Light-Sensitive Swelling of Isolated Frog Rod Outer Segments as an In Vitro Assay for Visual Transduction and Dark Adaptation

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ABSTRACT Frog rod outer segments swell slowly after being shaken from an excised retina into a modified Ringer's solution. The swelling has the following characteristics: (a) It is suppressed by illumination which bleaches only 500 rhodopsin molecules per outer segment per second. This is approximately the level required to saturate the in vivo receptor potential. (b) Light suppression is seen in NaCl but not in KCl solutions. (c) Dark swelling is labile and is enhanced by calf serum, low calcium concentrations, dithiothreitol, and cyclic nucleotide phosphodiesterase inhibitors. (d) Lowering the pH to 5.5 or removing magnesium reversibly reduces dark swelling to the same extent as illumination. (e) The amount of light required for maximal suppression of dark swelling increases approximately 10-fold if the calcium concentration is lowered by EGTA addition. (f) The effect of illumination is irreversibly abolished by antimycin and other inhibitors of mitochondrial electron transport. (g) A process analogous to dark adaptation in vivo can be observed: If 10–50% of the rhodopsin present is bleached and the outer segments are then kept dark, rapid dark swelling returns after a period of 15–45 min. This swelling is again sensitive to light. We tentatively ascribe the light suppression of swelling to the same decrease in sodium permeability which is observed on illuminating living receptor cells. The experiments suggest that outer segments retain their competence to perform both transduction and dark adaptation after their separation from the retina.

INTRODUCTION
The distinctive anatomy of vertebrate retinal rod cell outer segments makes them easily accessible for study. The photosensitive membrane system is housed in the rod-shaped organelles shown in Fig. 1. In the bullfrog (Rana catesbeiana) these are 6–8 μm in diameter, 50–80 μm in length, and composed of a plasma membrane which encloses a stacked series of 1,500–2,000 disc membranes. These outer segments attach to the receptor cell body by a ciliary stalk. Gentle shaking of the retina breaks this attachment, yielding a relatively pure and homogeneous suspension of the light-sensitive organelles.
FIGURE 1. Scanning electron micrograph of bullfrog retina. The top arrow (A) indicates the layer of outer segments, each rod outer segment being approximately 60 μm in length and 6–8 μm in diameter. The middle arrow (B) shows where outer segments have broken off at the ciliary connection leaving exposed the mitochondria-rich ellipsoid portion of the receptor cells. In the next shear plane (C) receptor cells are removed and neural layers of the retina are exposed. × 500.
The main protein of the disc membranes is the well-characterized visual pigment rhodopsin (1, 2). Because rhodopsin photochemistry has been extensively studied over the past 30 years (3, 4), the photon input to this system can be precisely quantitated. In the past few years several new chemical consequences of illumination have been uncovered: a rhodopsin phosphorylation reaction (5-8) and changes in the activities of enzymes controlling cyclic nucleotide levels in the cell (9-12). Photon absorption by rhodopsin ultimately causes sodium channels in the plasma membrane to close; this is generally thought to be effected by the release of an internal transmitter (perhaps calcium) from the discs (13, 14). The closing, measured as a decrease in sodium permeability ($P_{Na}$), depends not only on the light incident at a given moment, but also upon the history of illumination. Previous illumination appears to influence at least two separate control systems: a sensitivity mechanism which determines how much light is required to effect a $P_{Na}$ decrease, and another mechanism which may control the maximum change in sodium permeability ($\Delta P_{Na}$), the number of open sodium channels available for closing (15, 16).

The activity of rod receptor cells in living retinas can be monitored using electrophysiological techniques, but chemical changes or pharmacological effects frequently cannot be localized to a specific part of the receptor cells, or even to the receptor cells themselves. For this reason, we have chosen to work with isolated outer segments. Korenbrot and Cone have shown that osmotic behavior of these organelles is altered by illumination (17, 18), and their observations have led us to develop the simple qualitative method for monitoring light-dependent volume changes which is described below. The most important point made by this paper is that isolated outer segments perform both excitation and adaptation processes. We now have a reproducible in vitro assay for photoreceptor membrane function, an appropriate starting point for bringing together the chemistry and physiology of the system.

