Dynamics of Opsin, a Visual Protein

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Within a millisecond or so after a flash of light has been absorbed by the rhodopsin of rod cells, the first sign of neural excitation of the retina can be detected. In the vertebrate retina, this consists of a transient increase in the membrane potential of the rod. The potential develops and then decreases, the whole process taking a few hundred milliseconds if the stimulus is a short flash of moderate intensity. This brief change of potential is transmitted to higher order neurons in the retina and, just before the signal leaves the retina, it is encoded into a train of all-or-none action potentials. These "spikes" then travel up the optic nerve to the brain where "perception" of the stimulus occurs. The individual processes comprising what we call "vision" are all subjects of extensive investigations, and this Account must restrict itself to but one area, *viz.*, the rhodopsin reactions that go on in the first few milliseconds after light has been absorbed. The ultimate goal of our research is to determine how rhodopsin transduces light into the *first* neural event.

Ten years ago our laboratory began studying, simultaneously, the pigment reactions, "bleaching," and the neural response. The approach was aimed primarily at determining the rate and stoichiometry of the pigment reactions on the one hand and their neural correlates on the other. Figure 1 shows the receptor response. Its shape originally suggested that it may be tracing out the concentration of a hypothetical substance, "B," which is involved in the sequence of reactions, A → B → C. Initially an effort was made to see if any of the rhodopsin reactions had rates corresponding to the A → B step or to the B → C. The idea was to study the temperature dependence of the "rate of rise" of the response and obtain the activation parameters for the chemical reaction responsible for the production of the neural response. These were then to be correlated with their counterparts in the pigment reactions. Also, it was assumed that each molecule of pigment which absorbs light contributes a fixed increment of voltage to the response. Such an assumption was necessary if the "stoichiometry" of the neural response was to be learned and correlated with the pigment reactions. This kind of reasoning was later used with considerable success by others[1,2] in their experiments on the "early receptor potential" (a nonphysiological response), but it proved to be too simple for the neural event of interest here.

At the very outset, a serious problem was encountered because the stoichiometry of the pigment reactions themselves was ill-defined. For example, the relationship between pigment bleached and intensity was not understood. Consequently, it was decided that fundamental work such as this had to be undertaken and, reluctantly, the attempts at correlating the chemistry with the neurophysiology were temporarily set aside. That marked the start of several years of work devoted exclusively to understanding the rhodopsin reactions, and only recently our efforts have been turned again toward the problem of correlating the neurophysiology with the photochemistry. Most of what follows then will be a description of work on rhodopsin.

Wald and Hubbard[3] showed that the action of light on rhodopsin is to isomerize 11-*cis*-retinal to the all-trans configuration—a process which, presumably, occurs within a few picoseconds.[4] The isomerization of retinal is the signal to opsin, the protein component of rhodopsin, to undergo a sequence of thermal, or "dark," reactions, and most of us believe it is one of these reactions of *opsin* which is intimately involved in the first neural event. We base this belief primarily on temporal grounds: the photoisomerization of retinal is completed well before the first sign of neural activity appears but, on the other hand, the opsin reactions are going on at the same time that the neural event begins. In particular, there is a reaction, metarhodopsin I ⇌ metarhodopsin II, which is a likely candidate for the key opsin step. Because of its possible importance to vision, the meta-I–meta-II interconversion has been the center of much, but not all, of the work in our lab—especially in the last several years.

Effects of High Intensity: Photoreversal of Bleaching

That the stoichiometry of pigment reactions is complex was indicated by some of the early work of Hagins.[5] He showed that, regardless of its intensity, a short flash never bleached more than about 50% of the pigment. In an effort to explain this, a model (Figure 2) of the bleaching process was adapted from Grellman, *et al.*[6] Wavy-shafted arrows are photo-

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(1) R. A. Cone, *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 483 (1965).
(2) W. L. Pak, *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 493 (1965).
(3) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952).
(4) G. E. Busch, M. L. Applebury, A. A. Lamola, and P. Rentzepis, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2802 (1972).
(5) W. A. Hagins, *J. Physiol.*, **129**, 22 (1955).
(6) K. H. Grellman, R. Livingston, and D. Pratt, *Nature (London)*, **193**, 1258 (1962).

