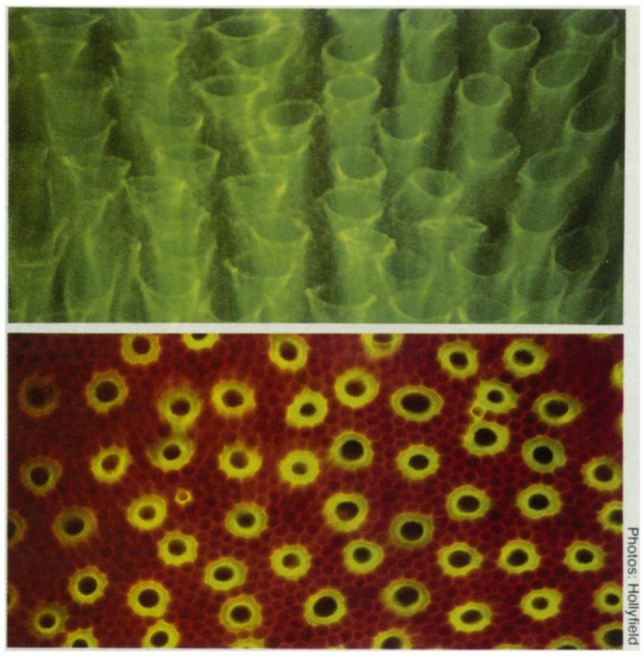
Eye Diving

A plunge into intercellular soup reveals a mysterious, multipurpose domain

By PETER L. WEISS



Photos: Hollyfield

Top, diaphanous sheaths in the subretinal "honeycomb" glow green with fluorescent dye. Their shape matches the thin-waisted form of the cone cells they encase. Bottom, a look into the holes of the honeycomb reveals a continous network of cone sheaths (yellow) and rod sheaths (red). The rods and cones that normally fill the holes have been removed from this sample.

At the back of the eye, about 10 droplets of gelatinous fluid separate the deepest layer of the retina from a black lining of pigment-rich cells. This miniature moat — thinner than a sheet of cellophane — went virtually unnoticed until the 1950s. Recently, however, it has captured the attention of a growing number of researchers, who report tantalizing clues that it plays critical roles in vision.

The very location of the moat points to one vital function. The nearest blood supply for the retina's light-detecting rods and cones lies well beyond it, forcing these cells to receive their nutrients and discard their wastes via the viscous waterway. And in order to do their job, the same photoreceptors rely on regular shipments of light-sensitive chemicals across the gel.

Medical evidence, too, suggests important roles for the gooey gap, more formally known as the subretinal space or interphotoreceptor matrix. This is precisely where damaged retinas detach, hinting that the moat's gummy contents normally "glue" the retina in place. And research into the most common form of inherited blindness — a disease called retinitis pigmentosa — shows several possible links to defects in the subretinal space.

Yet despite such clues, scientists still cannot say for sure what goes on there. "It is a region we all know is very important, but we don't know why or how," says Matthew M. LaVail, a cell biologist at the University of California, San Francisco (UCSF).

For explorers seeking to penetrate this dark continent of the eye, each new discovery seems to spawn new questions. For instance, scientists have found a castle of sorts in the waters of the moat: a spongy network of interlocking macromolecules. Rods and cones protrude into the network's form-fitting chambers like baby bees embedded in a honeycomb.

That discovery, described in 1986 by cell biologists Gregory S. Hageman and Lincoln V. Johnson of the California Institute of Technology in Pasadena, came as a surprise. Until then, researchers had assumed the subretinal space held only fluids.

But surprise turned to astonishment this summer when LaVail and Fumiyuki Uehara reported that in certain rats, the honeycomb sheaths seem to slide, or perhaps shrink, during shifts from darkness to light.

Hageman, now at the St. Louis University School of Medicine, argues that the motion may be an illusion, the result of other molecules "masking" the researchers' view of the sheaths. If it is real, however, the unexpected animation might somehow assist in moving ions, photosensitive chemicals and other substances across the gap, LaVail and Uehara suggest in the June 29 SCIENCE.

Scientists know that photosensitive molecules called retinoids — chemical cousins of vitamin A — embark on mass migrations when lighting changes dramatically. Under dark conditions, the rods and cones stockpile these molecules, which remain securely bound there as long as they maintain a certain shape. But when light strikes, the retinoids change shape and spring loose, heading across the subretinal space and into the black lining called the pigment epithelium. Cells in the pigment epithelium restore them to the "right" configuration, and the reshaped retinoids head homeward again to resume their light-detecting role in rods and cones.

LaVail and Uehara suspect that the light-triggered response they saw in the honeycomb sheaths — a motion they liken to the parting and closing of curtains — helps nudge retinoids back and forth across the moat.

