vessels which enter the implants and form branches with them. The pattern of vascularization in the implants differs from that seen in the in situ

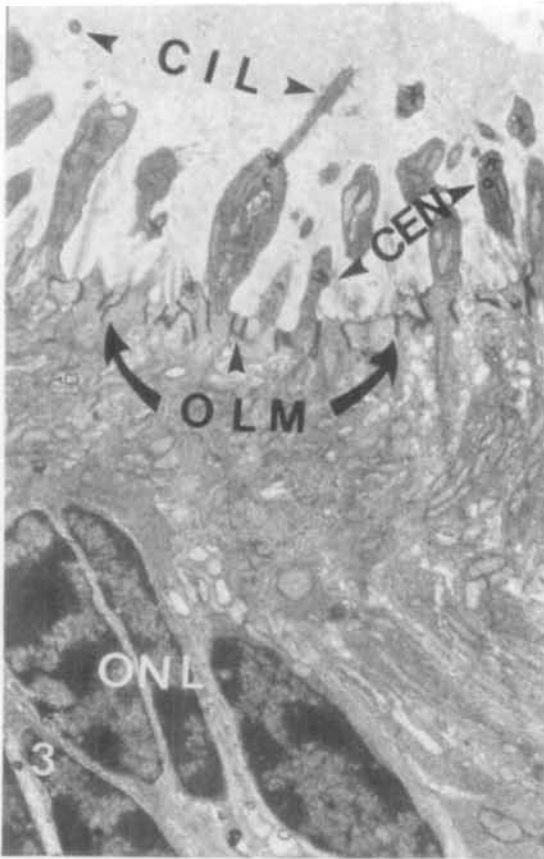


Fig. 3. Surface of a transplant (17 PTD). Inner segments project into the free space showing cilia (CIL) and centrioles (CEN); the outer limiting membrane (OLM) is clearly visible. The nuclei of the outer nuclear layer (ONL) are visible in the lower portion of the picture ($\times 5,000$).

retina. The vascular supply is more limited and lacks the layered distribution characteristic of the normal retina; instead the capillaries in the implant form tufts around the trunks of origin.

As described above, the embryonic retina used for transplantation is very immature; the usual cell proliferation and differentiation continue in the transplant in a parallel, although less complete manner, than those seen in situ. Survival times of 15 days or longer allow differentiation of a rudimentary layered arrangement which includes patches of inner and outer nuclear layers, as well as definite inner and outer limiting membranes and outer plexiform layers. Light and electron microscopic observations have identified a number of typical adult retinal features within the transplant. These include closely packed rod cells, which occasionally form rosettes within the mass of the transplant; inner segments, with their characteristically high mitochondrial population; ribosomes; and microtubules, which extend from the somas of the rod cells. At the upper portion of the inner segments, a basal body and a cilium are often present

(Fig. 3). Outer segments, when present, were stunted. In all transplants, there was a well-developed outer limiting membrane (Fig. 3) with baskets of Müller cell processes. Inner nuclear layer neurons were clustered in patches or islands. Numerous synaptic endings and both ribbon and conventional synaptic contacts (Fig. 4) are found in the plexiform layers. Well-developed retinal pigment epithelial cells developed in most transplants. Except for better developed outer segments, no clear-cut correlation was observed between the presence of pigment epithelium and the degree of differentiation of the neuroretina.

Transplants have been observed to develop and differentiate in all three groups of hosts. Those growing into rats of the same strain (group I), were very well tolerated by the host, even in animals allowed to survive up to three months after implantation. Implants in Lewis strain animals (group II) usually did not show inflammation, while implants in Fischer animals (group III) induced an intense reaction. Clinically, inflammation started in the form of vascular congestion in the iris; it could progress to a general hyperemia of the conjunctiva, with clouding of the media. The worst cases showed alterations such as