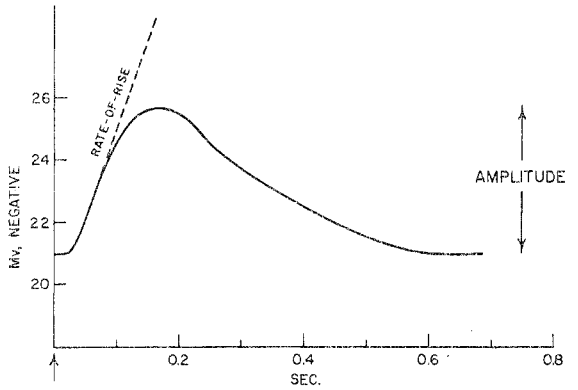

Figure 1. Simulated vertebrate receptor potential. A brief flash is given at $t = 0$ (arrow) and the receptor cell membrane potential transiently becomes more negative. The rate of rise of the potential reflects the rate of the pigment reaction responsible for the potential in the simplified discussion given in the text. The amplitude will be proportional to the number of pigment molecules undergoing the transduction reaction (also, in the oversimplified case).

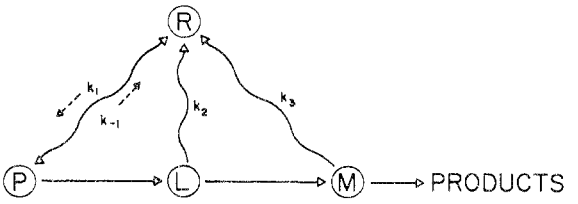

Figure 2. Mechanistic scheme for rhodopsin reactions. R is rhodopsin, P is prelumirhodopsin (bathorhodopsin), L is lumirhodopsin, and M is metarhodopsin. "Products" refers to all steps subsequent to the thermal decay of M. Wavy-shafted arrows indicate photochemical processes, straight-shafted arrows, thermal processes. The k's are specific rates.

reactions and straight-shafted arrows are thermal, "dark" reactions. Note that light starts the bleaching process by converting R into P, but more light, absorbed by P, L, or M, can reverse this. Two views of bleaching were developed[7] from this early model: (a) the rate of bleaching depends upon the net rate of accumulation of labile species; and (b) the number of labile molecules which accumulates in a certain period is the number which has absorbed an odd number of quanta by the end of that period. Both views, based on the photoreversibility of bleaching, lend themselves to concise mathematical formulation. The expected amounts of bleaching at various intensities, calculated according to kinetic and statistical formulas, gave very close fits to experimental data.

The process of photoreversal of bleaching results in unusual behavior of rhodopsin compared with simple photochemical systems. For example, one very high intensity, short flash will bleach less pigment than two flashes at half the intensity; four flashes at one-fourth intensity bleach still more. This is shown in Figure 3. As a result, the bleaching of rhodopsin is associated with a variable quantum efficiency, γ. For short (less than 1 msec) flashes γ depends upon intensity as shown in Figure 4. In the limit as the intensity approaches infinity, γ approaches 0.0, since only 50% of the pigment will bleach even though an infinite number of quanta are absorbed. For a while, the *extent* of bleaching was confused with the *quantum efficiency* of bleaching, and a value of $\gamma = 0.5$ was

(7) T. P. Williams, *J. Gen. Physiol.*, **47**, 679 (1964).

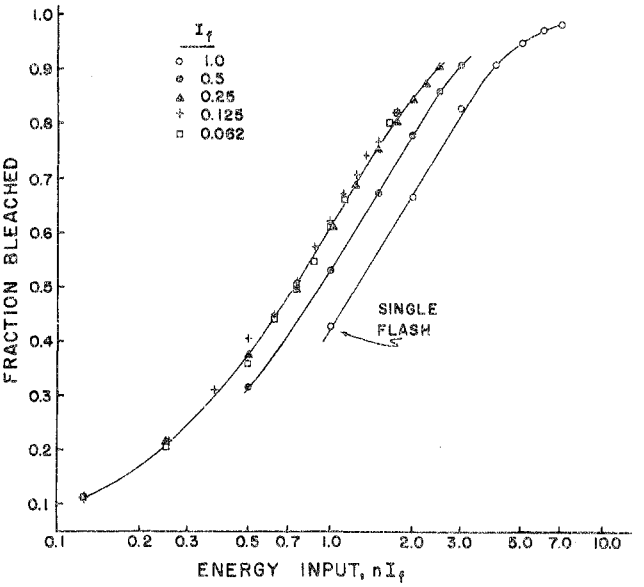

Figure 3. Bleaching with short flashes of various intensities. Energy input is defined as the number of flashes times the intensity per flash. The highest flash intensity is assigned unit value. Note that a decrease in flash intensity from 1.0 to 0.25 results in more rhodopsin bleached at any given value of energy input. For flash intensities of 0.25 and lower, the fraction bleached depends only on the total energy input.

