Effect of Illumination on the Membrane Permeability of Rod Photoreceptor Discs*

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ABSTRACT: Rod photoreceptor outer segment discs are free-floating intracellular membranous organelles which swell and contract in response to the osmotic pressure of the bathing medium.

Visual pigment$_{500}$ is an integral part of the disc membrane structure. Changes in disc volume were measured by weighing purified preparations of frog rod photoreceptor outer segments before and after illumination. Illumination of rod outer segments resulted in a 12–15% volume decrease over a period of 2 hr. This volume decrease was observed at 4°, but not at 27°. The light-induced volume decrease showed a graded response to variations of KCl and NaCl concentrations and was dependent upon the presence of ATP and $CaCl_2$ in the medium. Addition of ouabain to the medium led to a 46% inhibition of the light-induced volume decrease. These experiments were interpreted to show a permeability change of the disc membrane upon illumination. We suggest that intradiscal solute concentrations are maintained at a steady level by a dynamic equilibrium between passive ("leak") and active ("pump") ionic fluxes. Illumination, which causes conformational changes in visual pigment, probably changes the disc membrane in such a way that the passive flux component is enhanced, leading to solute diffusion down the chemical potential gradient. The resulting loss of solute from the intradiscal space leads to a volume decrease of the discs. We propose that the light-induced permeability change of photoreceptor disc membranes plays a physiological role in visual excitation.

Vertebrate rod photoreceptor cells can be divided structurally and functionally into three parts: a synaptic terminal; a cell body containing the nucleus, mitochondria, ribosomes, and other components of the cellular metabolic machinery; and an outer segment containing the light-sensitive system. The cell body and the outer segment are joined by a narrow connecting cilium. The rod outer segments are filled with a closely packed array of membranes which appear, in electron micrographs, as neatly arranged stacks of discs (Sjöstrand, 1961; Cohen, 1963). It was recently shown, in this as well as in other laboratories, that the rod outer segment discs are free-floating, membranous, intracellular organelles with an inside space separate and distinct from the photoreceptor intracellular space (Cohen, 1963; Brierley *et al.*, 1968; Heller *et al.*, 1971). Direct volume measurements (by weighing) in conjunction with electron microscopy have revealed that rod outer segment discs behave as osmometers when certain ions and nonelectrolytes are present in the bathing medium. The intradiscal space expands and contracts reversibly in response to the osmotic pressure of impermeant substances (Heller *et al.*, 1971). The rod outer segment disc membrane is impermeable to Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, (PO_4^{3-}), and sucrose, whereas it is freely permeable to ammonia and acetic acid (Brierley *et al.*, 1968; Heller *et al.*, 1971).

Visual pigments, the photosensitive conjugated proteins which mediate vision, are structural components of the outer segment disc membrane (Wald *et al.*, 1963; Blasie *et al.*, 1969; Hall *et al.*, 1969; Heller, 1969). For some time it has been known that retinyl, the prosthetic group of visual pigment$_{500}$, isomerizes from the 11-cis to the all-trans configuration upon illumination. Concomitantly the visual pigment apoprotein changes its conformation, as indicated by a variety of experimental techniques (Wald and Hubbard, 1960; Hubbard *et al.*, 1965; Heller, 1968).

Thus it is of interest to inquire if, as a result of illumination and the accompanying conformational change in the visual pigment molecule, changes occur in the properties of the rod outer segment disc membrane. In other words, does conformational change in a particular membrane structural protein lead to changes in the membrane as a whole?

