Dark Ionic Flux and the Effects of Light in Isolated Rod Outer Segments

JUAN I. KORENBROT and RICHARD A. CONE

From the Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218

ABSTRACT We have determined the permeability properties of freshly isolated frog rod outer segments by observing their osmotic behavior in a simple continuous flow apparatus. Outer segments obtained by gently shaking a retina are sensitive but nonideal osmometers; a small restoring force prevents them from shrinking or swelling quite as much as expected for ideal behavior. We find that Na^+, Cl^-, No_3^-, glycerol, acetate, and ammonium rapidly enter the outer segment, but K^+, $SO_4^=$, and melezitose appear impermeable. The Na flux is rectified; for concentration gradients in the physiological range, 2×10^9 Na^+ ions/sec enter the outer segment, but we detect no efflux of Na^+, under our conditions, when the gradient is reversed. Illumination of the outer segment produces a specific increase in the resistance to Na^+ influx, but has no effect on the flux of other solutes. This light-dependent Na^+ resistance increases linearly with the number of rhodopsin molecules bleached. We find that excitation of a single rhodopsin molecule produces a transient ($\sim$1 sec) "photoresistance" which reduces the Na^+ influx by about 1%, thus preventing the entry of about 10^7 Na^+ ions. At considerably higher light levels, a stable afterimage resistance appears which reduces the Na influx by one-half when 10^6 rhodopsin molecules are bleached per rod. We have incorporated these findings into a model for the electrophysiological characteristics of the receptor.

INTRODUCTION

Rods and cones in the vertebrate retina hyperpolarize in response to light (Bortoff, 1964; Tomita, 1970; Toyoda et al., 1970). In darkness, a steady ionic current flows between the inner and outer segments of the receptor cell and the effect of light is to reduce this "dark current." Extracellularly, the dark current flows from the inner segment to the outer segment, establishing a potential gradient along the receptor cell (Hagins et al., 1970).

The membrane characteristics which produce this current are not yet known, but several possibilities exist. For example, the membranes of the inner and outer segment may differ in ionic selectivities and the effect of light may be to alter the permeability to some ion. On the other hand, light may

inhibit active generators of dark current, or stimulate active current generators which oppose the dark current.

Light increases the resistance of the receptor membrane in rods (Toyoda et al., 1969) and also in cones (Baylor and Fuortes, 1970). However, it has so far been difficult to determine the cellular location of the resistance increase since intracellularly the receptors appear to be nearly isopotential (Murakami and Pak, 1970). Furthermore, it is not known whether the observed resistance increase can adequately account for the reduction in dark current.

Sillman et al. (1969) have suggested that in frog rods there is a light-sensitive Na resistance in the outer segment and that the potential to which the cell hyperpolarizes may be determined by a K battery. Baylor and O'Bryan (1971) have made the same suggestion for turtle cones. Arden and Ernst (1970), on the other hand, have suggested that under special conditions there is a light-sensitive nonspecific cationic resistance in the outer segment of pigeon cones, and a steady anionic resistance in the inner segment. More recently Yoshikami and Hagins (1971) have presented evidence which indicates the existence of a light-dependent Na^+ resistance in the plasma membrane of the outer segment of rat rods.

Many of the problems raised by these findings can be resolved by observing ionic fluxes in the isolated outer segment. Outer segments can easily be separated from the rest of the receptor, and several studies on their ionic permeabilities have been reported (Bowmaker, 1970; Brierley et al., 1968; Bonting and Bangham, 1967; Etingof et al., 1964; Heller et al., 1971). However, the results have been contradictory and not in agreement with electrophysiological data. In most of the investigations the outer segments were subjected to extended purification and measuring procedures which may have altered the ionic permeabilities since outer segments are known to undergo structural and, presumably, functional changes shortly after being separated from the retina (Sidman, 1957).

We report here a technique with which the ionic fluxes in isolated outer segments can be determined by their osmotic behavior. With this technique outer segments undergo a minimum of manipulation and measurements are completed within minutes after separation from the retina.