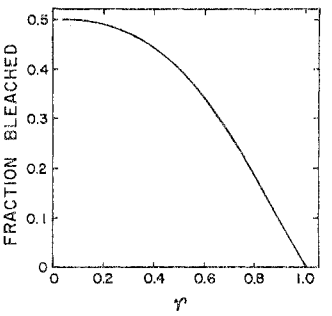

Figure 4. Calculated values of the quantum efficiency, γ, for different values of the fraction bleached with short flashes. Note that γ approaches zero and unity as the fraction bleached approaches 0.5 and 0, respectively.

assigned.[5] By coincidence, this is close to the presently accepted value of 0.7.[8]

The model in Figure 2 was successful at explaining experimental results if the early intermediates P, L, and M are completely photoreversible and if no "products" appear during the flash. Developments indicated that the bleaching process beyond meta-I (M in Figure 2) was quite complex.[9] Lumping this complexity into one term, "products," was possible only because short flashes were being considered in that early work and it was sufficient to assume that the flash was over before "products" were formed. Experimentally, P, L, and M appeared to be completely reversible, but it was assumed that "products" were photoirreversible. Actually, it was shown that the first of these products, metarhodopsin II, was partially photoreversible.[9] This result was verified even though no explanation could be given for the existence of an intermediate whose retinal was still attached to the original site in opsin but whose photoreversibility was only a fraction of that of other intermediates.[10] This meant that, in the transition

(8) H. J. A. Dartnall, C. F. Goodeve, and R. J. Lythgoe, *Proc. Roy. Soc. A*, **156**, 158 (1936).

(9) R. Matthews, R. Hubbard, P. K. Brown, and G. Wald, *J. Gen. Physiol.*, **47**, 215 (1963).

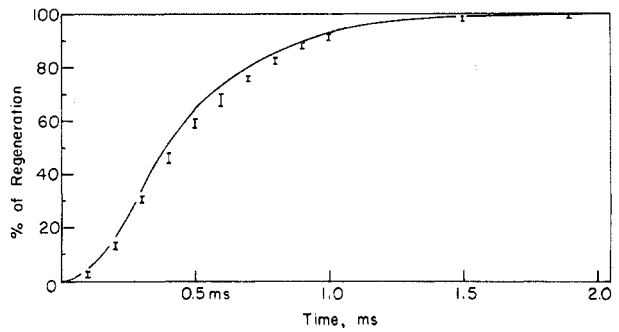

Figure 5. Rate of rhodopsin regeneration from photolysis of meta-rhodopsin I. Error bars are experimentally determined density changes at 530 nm. The solid curve is the integrated flash output.

from meta-I, opsin must be changing in some area remote from retinal so as to decrease the reversibility of meta-II. Since opsin was the object of our researches, we began to analyze carefully the meta-I–meta-II conversion in the hope of finding out what the protein was doing to cause this abrupt decrease in photoreversibility.

The earliest of these analyses was aimed at following the rate at which meta-I and meta-II regenerate stable pigment when they absorb light. The reasoning was that, if major conformational changes in opsin occur between meta-I and meta-II, these changes must be undone before meta-II can regenerate rhodopsin. This is exactly what was found.

B. N. Baker[11] showed that meta-I regenerated stable photopigment as fast as it absorbed light (*cf.* Figure 5). This indicates that the conformation of opsin is such that it can readily accept 11-*cis*-retinal and, therefore, it has not changed much compared with the starting conformation. However, when meta-II is flash-irradiated, only *ca.* 22% regenerates rhodopsin and it does this in the dark, *i.e.*, after the flash is over. This is summarized in Figure 6. One of the pathways involves a compound, meta-II′ whose spectrum was measured and which must contain the 11-cis isomer of retinal since it goes on to produce rhodopsin. The existence of the dark reaction of MII′ probably reflects a re-gathering of opsin around this newly formed 11-*cis*-retinal. Only when opsin has undone what went on during the production of meta-II does the spectrum of rhodopsin appear. If we could understand what goes on in this dark reaction, we would know the answer to a major question in this field: what environment does opsin provide to the retinal in order to give what we call the "rhodopsin" spectrum? Unfortunately, our attempts to understand this reaction have not been very fruitful. We have shown that the rate of the dark reaction is independent of pH, so it is unlikely that the reaction is just a simple protonation step. Only the temperature dependence of the rate gives a hint about the nature of the process. The activation enthalpy is 18 kcal/mol, and this leads to a value of the activation entropy of +11 eu at or near physiological temperatures. The latter figure suggests that rhodopsin has more degrees of freedom than meta-II. This is strange since meta-II is supposed to represent a rather disorganized state of opsin.[9] However, this interpretation may be more strange than correct since it

