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Vision: An Overview

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The visual process is complex. In order to be understood, it must be examined from many perspectives, for many phenomena observed in one area have their explanation in another. This interplay has provided the impetus for many chemical studies. The present issue of *Accounts* is devoted to the chemists' approach to investigating vision. Even under this one cloak, many varied tracks will become apparent.

A unified picture of the entire visual process will unfold only if the chemist digresses a moment into the realms of anatomy, biology, physiology, and even psychology. The literature is vast, and space forces us to be most selective in the topics considered. The interested reader is referred to any of the first seven references for more exhaustive discussion and the original literature citations.[1-7] We will provide in what follows a brief description of the general anatomy, photoreceptor systems, and chemical nature of the visual process.

General Anatomy

Figure 1 shows the major structural features common to all vertebrate eyes. The cornea, iris, and lens provide the necessary optics for focussing the light on the retina. Photoreceptors in the retina convert the light signals to electrical pulses, which the optic nerve then transmits to the brain.

The primary function of the cornea, besides its obvious protective role, is to bend incoming light to form an image on the retina. The lens has often been assigned that role and, while this is true for fish, it is not true for vertebrate eyes. In vertebrates, the lens is used to adjust the focussing of the cornea to accommodate near or far vision. The lens is suspended by the zonula, which keeps it under tension. For near vision, the ciliary muscle contracts, the zonula relaxes, and the lens springs back to assume a more convex form. The lens is built up in thin layers, like an onion. It continues to grow throughout life, the center being the oldest. The inner cells in the lens become more and more separated from the blood-

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(1) J. Field, H. Magoun, and V. Hall, "Handbook of Physiology", Vol. 1, American Physiological Association, Washington, D. C., 1959.

(2) H. Dartnall, "Handbook of Sensory Physiology", Vol. VII/1, Springer-Verlag, New York, N. Y., 1972.

(3) R. Rodieck, "The Vertebrate Retina", W. H. Freeman, San Francisco, Calif., 1973.

(4) B. Straatsma, "The Retina", University of California Press, Berkeley, Calif., 1969.

(5) C. Graymore, "Biochemistry of the Eye", Academic Press, New York, N. Y., 1970.

(6) G. Wald, P. Brown, and I. Gibbons, *J. Opt. Soc.*, **53**, 20 (1963).

(7) E. Abrahamson and S. Ostroy, *Prog. Biophys. Mol. Biol.*, **17**, 179 (1967).

stream giving oxygen and nutrient. Eventually they harden and die, and the lens loses its ability to accommodate.

The lens is transparent to visible radiation but absorbs strongly in the ultraviolet. There is an absorbance peak at 370 nm as well as strong absorbance below 300 nm. The latter is due to lens protein, while the former is attributed to two or more pigments. In time, the absorption extends into the blue and the lens "yellows with age". Consequently, one becomes progressively less sensitive to blue light. This may be the reason that so many artists switch from blue as a dominant color in their early works to red in their later ones. Removal of the short wavelengths reduces the chromatic aberration of the eye's optical system. If the lens is missing (e.g., after a cataract operation), the eye will perceive ultraviolet.

Light passes through the pupil, an orifice in the iris, enroute to the retina. It is popularly believed that the pupil changes in size to compensate for changes in light intensity. This is partly true but is not the main mechanism for such adaptation: the pupil can change in area by only a factor of 16, whereas vision works efficiently over a brightness range of about 10^5. The primary mechanism for light-dark adaptation is chemical and anatomical in nature. The pupil changes size to keep light rays on the central part of the lens, which has the best optical properties. When light levels are low the pupil opens, trading acuity for sensitivity. The pupil also closes for near vision to increase depth of field, much as increasing the *f* stop on a camera increases the focussing range. The pupil appears black because of the choroid, a pigmented, nutritive layer behind the retina. It increases acuity by absorbing light, preventing it from making multiple reflections inside the eye. In nocturnal animals, often this layer is reflective, and one can see an owl's eyes glow in the dark. Here again there is a trade-off between acuity and sensitivity.

