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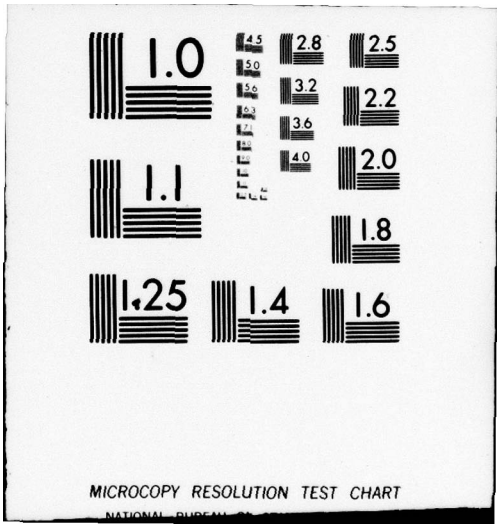
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(15) N00019-78-C-0306

LEVEL II

Final Report

on

Contract # N00019-78-C0306

entitled

(6) PRIMARY EVENTS IN VISION.

Naval Air Systems Command

(9) Final rept. May 1978 to May 1979

(12) 22

(10) Contractor: Aaron/Lewis

(11) 4 October 1979

Cornell Univ., Ithaca, NY

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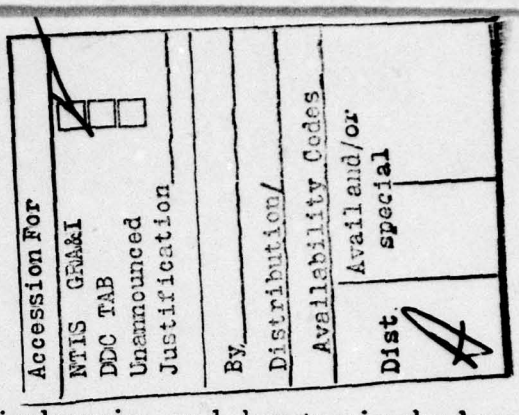

SUMMARY

Experiments on iodopsin and bacteriorhodopsin (which have thermal transitions from batho-iodopsin and batho-bacteriorhodopsin back to iodopsin and bacteriorhodopsin) suggest that light energy is stored as a result of the interaction of a photon with rhodopsin. Resonance Raman spectra of the batho intermediate in several rhodopsins indicate that the $-C=\overset{H}{\underset{+}{N}}-$ stretching vibration does not vary in going from rhodopsin to bathorhodopsin and this suggests that there is still a double bond between C_{15} and N, and a positive charge on the N after the photonic event. Furthermore the C-CH_3 vibration indicates that in bathorhodopsin there are changes in the vicinity of the CH_3 groups, and it is interesting to note that these carbons are tertiary centers which can stabilize charge. Finally, even though the $-C=\overset{H}{\underset{+}{N}}-$ vibration does not appear to be affected by the light event the C=C stretching vibration is significantly altered. These results indicate that light energy appears to be stored in a charge polarized state and at least in bacteriorhodopsin light emission is quenched in this state. Resonance Raman spectra obtained on the intermediates that follow the high energy batho form suggest a relocalization of C=C electron density in these later intermediates. The concluding event of this electron relocalization process is the release of the Schiff base proton which is detected by a reduction in the C=N vibrational frequency. The deprotonation of the Schiff base nitrogen occurs on the same time scale as the generation of a proton gradient in bacteriorhodopsin and the generation of a neural response in vertebrates. Thus it appears that release of the Schiff base proton may be critically tied to the role rhodopsin plays in energy transduction.

The deprotonation of the protonated Schiff base occurs in

all rhodopsins studied although in bacteriorhodopsin the deprotonation occurs apparently without a formal cis-trans isomerization. However the biological roles of bacteriorhodopsin and photoreceptor rhodopsins are completely different. Bacteriorhodopsin is a light driven proton pump while the role of vertebrate and invertebrate rhodopsins is essentially that of a quantum detector. In order to have a good quantum detector there must be irreversibility and we believe this is the role of cis-trans isomerization in photoreceptor rhodopsins. On the other hand in order to have a good energy converter, such as bacteriorhodopsin, conformational changes have to be minimized and reversibility has to be maximized. Thus the bacteria have evolved a molecule which can accomplish the release of the Schiff base proton reversibly without major changes in the conformation of the retinylidene chromophore and this enhances the biological role of bacteriorhodopsin to convert light energy into chemical energy.

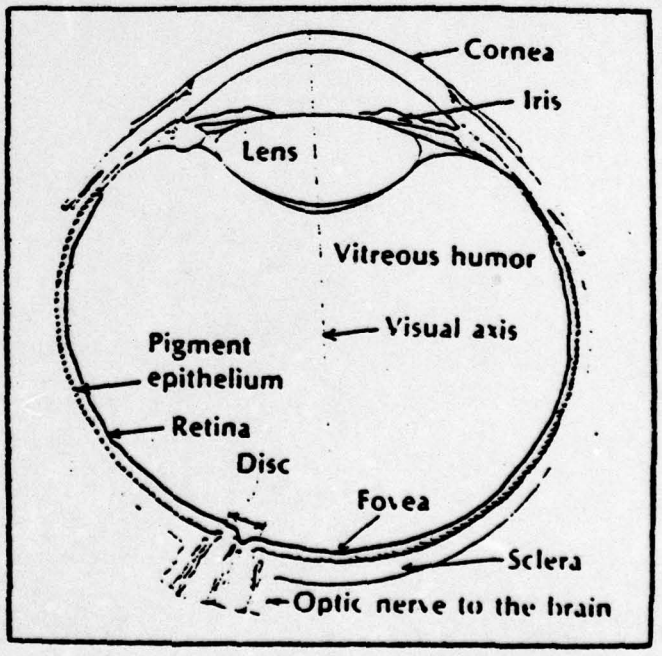

Fig.1. Cross section of a primate eye. Entering light is focused by the lens then passes through a clear, jelly-like substance (vitreous humor) onto the retina, a thin strip of tissue lining the back of the eyeball. The retina contains cone and rod photoreceptor cells. The cone cells, responsible for color vision, are centered in the fovea, a small indentation in the retina; the rod cells, responsible for black and white and dim light vision, are distributed throughout the rest of the retina.