**METHODS**

In preparation for each experiment, two dark-adapted frog retinas are obtained by dissection under dim red light in modified Ringer’s solution A, B, or C. Solution A contains 115 mM NaCl, 2 mM KCl, 10 mM HEPES (N-2-hydroxyethyl-piperazine-N'2-ethanesulfonic acid, Sigma Chemical Co., St. Louis, Mo.), 10% (vol/vol) calf serum, and 3 mM EGTA. No calcium or magnesium are added, but the serum introduces 0.18 mM Ca$$^{++}$$ and 0.08 mM Mg$$^{++}$$.

(Concentrations of free calcium and magnesium are calculated to be $<10^{-9}$ M and $1.6 \times 10^{-7}$ M, respectively.) Solution B is made by adding 3 mM dithiothreitol (DTT) to solution A. Solution C is made by adding 10$$^{-4}$$ M papaverine to solution B. The earliest experiments presented were performed with solution A, later solution B
was used, and most recently, solution C. Solution C contains supplements that we have found to enhance and stabilize in vitro physiology (see Results below). All manipulations are done at room temperature (~20°C).

Outer segments are removed from the retinas by gently shaking in 800 µl of the dissection solution; this treatment permits the plasma membrane to reseal around the disc membrane system. The shearing of outer segments and their fragments is illustrated by the scanning electron micrograph of Fig. 1. (While we have shown previously that washed and purified outer segments show light influenced swelling, the mechanical disruption involved in these preparations does diminish the magnitude of the light effects [5].) The suspension containing approximately 10⁶ outer segments and their fragments is divided into four to eight 100-µl portions. Each portion is then diluted to 25 ml (a 250-fold dilution) with either the same solution or with a "hyperosmotic Ringer's solution" in which the NaCl concentration is 215 mM instead of 115 mM. (In the latter solution outer segments initially shrink to approximately 80% of their original volume [17] and then slowly reexpand as in Figs. 6–14). At intervals, the volumes of approximately 20,000 outer segments in each 25-ml portion are determined by sampling with the particle size analysis system outlined in Fig. 2.

In this apparatus, outer segments are drawn through a 120-µm orifice by negative hydrostatic pressure (100 mm Hg), and then discarded into a reservoir. Subsequent measurements thus sample only outer segments which have not passed through the orifice. (Microscopic examination of the population being sampled indicates that outer segments retain their rod-like shape during the course of an experiment.) As each segment passes, a momentary change in the resistance between the electrodes occurs; this change causes a voltage pulse proportional to the volume of the segment. Each voltage pulse, after amplification and analog to digital conversion, is stored in a PDP8M computer. Data for a given suspension of outer segments is plotted as shown in Fig. 3. Approximately 20,000 outer segments are assigned to one of 64-vol classes; the figure shows the number of outer segments in each volume class versus the volume, expressed in cubic microns. In the experiment of Fig. 3, the volume distribution of the outer segments was also measured directly using photomicrographic techniques. The agreement between the direct and the electrical sizing methods is excellent. The number of outer segments counted in sequential sampling of the same

![Figure 2](image-url)
suspension can vary by 5%. Therefore, distributions (as in Figs. 3 and 5) are normalized to a constant total count. Then, for each volume distribution taken at a given time, an average volume is computed, and these data are used to plot the figures of this paper describing changes in the volume of suspended outer segments occurring over time.

We designed the particle analysis system and its accompanying computer program in collaboration with Particle Data, Inc., Elmhurst, Ill. It is now commercially available, and a more complete description of the apparatus can be obtained from that firm.

