On the Mechanism of the Visual Threshold and Visual Adaptation

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IT is a matter of common experience that the eye loses sensitivity in the light and regains it in darkness. These are the phenomena of light and dark adaptation. They have commonly been ascribed to the bleaching and resynthesis of visual pigments—rhodopsin in the rods, other pigments in the cones.

This hypothesis rests upon the observation that the visual pigments are engaged in reversible cycles of bleaching and regeneration in the retina, and upon the assumption that the visual sensitivity (1/threshold) falls and rises with their concentration. Hecht (*1*) at one time assumed that the concentration of visual pigments, rod and cone, is proportional to 1/log threshold. In this case, a large change in threshold might accompany a relatively small change in pigment concentration. Later it seemed better justified to assume that the concentration of visual pigment is proportional to 1/threshold—that is, to the visual sensitivity (*2*). Neither assumption succeeded in bringing the chemistry of visual systems into quantitative agreement with the properties of light and dark adaptation (*2, 3*). Yet the notion has persisted that the alterations of visual sensitivity in light and dark adaptation reflect principally changes in the concentration of the visual pigments and, hence, of their capacity to absorb light.

Some years ago, however, Granit and coworkers (*4, 5*) reported that large changes in the electrical response of the retina—the so-called *b*-wave of the retinal potential—in light and dark adaptation involve only minor changes in the concentration of rhodopsin. Measured in this way, much of light and dark adaptation seemed to depend primarily upon the first fraction of rhodopsin to be bleached and the last to be resynthesized. It was suggested that only a small fraction of the rhodopsin of a rod, perhaps concentrated at its surface, may be directly concerned with excitation and adaptation, and that the great bulk of the rhodopsin may represent a physiologically inert store from which the material at the active site is replenished by diffusion (*4*).

The opinion that a considerable light adaptation can be achieved with little bleaching of visual pigment has since been expressed a number of times. Rose (*6*), having analyzed the behavior of ideal "picture pickup" devices—artificial or visual—concluded that only a small fraction of the change of visual sensitivity in light and dark adaptation can be ascribed to changes in the capacity of the visual pigments to absorb light, that is, to bleaching. Baumgardt (*7*) and Pirenne and Denton (*8*) have calculated that at moderate illuminations too little light reaches the rods to produce a bleaching of rhodopsin commensurate with light adaptation. Hagins and Rushton (*9*) have shown that in the eye of the living albino rabbit, long-continued exposure to light as intense as 10^5 times the human absolute threshold causes no appreciable bleaching of rhodopsin. (This is not as surprising as it sounds; a light of this luminance—about 0.1 millilambert—causes also relatively little rise of threshold.) With further rise of intensity, the rhodopsin of the retina does bleach and may, indeed, bleach almost entirely (*9, 10*).

One of the most significant contributions to this development is the study by Hartline and coworkers of light and dark adaptation in single receptors of the eye of the horseshoe crab, *Limulus*. Here, although visual adaptation pursues a course much as in the vertebrate eye (*11*), it probably involves little bleaching of visual pigment (*12*). On the other hand, the number of quanta of light that must be absorbed to stimulate a visual response—the quantum demand—rises during light adaptation by amounts sufficient in themselves to account very nearly for the entire rise of threshold (*13*). Bouman and ten Doesschate (*14*) have presented evidence for a similar rise of quantum demand with light adaptation of the human eye.

Recently Rushton and Cohen (*15*) have given us the most explicit statement of this situation as it involves human vision. They state that exposure of the eye to a light which should have bleached only about 2 percent of its rhodopsin raises the visual threshold about 100 times.

Reluctant that anything—even a calculation—intrude upon so important a datum, I have performed the following experiment.

A replica of a human eyeball was blown from glass (Fig. 1). It has a short neck closed with a ground-glass stopper, through which it can be filled with water. It then mimics closely the optical system of the human eye (posterior focal length, 22.3 mm; front surface a spherical blister, like the cornea, of radius

* I should like to offer this paper to one of my former teachers, Otto Warburg, in this his 70th year. I know it will interest him that the organ of photosynthesis, the chloroplast, which possesses a microstructure much like that of a rod or cone, raises also functional problems closely related to those discussed here.

