COLORFUL VIEWS OF VISION

Scientists use their eyes, aided by fluorescent dyes and natural pigments, to map components of the visual system

BY JULIE ANN MILLER

A splash of color across a microscope field is a very satisfying piece ot data — especially to those scientists whose main interest is the study of vision. Some of the color is provided by dyes that give off yellow or blue-green fluorescence. Other color stems from the fluorescent properties of natural components of an eye.

"It was a mixture of serendipity and error," Francisco M. de Monasterio told SCIENCE NEWS when asked how he and colleagues discovered a new staining technique. De Monasterio, Stanley J. Schein and Edna P. McCrane report in the Sept. 11 SCIENCE that a dye called Procion yellow, when injected into a monkey's eye, is taken up by a surprisingly select group of cells. The dye fills only the cells that are most sensitive to blue light and, the scientists suspect, those cells that carry the message from the blue-sensitive receptor cells to the brain.

Primates, including people, have four types of light-sensing cells in their retinas. Each absorbs light best in a different part of the spectrum. One class is easily distinguishable from the others by its rod shape. The other types, all shaped like cones, have not been distinguishable, except by their preferential response to blue, green or red light.

The receptors that are most sensitive to blue light are of special interest both to scientists who study color vision and to clinicians. While the red- and green-sensitive receptors seem to be involved in many aspects of vision, the blue-sensitive cones are thought to be specialized for determinations of color. Clinically, monitoring the function of those cells may provide indications of retinal disease.

The error that played a role in the discovery of a way to stain only the blue-sensitive receptor cells occurred more than three years ago. De Monasterio was recording the electrical activity of single cells in a rabbit retina, and he was injecting Procion yellow into the cells so he could identify them after the experiment was finished. Accidentally he broke a micropipette filled with dye while it was in the retina. He noticed that many of the retinal cells took up the stain.

More recently de Monasterio, Schein and McCrane at the National Eye Institute were looking for a dye to stain the layer of retinal connections intermediate between the light-sensing cells and the brain. De Monasterio remembered his accident with Procion yellow, and also a report from other scientists that the dye stained parts of some amphibian light-sensing cells. Because they didn't have the other scientists' report handy, the NEI investigators arbitrarily selected a dose of dye. They chose one far higher than the other group in fact had used. But, Schein says, it was the perfect dose to reveal a new effect. Not only did the dye stain the intermediate layer as they had expected, but the entire bodies of some light-sensing cells took up the dye, creating bright yellow, cone-shaped silhouettes.

Luck entered into the development one more time, when de Monasterio cut a slice within a layer of retinal cells. The glowing yellow silhouettes appeared regularly spaced. This observation, which might not have been made had de Monasterio sliced the retina at a different angle, suggested that the stained cones are of a particular class, those most sensitive to blue light.

"The greatest serendipity of all was that both of us had worked on the physiology of the blue-sensing system," says Schein. "We were really primed to think about blue."

"We first joked that these might be blue cones. As the evidence of their retinal distribution accumulated, we had to take the idea more seriously," de Monasterio says.

Previous studies had determined some characteristics of the distribution of blue-sensitive cones. They are fairly widely spaced, comprising only about 10 percent of the total cone population. They are absent in the center of the eye, but most concentrated near the center. Their frequency diminishes toward the retina's periphery.

While pleased to have discovered a means to easily differentiate among the cones, the scientists found themselves facing a puzzling question. At first they asked why only the blue-sensitive cones picked up the dye. But because the dye is known to not cross cell boundaries, they soon turned the question around to ask, Schein says, "Why on earth did the dye get into any cells at all?"

The only clue that the scientists have so far is a quite different observation that sets blue-sensitive cones apart from the red- and green-sensitive varieties. In 1912 a German physician recognized that many types of retinal diseases lead to an acquired type of color blindness, suggesting dysfunction of only the blue-sensitive cones. Thus the blue-sensitive cones, for reasons yet unknown, appear to be most vulnerable to "retinal insult," a category that includes Procion yellow dye as well as a variety of diseases.