The answer to this question bears directly on the problem of visual excitation. Recent evidence obtained from the rat retina indicates that a steady dark current flows on the outside of the rod photoreceptor cells from the inner to the outer segment and that illumination leads to a reduction in this current (Penn and Hagins, 1969; Hagins *et al.*, 1970). Thus illumination of the rod photoreceptor seems to produce a change in the outer enveloping plasma membrane which ultimately decreases the dark current. It has been suggested that this phenomenon is due to a decrease in permeability of the enveloping membrane to Na^+ (Sillman *et al.*, 1969). There are no direct anatomical connections between rod outer segment discs and the outer enveloping membrane of the outer segment (Cohen, 1968). Yet, the disc membrane contains the visual pigment and, therefore, is the site of initial reaction to photic stimulation. If the changes in the current which are caused by light do indeed carry visual information to the photoreceptor synapse, then the excitation produced by light which is recorded by the visual pigment in the disc membrane must somehow be transferred to the outer

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enveloping membrane in order to be propagated. In the present report, we describe studies on light-induced changes in prepared rod outer segment discs. It is suggested that these changes might represent early stages of visual excitation.

Materials and Methods

The preparation of frog rod outer segments (ROS)[1] has been described elsewhere (Heller *et al.*, 1971). ROS were prepared fresh for every experiment. Forty to fifty retinas, yielding approximately 300 mg of ROS, were used in each experiment. Experiments were performed at 4° unless specified otherwise. Na_2ATP (Sigma) was dissolved in H_2O, adjusted to pH 7.2 with KOH and stored frozen until used. Ouabain (Sigma) was dissolved in H_2O and kept at 4°. All operations except weighing were performed under the dim illumination of a sodium lamp.

Purified ROS were resuspended in a preweighed 3.5-ml polycarbonate centrifuge tube by adding 3 ml of medium and vortexing for approximately 5 sec. The ROS were then centrifuged at 41,000*g* for 7 min. (The time was measured with a stopwatch from the start of acceleration to the time the centrifuge began to decelerate.) The clear supernatant was decanted and saved. The inside walls of the tube including the pellet rim were carefully dried with small pieces of lint-free paper. For the success of the experiments, it was crucial to dry the tube and pellet rim in a reproducible fashion. (When this was achieved, an ROS pellet could be repeatedly suspended and centrifuged down with the weight in successive spins varying by less than ±0.5%.) The dried centrifuge tube with the pellet was then placed in a light-tight, preweighed, lightweight aluminum container (Bering Cigars, Tampa, Fla.) and weighed with a Magni-Grad model FH microbalance (Ainsworth, Denver, Colo., rated sensitivity of 1 μg) using class S (g) and M (mg) weights. The weighing was performed at 22°. The procedure was then repeated by resuspending the pellet in the supernatant or in a new medium with the appropriate additions.

ROS in suspension were illuminated for 30 sec with a 120-W white light at a distance of 15 cm from the bulb. This was approximately twice the time needed to completely bleach the ROS suspension. In some experiments the ROS pellets were illuminated for 5 min at 500 nm with a monochromator (Bausch and Lomb, Model 338602) equipped with a tungsten light source, infrared filter, and an exit slit of 3 mm. This illumination was sufficient to bleach the ROS pellet completely. Once illuminated, the procedure, including drying the tube under dim sodium light and weighing in the light-tight container, continued as before.

Results

Effect of Illumination on ROS Disc Volume in the Presence of ATP at 4°. When ROS discs were suspended in a medium containing 7.5 mM ATP, 30 mM KCl, and 10 mM NaCl and repeatedly weighed in the *dark*, a very slow decrease in weight (volume) occurred over a period of several hours (Figure 1). During this time the pellet volume decreased

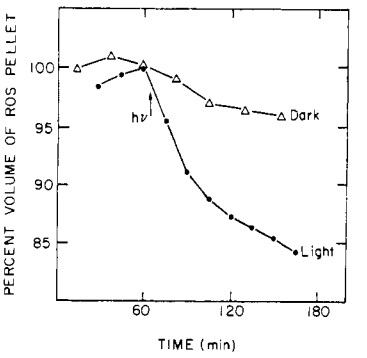

FIGURE 1: Volume of dark and illuminated ROS pellets in the presence of ATP at 4°. ROS discs were isolated as described in Materials and Methods. The ROS pellets were incubated in 30 mM KCl, 10 mM NaCl, 5 mM $CaCl_2$, 7.5 mM $MgCl_2$, 1 mM Tris-HCl buffer, pH 7.5, and 7.5 mM ATP at 4°. The total osmolarity of the medium was 148.5 mosmolar. The last point before illumination is defined as 100%.