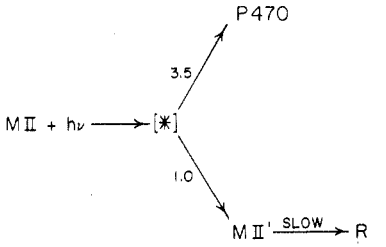

Figure 6. Proposed mechanism for the production of P470 and rhodopsin from meta-II. Meta-II absorbs a photon and enters the excited state indicated by the asterisk. P470 and MII′ are produced with relative quantum efficiencies of 3.5 and 1.0. MII′ slowly (in milliseconds) becomes rhodopsin.

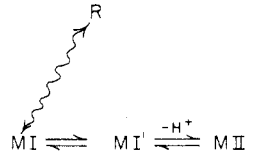

Figure 7. Schema of bleaching at elevated temperatures. R and MI are efficiently interconverted by light. MI enters a thermally determined equilibrium with MI′ which in turn must lose a proton in order to become MII. No photoreversal of MII is included because this schema pertains to a flash from which uv is excluded. No photoreversal of MI′ is shown because the extent to which it is reversible, although clearly less than MI, is not yet known.

Table I
Meta-I–Meta-I′–Meta-II Equilibria[a]

Temp, °C	MII/MI′ K	MII/MI′ ΔG, cal/mol	MII/MI′ ΔS, eu	MI′/MI K	MI′/MI ΔG, cal/mol	MI′/MI ΔS, eu
1	1.55	−238	+7.33	1.68	−282	+42.4
3	1.56	−243	+7.31	1.72	−298	+42.2
5	1.64	−273	+7.36	2.10	−408	+42.3
10	1.67	−290	+7.30	2.86	−610	+42.2
12	1.73	−310	+7.30	3.35	−685	+42.3
15	1.84	−348	+7.36	3.68	−735	+42.2
17	1.84	−348	+7.32	6.05	−1038	+42.7
18	1.86	−358	+7.32	5.04	−932	+42.2

[a] The values of the equilibrium constant were calculated from the concentrations of each species at the given temperature. The concentrations were measured directly in the case of MII and by the extent of photoreversal in the case of MI and MI′. The free energy was calculated from the equation $\Delta G = -RT \ln K$. The ΔG and the graphically determined values of ΔH were employed to yield the entropy by the equation $\Delta G = \Delta H - T\Delta S$.

derives from the application of transition state theory to a macromolecule. Obviously, we need to know more about the thermodynamic changes that accompany bleaching.

In the process of dissecting the interconversion of the metarhodopsins, it was proposed that two isochromic forms of meta-I exist: one which is readily photoreversible and the other not.[11,12] Since the two forms have identical spectra, it was solely on the basis of their photoreversibilities that they could be identified as separate species. This photoreversibility was shown to depend upon both temperature and pH. The irreversible form was called meta-I′ and it was concluded that meta-I′ is in thermal equilibrium with both meta-I and meta-II (*cf.* Figure 7). These thermal equilibria were studied, and their thermodynamic properties are listed in Table I.

The overall ΔH and ΔS values we obtained for the individual reactions are exactly the same as those of

(10) T. P. Williams, *Vision Res.*, 8, 1457 (1968).
(11) B. N. Baker and T. P. Williams, *Vision Res.*, 11, 449 (1971).
(12) T. P. Williams, *Vision Res.*, 10, 525 (1970).