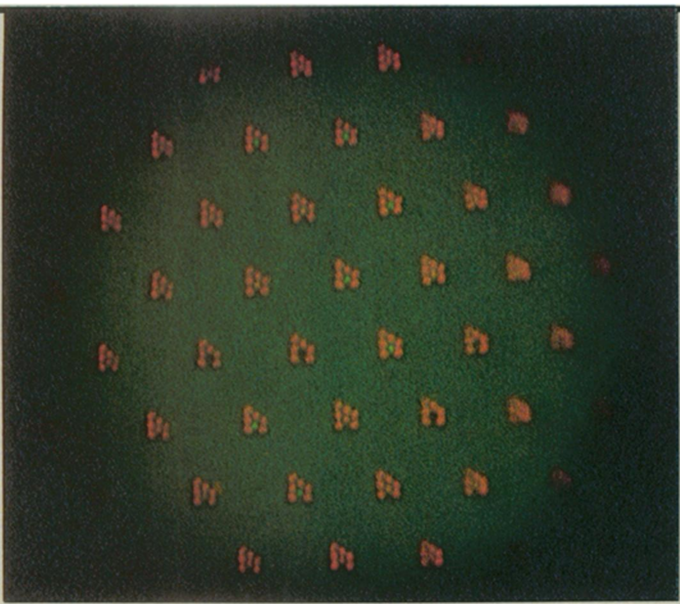
Because impaired functioning of the blue-sensitive cones may be a powerful indicator of retinal disease, the researchers see the dye study as an important step toward medical applications. The Procion yellow not only stains but it selectively kills the blue-sensitive cones. So some modification of the technique could produce a useful animal model of the acquired color vision disorder. "This model could be a tremendous help in extending diagnostic tests for retinal disease," Schein predicts. But first the scientists must circumvent such practical problems as the yellow staining of the eye's clear fluid by the dye.

The staining technique may also help scientists determine the complex circuitry of the retina. At a recent meeting de Monasterio and colleagues reported that a specific group of the intermediate cells connecting the photoreceptors and the brain are revealed by Procion yellow. From the size, shape and distribution of the stained cells, the scientists suspect they are the ones that carry and process signals from the blue-sensitive cones.

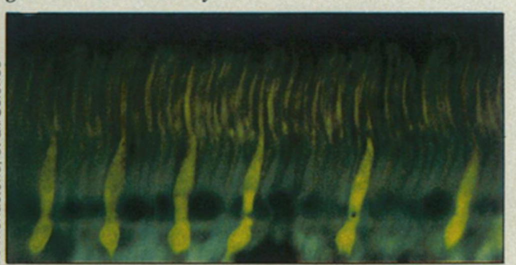
How the dye gets inside those cells is under examination. It may be that the entire "blue system," from the receptor cells to the brain, is exceptionally sensitive to chemicals such as the dye. Or it may be that the dye picked up by the cones is transported to the connecting intermediate cells. De Monasterio says that passage of the dye across the retina is not a matter of simple diffusion, and he suspects the non-nerve cells, the glial cells, are involved. Questions about how dyes get into cells are leading the scientists beyond satisfaction with the striking cell visualization technique. They are opening new and complex considerations of retinal physiology.

Not only are dyes adding fluorescent colors to visual studies, but natural pigments also are giving colorful results. Observations of the natural fluorescence of visual pigments have not been made extensively because it is a weak phenomenon. However, using new techniques of sample preparation and microscopy, a group of German scientists is taking advantage of the relationship between fluorescence colors and properties of natural pigments.

The compound eye of the housefly is the focus of these new fluorescence studies. Each unit of the fly retina has eight refract-