progressively with no sharp breaks. The total decrease in volume was approximately 5%. The largest change in volume between any two consecutive measurements was 1.7%, and the largest cumulative change of two consecutive measurements was 2.3%. On the other hand, when an ROS disc pellet incubated under identical conditions and in the same medium as above was illuminated for 30 sec with white light, there was a dramatic decrease in volume (Figure 1). The largest decrease in volume between two consecutive measurements was 4.5%, and it took place between the last dark measurement and the initial measurement following illumination. The largest volume change in two consecutive measurements, 9.3%, took place immediately following illumination. The total decrease in weight was 16%. Since the volume decrease in the dark during an equivalent period was 4%, the net volume decrease after illumination was 12%. The volume contraction following illumination seemed to have two components: a relatively rapid initial change in volume and a subsequent much slower change, over a period of an hour or so, stabilizing the ROS disc volume at a new lower level. If the rate of volume contraction is expressed as per cent change over time (min), then the initial change after illumination was $0.31\% \times \text{min}^{-1}$, compared to $0.072\% \times \text{min}^{-1}$ for the later change.

Illumination of an ROS pellet with 500-nm monochromatic light led to the same volume decrease obtained by illuminating with a white light source. This indicates that the light effect probably was due to a change in visual pigment_{500}.

Effect of Illumination on ROS-Disc Volume in Media of Different Osmolarities in the Presence of ATP at 4°. In the experiment described above, the total osmolarity of the suspending medium was 148.5 mosmolar with a KCl:NaCl ratio of 3:1. This is about half the normal tissue osmotic pressure. To investigate the effect of varying the total osmolarity of the medium on the light-induced volume decrease of ROS discs, the total osmotic pressure was changed while keeping the KCl:NaCl ratio constant. As shown in Figure 2, the largest light-induced volume decrease was observed when the osmolarity of the medium was 100 mosmolar, while the volume actually increased temporarily in media containing 300 mosmolar salts. The conclusion derived from this experi-

[1] Abbreviations used are: ROS, rod outer segment; ATP, adenosine triphosphate.

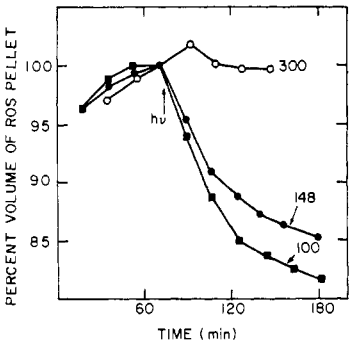

FIGURE 2: Effect of varying the total osmolarity of the medium. ROS pellets were incubated at 4° in 5 mM $CaCl_2$, 7.5 mM $MgCl_2$, 1 mM Tris-HCl buffer, pH 7.5, and 7.5 mM ATP with various amounts of KCl and NaCl. The concentrations were: 89 mM KCl and 29 mM NaCl (300 mosmolar); 30 mM KCl and 10 mM NaCl (148 mosmolar); 12 mM KCl and 4 mM NaCl (100 mosmolar).

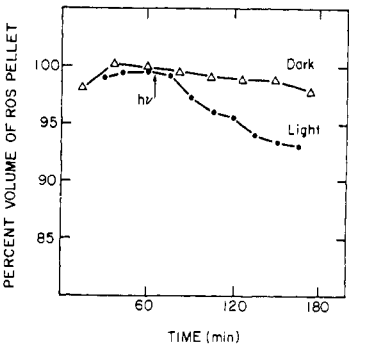

FIGURE 4: Volume of dark and illuminated ROS pellets in the absence of ATP at 4°. ROS pellets were incubated in 42 mM KCl, 12 mM NaCl, 5 mM $CaCl_2$, 7.5 mM $MgCl_2$, and 1 mM Tris-HCl buffer, pH 7.5. The total osmolarity of the medium was 148.5 mosmolar.

ment was that the light-induced volume decrease is dependent either on the total osmolarity of the medium as such, or on the concentration of a particular salt.