The retina is a thin sheet of interconnected nerve cells and light-sensitive cells, or "photoreceptors". The retina derives embryologically from the brain. Strangely, the retina is oriented in nearly all vertebrates in such a way that light impinging on it must pass through various layers of nerve cells, blood vessels, and supporting cells before it reaches the photosensitive receptors. The photoreceptors convert the light signal to a neural signal in a remarkable process called transduction.

Photoreceptor Systems

Anatomically, the eye appears to have two distinct photoreceptor systems: rods and cones. They differ in shape, number, distribution, and neurological connection.

Cones and rods in the human eye are about 0.05 mm long. Cones are roughly 0.005 mm wide at the base and are tapered so that the diameter at the tip is about half that at the base. The rods are cylindrical in shape and about 0.002 mm wide. Each rod contains 500 to 2000 flattened sacs called "disks", which, as studies with radioactive tracers have shown, are continuously being replenished at the base and disposed of at the top.[8] The lifetime of each disk is on the order of weeks and varies with the species. New disks are formed in a rod at the rate of about one every 40 min. Regeneration is vital to the visual process, for interference with the process, as in some hereditary disorders in rats, results in blindness. Cones have no disks. Instead, there are invaginations of the outer cone membrane. Cones do not appear to have a rejuvenating system like the rods. The cones are found predominantly in the fovea, with the rods complementing the distribution. There are many more rods than cones. In the human eye, there are about 10^8 rods and only about 5×10^6 cones. Generally, each cone is connected to a single neuron while several rods are hooked up together on a common neuron.

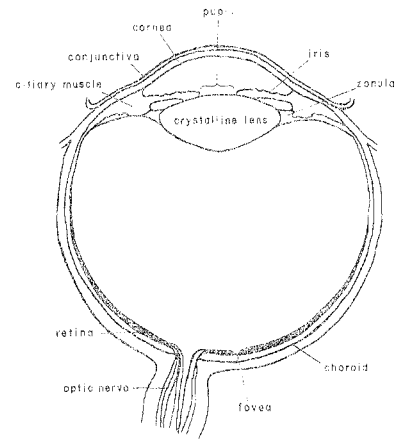

Figure 1. Vertebrate eye in cross section.

The dual receptor systems serve two functions. The rods are 10^2 to 10^3 times more sensitive and operate in dim illumination, or under so-called scotopic conditions. The cones work under bright (photopic) conditions and mediate color vision. The spectra of individual cones may be obtained using the technique of microspectrophotometry.[2] These methods reveal different spectra for different cones, providing a basis for color discrimination. Rods, on the other hand, all have identical spectra and are not involved in human color vision. In dim light, when the rods alone can function, one cannot discern colors, explaining the old adage, "At night, all cats are gray".

In moving from the center of the human retina to the periphery, one seems to travel back in evolutionary time. In the center, that is, at the fovea, there is full color vision of the highest acuity. Moving outward, color vision declines, as the density of cones decreases. Farther out, objects are not perceived, but only motion. (An opthalmologist must wave his or her hand when checking peripheral vision.) At the very edge, even motion is not perceived. However, stimulation will serve to initiate a reflex, turning the eye toward the motion. This last fact may help to explain tales of ghosts. One sees something out of the corner of the eye in the dark and turns to look. The image falls on the fovea. The fovea, being solely cones, is virtually blind in the dim light, and the moving object mysteriously disappears.

Chemical Basis of Visual Transduction

There has long been a great interest in the underlying chemical basis of visual transduction. A pigment in the outer segment of the rods was discovered

(8) R. Young, *J. Cell Biol.*, 33, 61 (1967).

by Boll, then carefully studied by Kuhne, over a century ago. Subsequently, other pigments were isolated from the cones. The history of the association of these pigments with the visual process is intriguing. Psychological as well as chemical evidence was brought to bear.

