# RETINAL TRANSPLANTATION:

## Will Blind Patients with Retinitis Pigmentosa be Able to See Again?

BY VAMSI K. GULLAPALLI, COCA DEL CERRO, AND MANUEL DEL CERRO

**Manuel del Cerro will give the Smith-Kettlewell Lecture at the Vision Science and Its Applications Topical Meeting this month. In this article based on his talk, he and his coauthors describe retinitis pigmentosa and explain how retinal transplantation might lead to better sight for those suffering from this disease.**

Take a sheet of white paper, hold it close to your face, and look through it. What do you see? Just that there is light in the room. Have someone switch off the light. Now you don't see any light. This is how little a person suffering from a blinding disease called retinitis pigmentosa (RP) sees when the disease has reached a late stage. Ophthalmologists describe this degree of vision as light perception. In this article, we'll discuss why and how people with this disease lose their sight, and whether eye surgeons can bring back some vision to RP patients.

## The human eye and RP

The human eye is often compared to a camera. Let's say we are looking at a beautiful painting. The light falling on the painting is reflected, and some of it enters the eye through a small opening called the pupil. A lens behind the pupil focuses the light onto the equivalent of the photographic film—the retina. The retina, which means "a net," records the features of the painting and sends messages to the brain. The brain, liking the image, makes the eyes move to get a better view and makes the lips move to form a smile of appreciation.

The retina has the important function of converting light that it detects into electric currents, which is vital as the brain only understands the language of electric currents, not that of light. Figure 1 is a simple diagram of the retina's complex structure. Once the light falls on the retina's "photoreceptors," these cells convert the light to chemical signals and send the signals to the bipolar cells, where the information is integrated. The information then travels to the ganglion cells where once again it is integrated. The ganglion cells finally send the information to the brain in the form of electrical signals.

If there is an interruption at any point in the steps outlined above, the person will have problems seeing. For example, if the lens is opaque like a cataract, light fails to enter the eye properly and vision is decreased. In RP—a condition that affects 1 in 4,500 people—the photoreceptors start dying (see Fig. 2). In recent years, the reason why this happens has become clearer. In a normal eye, there are two kinds of photoreceptors, *cones*, which are used to see in daylight, and *rods*, which are used in dim light (*e.g.*, a moonlit night or a movie theater). The rods have a protein called "rhodopsin" that is involved in the conversion of light to electrical currents. Rhodopsin is made up of 348 amino acids, the building blocks of proteins, arranged in the form of a chain. In RP, due to a genetic defect, one of the amino acids in the chain is different from the normal. This makes the rhodopsin molecule ineffective (unable to function), and over a period of time, the rods start dying. Initially, a person with RP will start stumbling against furniture in dim light. Soon, for unknown reasons, the cones start dying too. The region of the retina that is affected most is that which is involved in peripheral vision. As a result, the patient is able to see clearly what is directly in front of him, but unable to distinguish, while he is looking straight, things that are to the side, a state that is described as "tunnel vision." With time, even the central vision is lost and the patient is only able to perceive light, not objects. Ultimately, many patients become totally blind.