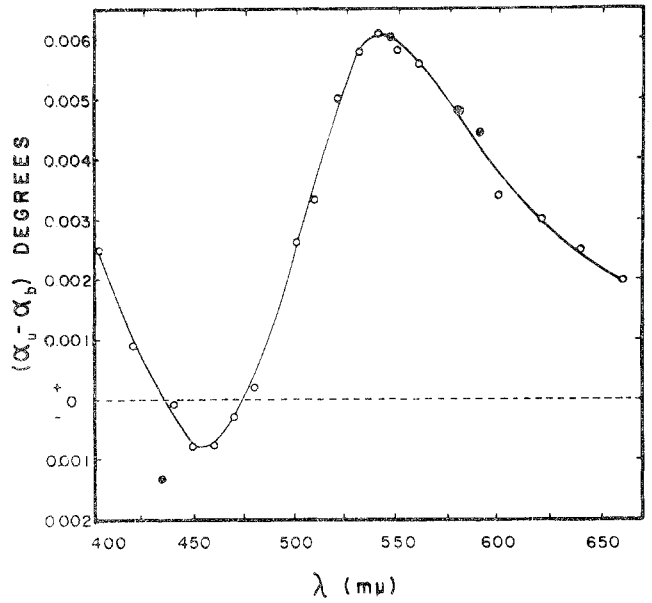

Figure 8. ORD differences of rhodopsin bleached in two different media. Open circles: rhodopsin extracted in 1% $CetMe_3NBr$; filled circles: rhodopsin extracted in 2% digitonin. Note the generation of a Cotton effect whose inflection is at 500 nm. This means that it is the 11-*cis*-retinal which produces this anomalous dispersion. Since retinal is itself not optically active, the asymmetry is being induced in it.

Matthews, *et al.*, who studied the meta-I–meta-II reaction without knowing the meta-I′ existed. This is gratifying since they used spectrophotometric methods for measuring the equilibria and we used a method which depended upon the photoreversibility of bleaching.

Isochromic forms of other intermediates may exist. After all, the spectral changes of the intermediates arise because of changes in and around retinal. Since retinal comprises only about 1% of the weight of rhodopsin, transient changes in opsin, remote from retinal, could easily go on and not effect spectral changes. Except in one case, it is difficult to develop "proof" for the existence of isochromic forms of the intermediates. The exception is the generally recognized existence of multiple "forms" of the intermediates which decay with concurrent rates. We have shown that these forms owe their existences to varying degrees of association with lipid (*vide infra*).

The foregoing review of high-intensity effects makes it clear that the conformation of opsin determines both the rate and extent of photoreversal of bleaching. It thus points out how photoreversal can be used as a tool to study opsin reactions.

We have developed these methods to yet a higher degree and have shown that opsin imposes stringent, time-dependent control over which isomer is formed *during* the photoreversal process.[13,14] The pattern of this selection is as follows: Only isorhodopsin (9-*cis*-retinal + opsin) is formed when bathorhodopsin (earliest intermediate) absorbs light, then the ratio of 9-cis to 11-cis falls to 60:40 in the "lumi" (second) stage of bleaching and then to 50:50 in the meta-I (third) stage. Finally, in the meta-II stage, the *only* stable pigment which can form is the 11-cis, rhodopsin. We have interpreted these results to mean that there exists a "template" within opsin which determines the quantum yield of isomerization. As bleaching proceeds, this template continuously changes from one which best fits 9-*cis*-retinal to one which only fits 11-*cis*-retinal. The exclusive selection of 11-cis at the meta-II stage is perhaps a sign that transduction has occurred and that opsin is now "ready" to recover from bleaching by dark-adaptation. We are left with the question: why should 9-cis be selected early in bleaching? If the "template" exists and if native rhodopsin contains 11-cis, why does not the template select 11-cis early in bleaching?

(13) T. P. Williams, B. N. Baker, and D. J. Eder in "Biochemistry and Physiology of Visual Pigments," Springer-Verlag, New York, N. Y., 1973, p 83.

(14) I. B. Federovich, V. Kuzmin, M. A. Ostrovskii, and T. P. Williams, unpublished results.

Nature of the Photoregenerated Material

Very early in our studies of the photoreversal process, the question arose about the nature of the photoregenerated material: was it the "same" as the original pigment? To try to answer this, we frequently ran parallel experiments with photoregenerated and native pigment. If the photoregenerated pigment were found to be grossly different from the starting material, studying the photoreversal steps would not tell us anything meaningful about *native* opsin. Fortunately, the experimental results described in this section indicate that photoregenerated pigment is indistinguishable from freshly extracted material. It must be pointed out, however, that no single test can constitute proof of this identity, but the entire battery of tests make a strong case for it.

For example, it was shown that the retinal of native rhodopsin exhibits "induced" asymmetry.[15] An optically inactive molecule like retinal can exhibit "induced" asymmetry if it is bound so extensively to a protein as to be rigidly fixed.[16] An anomalous Cotton effect was observed and was attributed to the main absorption band (λ_{max} 500 nm; Figure 8), and the optical rotatory dispersion of photoregenerated pigment showed just as much rotatory strength as did the original pigment; therefore, it was concluded that proper reattachment of retinal to opsin occurs during the photoreversal process.