Their experiments indicate that the sheath motion is accompanied by a bunching and unbunching of protein molecules that normally bind to retinoids. As the sheaths shift, they might pull the retinoid-laden proteins along with them, LaVail suggests.

That hypothesis, if confirmed, might solve a long-standing puzzle.

In 1982, three separate research teams studying the subretinal space's viscous fluid succeeded in isolating the specialized protein that binds to retinoids. This eagerly sought compound — called IRBP, for interphotoreceptor retinoid-binding protein — can ferry retinoids across the moat, says IRBP co-discoverer Alice J. Adler of the Eye Research Institute in Boston. The finding provided the first compelling evidence that specific moat molecules influence the crossing of substances vital to vision, as scientists had long suspected.

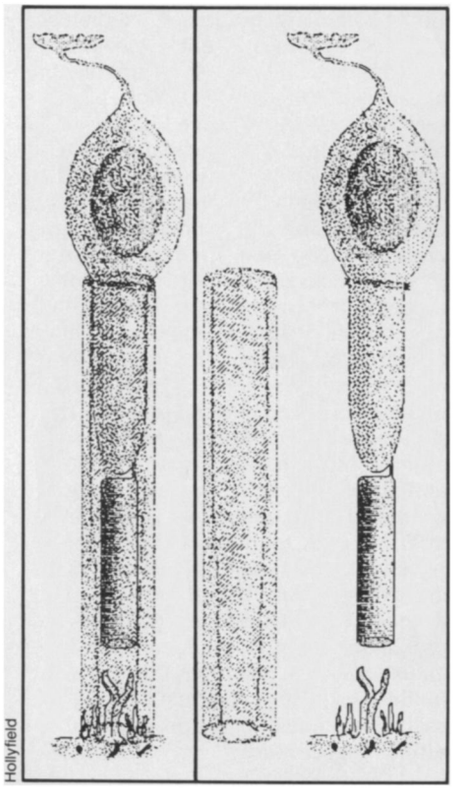
But a report published last year sug-

gested that the celebrated protein might slow, rather than speed, the passage of retinoids across the gap. Retinoids might actually cross the moat more rapidly *without* the protein ferries, assert cell biologists Ming-Tao P. Ho and Joe G. Hollyfield at Baylor College of Medicine in Houston, who tracked retinoid flow between artificial cell membranes in solutions with and without IRBP. They described their experiments in the Jan. 15, 1989 JOURNAL OF BIOLOGICAL CHEMISTRY.

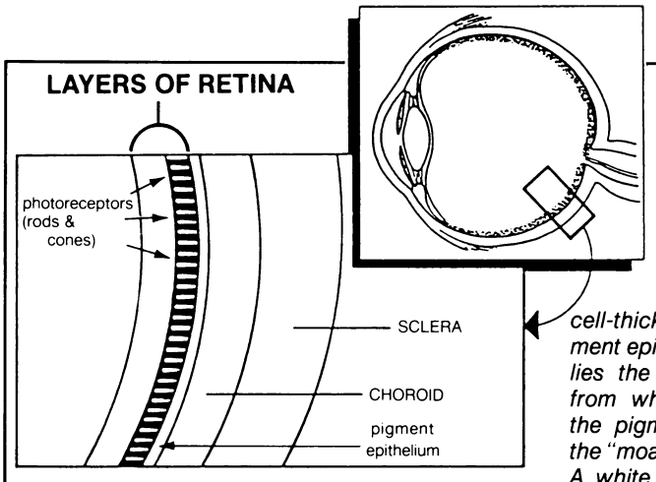
Adler now thinks the proteins might provide a temporary storage depot for retinoids, holding on to them until simple diffusion causes the light-sensitive loads to drift across the moat. Or perhaps the sheath motion carries them along. But all of this remains speculation. "Everything is open here," Adler stresses. "Nothing is set in stone."

Equally enigmatic is the honeycomb hugging the photoreceptor cells of the retina. Many scientists suspect that it constantly disintegrates and regrows. Indeed, experimental results hint that this regeneration process, combined with the honeycomb's elasticity, may be critical to proper photoreceptor alignment.

The network "acts like a rubber sheet if you pull on it," Hollyfield explains. When tugged at one corner, it gives a little throughout. By providing a flexible link from cell to cell, it might tether the photoreceptors together along the curving inner wall of the eye while aiming each at the best angle to catch light, Hollyfield and others suggest.



Sketch on left shows a cone cell in a sheath penetrated by spiky outgrowths of the underlying pigment epithelium. The structures appear separately on the right. Researchers suspect that the spikes' hold on the sheaths and the sheaths' grip on the cones serve to anchor the cones — and thus the retina — to the pigment epithelium.