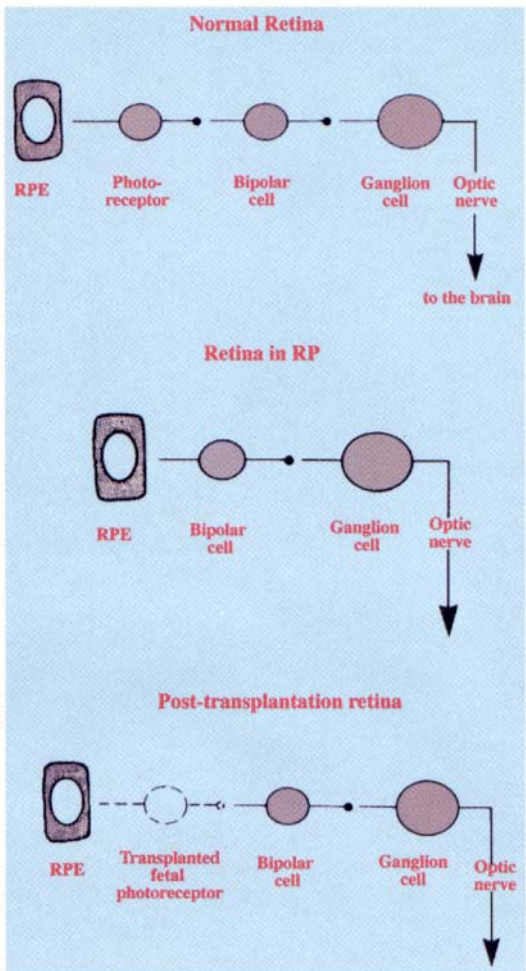


**Figure 1.** *Top:* The principal cells that make up the circuit that helps us to see are shown. The photoreceptor, stimulated by light, passes on the signals to the brain through the bipolar cell, the ganglion cell, and finally through the optic nerve. Residual light is absorbed by the pigment containing epithelium, preventing glare formation inside the eye (retinal pigment epithelium). *Middle:* In RP, the photoreceptors are lost due to genetic defects. Bipolar cells cannot be stimulated by light directly. The patient becomes blind. *Bottom:* When fetal photoreceptors are placed between the RPE and the bipolar cells, they mature and may connect with the bipolar cells, thus restoring the complete circuit.

## Treatment options

While there is still no cure for this particular condition, the last 10–15 years have seen the emergence of several forms of treatment that may prove beneficial. Nutritional supplements like vitamin A can slow the death of photoreceptors, but do not reverse the disease nor stop the photoreceptors from dying. Another approach is gene therapy. It is a simple-sounding idea: insert a normal rhodopsin gene into a photoreceptor with a defective rhodopsin gene so that the normal gene compensates for the defective gene. But scientists are still trying to figure out how to insert genes effectively into the photoreceptors (rods).

Another possible approach is to inject the eye with substances that might "rescue" dying photoreceptors. Several such substances exist, but they require large doses and their effect does not last. Worse, many of these substances cause blood vessels in the eyes to sprout new branches, which are often fragile and bleed, leading to further complications.

A futuristic-sounding way of treating RP, as well as some other retinal diseases, is to use an "artificial" retina. Such a device could be a microchip placed in the eye, attached to the ganglion cells. The chip would convert light signals to electrical currents and send these signals to the ganglion cells, thus completing the circuitry needed for vision to occur. A variation of this

approach is to mount a tiny camera on spectacles that a patient wears. The camera records images, converts them to digital signals, and transmits them either to a chip in the eye or directly to the brain. Unfortunately, none of these approaches has yet come close to human application or has been shown to be effective in blind animals.

## Retinal transplantation: Animal studies

In most patients with advanced RP or other retinal degenerations, the photoreceptors have died and so injection of growth substances or gene therapy is not effective. It is here that retinal transplantation—replacing lost cells with healthy cells—is an option. The problem is the retina is a nerve tissue, and unlike most other parts of the body, once fully developed, it does not repair itself if there is damage. In other words, damage to nerve tissue is more or less permanent.

We wondered back in 1983 if this problem could be bypassed using a retina that is still developing. If photoreceptors from a developing retina were injected into the eye, would they settle down and continue their development at their new location, making connections with the retina of the host, and completing the broken circuitry?

Thus the quest for a cure for RP using transplantation began. We had to first make sure the cells removed from fetal eyes and injected into the eyes of a living adult would survive and develop. For this, retina from fetal rats was removed and cut into small pieces or digested with enzymes to obtain a suspension of individual cells. These cells were injected into the subretinal space of a living rat's eye. When the injected eyes were examined after a month or so under a microscope, it was found that the cells had survived and continued to develop. This exciting beginning laid to rest doubts about the survival and continued development of retinal cells when transplanted into another eye, but begged the question: do these cells connect with the host and complete the retina-brain circuit?