The thermal stability of photoregenerated pigment is not different from native material. Figure 9 is an Arrhenius plot which provides the comparison.[17] Since thermal denaturations are cooperative effects, involving much, if not most, of the tertiary structure, this result indicated that the overall conformation of photoregenerated pigment is not different from the original rhodopsin.

The spectra of photoregenerated pigments (both 9- and 11-cis) are identical with their "fresh" counterparts. Denaturing agents destroy the spectra of these pigments. That photoreversal does not indicates the opsin must not be denatured.

We have also measured the kinetics of certain of the bleaching steps on the millisecond time scale and have found that they are the same whether "fresh" or photoregenerated pigment is used. Consequently, the dynamic properties seem unaffected by the photoreversal process.

In summary, then, we cannot distinguish *chemically* between native and photoregenerated pigment.

(15) T. P. Williams, *Vision Res.*, **6**, 293 (1966).

(16) L. Stryer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 1411 (1961).

(17) B. N. Baker and T. P. Williams, *Vision Res.*, **8**, 1467 (1968).

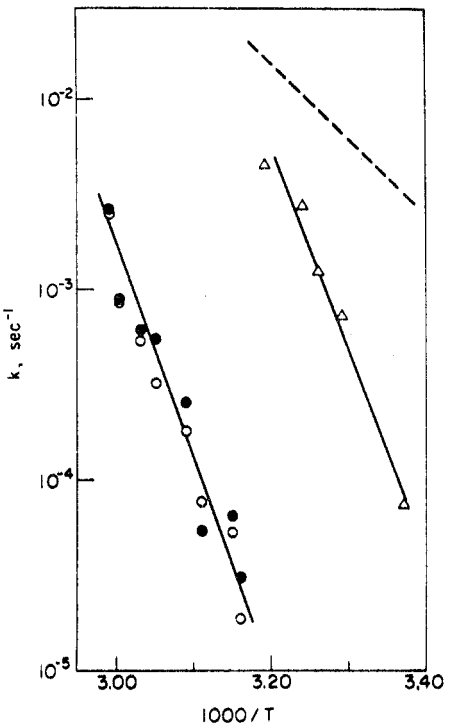

Figure 9. Arrhenius plots of rates of decomposition of native (open circles) and photoregenerated rhodopsin (filled circles), P470 (triangles), and metarhodopsin II (dashed line). The respective activation energies are 50, 48, and 20 kcal/mol.

The very interesting question remains, however: does the receptor cell distinguish between them? It has been shown that much photoreversal can occur in the eyes of intact rats and men. For example, the results of Dowling and Hubbard[18] suggest that, in the rat, photoregenerated pigment is physiologically active. Rushton,[19] however, has suggested that photoregenerated pigment in the eyes of man will not produce visual responses. This discord, if not simply due to a species difference, would seem to indicate that the matter is not settled.

The "Dark" Reactions of Bleaching

For many years it was commonly held that bleaching was a denaturation of opsin. The term, denaturation, implies a randomization of the tertiary structure of a protein and a destruction of the physiological viability of the molecule. Much of the evidence we have gathered over several years now leads us to feel that up to and including the meta-II stage (colorless, *i.e.*, bleached stage) the reactions of opsin are not random. To the contrary, it appears that opsin is doing something very specific and that these reactions do not destroy the viability of the opsin.

For example, opsin, stripped of its original retinal by bleaching with light, regenerates rhodopsin if it is simply incubated with 11-*cis*-retinal in the dark.[20] On the other hand, heat bleaching of rhodopsin results in a denatured opsin which does not regenerate pigment. This is an important distinction between heat and light bleaching and shows that light bleaching is not a denaturation in the usual sense of that term.

That the opsin of meta-II is not a random conformation is also evidenced by its selection of 11-*cis*-retinal in the photoreversal process.

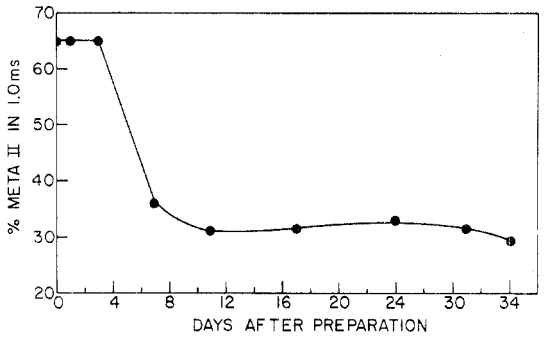

Figure 10. Meta-II production during the first msec as a function of age of the preparation. Frog rhodopsin in 2% digitonin, pH 6.5, 25°, bleached with a 2-msec flash from a Honeywell 65-C Strobonar through a Wratten 2E filter.