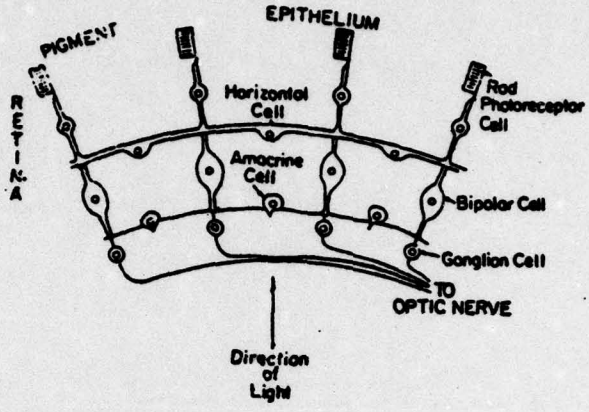

Fig.2. Cross section of the retina. After passing through a neuronal layer (ganglion cells, bipolar cells, amacrine cells, and horizontal cells) light impinges on the rod photoreceptor cells which absorb the light and generate a neuronal response. Light that passes through the retina finally strikes a pigmented layer called the pigment epithelium.

INTRODUCTION

The process of visual transduction is initiated when light is focused by the lens (see Fig. 1) onto the retina, a thin layer of tissue lining the back of the eyeball. Enmeshed in this tissue are two types of photoreceptor, or light transducing, cells: rods and cones. The rods are responsible for black and white and low light level vision; they are present in the eyes of vertebrates, sensitizing the entire retina. On the other hand, in primates cones are concentrated in only one small area of the retina, the fovea.

A cross section of the retina is seen in Fig. 2. Entering from the bottom, light pours through a series of neuronal layers. Their position notwithstanding, these layers do not take part in the light absorption which is the first step in the visual process but rather serve as an interface between the sensors (both rods and cones) and the optic nerve. Assembling and integrating the information from all of the cells, they transmit it in a sequence and form which is interpretable by the brain. Beyond the photoreceptor cells is the pigment epithelium. One of the roles of this layer of tissue is apparently chemical re-

be reversed to reactivate the sensors; the pigment epithelium functions as a recycling bath where this regeneration takes place [1]. It also has other important functions, such as decreasing scattering in the eye, especially at high light levels. It is the lack of pigment in this epithelial layer that accounts for the poor sight of albinos in bright light.

A cross section of a rod (photoreceptor) cell is shown in Fig. 3. Light enters the inner segment through its electrical connector, the synaptic terminal. The nucleus is, of course, the basic replicating element of the cell. The mitochondria are the energy source of the cell, manufacturing ATP (adenosine triphosphate). Although the production of ATP by the mitochondria is not related to the initial absorption of a photon, ATP may

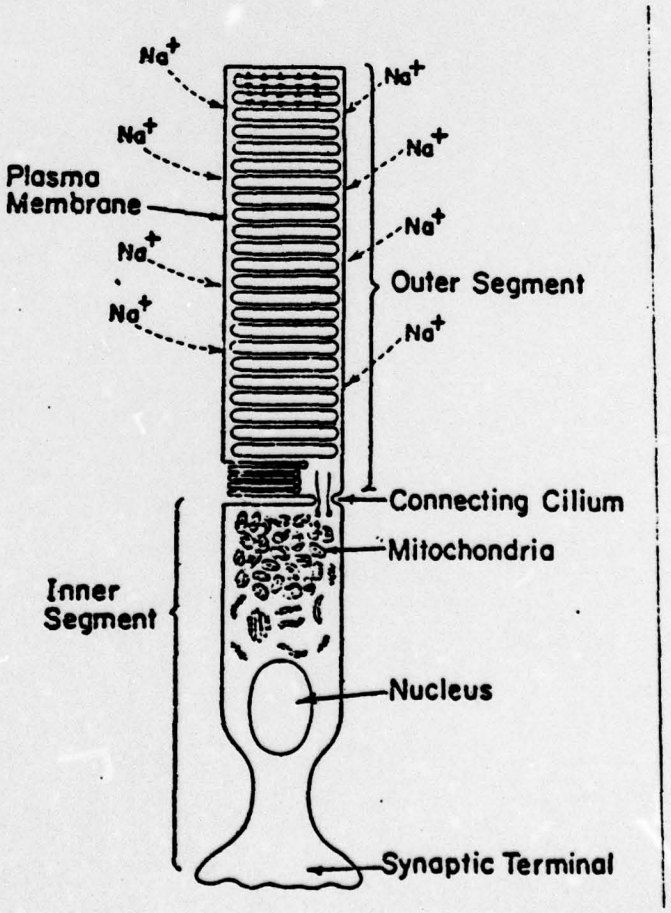

Fig.3. A schematic view of a rod cell in the eye of a vertebrate. The cell has an outer segment containing flat, pancake-like membranous discs in which are embedded the rhodopsin molecules (represented as triangles) in very high concentration. The outer segment is connected to an inner segment containing the nucleus and the mitochondria. The inner segment ends in a synaptic terminal. This connects the photoreceptor cell to the neuronal network which extends out of the retina through the optic nerve and eventually to the brain.

be instrumental at a later stage in the amplification of the energy of a single photon so it can be perceived by the brain. There is presently no concrete evidence to either support or refute this hypothesis, but Hagins and his coworkers have recently shown that the concentration of ATP does decrease after the rods absorb a photon [2]. What is certain is that photoreceptor cells consume large amounts of ATP and that the role of this ATP is not yet understood.