To distinguish between these possibilities, an ROS pellet was incubated in 148.5 mosmolar salts with sucrose added to bring the total osmolarity of the medium to 300 mosmolar. If the light-induced volume decrease is dependent on the osmotic pressure, no volume decrease should be observed in a medium of 300 mosmolar (Figure 2). As shown in Figure 3, the addition of sucrose to the medium did not affect the light-induced volume decrease observed in 148 mosmolar salt medium (Figure 1). Thus, it seems the volume decrease is affected by the presence of either KCl or NaCl, or both, but not by the total osmotic pressure of the medium.

Effect of Illumination on ROS-Disc Volume in the Absence of ATP at 4°. When an ROS pellet was repeatedly weighed in the dark at 4° in the absence of ATP, its weight stayed practically constant over a period of 2.5 hr (Figure 4). When a similar ROS pellet was incubated under identical conditions (namely, in the absence of ATP) and then illuminated, a small decrease in weight took place (Figure 4). The largest change in two consecutive measurements was a decrease of 2.0%. The total decrease in weight of the ROS pellet was 7.5%. The rate of volume decrease again exhibited two phases: an initial, relatively rapid decrease and a later, much slower one. The initial rate was 0.085% × min^{-1}, whereas the slower, later rate was 0.05% × min^{-1}. Thus, it can be seen that the volume decrease was smaller in the absence of ATP.

Effect of Temperature on the Volume Decrease of ROS Discs after Illumination. As described above, when ROS pellets were illuminated at 4° in the presence of ATP, the marked decrease in pellet volume amounted to a total of 16%. When the same experiment was repeated at 27° (in the presence of ATP) there actually was a small, temporary increase in weight following illumination. Thereafter the weight stayed constant (Figure 5). From this experiment, it was concluded that the process causing the volume decrease at 4° in the presence of ATP is either inoperative or overshadowed by some other process at 27°.

Effect of NaCl and KCl on the Volume Decrease of Illuminated ROS Discs. In the presence of 30 mM KCl, 10 mM NaCl, and ATP at 4°, a 16% decrease in the weight of ROS discs followed illumination (Figures 1 and 6). On the other hand, when the KCl in the medium was replaced by NaCl, the volume actually *increased* temporarily by 3.1% following illumination, only to decrease slowly later on (Figure 6). Replacing all the NaCl in the medium with KCl led to a light-induced volume decrease totaling 11.5% over a period of 2 hr. Still, the decrease in volume was smaller and slower in a

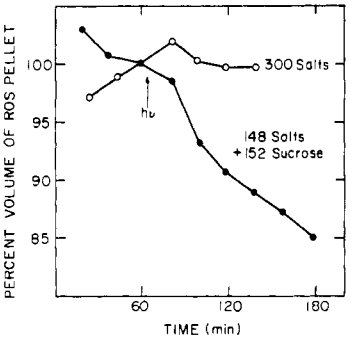

FIGURE 3: Effect of sucrose on the light-induced volume decrease of ROS discs. ROS pellets were incubated at 4° in salt media of either 300 mosmolar or 148 mosmolar (see legend, Figure 2). The latter medium contained, in addition, 152 mosmolar sucrose.

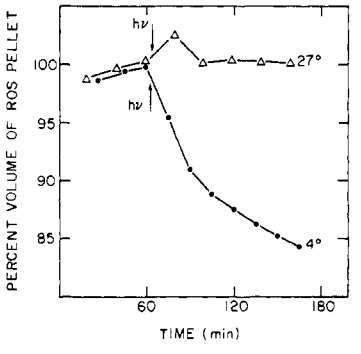

FIGURE 5: Effect of temperature on the volume decrease of ROS discs after illumination. ROS disc pellets were incubated in a medium containing 30 mM KCl, 10 mM NaCl, 5 mM $CaCl_2$, 7.5 mM $MgCl_2$, 1 mM Tris-HCl buffer, pH 7.5, and 7.5 mM ATP at either 4 or 27°. The total osmolarity of the medium was 148.5 mosmolar.