Measurements of the visual threshold (i.e., intensity of light needed to evoke a sensation) as a function of wavelength were made. Two curves were observed, depending upon whether the subject had been in dim or bright light conditions when the threshold measurements were being determined. The two threshold curves in dim and bright light were very similar to the absorption curves of the rod and cone pigments, respectively, as a function of wavelength. In bright light, the eye is most sensitive to red; in dim light, green. Thus, as dusk falls, the red rose is eclipsed by its green foliage. The change in relative brightness is known as the Purkinje shift. The similarity of the pigment absorption spectra to the threshold curves was compelling evidence that the pigments were of essential importance to vision.

Chemical interest then focussed on elucidating the structure of the pigment. It was long known that vitamin A deficiency was accompanied by visual deterioration. It was thus not too surprising when G. Wald, in the 1930's, found vitamin A in the retina.[9] Since then, this observation has been amply confirmed: vitamin A, or rather its oxidized form, called retinal, has been found in every visual system from squid to man. The structure of retinal is shown in Figure 1a of Abrahamson's Account. In a few species, vitamin A_2 is found (vitamin A_2 differs from A_1 only in having an additional double bond. The oxidized form of vitamin A_2, 3-dehydroretinal, is shown in Figure 1b of Abrahamson's Account).

All visual pigments studied consist of retinal bound to a protein generically called "opsin". Visual pigments derive their names from the types of opsins and retinals they contain. Thus, retinal A_1 combines with rod opsin to yield rhodopsin and with cone opsin to yield iodopsin. Similarly, retinal A_2 combines with rod opsin to yield porphyropsin and with cone opsin to yield cyanopsin. The opsin alone does not, of course, absorb visible light, and its color is imparted by the presence of the retinal.

One elegant piece of corroborating evidence identifying the crucial role of the pigments came again from a correlation of psychological and chemical effects. It is a matter of everyday observation that upon entering a dark room one can gradually see more clearly. This effect is known as dark adaptation and can be measured quantitatively. The curve representing this subjective phenomenon is rather characteristic. There is a rapid rise in sensitivity (sensitivity is defined as the reciprocal of the threshold) to high levels of light which reaches a plateau in about 5 min. Concurrently, there is a much slower recovery of sensitivity to very low levels of light. It takes about 30 min for the maximum sensitivity to return, that is, for one to become completely dark-adapted. The chemical analog can be observed when rod and cone opsins are mixed with retinal. The two types of pigments are spontaneously regenerated: the iodopsin rapidly and the rhodopsin slowly. The rise in the concentration of pigments and in the logarithm of the sensitivity has the same time dependence.[10]

Visual pigments in different species vary only in the nature of their opsins, not in their chromophores. The fact that pigments of different species exhibit different absorption spectra is attributed to variations in the protein moiety and hence in its interactions with the chromophore. Spectra of visual pigments are generally broad and structureless and are characterized by their wavelength of maximum absorption, λ_{max}. For different species, λ_{max} ranges from about 430 nm to 620 nm. A curious and not fully understood fact is that, if one plots the number of species with a given λ_{max} vs. λ, the curve shows that these absorption maxima tend to cluster about discrete wavelengths equally spaced at intervals of about 6.7 nm.[11]

Several recent studies have shown that the pigments are floating in membranes comprising the rod disks and cone invaginations. The pigments appear to move about freely on the surface of the bilayer membrane exhibiting both rotational and translational motions.[12,13] X-Ray studies indicate that much of the pigment lies outside of the membrane while part is anchored in the outer half of the bilayer.[14] This technique should soon enable one to monitor the actual changes in protein conformations in response to changing conditions. The native conformation and function of proteins associated with membranes depends upon the local environment. Recent successes in incorporating rhodopsin into synthetic membranes should enable the effects of different environments on proteins to be studied. Hubbell reports in his Account on his investigations of rhodopsin in synthetic, and hence chemically defined, membranes.