Photomicroscopy of suspended outer segments is performed using a Zeiss Universal microscope (Carl Zeiss, Inc., New York). To avoid bleaching outer segments, infrared illumination and infrared-sensitive film are employed, with an infrared image converter used for visualization. The lengths of outer segments, which are closely proportional to their volume (17), are obtained from measurements made on photographic negatives. Techniques for illumination of outer segment suspensions and for measuring rhodopsin bleaches are described in an earlier paper (1). Bullfrogs, 5–8 inches in length, are obtained from Jacques Weil Co., Rayne, La., and force-fed for 2 wk before use in experiments as previously described (5). All chemicals were obtained from Sigma Chemical Co., with the exception of A23187 (Eli Lilly & Co., Indianapolis, Ind.), SQ20-006 and SQ65-442 (E. R. Squibb & Sons, New York). In experiments using A23187, gramicidin D, valinomycin, or carbonylcyanide m-chlorophenylhydrazone, the compounds were first dissolved in ethanol and then added as 0.1% of the total volume of outer segment suspensions. This amount of ethanol, added alone, had no apparent effect on in vitro physiology.
RESULTS

Light-Inhibited Swelling in Isolated Outer Segments

The light-sensitive behavior of outer segments in suspension is summarized by the experiments of Fig. 4. Dark outer segments swell more rapidly than those which are continuously illuminated, in both isosmotic (Fig. 4 a) and hyperosmotic (Fig. 4 b) solutions. The following features of the light-de-
pendent swelling suggest that it is physiologically relevant: Dark swelling is suppressed by low levels of illumination-500 rhodopsin molecules bleached per outer segment per second (Fig. 4 c). This is only slightly higher than the level of 200 absorbed photons required to saturate the rod photoresponse of isolated retinas (13). Light-suppressible dark swelling is seen in NaCl but not in KCl solutions (Fig. 4 d); KCl solutions have the same effect as light suppression. This is an apparent mimicking of the ion requirements of the in vivo receptor potential (13). Finally a form of “dark adaptation” is expressed in these preparations (explained later in Fig. 12). An indication of the reproducibility of the data can be obtained by reference to the coinciding curves of Figs. 9 and 14 in which swelling of different portions of outer segments from one animal is monitored under similar conditions.

In the photomicrographic measurements summarized by Fig. 7 we determined that approximately 70% of the outer segments and outer segment fragments present performed light-sensitive swelling. The data of Fig. 5 a and b, obtained with the particle-sizing device, show the most extreme variation we have noted in the relative number of active outer segments in preparations from different animals. The symmetrical volume shifts seen in Fig. 5 a are consistent with virtually all of the outer segment fragments demonstrating light-suppressible swelling. In contrast, the volume distribution shift shown for another preparation in Fig. 5 b suggests that a smaller number of fragments, mainly larger ones, are influenced by illumination. The presence of inactive outer segments does not compromise the qualitative interpretation of the data presented here. However, their presence must be kept in mind when we begin to correlate chemical and physiological changes caused by illumination.

Illumination often does not completely suppress swelling of outer segments. The light-sensitive portion of this reaction is superimposed on “background” swelling; this is not ion specific, occurring in both NaCl and KCl Ringers’ solutions, and it can be partially suppressed by DTT (Fig. 6). The different figures in this paper reveal that background swelling is almost absent in some preparations and quite pronounced in others. In contrast, we find that the light-sensitive portion of swelling is more constant from preparation to preparation than background swelling.

When the external NaCl concentration is reduced to 40–50 mM, by replacing NaCl with KCl, light no longer suppresses swelling. This suggests that a sodium concentration gradient may be required for the expression of light sensitivity. (This point is dealt with further in the Discussion section.) An indication that the cation permeability of the outer segment may regulate swelling comes from the observation that cationophores enhance dark swelling: the Na+, K+, H+ ionophore, gramicidin D (10^{-7} M) has a 10- to 20-fold effect; the K+ ionophore valinomycin (10^{-7} M) has a two- to fourfold
effect in solution B made with 115 mM KCl replacing NaCl; but the proton ionophore carbonylcyanide m-chlorophenylhydrazone has no effect.

