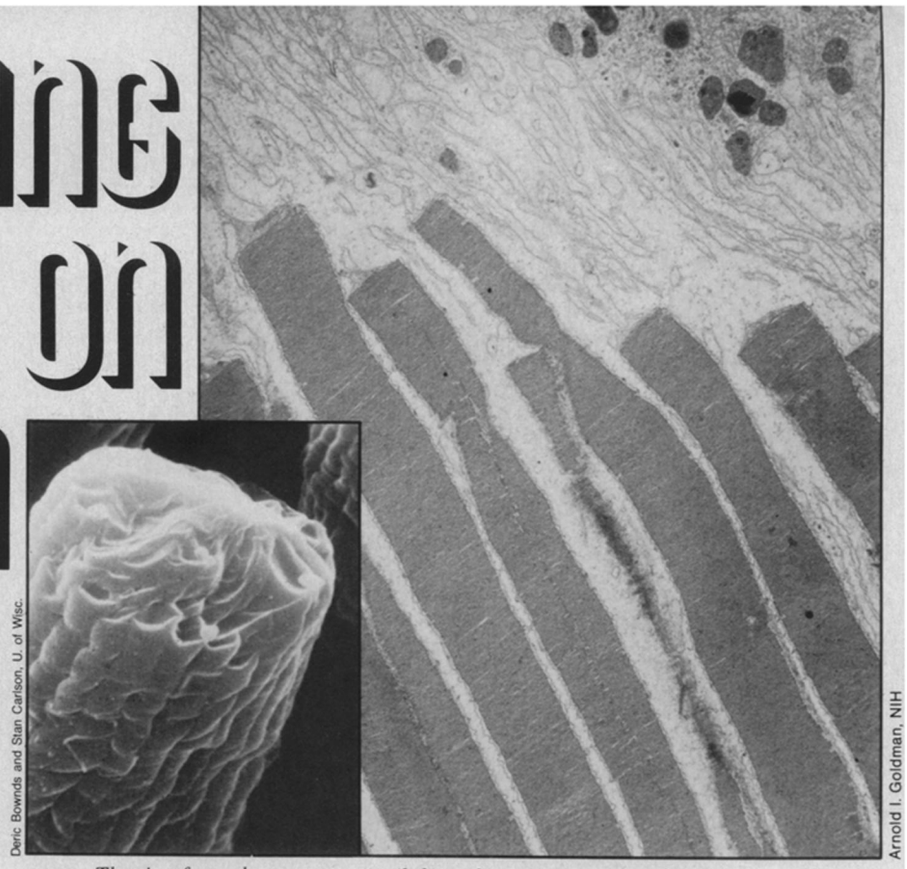
SHEDDING LIGHT ON VISION



Deric Bownds and Stan Carlson, U. of Wisc.

Arnold I. Goldman, NIH

The tip of a rod outer segment (left); a few outer segments from a rod.

Researchers are learning ever more about the primary event in vision: how light excites rods and cones in the retina of the eye. But many details of this incredibly complex event still elude discovery

BY JOAN AREHART-TREICHEL

In 1876, a young German scientist, Franz Boll, wanted to learn how light triggers vision. He removed the retina from the eye of a frog that had become accustomed to the dark and examined it as it was exposed to light. He saw that a reddish pigment in the retina turned yellow and eventually went colorless (bleached). His discovery turned out to be the detection of visual pigment, a disclosure that opened more than a century of research on how visual pigment initiates vision.

For today, scientists know that the primary event in vision, in both humans and animals, consists of a light pattern from an object being received by the visual pigment in the rods and cones (photoreceptor cells) of the retina. The pigments are bleached, change their configurations and electrically excite the photoreceptors. The photoreceptors then excite the optic nerve. The optic nerve signals nerves in the visual cortex of the brain. The visual cortex processes, and passes on to higher brain centers, elements of the image of what the eye has seen.