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about 6 mm). This "water eye" was placed behind a screen that bears a disk pierced with circular holes of various sizes to serve as pupils. It faced a large opal glass plate, illuminated from behind with white light. Close against the back of the water eye, which was slightly flattened, a small absorption cell, 10 by 2 by 2 mm, was held by a clip. The cell contained a solution of cattle rhodopsin, to which hydroxylamine (0.1*M*) had been added. This reagent binds the retinene liberated by bleaching in the form of retinene oxime, so preventing any regeneration of rhodopsin (*16*). Furthermore, retinene oxime, unlike retinene itself, has no appreciable absorption at 500 mμ, where the extinction of rhodopsin was measured; so in the presence of hydroxylamine all changes in extinction at 500 mμ involve rhodopsin alone. In this arrangement, I measured the bleaching of rhodopsin, using a 3-mm pupil, the water eye facing the opal glass plate set at a luminance of 324 millilamberts (300 ft-lamberts), at a distance of 7.3 cm. These measurements are shown in Fig. 2.

A dark-adapted subject (R. H.) was now placed with her eye in the position formerly occupied by the water eye, looking through a 3-mm artificial pupil, with all other conditions exactly as before. On separate occasions, she was exposed for 5 sec to luminances of 10, 324, and 1008 millilamberts, and her dark adaptation was measured. These data are shown in Fig. 3.

It is important for our purpose to know the visual threshold at zero time, the moment of turning off the adapting light (log I_0). To estimate this, the log threshold at complete dark adaptation (log I_f) was subtracted from all the measured values of log threshold (log I_t). It was found that $1/(\log I_t - \log I_f)$ plotted as a function of time yielded reasonably straight lines. These cut the zero time axis at $1/(\log I_0 - \log I_f)$, from which values of log I_0 are readily obtained. They are shown in Fig. 3 with crosses.

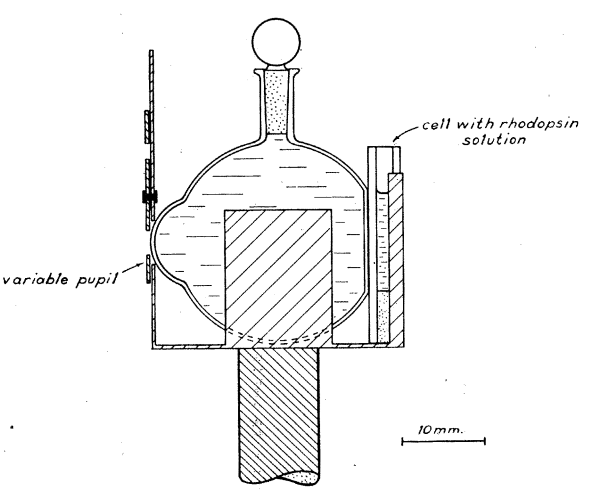

Fig. 1. Water model of the human eye. The "eyeball," blown from thin glass and filled with water, has a radius of 13 mm and bears on its front surface a "corneal" bulge of radius 6 mm. The brass holder bears on its face a rotatable disk pierced with circular openings of various sizes to serve as pupils. At the back of the "eyeball," which is slightly flattened, may be placed a thin glass cell holding rhodopsin solution.

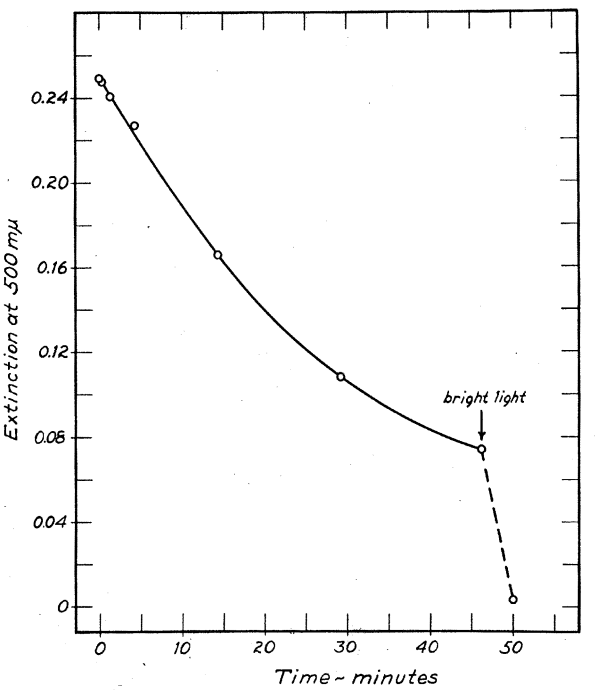

Fig. 2. Bleaching of rhodopsin in the "water eye." A solution of cattle rhodopsin containing 0.1*M* hydroxylamine was exposed to diffuse white light of luminance 324 millilamberts through a 3-mm pupil, and its bleaching was followed by measuring the fall in extinction at 500 mμ. After 47 min, the remaining rhodopsin was exposed to intense light to complete the bleaching.