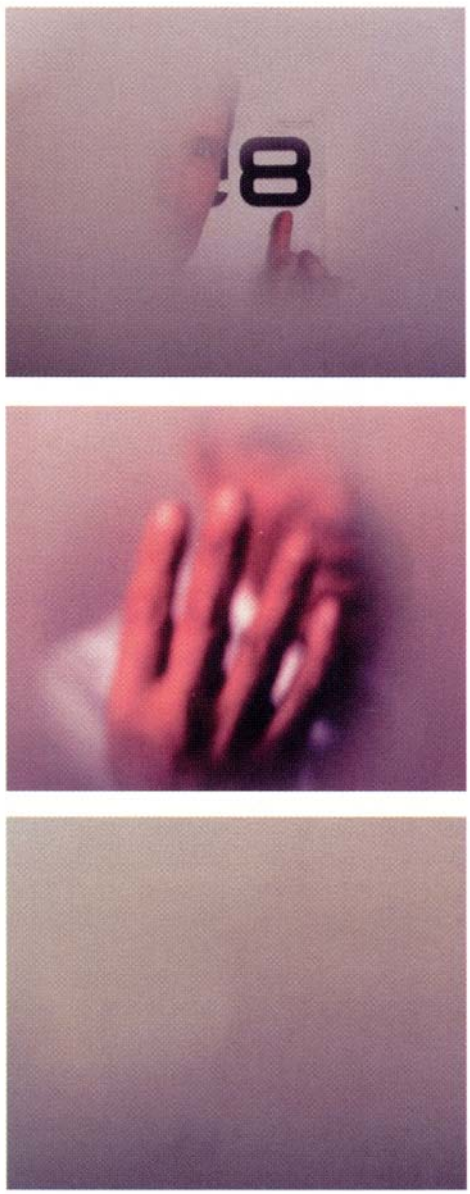

**Figure 2.** *Top:* The gradual deterioration of vision in RP patients begins with difficulty in seeing objects in dim light. Soon, the peripheral part of the vision becomes hazy and the patient can only see clearly what is directly in front of him. *Middle:* After a while, even this starts to become hazy and he is only able to count the fingers close to his face. *Bottom:* With time, all he can see is whether there is light in the room or not. In the final stages of the disease, he cannot even see this; everything is pitch black.

After some years of preliminary retinal transplantation work, a rat eye transplanted with fetal retina was being examined in a darkened microscope room under an electron microscope. The tissue, magnified 10,000x, revealed that the transplant had connected, and that there were synapses ("connections" through which two nerve cells communicate) connecting the grafted cells to the host! The reaction of the observers was a modern version of the Archimedes's "Eureka!" (except, of course, there was no nakedness).

Exciting no doubt to the scientists, but for the human patient the question remained, "would this help them see again? Would the synapses improve their vision?"

To determine if vision improved in blind rats after transplantation, a test was designed based on a well-known observation: rats (humans, too) are startled by sudden noise. In a dark room, this reaction lessens if there is a flash of light preceding the noise, a process called reflex inhibition. If, however, the rats are blind, they obviously don't see the flash, and they don't experience reflex inhibitions.

A group of normal rats were tested, and their responses to a loud noise both with and without a proceeding light flash was recorded. The rats were then blinded by exposing them to continuous light, which destroyed their photoreceptors, and the test was repeated. Because the rats were blind, they did not see the light flash and did not have a lessened startle response.

The rats were transplanted with fetal retinal cells, and tested again fortnightly. The results, conveyed through a phone call from the laboratory on a Thanksgiving morning, was that the rats could see! Needless to say, the turkey never tasted better. The rats showed less startle, meaning they could see the light flash preceding the loud sound!

## Human trials

As with the testing of any new drug or treatment procedure, before attempting to answer the question of