Since Boll's time, and particularly since the 1930s, vision researchers have unraveled a number of mysteries about light excitation of photoreceptors. And they are learning more each year, as the 1977 spring meeting of the Association for Research in Vision and Ophthalmology revealed to a room-packed audience of bathing-attired vision scientists. (The meeting was held in hotels along Lido Beach, Sarasota, Fla.) Still, vastly more needs to be unmasked about this universal human and animal event, particularly as investigators probe ever deeper into the molecular events of photoreceptors. And even if photoreceptors are ever completely understood, an enormous amount still remains to be learned about the subsequent events of the visual process.

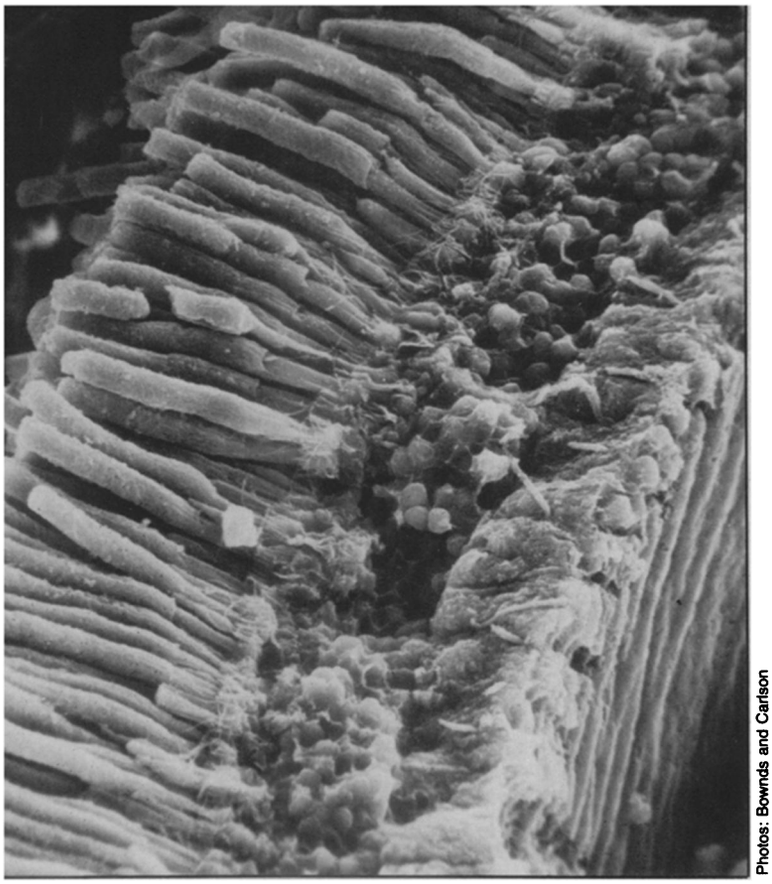
Most 20th-century research on photoreceptors has concentrated on rods—those photoreceptor cells that provide night vision, in contrast to cones, which are photoreceptor cells that provide daytime and color vision. The rod is a highly specialized nerve cell. It consists of a cell body (inner segment) complete with nucleus, cytoplasm and other normal cell equipment. It has an axon that carries a nerve impulse to cells known as bipolar cells, which in turn hook up to the optic nerve. This axon is shorter than the axon of a regular nerve cell and conveys the visual signal with graded responses rather than with the usual "action potential." But what really makes the rod unique is a clump of membranous material coming off its cell body at the opposite end from where the axon sprouts from the cell body. This appendage is known as the outer segment of the cell. In this segment reside the light-sensitive pigment molecules.

So far, light-sensitive pigment research has concentrated on one particular rod pigment called rhodopsin. The pigment consists of two chemicals—a protein called opsin and a chromophore (color-bearing) molecule of vitamin A aldehyde called retinal. George Wald of Harvard University made this discovery in 1933. What was so remarkable about it was its universality. All light-sensitive pigments serving humans and animals seem to be built on the same plan: Each contains opsin and retinal.