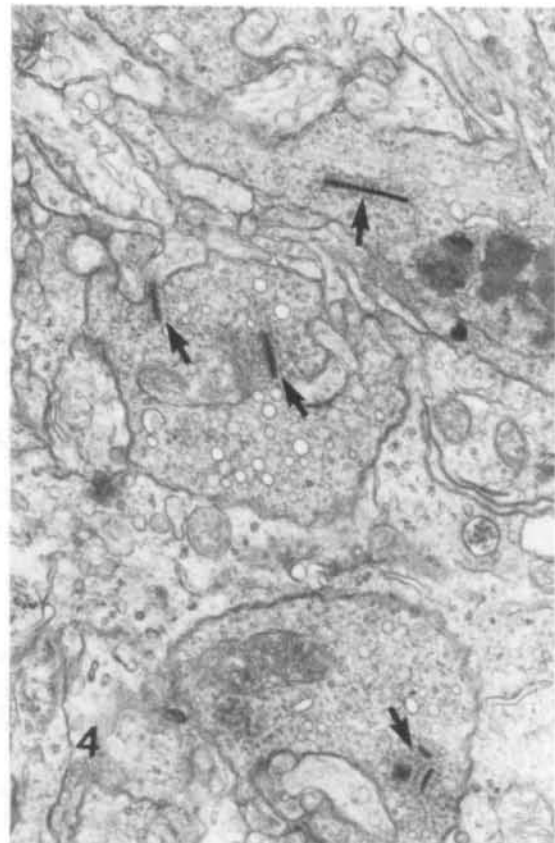


Fig. 4. The outer plexiform layer of a 17 PTD transplant showing intermingled neurites and nerve terminals containing synaptic vesicles and one or more synaptic ribbons (arrows) ($\times 20,000$).

anterior chamber hemorrhages, cataractous changes, vascularization and opacity of the cornea. Histologically, the cases of severe inflammation showed the transplant surrounded and infiltrated by macrophages. Macrophages were also plentiful in the subretinal space of the host retina and were a major component of a granulomatous tissue found throughout the vitreal cavity. Only two eyes in our series of 41 transplants reached this degree of inflammation.

Discussion. Intraocular transplants, it should be noted, have been carried out for more than a hundred years (reviewed by Faldino⁷). During this time, a bewildering variety of tissues, embryonic and adult, normal and tumoral, have been implanted in the anterior chamber of mammalian eyes^{8,9}; but, with a single exception,¹⁰ intraocular retinal transplants have been overlooked.

On the other hand, it has been shown that under favorable conditions embryonic retinal transplants into the CNS grow and are able to send fibers to specific visual centers of the host brain. This observation indicates that considerable plasticity exists in the postnatal visual system, a fact that prompted us to study the effects of transplanting embryonic retina into adult eyes.

It is of interest to compare the growth and differentiation of embryonic retinae transplanted into the anterior chamber with that seen to occur *in vitro*,¹¹ or in transplants into the developing CNS.^{12,13} In the first case,¹¹ parts of the culture developed histotypic organization, which included differentiation of all cell and synaptic types. Abortive outer segments did develop, although the pigment epithelium was excluded from the explanted tissue. Transplants of fetal retinae into the brains of newborn rats^{12,13} have shown comparable differentiation of cell types, synaptic contacts, and photoreceptors. The same degree of differentiation occurs in our anterior chamber transplants. Thus, it can be said that if the immature retina, whether embryonic or neonatal (as in the LaVail and Hild experiments¹¹), is placed in a milieu that supports growth, histotypic differentiation will occur in the presence or absence of either pigment epithelium or vascularization. It is possible then that the inductive effects of the pigment epithelium (reviewed by Coulombre¹⁴) are exerted at an earlier developmental stage than that used for transplantation or culture.

The only previously published study of intraocular retinal transplants¹⁰ was limited to implanting mothers with retinae obtained from their own fetuses. The present observations extend the scope of that early work by showing that embryonic retina can be implanted into the eyes of adult hosts unrelated to the donor or even of a different strain. The success of

these first experiments may bring closer the possibility of using embryonic retinal transplants to replace populations of neurons lost in damage to the mature retinae.

Key words: retina, transplant, rat, eye, nervous tissue

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