Measurement of the bleaching of rhodopsin under one such set of conditions yields information under other conditions, since the rate of bleaching is directly proportional to the intensity of the light (*17*) and is independent of the temperature (*18*).

Table 1 brings together the results of this experiment. The first two columns show the conditions of irradiation; the third, the rise of visual threshold over the dark-adapted condition; and the fourth, the percentage bleaching of rhodopsin, measured in the water eye.

The exposure of the eye for 5 sec to 10 millilamberts raises the visual threshold about 8.5 times and bleaches 0.006 percent of the rhodopsin. Exposure of the eye to 324 millilamberts raises the threshold about 480 times and bleaches 0.2 percent of the rhodopsin. Exposure to 1008 millilamberts raises the threshold 3300 times and bleaches 0.6 percent of the rhodopsin. It should be noted that the conditions in the water eye all favored maximum bleaching. The transmission of light by this device is greater than by the eye; and the presence of hydroxylamine in the rhodopsin solution blocked all regeneration. I see no escape from the conclusion that a high degree of light adaptation can

Table 1. Human light adaptation and bleaching of rhodopsin. Comparison of the rise of visual threshold (rod) caused by exposure to lights of various luminance, with the percentage bleaching of rhodopsin measured under the same conditions in the water eye.

Light adaptation				
Luminance (millilamberts)	Duration (sec)	Rise in dark-adapted threshold	Percentage bleaching of rhodopsin	Number of rhodopsin molecules bleached per rod
10	5	× 8.5	0.006	1,200
324	5	× 480	.19	40,000
1008	5	× 3300	.59	120,000

be achieved with very little bleaching of rhodopsin.

How many molecules do these small amounts of rhodopsin represent? A parafoveal human rod measures about 1.8 by 44 μ (*19*). The extinction of its rhodopsin at 500 mμ may be taken to be about 0.05. Some years ago, I estimated it to be about 0.12 on the basis of extractions of rhodopsin from retinas of other mammals (*20*); and recently Crescitelli and Dartnall (*21*), having extracted the rhodopsin from a single human retina, estimated that, if spread in an even layer over the retinal surface, it should have an extinction of 0.016. Judging by past experience with such methods of estimation, this is probably 2 to 5 times too low. I have taken 0.05 as a conservative value, which might almost equally well be too high or too low by a factor of 2. It corresponds to an absorption at 500 mμ of 10 percent.

From this value of the extinction, the volume of the rod, 112 μ^3, and the molar extinction of rhodopsin, 40,600 (*22*), we can calculate that a human rod contains about 3×10^{-17} mole, or about 18 million molecules of rhodopsin. Putting this together with the values of percentage bleaching shown in Table 1, we obtain estimates of the numbers of rhodopsin molecules bleached per rod (Table 1, column 5). We see that the exposure of the eye to 10 millilamberts for 5 sec, in raising the threshold about 8.5 times, bleaches at most 1200 molecules of rhodopsin per rod.

One can reach much the same conclusion in an independent way. A luminance of 10 millilamberts viewed with the natural pupil results in a *retinal* illumination of 1.9×10^{-6} lumen/mm² (*23*; also *7, 8*). For rod vision, by definition, 1 lumen at wavelength 507 mμ is equivalent to 1.37×10^{-4} cal/sec, or to 1.44×10^{15} quanta/sec. At this luminance, therefore, each square millimeter of retina receives $(1.9 \times 10^{-6}) \times (1.44 \times 10^{15}) = 2.7 \times 10^{9}$ quanta/sec. The cross-sectional area of the outer segment of a human rod is about 2.5×10^{-6} mm². Therefore, a single rod has incident upon it 6800 quanta/sec. If, as we suppose, about 0.1 of these are absorbed, the rod absorbs about 680 quanta/sec. With a 3-mm artificial pupil, as in my experiment, in place of the natural pupil, which at this luminance is about 4.32 mm, this value is reduced by the ratio of the pupil areas, 9/18.7, to 330 quanta absorbed per second, or 1650 quanta in 5 sec. All things considered, this value is in good agreement with the estimate in Table 1, that such an exposure bleaches about 1200 molecules of rhodopsin per rod.