The part of the cell where the photon is absorbed is known as the outer segment. The light energy is absorbed by a molecule called rhodopsin which converts, within milliseconds, the photon's energy into the chemical species responsible for generating a neural response. Several thousand rhodopsin molecules are embedded in the membrane of a few hundred Frisbee-like discs which are stacked within the rod's outer segment. Surrounding but not connected to the discs is a plasma membrane. Unexposed to light Na^+ ions permeate the plasma membrane giving rise to a "dark current" which is interpreted by the brain as darkness. When even a single photon strikes a rhodopsin the signal is somehow amplified and the flow of Na^+ ions is blocked, causing an electrical imbalance (hyperpolarization) across the plasma membrane. The resulting neural response is finally detected by the brain as vision [3].

What is not fully understood is all of the steps between absorption of the photon and generation of the neural response. Despite the fact that a fundamental breakthrough in research in this area occurred over 40 years ago, an accepted theory to identify and explain the entire train of events is yet to be offered.

Harvard was the scene of much of the pioneering work in visual transduction. There George Wald, who had extracted rhodopsin from eyes in the first of many successful experiments designed to elucidate the visual process, was able to follow (mainly through absorption spectroscopy) changes in the rhodopsin molecule when it absorbed a photon [4].

Figure 4 summarizes part of the knowledge gained by Wald and coworkers, Abrahamson and coworkers [5a,b] and many others [6]. Rhodopsin (mol. wt. ~36,000) is composed of a membrane glycoprotein matrix called opsin and a form of vitamin A called

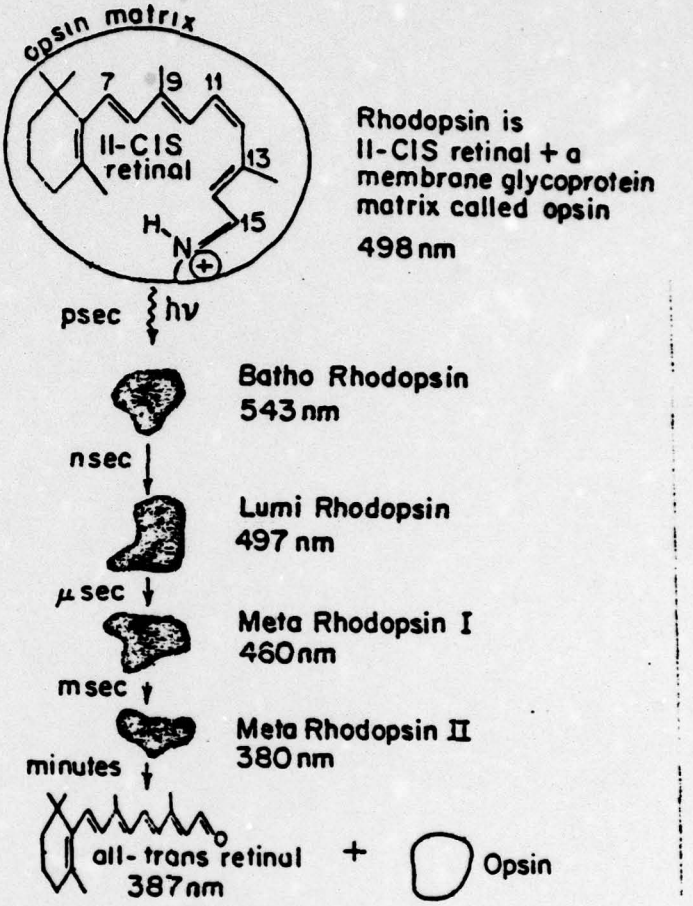

Fig.4. The chemical composition and the light-induced conformational changes of rhodopsin, the principal light absorber in the eye. Rhodopsin is a large molecule consisting of a small (45-atom) unit called retinal embedded in a matrix called opsin. The center of photochemical activity is the double-bonded (12-atom) segment of the retinal and those atoms electronically coupled to this region of the molecule. It is the vibration of these atoms that is selectively observed in the resonance Raman spectrum of rhodopsin.

retinal. All rhodopsins studied have this same form of vitamin A but they differ in the composition of their opsin. In fact all photoreceptor rhodopsins have even the same isomeric form of retinal, the 11-cis isomer. However this primacy of the 11-cis isomer was recently broken when a rhodopsin-like protein was discovered in a bacterium called Halobacterium halobium [7a,7b]. This rhodopsin-like molecule called bacteriorhodopsin has been shown to be a critical element in the bacterium's bioenergetics [8].

The vitamin A (retinal) component of rhodopsin is the site of photon absorption. The retinal is connected to the opsin through a Schiff base linkage. Here the retinal, which is an

aldehyde with a $-\overset{H}{C}=O$ end group, is complexed to the ε-amino (NH_2) group of a lysine residue of the protein to form the retinylidene chromophore of rhodopsin [9].

When Wald extracted retinal from its opsin matrix in the dark he found surprisingly by chromatography and absorption spectroscopy that it was twisted in a constrained 11-cis form. (Linus Pauling had felt that this cis form was so severely strained as to altogether preclude its existence.) Exposure to light, Wald found, finally produced in the last step of the reaction (see Fig. 4) the trans form. Although the isomeric configuration of the retinylidene chromophore in each of the transient intermediates (see Fig. 4) could not be analyzed, it was quite plausible to conclude that light triggered the cis-retinal back to its unstrained trans form. To Wald this isomerization constituted the entire primary photonic event: light was but the catalyst providing the small amount of energy needed to spring the already energized molecule open.