At the very back of the retina, the moat-like subretinal space (black zone in diagram) surrounds the light-sensing rods and cones (white bars). Just behind the space is a single-cell-thick lining called the pigment epithelium. Beyond that lies the blood-rich choroid, from which nutrients cross the pigment epithelium and the "moat" to reach the retina. A white covering called the sclera forms the tough outer wall of the eye.

One might expect the form-fitting sheaths to restrict the movement of the photoreceptors they encase. But Jay M. Enoch, a biophysicist at the University of California, Berkeley, has demonstrated in humans that cone cells can change their orientation considerably.

Enoch chemically dilated volunteers' pupils and fitted them with contact lenses coated with a false "iris" encircling an equally false, off-center "pupil." After three days, tests showed that many of the cones had realigned themselves to point in the direction of the misplaced light source. Enoch, who reported these results in 1981, now plans an experiment to see how photoreceptors respond to two out-of-place pupils.

Hollyfield speculates that the honeycomb adapts to the cones' new alignment through regeneration, replacing old sheaths with new ones pointing toward the false pupil. But where do the replacement materials come from, and where do the discarded materials go? So far, attempts to trace the origin of the honeycomb's interlocking macromolecules have proved inconclusive. Moreover, as Hollyfield puts it, "we don't know who uses up the unwanted matrix."

Why does scientific uncertainty hover so thickly around this paper-thin gap? For one thing, the subretinal space contains an unexpected bonanza of substances and structures. It's like a bottomless suitcase: The more you try to unpack it, the more new contents it seems to yield.

Most recently, researchers have come across "large quantities" of a substance called basic fibroblast growth factor (bFGF) in the gel. Hageman, who announced the discovery in July at the Stockholm (Sweden) Symposium on Retinal Degeneration, says this suggests two more functions for the gel: retinal repair and cell differentiation.

In skin, Hageman notes, bFGF molecules "stand guard like soldiers," waiting to rush to the assistance of injured cells. In the retina, the growth hormone might also serve as a toner to help still-healthy photoreceptors stay in functional shape, he proposes. Both rods and cones — whose cell membranes carry receptors for bFGF — show dramatic structural and functional differences from one end to the other. This polarization is critical to their light-sensing function. In order to maintain it, says Hageman, the cells might depend on cues from bFGF molecules docked at their receptors. Such signals might "keep [the rods and cones] from changing into little round cells" that could no longer detect light, he suggests.

The technical difficulties of probing the gel have also hampered investigators. The nearly transparent fluid played hide-and-seek with early retina researchers: Although they first glimpsed it in 1855, scientists debated its existence for the next 50 years. Another half-century passed before biologists developed the necessary chemical stains to reveal some of its contents. Today, the scant amount of gel obtainable from an eye and the ponderous steps needed to study its dynamic behavior *in vivo* continue to frustrate efforts to clarify the gap's functions. "It doesn't give up its secrets very easily," Hollyfield says.

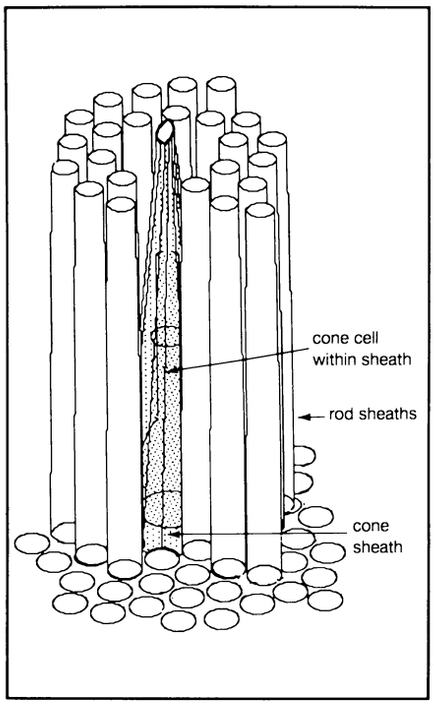
Explorers continue to make headway, however. Recently, they have dismantled the honeycomb walls — around the cones, at least — in hopes of resolving a sticky issue. Since the late 1960s, scientists have hypothesized that the viscous blend in the subretinal space somehow glues the retina to the underlying pigment epithelium, but the specific

anchoring sites remained elusive. Several studies now point to cone sheaths as the likely candidate.

The sheaths of the honeycomb network keep a firm grip on the rods and cones they encase. And at the other side of the moat, twisted strands extend from the pigment epithelium and screw themselves into cone-containing sheaths. (Rod sheaths show little evidence of such attachment.) Researchers have also detected a number of glue-like molecules near the anchoring strands.