When outer segments are sheared from the retina into a minimum volume (≈10 μl) and made hyperosmotic, swelling is relatively rapid (Fig. 7). Under these conditions, a 6% difference in volume between dark and illuminated outer segments develops within 5 min. In contrast, the more dilute and aged preparations studied in this paper require 15–90 min for the same result.
Thus it appears that dark swelling decays on aging and dilution. This effect has also been noted by C. Wormington in R. A. Cone's laboratory (personal communication).

The decay of dark swelling can be partially reversed by a variety of conditions (Figs. 8 and 9). Most important is the presence of calf serum (Fig. 8 a) which stabilizes the structure of outer segments and prevents them from adhering to glass. When 0.5% gelatin is substituted for serum dark swelling and its suppression by light can be observed for only 20-30 min before the outer segments lose activity. Because we wish to follow volume changes over longer periods of time, especially in dark-adaptation experiments, serum is routinely used. In many, but not all, experiments we have observed that dark swelling is stimulated by reducing conditions: addition of 1 mM DTT or bubbling argon through the medium.

Lowering the concentration of calcium to less than $10^{-3}$ M, by addition of 3 mM EGTA, also enhances dark swelling (Fig. 8 b). Calcium ion suppress-
Figure 8. Calf serum and EGTA increase dark swelling. Removal of either serum (a) or EGTA (b) from hyperosmotic solution A decreases dark swelling.

sion of osmotic swelling has also been noted by Korenbrot and Cone, using different experimental conditions (17). In our preparations the suppression of dark swelling by illumination is small and sometimes absent if EGTA is not added. EGTA has the further effect of increasing the amount of illumination required to suppress dark swelling (Fig. 9). Yoshikami and Hagins, studying a perfused retina preparation, have also reported that lowering calcium decreases sensitivity (19).

A striking increase in dark swelling is observed when $10^{-4}$ M papaverine is added to the medium (Fig. 10). Papaverine, a cyclic nucleotide phosphodiesterase inhibitor, might be expected to inhibit degradation of either cyclic AMP or cyclic GMP. The same enhancement of dark swelling is caused by two other compounds which inhibit the same enzyme, SQ20-006 ($5 \times 10^{-4}$ M) and SQ65-442 ($10^{-4}$ M). Because papaverine is thought to increase intracellular levels of cyclic AMP or cyclic GMP, we expected to mimic its effects by direct addition of these compounds or their dibutylryl derivatives in millimolar levels. However, dark swelling was enhanced in less than half of the experiments tried. We presume therefore that permeability barriers prevent these compounds from readily entering the outer segment.
We have found that not only illumination but also several chemical perturbations can reversibly decrease dark swelling. Lowering the pH of outer segments in solution from 7.5 to 5.5 by HCl addition "mimics" the effect of illumination by suppressing swelling. If the pH is returned to 7.5, rapid dark swelling returns, and it can be suppressed by illumination. Comparable data have been obtained by Wormington under different experimental conditions (personal communication). ¹

A similar result, suggesting a role for magnesium ions, is obtained in experiments using the Ca⁺⁺-Mg⁺⁺ ionophore A23187 (20). Addition of 10 μM A23187, 3 mM EGTA, and 3 mM EDTA, meant to remove calcium and magnesium ions from the outer segments, causes swelling to decrease to the level normally observed during illumination (Fig. 11). (Addition of EGTA and EDTA without the ionophore does not have this effect.) The suppression of swelling can be reversed, with a return of light sensitivity, if 3 mM Mg⁺⁺ is added back to the suspension, the addition of calcium not being required. This suggests a magnesium requirement for dark swelling. Curiously, the addition of A23187 and EGTA, meant to remove mainly calcium from the outer segments, has no effect (Fig. 11). We draw no conclusion from this, because we have not determined whether calcium is in fact completely removed by this treatment. Hagins and Yoshikami have shown that another calcium ionophore, X537A, can influence sensitivity in vivo (21).