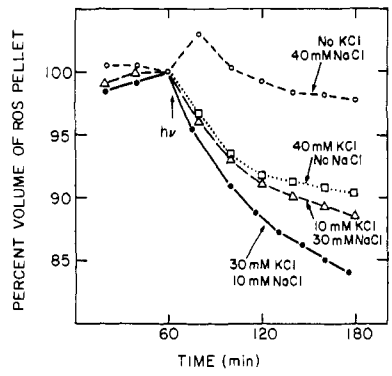

FIGURE 6: Effect of NaCl and KCl on the volume decrease of illuminated ROS discs. ROS pellets were suspended at 4° in media containing 7.5 mM ATP, 5 mM $CaCl_2$, 7.5 mM $MgCl_2$, 1 mM Tris-HCl buffer, pH 7.5, and variable amounts of NaCl and KCl. Total osmolarity of media was 148.5 mosmolar. ATP was a mixed Na and K salt.

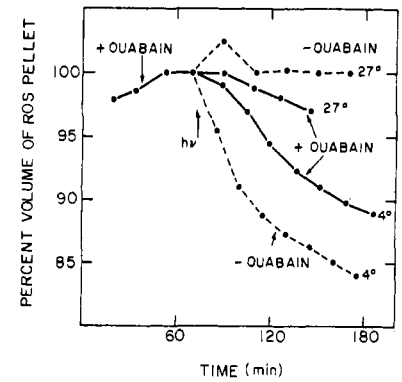

FIGURE 7: Effect of ouabain on the volume decrease of ROS discs after illumination. ROS pellets were incubated in a medium containing 30 mM KCl, 10 mM NaCl, 5 mM $CaCl_2$, 7.5 mM ATP, 7.5 mM $MgCl_2$, 1 mM Tris-HCl buffer, pH 7.5, and 0.1 mM ouabain, either at 4 or 27°. Total osmolarity of the media was 148.5 mosmolar. ROS volume decrease after illumination in the absence of ouabain is plotted for comparison.

medium that contained only KCl than in a medium that contained both KCl and NaCl at a concentration ratio of 3:1 (Figure 6). Reversing the molar concentration of KCl and NaCl (1:3) led to a volume decrease following illumination that was closer to that observed with only KCl in the medium (Figure 6). This series of experiments shows that the ROS volume decrease following illumination is maximal in the presence of both KCl and NaCl (3:1). No volume decrease is observed in the absence of KCl. On the other hand, there is a considerable volume decrease in the absence of NaCl. It should be pointed out that all media contained 15 mM Na^+ and 15 mM K^+ as ATP counterions so the media were never potassium or sodium free.

Effect of Ouabain on ROS Discs. Addition of 0.1 mM ouabain to an ROS pellet in the dark at 4° in the presence of ATP and other salts did not cause any changes in the pellet volume (Figure 7). Illuminating the pellet (at 4°) resulted in a volume decrease considerably slower and smaller than in the absence of ouabain (Figure 7). The initial rate of change in the presence of ouabain was 0.115% × min^{-1} as compared with 0.31% × min^{-1} in its absence, whereas the later changes were 0.068% × min^{-1} and 0.072% × min^{-1}, respectively. The total volume decrease was 10.5% as compared with 16%. Since over an equivalent period of time, the volume decrease of ROS suspended at 4° in the dark was 4% (Figure 1), the actual inhibition caused by ouabain was 46% of the full effect of illumination. Suspending an ROS pellet at 27° in the presence of ouabain and illuminating it led to a slow volume decrease (Figure 7). Thus ouabain diminishes but does not abolish the effect of light on ROS disc volume at 4°. Ouabain seems to have no effect on ROS discs in the dark and little effect on the response to light at 27°.