Subsequent work, ours and that of others, has extended that first premise. We have suggested that the isomerization is energized by the light, not simply triggered by it [10]. It is known that there are at least five initial steps in the process of vision. In the first step an intermediate species called bathorhodopsin is produced in less than 6 psec after the absorption of light by rhodopsin [11,12], and after a lifetime of about 30 nsec at physiological temperatures it decays to lumirhodopsin [11].

The transformations of lumirhodopsin to metarhodopsin I and II take place in μsec and msec, respectively, as absorption flash spectroscopy has shown [5a,b]. In vertebrates the neural response begins during the milliseconds between the formation of metarhodopsin I and of metarhodopsin II. Taking minutes, the final change of opsin to free trans-retinal is considered ancillary to vision. It appears to be the beginning of the chemical recycling phase. Once a retinal molecule is struck by a photon it is deactivated, and enzymatic reactivation (regeneration) of the cis form can be very slow. There are always, however, enough rhodopsin molecules on hand so that vision can proceed without interruption.

Through resonance Raman spectroscopy we have been able to

probe the electronic and molecular structure of the retinylidene chromophore (the center of photochemical activity in vision) by following changes in its vibrational spectrum. Where other forms of spectroscopy – nuclear magnetic resonance, electronic paramagnetic resonance, circular dichroism, fluorescence, and absorption – have met with at best limited success in further elucidating the process of vision, resonance Raman spectroscopy is proving to be a powerful new tool in the study of the various steps in visual excitation.

RESONANCE RAMAN SPECTROSCOPY

Resonance Raman spectroscopy is essentially a specialized branch of Raman spectroscopy, which was discovered independently by C. V. Raman in India and L. I. Mandelshtam and G. S. Landsberg in Russia about 50 years ago. Raman spectroscopy is the *inelastic scattering of light off* the vibrational energy levels of molecules. Classically, to gain a physical appreciation of Raman spectroscopy one considers photons interacting with a vibrating molecule. The photons exchange their momenta with the various vibrational modes, and this causes the molecule to vibrate with larger amplitudes and at higher energy (Stokes scattering). The resulting photons are scattered with less momentum, less energy, lower frequency, and longer wavelength. The differences between the unchanging frequency of the incident photons and the frequencies of the scattered photons are precisely the vibrational frequencies of the molecule under study. Because only one photon in a million is scattered in such an inelastic event the application of the laser, a high-powered, spatially coherent, single-frequency light source, has revolutionized the technique. There are, however, certain major problems with Raman spectroscopy as described above. Firstly, the spectrum one obtains from such an experiment is essentially nonselective. All of the active vibrations of the molecule are observed, and this would result in the spectrum of a 36,000-molecular-weight protein which is quite difficult to interpret. Secondly, Raman scattering even with a laser is still a very weak process and thus insensitive. In essence macromolecules

which is well out of the physiologically relevant concentration range of most proteins and nucleic acids.

Both these problems, selectivity and sensitivity, are overcome in a resonance Raman experiment. In this specialized form of Raman spectroscopy recently developed tunable lasers allow selection of an incident laser frequency which is in resonance (near-coincidence) with the electronic absorption of a particular molecular entity such as the retinylidene chromophore of rhodopsin. In the scattered light the ground state vibrational spectrum of the chromophore (at concentrations of 10^{-5} or less) is selectively observed above the background of surrounding vibrations from the membrane glycoprotein (opsin) matrix. This selectivity and sensitivity of resonance Raman spectroscopy, along with the advantages of non-resonance Raman – the weak to nonexisting Raman spectrum of water which obliterates ir vibrational spectra in aqueous media and the experimental flexibility of simply scattering a laser beam 1μm or less in diameter from particular areas of even opaque samples – makes this a new biophysical tool with a great deal of potential.

The serious disadvantage of this technique is fluorescence. Because fluorescence and Raman spectra are often superimposed, and because the intensity of the former is frequently much greater, fluorescence often presents a serious obstacle to Raman studies. Many photobiological systems, however, are found to fluoresce either very weakly or not detectably. Of course this should be expected intuitively: absorbed photons are thereby preserved for photochemistry, not reemitted where they may interfere. Thus, from an experimental standpoint these photobiological systems are ideal for the application of Raman spectroscopy. Furthermore, it has been shown [13,14] that the ground state vibrational modes that are most intense in resonance Raman spectroscopy are the ones that exhibit large nuclear excursions between the ground and excited state. In summary then photobiologically speaking resonance Raman spectroscopy not only selectively probes the center of photochemical activity in its natural membrane environment, but it also singles out the specific vibrational modes that lead the molecule along its photochemical pathway.

UNANSWERED QUESTIONS

The process of visual transduction in rod cells can actually be subdivided into two related and unanswered quations. First, how is light energy absorbed and stored by rhodopsin? Second, how is the light energy converted into the chemical species which mediates the transport of sodium ions across the plasma membrane to generate the hyperpolarization?

To begin to answer these fundamental questions through resonance Raman spectroscopy we felt that it was important to clarify the chemical constitution of rhodopsin. We obtained the first resonance Raman spectrum of the retinylidene chromophore in an extract of bovine rhodopsin [15,16]; more recently we have obtained the spectrum with a live eye preparation [17] and with bacteriorhodopsin [18]. By selectively observing the carbon-nitrogen vibration above the background of surrounding molecular species we were able to demonstrate that the Schiff base is protonated. Although this contradicted an earlier suggestion, subsequent workers have also found that the Schiff base linkage is indeed protonated [19-22]. This observation is significant, for if the linkage were unprotonated it would dramatically alter the photochemical basis of visual transduction. In fact, work in our laboratory has shown that this observation may assume greater importance in the later steps of visual transduction.