The binding of the chromopore to the opsin is through a Schiff base linkage to the protein. Thus, the aldehyde group on the retinal undergoes a condensation reaction with an amino group on the protein as

$$C_{19}H_{28}C{=}O + H_2N\text{-opsin} \rightarrow C_{19}H_{28}C{=}N\text{-opsin} + H_2O$$

Retinal will spontaneously condense with a number of simple amino acids. These compounds all have a λ_{max} of about 360 nm, far to the blue of rhodopsin. The spectra of the model compounds are shifted to about 440 nm when the Schiff base is protonated and even further to the red in highly polarizable solvents. On this evidence, the chromophore in rhodopsin is believed to be protonated and in a local environment that is highly polarizable.

One important aspect of the chromophore has so far not been considered. Because of its numerous double bonds, retinal can exist in many isomeric forms. The Account by Honig et al. considers from the theoretical point of view the conformational and spectroscopic properties of the retinal isomers. Interestingly, of the several stable isomers of retinal known, only the 11-cis is found in natural pigments.[15] This was deduced by determining the iso-

(9) G. Wald, *Nature (London)*, **132**, 316 (1933).

(10) G. Wald, P. Brown, and P. Smith, *J. Gen. Physiol.*, **38**, 623 (1954-1955).

(11) H. Dartnall and J. Lythgoe, *Vision Res.*, **5**, 81 (1965).

(12) C. Gitler, *Annu. Rev. Biophys. Bioeng.*, **1**, 51 (1972).

(13) M. Edidin, *Annu. Rev. Biophys. Bioeng.*, **3**, 179 (1974).

(14) B. Honig and T. Ebrey, *Q. Rev. Biophys.*, in press.

meric form of the chromophore after pigments were gently heated. Under these conditions the opsins denature, releasing their chromophores. The conditions were mild enough to preclude thermal isomerization. In all cases, only the 11-cis isomer was released.

Much effort has been directed at understanding the basic photochemistry of rhodopsin. When rhodopsin solutions are exposed to light, their color changes from red to yellow. It has become customary to refer to this change as "bleaching". In the bleaching of vertebrate rhodopsin, the chromophore becomes detached from the opsin. The first clue as to the bleaching of light was the observation that the chromophore after bleaching with light was in the all-trans form.[16] Since the previous studies had shown that rhodopsin initially incorporated 11-*cis*-retinal as the chromophore, it was concluded that the light acted to induce isomerization. Such cis–trans isomerizations in unsaturated compounds constitute a well-studied class of photochemical reactions.

The photolytic bleaching reaction is not simple. Many intermediate species have been observed, each characterized by its own absorption spectrum. Classically, the bleaching reaction was studied by lowering the temperature and slowing the reaction. Each intermediate was stable below a certain temperature, as indicated in Figure 2 of Abrahamson's Account. Modern technology has permitted kinetic investigations at physiological temperatures, and these studies have confirmed the presence of intermediates.[17] Abrahamson's Account discusses the dynamics of events following the absorption of a photon.

Only recently have chemists made headway in understanding how a nervous impulse is initiated by light. In the absence of light there is a current from the inner segment to the outer segment of the rod.[18] This so-called dark current is due to a flow of Na^+ ions. Light impinging on vertebrate rods causes the dark current to decrease because of a decrease in the permeability of the membrane to Na^+ ions. The change in membrane resistance is proportional to the intensity of light. A synapse at the base of the rod senses changes in current and responds by sending this information through the neural network for ultimate processing in the visual cortex of the brain. The precise role of rhodopsin in initiating the entire process is not understood. Williams addresses himself to this question in the final Account.

A chemist categorizes a chemical process in a living organism as "understood" only after its successive stages, intermediate compounds, and accompanying enzymes have all been elucidated. The ultimate aim is to describe each step by a chemical equation and to imitate the process *in vitro*. The chemistry of vision is not yet understood to this degree of detail and sophistication; vision remains an active area of research. The present issue of *Accounts* will delineate the present state of our knowledge of this fascinating field from several different perspectives.

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(16) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 673 (1968).
(17) G. Busch, M. Applebury, A. Lamola, and P. Rentzepis, *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 2802 (1972).
(18) W. Hagins, *Annu. Rev. Biophys. Bioeng.*, **1**, 131 (1972).