¹ This work has now been reported. Worthington, C. M., III, and R. A. Cone. 1975. Ca⁺⁺ and H⁺ dependence and ionic selectivity of the light-regulated Na⁺ channel in rod outer segments. Biophys. J. 15(2, Pt. 2):171a.
Addition of 5 × 10^{-6} M antimycin to an outer segment suspension completely abolishes light suppression of swelling. Either oligomycin (10^{-4} M) or rotenone (10^{-5} M) alone is partially effective, and completely effective when supplemented with 5 × 10^{-2} M sodium cyanide. Sulfhydryl reagents also irreversibly block the effect of illumination. Iodoacetamide, N-ethyl-maleimide, and mersalyl are effective at 1 mM levels, and rapid swelling of the outer segments is caused by the latter two. We have not established that the mitochondrial inhibitors actually interact with mitochondria present in the preparation. Isolated outer segments which perform light-sensitive swelling have detached from the mitochondria-rich inner segment of the receptor cell (see Fig. 1). They might, however, retain a small number of residual mitochondria. An alternative possibility is that the outer segments contain a "mitochondria-like" pathway.

**Dark Adaptation**

Dark adaptation as it is expressed in these preparations is illustrated by Fig. 12. In these experiments, outer segments are either kept continuously in the
dark, illuminated briefly to give a 20–30% rhodopsin bleach, or illuminated continuously. Swelling is monitored in each condition. It is found that after brief illumination, followed by dark incubation, outer segments swell slowly for 20 min (as if they were still being illuminated), and then begin the more rapid swelling characteristic of dark outer segments. This rapid swelling can again be suppressed by illumination (Fig. 12).

The time required for dark adaptation increases with increasing rhodopsin bleaches (Fig. 13). Thus dark swelling returns 15–20 min after a 30% bleach and 30–45 min after a 60% bleach. While the process which restores dark swelling is influenced by the amount of rhodopsin initially bleached, we find no correlation between dark adaptation and the decay of rhodopsin intermediates (22). Further, the return of dark swelling and its suppressibility by light does not depend on rhodopsin resynthesis, for spectrophotometric measurements reveal no rhodopsin resynthesis in these diluted outer segment suspensions.

The dark-adaptation mechanism is more labile than dark swelling, for in some preparations, even though light-suppressible dark swelling can persist for several hours, dark swelling does not recover after a large rhodopsin bleach. Further, dark adaptation is more rapidly lost than dark swelling as

![Figure 12](image-url)
outer segment suspensions age (5). We have found in several experiments that the process can be maintained by reducing conditions, adding argon and 1 mM DTT.

A correlation with in vivo studies is shown by the experiment of Fig. 14, which demonstrates that return of rapid dark swelling during dark adaptation occurs more rapidly than return of light sensitivity. Illumination of dark outer segments to bleach $5 \times 10^3$ rhodopsins per outer segment per second

![Figure 13](image1.png)

**Figure 13.** Time required for dark adaptation increases with rhodopsin bleaching. Four portions of outer segments were illuminated as indicated, each was then diluted with 25 ml of hyperosmotic solution C, and swelling was monitored.

![Figure 14](image2.png)

**Figure 14.** Light sensitivity returns more slowly than dark swelling during dark adaptation. In this experiment outer segments are divided between six beakers containing hyperosmotic solution A, and each beaker is illuminated as shown. Normal light suppression of dark swelling is observed ($\times-\times-\times-\times$, dark; $\circ-\circ-\circ-\circ$, illuminated), and half suppression is caused by light bleaching $5 \times 10^8$ rhodopsin molecules per outer segment per second ($\times-\times-\times-\times$). This level of illumination fails to suppress swelling after recovery from a 20% bleach (cf. $\square-\square-\square$ and $\triangle-\triangle-\triangle$), a level of bleaching $5 \times 10^9$ rhodopsin molecules per outer segment per second will suppress swelling ($\bullet-\bullet-\bullet-\bullet$).
causes half-maximal suppression of dark swelling (as in Fig. 9 a). If, however, the outer segment suspension is illuminated to bleach 20% of the rhodopsin present, left in the dark until dark swelling is restored, and then irradiated with this same level of illumination, no suppression of this swelling is observed. The preparation now requires a brighter light, bleaching $5 \times 10^4$ rhodopsins per outer segment per second, to suppress swelling. Thus dark adaptation can be divided into two steps: return of dark swelling and restoration of light sensitivity.