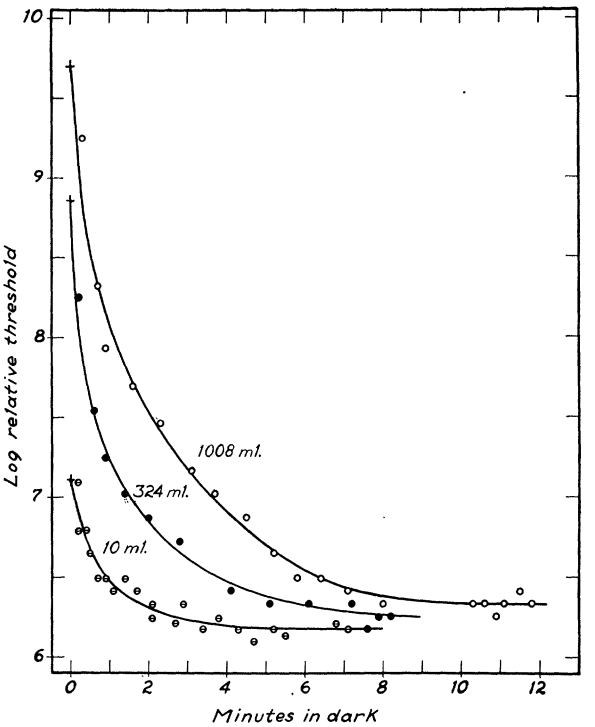

Fig. 3. Dark adaptation following 5-sec exposures to diffuse white light of luminance 10, 324, and 1008 millilamberts. A 3-mm artificial pupil was employed during light adaptation. The test field for the dark adaptation measurements subtended an angle of 1° with the eye and was fixated 10° below a small fixation point. The test light was of wavelength 436 mμ and was exposed for flashes of ⅛ sec. Thresholds in arbitrary units. Each dark adaptation begins with a threshold at zero time, obtained by extrapolation from the measurements as described in the text, and marked in the figure with a cross.

What do these relationships mean?

The outer segment of a rod or cone is a layered structure. That it is made up of transverse layers of microscopic dimensions has been known since the work of Max Schultze (*24*). In a frog rod, the layers may be very regularly spaced, a single cycle of alternate light and dark bands having a thickness of about 420 mμ (*25*). Studies with the polarizing microscope have shown that there must also exist a submicroscopic layering, of dimensions small compared with the wavelengths of visible light and associated apparently with alternate layers of protein and lipid, both highly oriented. This submicroscopic structure is believed to be responsible for the strong negative form birefringence observed in the intact rod [W. J. Schmidt (*26*)].

Some years ago, I suggested that rhodopsin, itself a lipoprotein composed of the protein opsin united with a carotenoid prosthetic group, might be situated

in the rod in the interfaces between the protein and lipid layers, with opsin in the protein and the carotenoid projecting into the lipid. In this case, the interfaces between the layers should be composed in part of rhodopsin; and one could estimate that a frog rod contains enough rhodopsin to form a large portion of such structures. It was suggested that the effect of light on rhodopsin is to disrupt such an interface, and that this in itself might be the source of rod excitation (*25, 27*).

Sjöstrand (*28*) has now revealed in the electron microscope the submicroscopic structure of certain rods and cones. The entire length of a rod (guinea pig, perch) appears to consist of a pile of regularly spaced membranes, associated in pairs, and consisting primarily of protein. The location of the lipids has not yet been determined. A guinea pig rod contains 1400 such membranes, each 4.1 (3.5 to 4.7) mμ thick (*29*). In a perch rod, there are about 2800 such membranes, each 8.0 ± 0.2 mμ thick. A perch cone contains about 800 single membranes, each about 16.6 mμ thick.

Hubbard (*30*) has recently shown that cattle rhodopsin has a molecular weight of about 40,000. Assuming the usual protein density of 1.4, this corresponds to a molar volume of 28,600 cm^3. A single rhodopsin molecule, therefore, has a volume of 47.7 $m\mu^3$. If spherical, this corresponds to a diameter of 4.5 mμ; if cubical, to a diameter of 3.6 mμ. That is, the diameter of a rhodopsin molecule is very close to the thickness of a membrane in a guinea pig rod. A membrane in a perch rod is twice this dimension; a membrane in a perch cone, 4 times this dimension.