Table II
Effect of Delipidation on Meta-II Kinetics: Cattle Rhodopsin

	$k_{4°}$, msec^{-1}	$k_{15°}$, msec^{-1}	$E_a \approx \Delta H^*$, kcal	A, msec^{-1}	ΔS^*, cal/deg
"Normal" fast form	0.137	0.897	27.6	8.71×10^{20}	49.0
"Normal" slow form	5.36×10^{-3}	3.68×10^{-2}	27.7	3.82×10^{19}	42.8
Delipidated (only slow form appears)	2.30×10^{-3}	3.16×10^{-3}	5.47	43.3	−39.3

Another indication that the opsin reactions are directed toward a specific end comes from our studies on the lipids of rhodopsin.[21] We have shown that the presence or absence of lipids dramatically affects the rate of the dark reactions. The dark reactions of particular interest were, again, the interconversions of the metarhodopsins. Lipids were removed by chemical means or by ageing; the latter probably removes the lipids by a peroxidation reaction. An example of the ageing effect is shown in Figure 10. Notice the rapid fall of the rate of meta-II accumulation at about 4 days after preparation of the pigment solution. There was a precipitation of lipids from the solution that coincided with the decrease in rate. When lipids were removed by chemical extraction[22] a more pronounced effect was observed. Table II shows this. Two "forms" of meta-II are observed in "normal" (nondelipidated) pigment: a fast and a slow form. When lipids are removed, virtually no fast form is seen, but incubating delipidated rhodopsin with phosphotidylethanolamine restores the appearance of a fast form. This is shown in Figure 11. Indeed, difference spectra show that delipidated rhodopsin does not arrive at the meta-II stage but, rather, accumulates as meta-I. Shichi[23] has shown that delipidated rhodopsin is *readily* denatured and, taken together with our result, this indicates that the conversion of meta-I to meta-II is not a randomi-

(18) J. E. Dowling and R. Hubbard, *Nature (London)*, **199**, 972 (1963).
(19) W. A. H. Rushton, *Nature (London)*, **199**, 971 (1963).
(20) G. Wald and R. Hubbard, *Proc. Natl. Acad. Sci., U.S.A.*, **36**, (1950).
(21) T. P. Williams, B. N. Baker, and J. H. McDowell, *Exp. Eye Res.*, in press.
(22) M. Zorn and S. Futterman, *J. Biol. Chem.*, **246**, 881 (1971).
(23) H. Shichi, *J. Biol. Chem.*, **246**, 6178 (1971).

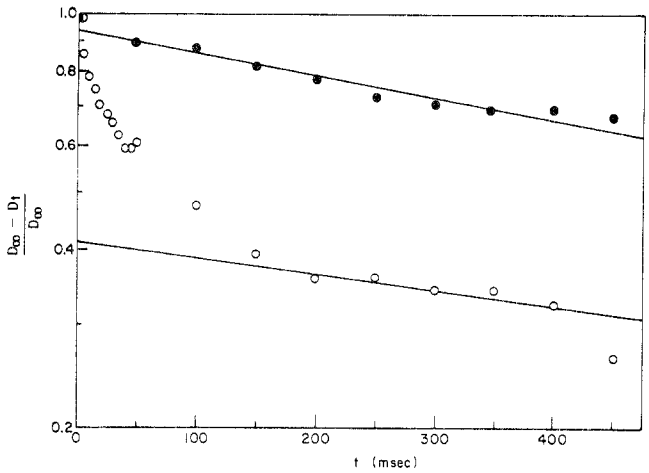

Figure 11. Integrated, first-order rate plot of meta-II production. Cattle rhodopsin in 2% digitonin, pH 6.5, 11°, bleached with a 2-msec flash from a Honeywell 65-C Strobonar through a Corning 3-72 filter. Closed circles are data from a delipidated preparation; open circles are the same delipidated preparation incubated with phosphotidylethanolamine.

zation of opsin but is so specific as to require lipid as "cofactor." Thus, these experiments with lipids have not only provided an answer to the long-standing question regarding the multiple "forms" of the intermediates but have contributed more evidence that the opsin reactions are not denaturation steps.