BIOENERGETICS OF THE VISUAL PROCESS

The energetics of the interaction of a photon with rhodopsin can be expressed in two divergent ways as depicted in Fig. 5. Assuming that the primary photonic event in vision is a simple cis-trans conversion, then based on previous investigations of cis-trans isomerizations in other systems it would be necessary to assume that bathorhodopsin is at a lower energy than the initial cis-rhodopsin (see Fig. 5A). The alternative scheme of events seen in Fig. 5B, however, appears to us to represent what actually happens in vision. Here part of the energy of the 2 eV photon is absorbed by the cis-rhodopsin and is stored

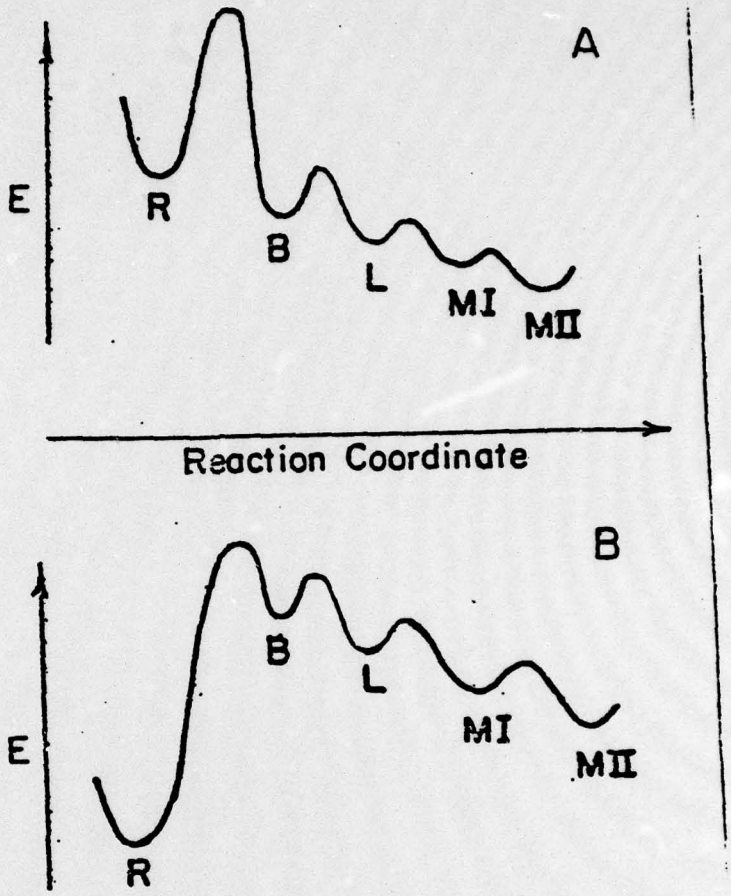

Fig.5. Diagrams depicting two possible schemes for the relative energies of rhodopsin (R) and the intermediates (batho, B; lumi, L; meta I, MI; and meta II, MII) generated thermally as a function of time after light is absorbed. It can be shown that the lower diagram is correct. Here the part of the energy of the photon absorbed by rhodopsin in the first step is stored in B (the batho intermediate). This energizes the subsequent steps in the process.

transduction as one more example of cellular energy transduction.

Data in an early paper by Wald and coworkers support this latter hypothesis. In this work the anomalous behavior of iodopsin (a chicken rhodopsin) was reported. In all other rhodopsins studied the six-step reaction depicted in Fig. 4 proceeds unidirectionally as shown. The thermal sequence can be slowed – even halted – if the temperature is reduced enough, but at physiological temperatures the reaction is ordinarily a one-way street proceeding inexorably towards the trans form. With iodopsin, though, Wald observed an anomaly. When its bathorhodopsin was formed photolytically at 77°K (where it is stable indefinitely) and then heated, it reverted to the cis-

Fig. 5B can explain this anomaly in that the batho form is at a higher energy than the cis-retinal [10]. The fact that no other rhodopsin has been found to exhibit this behavior is explainable, too. The energy "well" is too deep, and the thermodynamic barrier is too great, for the reaction to be reversed.

This explanation has been further supported by the spectral properties of the recently discovered bacterial rhodopsin which behaves similarly [10]. Independent of our work, and outside the scope of this lecture, Rosenfeld et al. [23] have concluded from an elegant kinetic argument that all bathorhodopsins are at higher energies than the cis form.

EXPERIMENTAL METHODS

Although resonance Raman spectroscopy did not contribute materially to this theory regarding the energetics of the photochemical reaction, it can reveal the mechanism by which the energy is stored and subsequently converted into the chemical species that generate a neural response. One type of resonance Raman experiment consists of photolyzing the rhodopsin and probing the resonance Raman spectrum of the photostationary mixture produced at low temperatures with a single laser beam [18,19]. Alternatively a dual laser beam technique can be employed [19]. In this method one laser frequency is used to excite the photochemistry while a second laser beam at another frequency is used to probe the resonance Raman spectrum of a particular species. In either case, depending on the temperature and the exciting and probing laser wavelength, a photostationary mixture of rhodopsin and its thermal intermediates is produced. Varying the temperature and wavelength changes the relative concentration of the intermediates, and spectral bands can be assigned to the various intermediates seen in Fig. 4. To obtain the resonance Raman spectrum of such a photostationary mixture the laser light inelastically scattered off the sample is focused into a double monochromator which is computer controlled through circuitry designed at Cornell [24]. The light scattered at wavelengths which are different to the

detected with a low dark count cooled photomultiplier. The counts recorded by the photomultiplier at each wavelength setting of the monochromator as the monochromator is driven to lower energies away from the incident laser frequency can then be plotted as a function of wavelength.

The resonance Raman spectrum of the retinylidene chromophore of rhodopsin and its thermal intermediates consists of several bands which are associated with the ~17 atoms either directly or indirectly connected to the conjugated portion of the molecule. Where most of these bands originate has not yet been determined but, fortunately, three of the vibrational modes have been unequivocally identified – they correspond to the C=C stretching frequency [21,25-28] (the most intense vibrational mode in the spectrum), the $-C=\overset{H}{\underset{+}{N}}-$ stretching vibration (which was identified by deuterating the Schiff base nitrogen [18,19]), and the C_9-CH_3 and C_{13}-CH_3 stretching vibrations (which were assigned using chemically modified retinals [29]).