From the molar extinction of rhodopsin, 40,600 (*22*), and the dimensions of a rhodopsin molecule, one can calculate that the extinction at 500 mμ of a monomolecular layer of rhodopsin, assuming spherical molecules tightly packed, is 4.25×10^{-4}. Suppose for a moment that the membranes of a guinea pig rod consist entirely of such monomolecular layers of rhodopsin. Then the rod would have a total extinction of $1400 \times 4.25 \times 10^{-4} = 0.60$. This is almost certainly too high, but probably by not more than 3 to 6 times. What it means is that the membranes of this rod, though not entirely rhodopsin, are probably 1/3 to 1/6 rhodopsin.

Again, the outer segment of a frog rod has a dry weight of about 3.5×10^{-10} g (*30*). About 35 percent of this is lipid. The nonlipid portion of the rod, therefore, weighs about 2.3×10^{-10} g. Hubbard (*30*) estimates that the extinction of this rod is about 0.50. Since its dimensions are about 6 by 50 μ, a rod contains about 3.5×10^{-15} mole of rhodopsin. If the entire nonlipid dry weight of the rod were rhodopsin, this substance would have a molecular weight of 66,000. This is, therefore, the maximum value that can be assigned to the molecular weight of frog rhodopsin.

On the other hand, we can be reasonably sure that the entire nonlipid moiety of the rod is not rhodopsin, and that this maximal value is almost surely too high. Accepting, instead, for frog rhodopsin the somewhat smaller value that Hubbard found for cattle rhodopsin, 40,000 (*30*), each frog rod should contain $3.5 \times 10^{-15} \times 40{,}000 = 1.4 \times 10^{-10}$ g of rhodopsin. This is about 61 percent of the nonlipid dry weight, or about 40 percent of the total dry weight of the rod. On a quite different basis, Hubbard has estimated rhodopsin to account for about 35 percent of the dry weight of a frog rod. It also accounts for about 14 percent of the dry weight of a cattle rod, or about 22 percent of the nonlipid dry weight.

The burden of these computations is that rhodopsin constitutes a large part of the protein of the rod and, hence, that it must also form a large part, and in some cases the major part, of Sjöstrand's membranes.

A further essential element in our argument is that *a dark-adapted rod may be stimulated by the absorption of a single quantum of light* (*7, 31*). One quantum of light is absorbed by 1 molecule of rhodopsin; and a rod is so peculiarly constructed that this change in a single molecule of rhodopsin can excite it.

This datum has another important consequence. Since *any* molecule of rhodopsin in the dark-adapted rod can produce this effect, *none of the rhodopsin of the rod can be inert.* All of it must be equally able to contribute to excitation.

I should like to propose the following hypothesis.

The threshold of a dark-adapted rod depends upon its entire content of rhodopsin. The rod, however, is a compartmented structure, which can undergo, compartment by compartment, a stepwise response. Each compartment contains a considerable quantity of rhodopsin, any molecule of which, on absorbing a quantum of light, discharges the compartment. If there are n_0 compartments, when n_x of them have each absorbed at least 1 quantum of light, the threshold has risen $n_0/(n_0 - n_x)$ times. Thus, a large rise of threshold is achieved with very little bleaching of rhodopsin —a minimum of 1 molecule per compartment. A compartment on absorbing a first quantum of light makes its whole contribution to the response and cannot contribute again until all its rhodopsin is restored. The remaining rhodopsin of this compartment, though rendered temporarily inert for excitation, absorbs light, just as before. Such absorption can have no other effect than to delay the eventual recovery of the compartment.

What are the compartments? Enough has been said to suggest strongly that they have a close relationship to Sjöstrand's membranes. There seems little place for the rhodopsin of a frog or mammalian rod other than in the membranes; and in dimensions and numbers, the membranes satisfy very well the requirements of our hypothesis. Yet Sjöstrand finds both single and double membranes and, perhaps, substructures within the thicker single membranes. Furthermore, the rods of frogs and other amphibians exhibit a coarser, microscopic layering and longitudinal striations which may be fibrillae (*25*), no evidence of either of which appears in Sjöstrand's figures. Although I think it very probable that the compartments are closely associated with Sjöstrand's membranes, it is

too early yet to identify them; and I shall go on for the present referring to them simply as compartments.