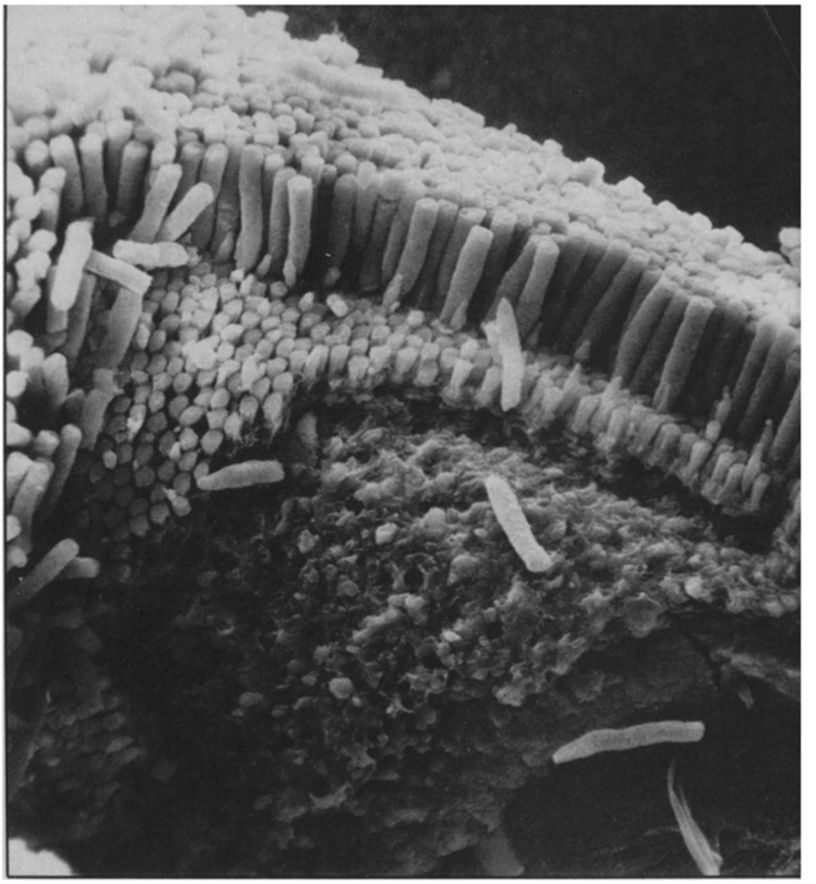
After light strikes rhodopsin, Wald and his co-workers observed, retinal changes its geometric configuration. This change, he pointed out, constitutes the primary event in visual excitation—and 40 years later it still looks as if he was right. In fact, because his discoveries of the 1930s set the stage for photoreceptor advances during the latter part of the 20th century, he was awarded a Nobel Prize for Physiology and Medicine in 1967.

This conformational change, evidence reported at the spring vision research meeting by David R. Pepperberg of Purdue University suggests, then leads to the bleaching of rhodopsin—just as Boll observed a century ago. And as Deric Bownds and his colleagues at the University of Wisconsin related at the meeting, rhodopsin bleaching is also accompanied by the phosphorylation of rhodopsin—rhodopsin receives a phosphate during the bleaching process.

Now, what happens next in photoreceptor excitation by light? A number of researchers are showing that levels of cyclic GMP, a chemical that works as an intracellular messenger in many kinds of cells, rapidly drop in the rod outer segment after a flash of light. Many investigators suspect that cyclic GMP may somehow regulate the permeability of ions passing in and out of the outermost (plasma) membrane of the rod outer segment. In fact, as Richard A. Cone of Johns Hopkins University stresses, when a single rhodopsin molecule captures a photon (discrete unit) of light, numerous



Rod outer segments synapsing with other cells in retina.