Effect of $CaCl_2$ on the Volume Decrease of Illuminated ROS Discs. Illuminating an ROS pellet suspended in a $CaCl_2$-free medium resulted in an initial temporary increase in volume followed by a slow decrease (Figure 8). The absence of $CaCl_2$ in the medium thus mimics the effect of illumination on an ROS pellet suspended in a KCl-free medium (Figure 6).

Discussion

A previous study from this laboratory has shown that the ROS preparation utilized in the present study was composed of ROS fragments of various sizes (Heller *et al.*, 1971). The ROS fragments contained from a few up to several hundred discs and, in addition, vesicles of various sizes. The vesicles were most probably derived from individual discs. On the basis of electron micrographs of random sections, the ROS preparation was judged to be approximately 90% pure (Heller *et al.*, 1971). The membrane enveloping the outer segment was either torn or entirely missing, and the discs were consequently in direct contact with the bathing medium. The intradiscal volume could be directly controlled and manipulated by changing the osmotic pressure of the medium and could be measured with great accuracy by weighing (Heller *et al.*, 1971).

The present series of experiments has shown that when ROS discs were kept in the dark in a medium of given osmolarity, the intradiscal volume stayed constant or decreased at a slow rate. The disc volume is a reflection of a dynamic equilibrium between the osmotic pressure of the intradiscal and the

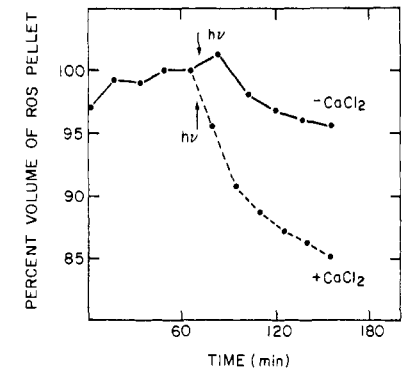

FIGURE 8: Effect of $CaCl_2$ on the volume decrease of illuminated ROS discs. An ROS pellet was incubated in 35.6 mM KCl, 11.9 mM NaCl, 7.5 mM $MgCl_2$, 7.5 mM ATP, and 1 mM Tris-HCl buffer, pH 7.5 at 4°. The medium osmolarity was 148.5 mosmolar. The effect of illumination on a pellet suspended in a medium containing $CaCl_2$ is shown for comparison. (For experimental details, see Figure 1.)

interdiscal space ("medium"). The constancy of the intradiscal volume means that the osmolarity of the intradiscal and interdiscal fluids was maintained; that is, the selective permeability of ROS disc membranes was preserved.

The initial observation in the present study was that frog ROS discs decreased in volume after illumination with white light when incubated at 4° in the presence of ATP, NaCl, KCl, $CaCl_2$, $MgCl_2$, and Tris buffer (Figure 1). The volume change occurred in two phases: initially a rapid volume decrease, followed by a slower change. The maximal volume decrease of a whole ROS pellet was approximately 16%. Since 38% of the pellet volume is interstitial (interdiscal) space (Heller *et al.*, 1971), the intradiscal volume decrease was actually 26%. The intradiscal volume decrease indicates a decrease of the osmolarity inside the disc, since the osmolarity of the relatively large volume of bathing medium remained constant throughout. This implies that as a result of illumination the permeability of the disc membrane changes in such a way that there is a net loss of osmotically active substances. Using figures obtained in a previous study (Heller *et al.*, 1971), a 16% *decrease* in volume of an ROS pellet suspended in 150 mosmolar NaCl can be obtained by increasing the osmolarity of the *medium* from 150 to 300 mosmolar. Because the osmolarity of the medium in the present series of experiments remained constant, the decrease of intradiscal volume was due to net loss of solute from this space. Remembering the linear relationship between osmotic pressure and solute concentration, a *change that is simulated by doubling the osmotic pressure of the medium is equivalent to halving the intradiscal solute concentration.* It is assumed that the interstitial (interdiscal) space is identical in all ROS pellets; therefore, in the above calculation, the change of intradiscal volume as a function of the osmolarity of the medium is obtained directly by experiment (Heller *et al.*, 1971).