The absorption of a first quantum of light by a dark-adapted rod—one in which all the compartments are intact—causes a first response. For a second quantum of light to excite, it must be absorbed in a new compartment. If absorbed in an already discharged compartment, it is wasted. The more compartments discharged, the more quanta absorbed to no avail. Therefore, the quantum demand begins at once to rise. Having been 1 in the dark-adapted rod, it rises to higher and higher values as more compartments respond. If there are n_0 compartments and n_x have been discharged, the quantum demand has risen $n_0/(n_0 - n_x)$ times—the same law that governs the rise of threshold. That threshold and quantum demand rise together in light adaptation has been demonstrated by Hartline *et al.* in the *Limulus* photoreceptor (*13*); and some evidence exists of a comparable phenomenon in the human eye (*14, 15*).

The absorption of the first quantum of light by a dark-adapted rod should raise its threshold $n_0/(n_0 - 1)$ times. When half of the compartments have been discharged, the threshold should have doubled; when 9/10 have been discharged, the threshold should have risen 10 times. When only two compartments remain intact, the absorption of a quantum by one of them doubles the threshold. Finally, the discharge of the last compartment projects the threshold to infinity; the rod is now inexcitable.

In these terms, Table 1 shows that a 5-sec exposure of the eye to 10 millilamberts leaves, on the average, $1/8.5 = 0.12$ of the compartments of the rod intact; 88 percent have been discharged. This has involved the bleaching of about 1200 molecules of rhodopsin. This number is in good accord with the suggestion that the compartments may be identical with Sjöstrand's membranes or membrane pairs.

It has already been noted that the absorption of light by an already discharged compartment causes no further response, nor does it raise the threshold further—it is already infinite—but it delays the eventual repair of that compartment. That is, such extra absorption of light and bleaching of rhodopsin in the compartments has the effect, not of raising the threshold of the rod, but of delaying its recovery—of slowing its subsequent dark adaptation.

It is well known that, in general, the higher the light adaptation, the slower the dark adaptation that follows (*32*). Figure 3 shows an example of this phenomenon. It is found equally in rods (*32*), cones (*33*), and single receptors of the *Limulus* eye (*11*).

After high light adaptation, most of the rhodopsin regenerated should go to restore the rhodopsin content of badly depleted compartments; but no compartment comes back into function until its *last* rhodopsin molecule has been regenerated. It is, therefore, the last stages of rhodopsin synthesis that should be most effective in restoring the compartments to function and so causing the fall in visual threshold that we measure as dark adaptation.

I think that this explains the observation by Granit *et al.* (*5*) that, following high states of light adaptation, dark adaptation measured electrophysiologically lags markedly behind the regeneration of rhodopsin. These workers found that, on shutting off a strong adapting light, which had bleached most of the retinal rhodopsin, the latter began to regenerate at once; but the *b*-wave of the retinal potentional remained very small (frog) or wholly absent (cat) until the rhodopsin had reached about 40 to 50 percent of its maximal concentration. Thereafter it rose rapidly. This is the behavior expected on the basis of the present hypothesis.

What of the cones? Large as rhodopsin looms in the microstructure of a rod, it occupies a correspondingly small position in the microstructure of a cone. Indeed, if it were necessary to bleach much pigment to stimulate a photoreceptor, it would be difficult to understand how a cone functions at all. The realization that stimulation is the business of single molecules of visual pigment absorbing single quanta of light disposes of this difficulty.

A cone, which possesses very little visual pigment and hence a small probability of absorbing light, has a correspondingly high threshold. If, as we have supposed in rods, the visual pigment of cones is distributed among a large number of compartments —perhaps the single membranes photographed by Sjöstrand—this arrangement has important consequences. Our general hypothesis remains unchanged; but in a structure that contains very little visual pigment it involves new considerations.

Consider the limiting case of a cone in which each compartment contains a single molecule of visual pigment, which by absorbing a quantum of light discharges it. Then in all stages of adaptation this cone would have a quantum demand of 1—each quantum absorbed would involve a new compartment and would result in a response. Also, the sensitivity of such a cone would at all times be proportional to its concentration of visual pigment. When half of the pigment had been bleached, half of the compartments should have been discharged, and the threshold should have doubled. Furthermore, in such a receptor, there would be no such slowing of dark adaptation with increase in light adaptation as we have described in the rods. For each molecule of visual pigment resynthesized, one compartment would come back into function; and the rate of dark adaptation would measure simply and directly the rate of synthesis of visual pigment.