To test the adhesion hypothesis, Hageman and Howard S. Lazarus of St. Louis University disrupted cone sheaths in pigs by injecting the subretinal space with xyloside – a sugar known to inhibit synthesis of the main macromolecule in these sheaths. This caused the retina to detach from the pigmented lining, they report. Their finding, presented at the Stockholm symposium in July, indicates that the cone sheaths not only hold the retina in place, but also require constant renewal from whatever source manufactures the macromolecules, Hageman says.

Hageman launched a second chemical assault in a study conducted with Xiao-Ying Yao and Michael F. Marmor of Stanford University. In test rabbits, the researchers injected the subretinal gap with enzymes that selectively degrade cone sheaths. In control rabbits, they injected a nondegrading fluid. The control rabbits' retinas remained attached except at the point of injection. But in the test rabbits, a widening circle of cone-sheath disintegration and retinal detachment grew outward from the injection site over a three-day period, the team found. A report on their work will appear in the October INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE.



Hollyfield

Sketch of a cone sheath (center) surrounded by rod sheaths. The two types of sheaths bind together to form a honeycomb structure. The cone sheath's tapered end (top) faces the pigment epithelium.

Scientific interest in the miniature moat continues to grow as new discoveries stir curiosity and attract recruits to the field. "Now we have a critical mass" of researchers focusing on the subretinal gap, Hollyfield says.

Many are particularly intrigued by LaVail's report of shifting rod sheaths. To Hollyfield, that phenomenon implies that materials in the subretinal space "are very actively involved somehow in the functioning of the photoreceptors themselves." Confirmation of such involvement, he says, might even change the way researchers approach the physiology of vision in general.

"Physiologists are going to have to start thinking about more than what is in the [retinal] cell," Hollyfield says. "They are going to have to start thinking about what is outside the cell if they want to have a full understanding of photoreceptor function." □

Retinitis pigmentosa: Filling an information gap

Clumsy fielding in a twilight Little League game, or a "careless" bike accident at dusk, could offer the first clues. Retinitis pigmentosa – a hereditary disease affecting about 100,000 people in the United States – typically begins by eroding night vision during childhood or adolescence. Tunnel vision or complete blindness commonly follows as the retina, for no known reason, slowly degenerates.

The search for a treatment or cure has drawn a blank. However, efforts to determine the cause of the disease are beginning to pay off. Molecular biologists this year located genes associated with some forms of the disease. And researchers studying the subretinal space at the back of the eye have uncovered other potentially important leads.

The retina coats the curving back wall of the eye like a transparent layer cake consisting of different cell types. Directly behind it lies the gel-filled subretinal gap, and behind that lies the pigment epithelium, a single-cell-thick lining of black, pigment-rich cells. Retinitis pigmentosa destroys the photoreceptor cells that protrude into the subretinal space from the retina's hindmost layer. The rod cells usually go first, sometimes followed by the cones. Often, the pigment epithelium also deteriorates.

Scientists have speculated that 20 to 30 different hereditary disorders fall under the heading of retinitis pigmentosa. These range from barely perceptible (and frequently undiagnosed) loss of vision to potentially deadly syndromes with symptoms such as blindness, deafness and mental retardation.

Investigators of retinitis pigmentosa take great interest in the narrow space between the retina and the pigment epithelium. Indeed, organizations seeking cures for blindness, including the National Eye Institute and the National Retinitis Pigmentosa Foundation, frequently fund studies of the subretinal space in hopes of better understanding retinal disease and eventually finding a way to fight it. Accumulating evidence from such research indicates the gap is important to retinal function in general, and possibly to retinal degeneration in particular.

In 1981, cell biologist Matthew M. LaVail and his co-workers at the University of California, San Francisco, showed a suggestive and possibly causal link between changes observed in the subretinal space and a subsequent death of photoreceptor cells. In rats with a form of hereditary retinal degeneration, they found that the gap accumulated an unusual buildup of photoreceptor fragments. (In normal rats, such fragments slough off daily but are immediately digested by the pigment epithelium.) The buildup of undigested fragments began as much as a week before photoreceptors started to die. LaVail and others continue to investigate why the debris piles up and how its accumulation might harm photoreceptors.

This year, he and Fumiyuki Uehara presented two new findings. Normal rats show a shifting motion in the photoreceptor-hugging sheaths that permeate the subretinal space, apparently triggered by changes in lighting, they reported in the June 29 SCIENCE. In rats with the inherited disease, however, the shifting ceased as retinal degeneration progressed. Uehara reported the latter finding last spring in Sarasota, Fla., at a meeting of the Association for Research in Vision and Ophthalmology.

Uehara, now at Kagoshima University in Japan, cautions that the rats' retinal degeneration at best only loosely approximates retinitis pigmentosa in humans. Still, he ventures, "if we can inhibit this [loss of response], it may be useful for preventing the progression of retinal degeneration." – *P.L. Weiss*