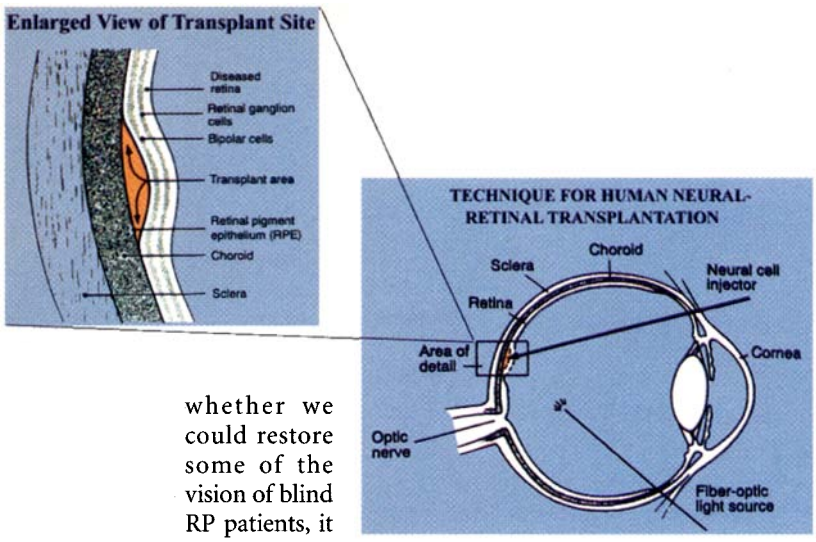


**Figure 3.** To transplant the retinal cells, the surgeon passes three thin probes into the eye. One of them is a fiber optic light that helps the surgeon to see inside the eye. The second is an instrument that is used to inject the cells. The third instrument, not shown here, is used to cut through the jelly-like vitreous to make a clear path. At that point, the surgeon passes the injector into the eye, and through a small hole in the retina, injects the cells under the retina (inset).

whether we could restore some of the vision of blind RP patients, it was very important to demonstrate that the procedure was safe. A collaborative effort was planned between the lab headed by Manuel del Cerro at the University of Rochester and teams of retinal surgeons headed by Eugene de Juan at Johns Hopkins University in Baltimore and by Taraprasad Das at L.V. Prasad Eye Institute in Hyderabad, India. The plan: to transplant fetal neural retina obtained from voluntarily terminated pregnancies into advanced RP patients who had little vision left. If the procedure led to complications, there would be little loss of vision. The goal was to evaluate the procedure to determine whether it was safe enough for general application in humans.

The first human transplantation began a little unexpectedly. While waiting for fetal tissue to become available, de Juan was in Rochester spending some time at del Cerro's lab. During this trip, some fetal eyes were received, and del Cerro demonstrated dissecting out the retina. As he delicately isolated it from the rest of the eye, de Juan suggested transplanting immediately. Cancelling all appointments, the team flew to Hopkins, where they met the first patient in a previously prepared list of possible candidates.

The procedure was simple, but delicate. Tiny pieces of fetal retina are loaded into a syringe. Meanwhile, three small holes are made in the sclera (see Fig. 3), through which three different, thin instruments are passed. One is a fiber optic light source, which illuminates the inside of the eye. Another cuts and cleans up the jelly-like vitreous, making it easy to work inside the eye. And the third sucks out the vitreous. Once the path is cleared, a small opening is created in the retina for the syringe. About one-tenth of a milliliter of retinal cells is injected under the retina, lifting it up slightly. Once this is done, the instruments are removed and the openings in the sclera are closed.

Over the next three years, 14 patients in Hyderabad and 7 at Hopkins were transplanted to determine the safety of the procedure. Only one patient developed a surgical complication, wherein the retina became detached. Except for that detachment, no complications have been observed. Particularly important is the fact that there has been no rejection of foreign tissue (the fetal retina) in any of the patients for up to four years now. It is clear from these results that transplantation is indeed a procedure that can be done safely in humans.

## Will blind men see again?

Although the first aim of these 22 transplants was to test the safety of the procedure, it is natural to wonder "Did it work?" Of the seven patients from Hopkins, three reported subjective improvement in their vision, though this improvement later disappeared. Of the 14 patients who underwent transplantation in India, eight have reported no change in their vision; three who could only perceive light before, can now make out a hand moved in front of their face; in the case of the patient mentioned whose retina detached, vision dropped from detecting hand motion to only perceiving light; and one patient who could only perceive light before, when tested eight months after surgery, was able to read the chart used to test vision. His vision is now 20/200, and he was so delighted by the result, he asked that his other eye be operated on.

However, medical researchers are not as easily excited, since this patient's vision change is subjective. It takes more rigorous evidence than that to convince a scientist before it can be said that this surgery will bring back vision. Nevertheless, it certainly provides the impetus to go further to find out if it really works. If it does, the procedure could be used to treat other retinal diseases like age-related macular degeneration, a condition in which photoreceptors are lost due to another defect in one of the retinal cells.

Currently, sophisticated instruments and methods are being assembled to evaluate the next group of RP patients who will undergo transplantation. These tests, which will not be influenced by patient bias, should answer the question, "Can we bring back vision?" That day may not be very far off.

## Further reading

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4. T.P. Das *et al.*, "The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: Results of a long-term safety study," Experimental Neurology, May 1999.

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