Photos: Bownds and Carlson

Top to bottom: Outer segments, inner segments, bipolar cells.

sodium channels in the rod outer segment are switched off. So the drastic decrease in cyclic GMP levels implies that cyclic GMP may well be the "gatekeeper" of rod outer-segment permeability to ions. Calcium is also involved in this role, reports G.B. Arden of the Institute of Ophthalmology in London.

Whether cyclic GMP, calcium or other cytoplasmic chemicals are or are not involved in mediating rhodopsin's effects on ion permeability in the rod outer segment, another thing is for sure: Light and changes in the shape of rhodopsin do set off electrical currents in the segment, according to recent research by D.A. Baylor of Stanford University School of Medicine. Thus one can fairly well conclude that after light strikes rhodopsin, the rod outer segment is altered electrically and that this alteration constitutes a change in the electrical potential of the rod. The electrical potential then passes an electrical signal to the bipolar cells, the optic nerve and ultimately to the visual cortex of the brain.

But there's a catch. As Cone explains, rhodopsin is rapidly altered by light, and most nerve cells also act extremely fast; they pass electrical impulses to each other in milliseconds. But the electrical responses in rods are terribly sluggish—hundreds to even thousands of times slower than those of nerves, occurring in seconds. "The entire human visual system is limited by the slow response of rods," says Baylor. "You can walk but not run in the woods at night because it takes your rods such a long time to respond to each photon of light." Why are these outer segment rods so dilatory in reacting to light?

"We may soon know," he replies, because some theoretical calculations he and his colleagues are doing suggest that the "gatekeeper" molecules (cyclic GMP? calcium?) are themselves laggards, diffusing slowly and taking a long time to find the sodium channels in the plasma membrane of the outer segment. Other researchers are probing ever deeper into rhodopsin in hopes of further baring the details of visual excitation.

For instance, Paul A. Hargrave of Southern Illinois University reports that rhodopsin appears to be a fair-sized protein (about 40,000 daltons in weight), firmly embedded in the plasma membrane of the rod outer segment and that it has sugars attached to it at three sites. Although sugars are linked to many proteins in the bloodstream, only a few membrane proteins have sugars hooked to them. Hargrave and other scientists are now trying to determine the sequence of rhodopsin's 350 amino acids.

Paul J. O'Brien and Arnold I. Goldman of the National Eye Institute in Bethesda, Md., are studying the synthesis of rhodopsin. They have found that the sugars incorporated into rhodopsin are made in the cytoplasm of the rod cell body. The sugars are attached to opsin as it rolls off the ribosomes in the cell body cytoplasm. (Amino acids are always made into proteins on ribosomes.) The sugars may also be linked to opsin in a region of the cytoplasm known as the Golgi complex. In any event, once the glycoprotein is made, it migrates into the rod outer segment, where a chromophore (retinal) is added to it. *Voila!* A finished rhodopsin molecule ready to get down to the vision business.

Still other investigators, such as Allen Kropf of Amherst College, are trying to learn more about cone vision—for example, what causes light-sensitive pigments in the cone to absorb different portions of the light spectrum and hence provide color vision? Kropf's results suggest that, although all color pigments consist of the same opsin and retinal molecules, they are sensitive to different portions of the light spectrum because specific chemical interactions take place between opsin and retinal in each color pigment.

As for Wald, he continues to inspire his fellow vision researchers in their quest to understand the visual process. At the spring vision meeting, he hypothesized that rhodopsin may bind to calcium or other ions in the rod outer segment and suggested that his colleagues explore this possibility. If such binding indeed takes place, he says, then it might well control ion fluctuation through the rod outer segment and in turn the membrane's electrical potential.

"I am deeply assured that the things we are doing in this field are moving it ahead," Wald told his colleagues. Nonetheless, there are worlds more to be discovered about vision, and vision pioneer Wald is the first to admit it: "I have spent my life researching vision, but I haven't a prayer of an idea how we see. Consciousness is where it all begins and ends. So one can feel quite humble in this field, even in moments of great elation." □