If, as we have calculated, the ROS discs lose about half of their osmotically active solutes following illumination, does this mean they become completely "leaky" and permeable? In a previous study (Heller *et al.*, 1971), we found that the linear relationship between the ROS disc volume and the inverse osmotic pressure holds in both dark and illuminated preparations. This was interpreted to show that disc membranes of illuminated ROS retain their semipermeability. Thus, it is not simply that illuminated ROS discs become "leaky," losing part of their solute complement; the solute loss occurs in spite of other mechanisms that maintain the disc membrane semipermeability and solute segregation.

The observation that illumination of ROS discs in a medium containing ATP and salts (total 148.5 mosmolar) at 4° causes a net 12% volume decrease led us to investigate further the effect of several parameters on this volume change. The experiments reported in this paper can be summarized as follows.

1. The presence and relative extent of the volume decrease was a function of the salt concentration in the medium. *Lowering* the salt concentration (with a constant 3:1 ratio of KCl:NaCl) *increased* the relative magnitude of the volume decrease (Figure 2). This was a specific effect due to change of salt concentration and was not an effect due to the total osmotic pressure of the medium, since addition of sucrose did not change the light-induced volume decrease (Figure 3).

2. The volume decrease was completely dependent on the presence of $CaCl_2$ in the medium (Figure 8) and partially dependent on the presence of ATP (Figure 4).

3. The volume decrease was only observed at 4°. At 27° there was a small transitory increase (Figure 5).

4. The largest volume decrease took place with a 3:1 molar ratio of KCl to NaCl in the medium. Other ratios of KCl to NaCl resulted in a smaller or no volume decrease (Figure 6).

5. The addition of 10^{-4} M ouabain to the medium at 4° led to volume decrease which was slower and 46% smaller than the effect observed in its absence (Figure 7).

We have incorporated these findings and previously published data into the following working hypothesis concerning rod–disc structure and function. The disc membrane encloses a space which is separate from the photoreceptor intracellular space. We assume that the interdiscal (intracellular) space is high in K^+, while the intradiscal space (which is derived from the intercellular space by invagination of the plasma membrane) is high in Na^+. The different solute concentrations in the interdiscal and intradiscal spaces are kept at a steady level by a dynamic equilibrium between passive and active ionic fluxes. The passive flux, or diffusion, moves Na^+ from the intradiscal to the interdiscal space while moving K^+ in the other direction. The active flux, or pump, transports the same ions in the opposite direction, against their chemical potential gradients. The levels at which the concentrations of K^+ and Na^+ stabilize in the dark is determined by the interplay between the disc membrane permeability and the pump activity. Illumination probably affects the permeability (diffusion) part of this ionic balance by causing conformational changes in a structural component (visual pigment) of the membrane. The increased permeability facilitates the passive diffusion and the pump has to do more work if the ions are to be kept at their former level. Lowering the temperature slows down the active pump enough to result in a net solute loss from the intradiscal space through diffusion. This in turn leads to the observed osmotic contraction of disc volume. The effects of varying the KCl:NaCl ratio of the medium can probably be explained in two ways. First, increasing the NaCl concentration in the medium decreases the gradient between intradiscal and interdiscal Na^+ concentration and the pump is able to maintain the ionic concentrations even at 4°. On the other hand, the pump probably has an optimal concentration of KCl and NaCl in the medium for full activity. Thus, the pump activity at 30 mM KCl and 10 mM NaCl might be different from that at 30 mM NaCl and 10 mM KCl. In addition, the permeability of membranes to a particular ion is in some cases a function of the concentration of this ion in the medium (Grundfest, 1967). The above explanations are probably not mutually exclusive. The necessity of having $CaCl_2$ in the medium in order to observe the volume decrease is probably due to the general effects of Ca^{2+} ions on membrane structure and permeability (Morrill and Robbins, 1967; Kleinzeller *et al.*, 1968). The absence of Ca^{2+} might diminish or prevent the light-induced permeability increase of disc membranes. The observations that the lack of ATP or presence of ouabain decreases light-induced volume contraction are difficult to reconcile with the proposed hypothesis. If it is true that the volume decrease can only be observed at 4°, because at this temperature the pump activity is sufficiently slowed down, then the lack of ATP would be expected to have the same effect as lowering the temperature.