Other results concerning the directionality of opsin reactions have been obtained by J. H. McDowell in our laboratory. He has been studying the oxidation states and reactivity of sulfur in opsin. In "normal" (nondelipidated) rhodopsin he has shown that there is a disulfide bridge and two reactive sulfhydryls. Upon exposure to light, the original disulfide breaks and a new one forms. This is shown schematically in Figure 12. The free-energy change associated with the new disulfide bridge must be considerable (but not measurable at present) since it forces the "TAG" (Ellman's reagent) off the sulfur atom (S_4 in the figure). In addition, the new bridge (S_1–S_4) must be broken and the old one (S_1–S_2) re-formed before opsin will accept 11-cis in dark regeneration. Thus, McDowell has shown that the role of sulfur in the bleaching process is very specific and not just a simple unmasking of sulfhydryls as once had been proposed.[24]

Summary

A major problem in the study of sensory systems lies in understanding the nature of the transduction step. In the visual system, it appears that the pigment protein, opsin, may be the important mediator between the action of light and the first neural event. Studies of the visual pigment reactions are for the most part based on the spectral changes that occur when rhodopsin absorbs light. But doing so emphasizes the changes that occur in only a limited region of opsin and, consequently, methods have had to be developed for studying opsin in general. Among these, described in this account, is the phenomenon of photoreversal of bleaching. Our lab has developed this into a useful tool and through its use has found that opsin: (1) At the meta-I stage has the correct shape to immediately accept 9- or 11-*cis*-retinal; (2) at the meta-II stage must undergo considerable change in its shape before it can accept 11-*cis*-retinal; and (3) exerts profound, time-dependent control over the retinal isomer which it will accept.

We have also shown that photoregenerated pigment cannot be distinguished from "fresh" pigments by any of the tests we know how to apply.

The bleaching pathway is traversed by multiple "forms" of the intermediates, the existence of some of these being due to the degree of association of opsin with phospholipid. The "faster" forms are those associated with more lipid. Other "forms" exist and isochromic meta-I is an example of these.

Bleaching to the meta-II condition is "directional" and not just a randomization of opsin: lipid is necessary to achieve the meta-II state and a disulfide exchange occurs which is undone when opsin is incubated with 11-*cis*-retinal in the dark.

We believe that the metarhodopsin stage of bleaching is a prime candidate for implication in transduction, and more experiments are going on in our lab to test this candidacy. Having learned something about the opsin reactions described here, we are developing new hypotheses to test. These include temperature effects on the ratio of fast and slow forms, the role of sulfhydryls and disulfides, and the possible involvement of isochromic changes in opsin.

Perception of the environment begins at the interface between the outside world and the organism. Literally, tiny bits of the physical surroundings, stimuli, impinge upon the sense organs, regions of specialization which are strategically located and designed to receive the stimuli. When the interaction of a sense cell with a stimulus results in a change in the electrical properties of that cell, we say that transduction occurs. How this happens is, as yet, not understood in any of the sensory systems. But, given the high rate at which new knowledge is being generated in so many places all over the globe, it seems reasonable to predict that a fairly detailed understanding of visual transduction will be available in 5 to 10 years.

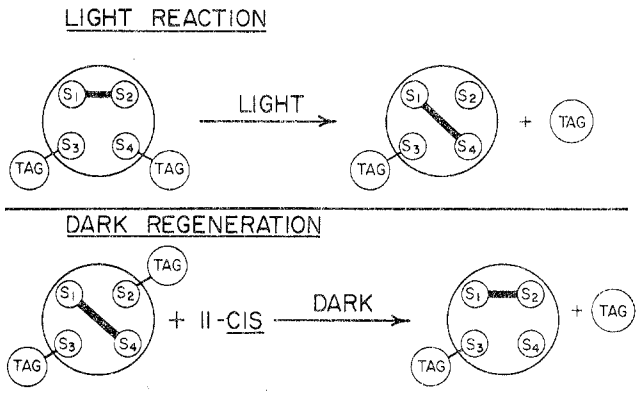

Figure 12. Schema for explaining the light and dark reactions of sulfur in rhodopsin. In the upper half of the figure the pigment is tagged with Ellman's reagent and during bleaching a disulfide exchange occurs which forces one of the tags off its sulfur site. In the lower half of the figure, bleached opsin is tagged and then incubated with 11-*cis*-retinal. During regeneration of rhodopsin, a tag is forced off, completing the cycle of disulfide exchanges.

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(24) G. Wald and P. K. Brown, *J. Gen. Physiol.*, **35**, 797 (1952).