THE SPECTRUM OF RHODOPSIN

Although most of the resonance Raman spectrum of the retinylidene chromophore has not yet been assigned, we have found that the spectrum of rhodopsin can be modeled with the resonance Raman spectrum of an 11-cis protonated Schiff base. This is significant because such data has a considerable bearing on the explanations that have been given for the color of visual pigments. As we have recently pointed out [30] there have in general been two differing theories which have tried to explain why the retinylidene chromophore in opsin absorbs, as a rule, significantly to the red of the free chromophore. One model predicts that this "red shift" is caused by excited state charge stabilization [31,32] while an alternate theory suggests that the "red shift" is induced by ground state destabilization [33,34]. Our experimental data show that the ground state resonance Raman spectrum of the retinylidene chromophore can be modeled by the vibrational spectrum of the free chromophore. This indicates that the ground state of the retinal is not seriously perturbed by opsin. Thus the color of visual pigment

by the opsin matrix.

THE C=C AND C-CH_3 STRETCHING VIBRATIONS

The vibrational modes that have been assigned are already providing detailed molecular and electronic information on the primary events in visual transduction. Both the C=C and the C-CH_3 stretching frequencies are significantly altered when light interacts with rhodopsin producing the batho intermediate – the primary photochemical product. We have analyzed this data to mean [10] that light polarizes the electron density in the chromophore and the protein matrix stabilizes this high energy charge polarization in the retinal. In the subsequent intermediates which follow the charge polarized batho state the frequency of the C=C stretching vibration indicates that a process of electron relocalization occurs. After comparing these data with the weak emission spectra of the retinylidene chromophore observed in our laboratory (with our tunable laser Raman spectrometer which is also an exceptional fluorimeter) [35] we have been able to suggest that the batho intermediate is similar in nature to the excited state of the retinylidene chromophore of rhodopsin from which it evolves.

KINETIC RESONANCE RAMAN SPECTRA OF THE C=C STRETCH AND C=N STRETCH

We can now study the state of protonation of the Schiff base linkage as a function of the electron polarization and relocalization process described above. This can be accomplished either under photostationary conditions, as already discussed, or with the recently described kinetic resonance Raman spectroscopy (KRRS) [36]. In Fig. 6 KRRS follows the electron polarization-relocalization process and the state of protonation of the Schiff base by monitoring simultaneously the C=C and $-C=\overset{H}{\underset{}{N}}^{+}-$ stretching frequencies. The resonance Raman spectrum of bacterial rhodopsin in Fig. 6B is compared to a steady-state spectrum (Fig. 6A) obtained under conditions where the predominant species are bacterial rhodopsin (bR_{570}) and an intermediate

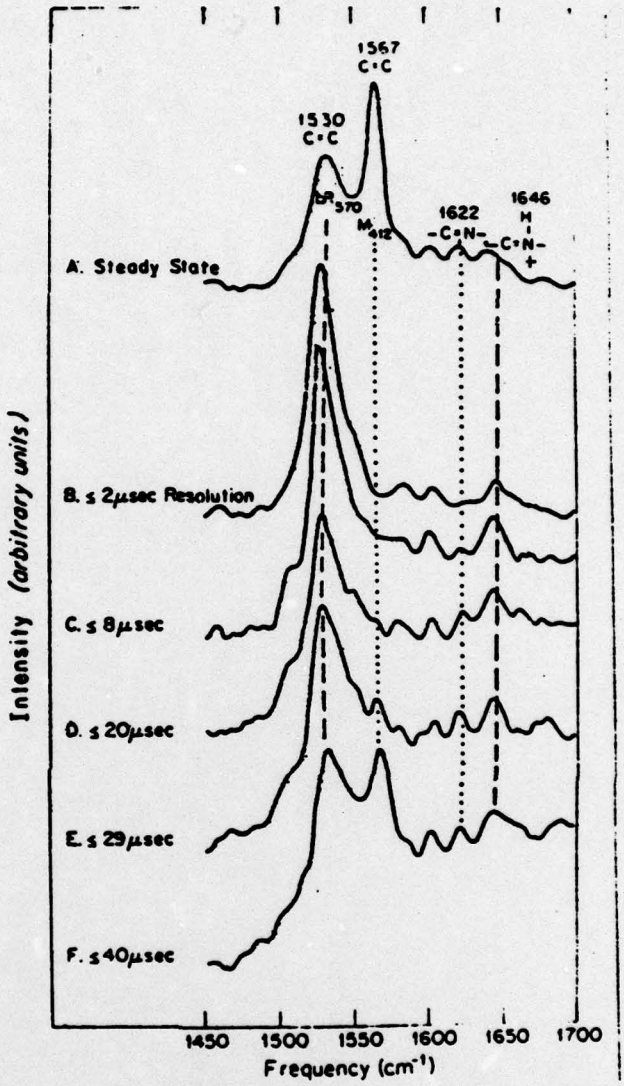

Fig.6. Steady state and kinetic resonance Raman spectra of bacterial rhodopsin over a limited frequency region. These spectra illustrate the dynamics of the deprotonation of the Schiff base linkage as a function of the polarization and relocalization of electron density in the C=C bond.

shown [18] by deuterating the Schiff base that the band at 1646cm^{-1} was due to the protonated Schiff base linkage and this band, together with the C=C stretch at 1530cm^{-1}, arose from bacterial rhodopsin [18]. In addition, our investigations had shown that the band at 1622cm^{-1} (the carbon-nitrogen vibration for an unprotonated Schiff base) and the C=C stretch at 1567cm^{-1} corresponded to the M_{412} intermediate. The KRRS in Fig. 6B-6E were obtained with a laser frequency which maximized the resonance enhancement of the M_{412} intermediate. As is clearly seen in these spectra the deprotonation of the Schiff base

occurs in several milliseconds before the onset of the C=C stretch of the M_{412} intermediate. This indicates that the deprotonation of the Schiff base is probably a direct consequence of the relocalization process (producing the intermediate in bacterial rhodopsin corresponding to Meta I), which follows the formation of the electron-polarized high-energy batho form. Thus the deprotonation of the Schiff base appears to be a result of the primary photochemistry. Similar conclusions about the protonation of the Schiff base linkage have been obtained on photoreceptor rhodopsins where the deprotonation of the Schiff base in bovine rhodopsin seems to occur on the same time scale as the generation of a neural response between metarhodopsin I and II.