DISCUSSION

In several respects the data we report here are similar to those obtained by Korenbrot and Cone (17). Both laboratories observe dark swelling which is suppressed by low levels of illumination if sodium is the major cation present. The light-sensitive swelling that we describe, however, is slower and is observed both in isosmotic and hyperosmotic solutions. Korenbrot and Cone used outer segments freshly shaken from a retina into a small volume, and then rapidly performed hyperosmotic shock with a mixing device which subjected the outer segments to turbulent flow. Under these conditions dark outer segments recovered 2% of their normal volume per second after initial shrinking caused by hyperosmotic shock. In contrast, the outer segments studied in this paper were diluted, their swelling monitored minutes to hours after detachment from the retina, and swelling proceeded at a rate of 0.05–1% of their starting volume per minute. Thus it appears that dilution and aging slow the swelling of dark outer segments, even though the suppression of this swelling by light retains ion specificity and high light sensitivity. This suggestion is reinforced by the data of Fig. 7, which demonstrate that dark swelling is more rapid if observed within minutes after outer segments are sheared from the retina into a very small (~10 µl) volume.

In experiments using hundreds of frogs at different seasons of the year, we repeatedly obtain data on “slow” swelling as shown in the figures of this paper, but have difficulty in repeating both the observations in Fig. 7 and also those reported by Korenbrot et al. in their second paper (18). We find that outer segments from different animals swell variably immediately after they are shaken from a retina into a small volume. This variability between preparations, as well as differences in technique, may account for the failure of Cobbs and Hagins (23) to obtain data of the sort shown by us (Fig. 7), and by Cone’s laboratory (17, 18).

The most simple explanation of the swelling we observe is that it is accounted for by an increase in the volume of the aqueous compartment between the outer segment disc and plasma membranes. Korenbrot et al. (18) have shown in freeze-fracture studies that rapid osmotic volume changes are
confined to this compartment. The fact that outer segments show light-sensitive swelling only if sodium is the major cation present further suggests that influx of sodium chloride across the plasma membrane is responsible for at least part of the observed dark swelling. We would assume then that the driving force for sodium entry is provided by a sodium concentration gradient. This gradient would arise because the concentration of sodium trapped in the outer segments after they break from the retina is likely to be significantly lower than 115 mM, the concentration of sodium in the suspending medium. The suppression of swelling by illumination would be caused by a decrease in sodium permeability ($P_{Na}$). It is known that illumination in vivo decreases the sodium permeability of the plasma membrane (13). (It is also possible that the appearance of impermeant particles inside the outer segment could generate an osmotic gradient causing sodium chloride entry [see below].)

If light-sensitive swelling is regulated by permeability it should be influenced by altering the concentration gradients of the relevant ions. We in fact observe that when NaCl is progressively replaced with KCl (leaving the Ringer's solution osmolarity constant at 235 mosM), outer segments swell at the same rate in the light and dark when the external NaCl concentration is 40–50 mM. This suggests a requirement for a sodium concentration gradient. Because the background swelling (mentioned in the third paragraph of the Results section) is also influenced by changing NaCl concentration we cannot draw a correlation between dark swelling and the magnitude of the NaCl gradient. (We have attempted to demonstrate NaCl influx directly by examining $^{22}$Na uptake by outer segments, but technical problems have blocked these efforts.)

Further evidence that ion permeation might control swelling also comes from observing the effects of ionophores (24). The swelling rate of outer segments in solution B made with 115 mM KCl replacing NaCl is increased by a factor of 2–4 when the K+ ionophore valinomycin ($10^{-7}$ M) is added. Gramicidin D ($10^{-7}$ M), which facilitates movement of Na+, K+, or H+ ions across membranes, increases dark swelling by a factor of 10–20. This effect is probably not caused by H+ movements, for the addition of the H+ ionophore carboxylicanide m-chlorophenylhydrazone with gramicidin, has no additional effect on dark swelling.