I do not suppose that a cone exists with the properties just described; but the consideration of such a limiting case helps to clarify an important relationship. We see that many of the special complications of light and dark adaptation in the rods come directly out of their high concentrations of visual pigment. This is the particular source of disparities in their behavior from properties predicted by the simplest of photochemical theories. A receptor that possessed 1 molecule of visual pigment per compartment would

follow in detail the state of its photochemical system. A rod—entirely because it is highly overloaded with visual pigment—exhibits all the special phenomena we have considered: low threshold; rise of quantum demand with light adaptation; dependence of light and dark adaptation primarily upon the first fraction of visual pigment to be bleached and the last to be resynthesized; and the slowing of dark adaptation with the degree of bleaching achieved in light adaptation. For this reason, we can expect that the more dilute the visual pigment in a receptor, the less conspicuous should be all these properties. In general, therefore, they should be less conspicuous in cones than in rods.

Where do these considerations leave our concept of the visual threshold and visual adaptation?

In this discussion, I have called upon nothing but the chemistry of the outer segments of the rods and cones and their microstructure. I have no conviction that this is all, yet for the present it may be enough. The visual pathways include nerves, synapses, and a portion of the brain; and in time we should know what part these structures play in determining visual thresholds and adaptation. A number of special conditions are already known—anoxia, acidosis, and alkalosis—that cause fluctuations in the threshold of the completely dark-adapted eye, owing presumably to changes central to the photochemical systems of the receptors (*34*). The entire visual apparatus, however, hangs upon the initial action of light on the photosensitive pigments of the rods and cones, and it seems probable that under all ordinary conditions the pigments are the major determinants of visual threshold and adaptation.

We emerge, therefore, with a photochemical view of the threshold and its changes, a photochemistry, however, not as one would find it in solution, but intimately interwoven with the microstructure of the receptors. Once it had been shown that a rod can be excited by the absorption of a single quantum of light, this further development was inevitable. A process that depends upon single quanta must be considered chemically in terms, not of moles, but of molecules; and it is only within a superbly organized structure that 1 quantum acting upon 1 molecule could have so large an effect.

What I regard as most important in this situation, however, is the opportunity it offers to consider together the structure, chemical composition, and function of an excitatory tissue. Only at grosser levels of analysis can anatomy, chemistry, and physiology be dealt with separately. At the molecular level they are one and must be so regarded.

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29. Sjöstrand (1953) speaks of this thickness as 30 A (3 mμ); but since, as he says, this is at the limit of resolution of his thinnest sections, the thickness of the membrane is measured most accurately by subtracting the thickness of a membrane pair, measured from center to center of the membranes (mean 105 ± 2 A), from the total thickness of such a pair (mean 146 ± 4 A). It is this difference that I have, therefore, taken as the best value.
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31. S. Hecht, S. Shlaer, and M. H. Pirenne, *J. Gen. Physiol.* **25**, 819 (1942); M. A. Bouman and H. A. van der Velden, *J. Opt. Soc. Amer.* **37**, 908 (1947); M. H. Pirenne, *Brit. Med. Bull.* **9**, 61 (1953).
32. G. Wald and A.-B. Clark, *J. Gen. Physiol.* **21**, 93 (1937–38). This consideration does not obviate, but adds to, another source of slowing of dark adaptation, described by Wald and Clark, and associated particularly with the *duration* of light adaptation. After short exposures to light, whatever its intensity, dark adaptation depends largely upon the regeneration of rhodopsin from retinene and opsin and is relatively rapid. After long exposures, most of the retinene has been reduced to vitamin A, and much of the vitamin A has diffused out of the retina. Dark adaptation is then relatively slow, owing to the necessity to recapture the vitamin A that has left the retina and to reoxidize it to retinene.
33. F. A. Mote and A. J. Riopelle, *J. Gen. Physiol.* **34**, 657 (1950–51); J. F. Schouten and L. S. Ornstein, *J. Opt. Soc. Amer.* **29**, 168 (1939).
34. G. Wald *et al., J. Gen. Physiol.* **25**, 891 (1941–42).