The data show the opposite: lack of ATP diminishes rather than enhances the light-induced volume decrease. The same can be said about ouabain. We know of no simple explanation that would reconcile all the experimental observations. It is, of course, possible that more than one mechanism leads to the same final result (disc volume decrease), and that ATP and ouabain might act by modifying the passive diffusion from the intradiscal space by affecting the light-induced conformational change.

Despite the difference in time scale between the light-induced volume decrease of photoreceptor discs reported in this paper and the time it takes to perceive a visual image, we feel that our data have a bearing on the physiological mechanism of visual excitation. We have shown that illumination changes the overall permeability of photoreceptor discs, that this change is not simply a loss of semipermeability, and that there seems to be a pump component present. It is conceivable that, *in vivo*, illumination results in a sudden localized ionic surge from the photoreceptor disc. The ionic surge in turn affects the permeability of the enveloping plasma membrane leading to the observed change in the dark current (Penn and Hagins, 1969; Hagins *et al.*, 1970; Sillman *et al.*, 1969). It is well known that one or a few photons can evoke a visual image. Thus, it is possible that a small sudden change in the permeability of a single disc can trigger visual excitation. The experiments reported in this paper bleached the total complement of visual pigment and thereby transformed all the discs. A considerable net loss of ions from the intradiscal space had to take place before we could observe a change in volume. This change, probably, is many times larger than the physiological change needed to evoke a receptor potential. The significant contribution of these studies, we feel, is the demonstration that light-induced permeability changes can occur in photoreceptor discs.

References

Blasie, J. K., Worthington, C. R., and Dewey, M. M. (1969), *J. Mol. Biol. 39*, 407.

Brierley, G. P., Fleischman, D., Hughes, S. D., Hunter, G. R., and McConnell, D. G. (1968), *Biochim. Biophys. Acta 163*, 117.

Cohen, A. I. (1963), *Biol. Rev. 38*, 427.

Cohen, A. I. (1968), *J. Cell Biol. 37*, 424.

Grundfest, H. (1967), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 26*, 1613.

Hagins, W. A., Penn, R. D., and Yoshikami, S. (1970), *Biophys. J. 10*, 380.

Hall, M. O., Bok, D., and Bacharach, A. D. E. (1969), *J. Mol. Biol. 45*, 397.

Heller, J. (1968), *Biochemistry 7*, 2914.

Heller, J. (1969), *Biochemistry 8*, 675.

Heller, J., Ostwald, T. J., and Bok, D. (1971), *J. Cell Biol. 48* (in press).

Hubbard, R., Bownds, D., and Yoshizawa, T. (1965), *Cold Spring Harbor Symp. Quant. Biol. 30*, 301.

Kleinzeller, A., Knotkova, A., and Nedvidkova, J. (1968), *J. Gen. Physiol. 51*, 326S.

Morrill, G. A., and Robbins, E. (1967), *J. Gen. Physiol. 50*, 781.

Penn, R. D., and Hagins, W. A. (1969), *Nature (London) 223*, 201.

Sillman, A., Ito, H., and Tomita, T. (1969), *Vision Res. 9*, 1443.

Sjöstrand, F. S. (1961), *in* The Structure of the Eye, Smelser, G. K., Ed., New York, N. Y., Academic, p 1.

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

Wald, G., and Hubbard, R. (1960), *Enzymes 3*, 369.