N. Franceschini, et al./Science

Elements of a housefly compound eye (left) emit red, green or no fluorescence when illuminated with blue light. The fluorescence reflects the different natural pigments of the eye. Scale bar is 30 microns. Procion yellow dye stains the outer segments of all cones in monkey retina (below), but only the inner segments of a few. The yellow silhouettes are fairly regularly spaced. The other cone inner segments are stained with a blue-green fluorescent dye.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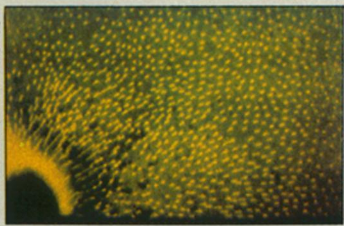
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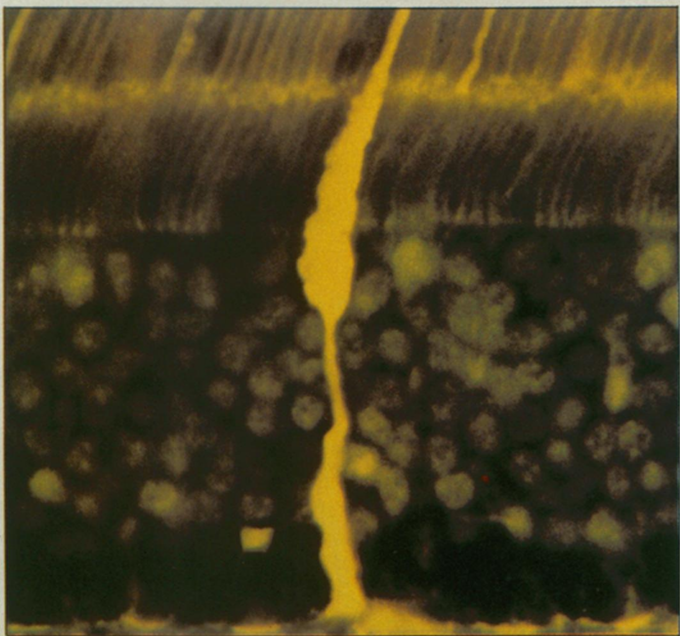
Procion-stained cone inner segments (immediate left) are most concentrated near the center of the retina, less abundant toward the periphery. Solid yellow band at lower left is cone outer segments.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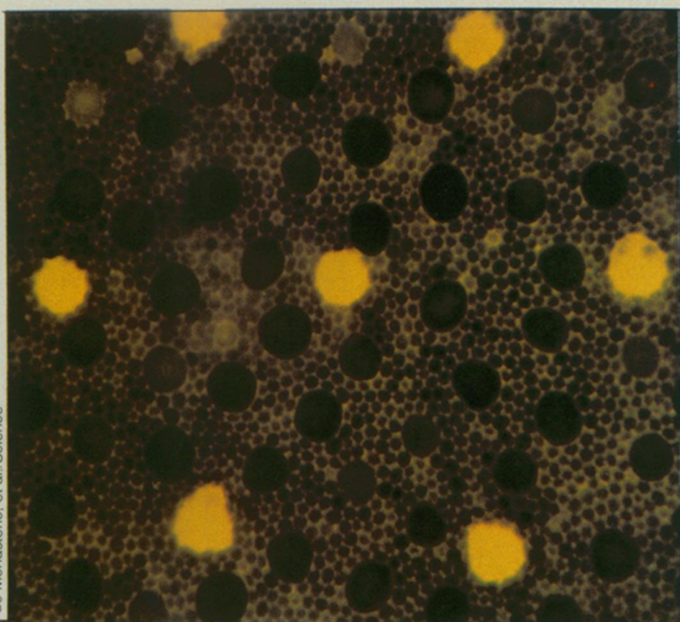
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A yellow silhouette of a single cone among many cones (far left, center) unstained except for their outer segments was the surprising result of experiments with Procion yellow dye. The yellow-stained inner segments of selected cones (far left, bottom) form a nearly perfect hexagon around a centrally positioned stained cone. The position of these cones, packed among unstained ones (the dark round structures) suggested that Procion yellow dye may specifically stain the cones most sensitive to blue light. The finer cobblestone pattern is due to other unstained photoreceptors, the rod cells.

ing elements, called rhabdomeres. N. Franceschini, K. Kirschfeld and B. Minke of the Max Planck Institute for Cybernetic Biology in Tübingen report, also in the Sept. 11 SCIENCE, that the rhabdomeres emit different colors when excited by blue light. In each unit, the six rhabdomeres arranged in the outer ring emit red light. The central rhabdomeres can appear green, red or black. Usually 70 percent of these central rhabdomeres of a housefly retina fluoresce green, and 30 percent don't fluoresce at all and so appear black. The red fluorescing central rhabdomeres have only been found in one portion of the male fly's eye.

"Each fluorescence color appears as a natural color tag, which can henceforth be used reliably to map out the various spectral types of the retina *in vivo,*" Franceschini and colleagues say. They point to the red-fluorescing central rhabdomeres as a unique example of sex-specific retinal organization. The non-fluorescing rhabdomeres seem to be receptors for ultraviolet light, and the green-fluorescing ones seem to sense both ultraviolet light and the "tail-end" of the blue part of the spectrum. Analyzing the intensity of fluorescence emission under different conditions can give clues to how pigment molecules respond to light.

The scientists conclude, "Even though fluorescence emission may represent but a spillover of excitation energy, it may shed new light on the conformational changes of various molecules involved in the generation of the bioelectric signal."□