Although mechanisms other than a $P_{Na}$ change can be suggested for the light-sensitive swelling, serious problems arise with each. One possibility is that illumination might inhibit active sodium transport responsible for the swelling of outer segments. We have found, however, that 1 mM ouabain, the conventional inhibitor of sodium-potassium-activated ATPase, has no effect on the system. Korenbrot and Cone also failed to observe an effect of ouabain on light-sensitive swelling (17). Another potential mechanism would
have light reducing the driving force of an internal restoring force (acting like a spring) which is responsible for dark swelling. If this explanation were correct, as Korenbrot and Cone point out (17), the light suppression of swelling should be observed in any permeant solute, such as ammonium acetate; this is not observed in either their experiments or ours.

Perhaps the most serious alternative to the plasma membrane permeability change model is the possibility that light suppresses the generation of osmotically active particles inside the outer segment plasma membrane. Such particles might appear, for example, by leakage from the inside to the outside of discs. The presence of these impermeant particles could generate an osmotic gradient causing water and probably sodium chloride entry. If light inhibited the appearance of these particles, this entry, and swelling, would be slowed. Thus particle generation, not sodium permeability, would be controlling the rate of dark swelling.

If this is the correct model, generation of internal solute must be inhibited by very low light levels, and this inhibition must be a continuous function of illumination over four log units of light intensity. (Dark swelling as a function of intensity is described in more detail in a following paper.) Further, generation of this internal solute would be a function of external sodium chloride concentration, be stimulated by papaverine, and show dark adaptation. These characteristics seem most descriptive of what we know about the plasma membrane $P_{Na}$ mechanism of the living cell, and thus we assume that $P_{Na}$ is more likely to be a rate-limiting factor controlling swelling of outer segments in sodium-containing solutions. If this is the case, the partial suppression of dark swelling caused by illumination in all these experiments provides us with a qualitative measure of $\Delta P_{Na}$, the decrease in permeability caused by illumination. It is important to note that this interpretation holds whether the driving force for sodium chloride entry is provided by a sodium concentration gradient or by the steady appearance of the osmotic particles mentioned in the preceding paragraph.

A further point, already mentioned above, is that the dark swelling meant to reflect $P_{Na}$ decays after outer segments are shaken from the retina and diluted. This change suggests either the loss of a component(s) important in keeping open sodium channels, or the generation of an inhibitor of these channels. While we find that dark swelling is enhanced by serum, low calcium, and dithiothreitol, the most dramatic increase is seen when papaverine, or other cyclic nucleotide phosphodiesterase inhibitors, are added. These drugs would be expected to increase intracellular levels of cyclic GMP or cyclic AMP. These effects lead us to consider the possibility that intracellular cyclic nucleotide levels regulate ion permeability. (This issue is discussed in a following paper.)

One of the most striking findings from these "in vitro" studies of outer
segment function is that dark swelling can be abolished by large rhodopsin bleaches, but then returns after a period of time in the dark. We refer to this as dark adaptation because it resembles the process observed in living cells. This dark adaptation appears to occur in two phases. First, dark swelling is restored, perhaps as sodium channels are reopened. Then sensitivity, which can be thought of as reflecting the amount of light needed to close the channels, apparently recovers more slowly. Electrophysiological recordings from receptor cells have suggested that recovery of sensitivity lags recovery of the maximum response amplitude (15, 16). This corresponds to our observations if we assume that maximum response amplitude in vivo reflects the maximum sodium permeability change which can be caused by illumination. In a following paper we demonstrate more clearly that the mechanisms which regulate permeability and light sensitivity can be dissociated and studied separately.

Taken together, the observations of this paper demonstrate that rod outer segments can retain much of their functional competence for several hours after their detachment from the retina. Having probed the in vitro physiology and pharmacology of this system to obtain clues on what pathways might be important, we are now trying to effect correlations between directly measured chemical changes and time-dependent and light-dependent changes in sodium permeability.

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