Thus, as is seen in Fig. 7, the interaction of a photon with rhodopsin can be described as an electron polarization process which stores part of the photon's energy to energize the later

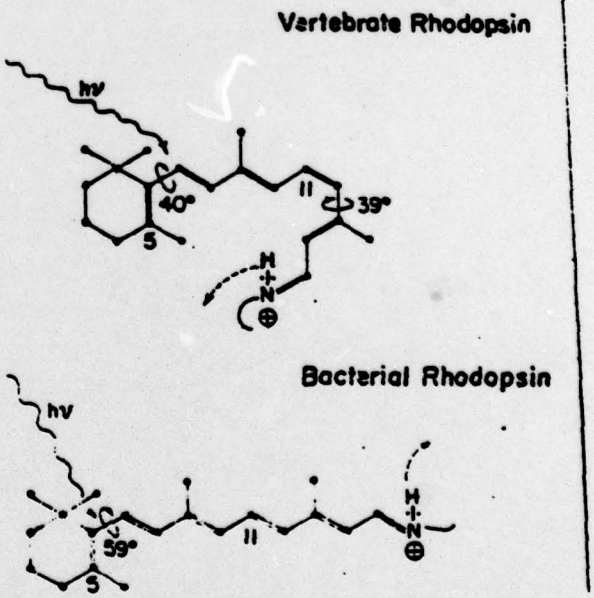

Fig.7. In both vertebrate and bacterial rhodopsin absorption of a photon eventually causes a deprotonation of the Schiff base linkage. Vertebrate rhodopsin, however, is initially in an 11-cis conformation and converts to an all trans conformation before the loss of a proton. Bacterial rhodopsin, on the other hand, is trans initially and is also trans when the deprotonation occurs, it is suggested that the 11-cis to trans isomerization is essential in all photoreceptor rhodopsins to prevent reversibility of the reaction which is fundamental to the operation of a good quantum detector. Such irreversibility would be a detriment to bacterial rhodopsin which is an energy

relocalization of electron density which leads to the ejection of the Schiff base proton [10]. Furthermore, in all photoreceptor rhodopsins the stored photon energy also energizes the formal cis-trans isomerization of the retinylidene chromophore. Bacterial rhodopsin has identical spectral properties to all other rhodopsins, even though the chromophore has been shown to be initially trans [7a,7b,8] and we have recently demonstrated using KRRS that the chromophore in Meta II (M_{412}) is also all-trans. This suggests that bacterial rhodopsin can accomplish the electron polarization, relocalization and the proton ejection without a detectable isomerization. In addition it has also been shown that in going from protonated bacterial rhodopsin to unprotonated M_{412} the bacterial system misses one of the intermediates in the vertebrate sequence [37,38]. Furthermore the chromophore of the bacterial rhodopsin does not detach from the matrix as shown in Fig. 4 but instead this is the only rhodopsin-like system that can regenerate itself thermally in the dark without the help of an enzymatic process a few milliseconds after M_{412} is produced [37,38]. At first all of these aspects of bacterial rhodopsin were quite puzzling. It suggested that bacterial rhodopsin (presumably a primitive form of rhodopsin) could accomplish more effeciently all the principal spectral transitions seen in photoreceptor rhodopsins. If, however, these spectral similarities are analyzed in the context of biological differences between the rhodopsins, then a very fundamental understanding of the nature of the 11-cis to trans isomerization in vision results.

THE 11-CIS TO TRANS ISOMERIZATION

To appreciate the role of the cis-trans isomerization in vision let us consider the biological role of bacterial rhodopsin. W. Stoeckenius and D. Oesterhelt [7a], who discovered bacterial rhodopsin in 1971, have shown that this rhodopsin-like protein is an energy converter for the bacterium [8]. When the bacteria are grown under anaerobic conditions they inhibit the normal mechanisms for energy transduction (which require oxygen) and generate a rhodopsin-like membrane protein

rhodopsin absorbs light energy for the bacterium, ejects the Schiff base proton, and forms a proton gradient across the bacterial cell membrane [18]. The proton gradient participates in the formation of ATP in the bacterium [39]. Thus bacterial rhodopsin is a light-driven proton pump. Its biological function is to convert light energy into chemical energy. On the other hand, photoreceptor rhodopsins are not energy converters but quantum detectors, and photoreceptors are exceptional quantum detectors, capable of detecting a single photon. For a good quantum detector there must be irreversibility, and this we believe to be the role of the 11-cis to trans isomerization in all photoreceptor rhodopsins [10]. The bacterial rhodopsin, however, has to optimize energy conversion, so the bacteria have evolved a rhodopsin-like protein which minimizes endoenergetic conformational changes and maximizes reversibility [10]. In so doing it sacrifices the high quantum efficiency (~65%) of photoreceptor rhodopsins by approximately a factor of two.

FUTURE RESEARCH

Future research will have to focus on the role of the ejected proton in the cellular energy transduction which produces a neural response. In bacterial rhodopsin it is clear that the Schiff base proton plays an important part in stimulating the formation of a proton gradient. It is tempting to suggest, based on the spectral similarities, that rhodopsin in photoreceptor cells also acts as a light-driven proton pump. Unfortunately no evidence exists that supports this hypothesis, and it has already been pointed out that bacterial and photoreceptor rhodopsins have widely differing biological functions. Therefore, it is quite possible that bacterial rhodopsin is only a good model system for the photochemistry which is probably similar in all rhodopsins. Contrarily, the proton ejection by the photoreceptor cells may be only the first step in a complicated sequence of reactions which ultimately results in calcium ions' mediation of sodium transport across the plasma membrane. It seems probable that an important element

words, the decrease in ATP after the absorption of a photon [2] may (in some as yet undetermined way) be the bridge between the ejected proton in the disc membrane and the generation (by calcium ions) of the hyperpolarization across the plasma membrane which eventually leads to a neural response.

REFERENCES

1. C. H. Baumann, in H. J. A. Dartnall (Editor), Handbook of Sensory Physiology, Vol. VII/1, Springer-Verlag, Berlin, 1972, Chap 10, p. 395.
2. W. E. Robinson, S. Yoshikami, and W. A. Hagins, Biophys. J., 15(1975)169a.
3. S. Yoshikami and W. A. Hagins, in H. Langer (Editor), Biochemistry and Physiology of Visual Pigments, Springer-Verlag, Berlin, 1973, p. 245.
4. G. Wald, Science 162(1968)230.

5a. E. W. Abrahamson and S. E. Ostroy, Progr. Biophys. Molec. Biol. 17(1967)179.

5b. E. W. Abrahamson and J. R. Wiesenfeld, in H. J. A. Dartnall (Editor), Handbook of Sensory Physiology, Vol. VII/1, Springer-Verlag, Berlin, 1972, Chap 3, p. 69.

6. T. G. Ebrey and B. Honig, Quart. Rev. Biophys. 8(1975)129.

7a. D. Oesterhelt and W. Stoeckenius, Nature (New Biology) 233(1971)149.

7b. L. Y. Jan, Vision Res. 15(1975)1081.

8. R. Henderson, Ann. Rev. Biophys. and Bioeng. in press 1977.
9. D. Bownds, Nature 216(1967)1178.
10. A. Lewis, Biophys. J. 16(1976)204a.
11. G. Busch, M. Applebury, A. Lamola and P. Rentzepis, Proc. Natl. Aca. Sci. USA 69(1972)2802.

12. E. P. Ippen, C. V. Shank, A. Lewis and M. Marcus, Science 200(1978)1279.
13. A. Warshel and M. Karplus, J. Am. Chem. Soc. 96(1974)5677.
14. A. Y. Hirakawa and M. Tsuboi, Science 188(1975)359.
15. A. Lewis, R. Fager and E. W. Abrahamson, J. Raman spectrosc. 1(1973)465.
16. A. Lewis and J. Spoonhower, in S. Chen and S. Yip (Editors), Spectroscopy in Biology and Chemistry, Academic Press, New York, 1974, Chap 11, p. 347.
17. A. Lewis, Fed. Proc., Fed. Am. Soc. Exp. Biol. 35(1976)51.
18. A. Lewis, J. Spoonhower, R. A. Bogomolni, R. H. Lozier and W. Stoeckenius, Proc. Natl. Acad. Sci. USA 71(1974) 4462.
19. A. R. Oseroff and R. H. Callender, Biochemistry 13(1974) 4243.
20. R. Mathies, A. R. Oseroff and L. Stryer, Proc. Natl. Acad. Sci. USA 73(1976)1.
21. R. H. Callender, A. Doukas, R. Crouch and K. Nakanishi, Biochemistry 15(1976)1621.
22. R. Mendelsohn, Biochim. Biophys. Acta in press 1976.
23. T. Rosenfeld, B. Honig, M. Ottolenghi, J. Hurley and T. G. Ebrey, Proceedings of IUPAC Photochemistry Meeting, Aix en Provance, July 1976, to be published.
24. G. Perreault, R. Cookingham, J. Spoonhower and A. Lewis, Appl. Spectrosc. in press.
25. L. Rimai, D. Gill and J. Parsons, J. Am. Chem. Soc. 93 (1971)1353.
26. D. Gill, M. Heyde and L. Rimai, J. Am. Chem. Soc. 93 (1971)6288.
27. M. Heyde, D. Gill, R. Kilponen and L. Rimai, J. Am. Chem. Soc. 93(1971)6775.
28. R. Cookingham, A. Lewis, D. Collins and M. Marcus, J. Am. Chem. Soc. 98(1976)2759.
29. R. E. Cookingham, A. Lewis and A. Kropf, submitted to J. Mol. Biol.
30. M. Sulkes, A. Lewis, A. Lemley and R. E. Cookingham, Proc. Natl. Acad. Sci. USA in press, Dec. 1976 or Jan. 1977.

31. A. Kropf and R. Hubbard, Ann. N. Y. Acad. Sci. 74(1958) 266.
32. B. Honig, A. D. Greenberg, U. Dinur and T. G. Ebrey in press, Biochemistry.
33. C. S. Irving, G. W. Byers and P. A. Leermarkers, Biochemistry 9(1970)858.
34. P. E. Blatz, Photochem. Photobiol. 15(1972)1.
35. A. Lewis, J. P. Spoonhower and G. J. Perreault, Nature 260(1976)675.
36. M. Marcus and A. Lewis, Science in press.
37. R. H. Lozier, R. A. Bogomolni and W. Stoeckenius, Biophys. J. 15(1975)955.
38. M. C. Kung, D. Devault, B. Hess and D. Oesterhelt, Biophys. J. 15(1975)907.
39. E. Racker and W. Stoeckenius, J. Biol. Chem. 249(1974) 662.