MATERIAL AND METHODS

Biological Material All the experiments reported here were performed on rod outer segments isolated from retinas of adult leopard frogs (*R. pipiens*), except for one series of experiments on albino rats (*R. rattus*). The frogs were kept in constant darkness and killed by decapitation immediately preceding the experiment. The rats, which were also kept in constant darkness, were killed by intraperitoneal injection of pentobarbital (300 mg/kg). The eyes were enucleated and the retinas were gently dissected free of the pigment epithelium under dim red light. This procedure took ap-

proximately 4 min. With the use of photomicrography and a simple continuous flow apparatus we followed the time-course of volume changes of outer segments following an osmotic shock.

Continuous Flow Apparatus In a Lucite block (6 × 7 × 1 cm) three channels were drilled so as to form a T junction (Fig. 1). A suspension of outer segments flowed into the junction through one of the opposing arms of the T, while a nonisosmotic solution flowed in through the other. These two solutions mixed at the junction and flowed out through the third channel, which led to an exit point on the surface of the block. This channel was 1 cm long and 380 μ in diameter. At the exit point a duct was made by cutting a 0.2 mm wide slit in a single layer of Parafilm (American Can Company, New York), which was aligned with the exit. The slit was covered with a 22 × 60

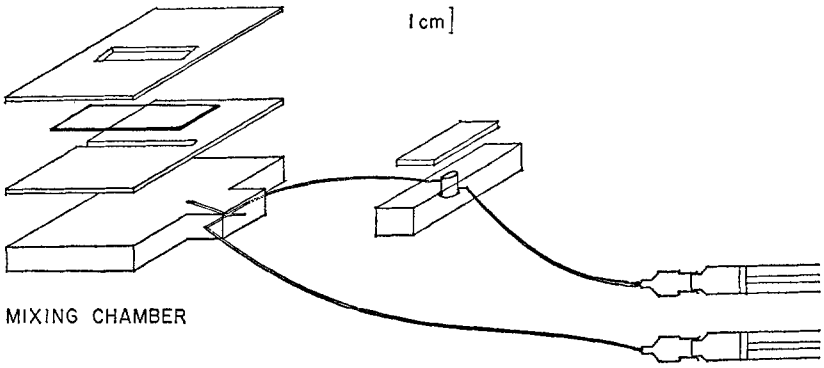

FIGURE 1. Diagram of the continuous flow apparatus. The Parafilm, cover slip, and Lucite clamping sheet are presented in their order of assembly. They are held together with nylon screws. The T junction has a fourth channel which is occasionally used to help eliminate air bubbles in the mixing chamber. The loading well is tightly sealed with a Lucite top. The upper syringe and the loading well contain the incubating solution; the lower syringe contains the test solution.

mm cover slip, and clamped by a thin sheet of Lucite. (This assembly was cleaned and prepared anew for every experiment.) The assembled Lucite block was mounted on a microscope stage and the ×10 objective of the microscope was focused at the exit point of the mixing chamber. A Polaroid camera (ED-10, Polaroid Corporation, Cambridge, Mass.) was mounted on the microscope with a ×15 ocular. Photographs were obtained using a xenon arc flash with a 0.1 msec duration (Lightning-Lite, Lakewood, Ohio) filtered through Schott filters, types BG-18 and OG-2 (Schott und Gen., Jena, Germany), which passed light between 550 and 700 nm.

With the use of dyes, and also isolated outer segments, we found that mixing was essentially complete shortly after the solutions combined at the T junction. Therefore, the rate at which the outer segments advanced from the mixing point to the exit point, where they were photographed, defined the time that elapsed after the osmotic shock.

The suspension of outer segments was prepared under dim red light by gently shaking the isolated retina in a 0.06 cc loading well. The shaking action was just

sufficient to break the narrow ciliary stalk that connects outer and inner segments so that nearly all of the outer segments in the suspension were intact and unfragmented. The loading well was connected to the mixing chamber by a piece of 25 cm long polyethylene tubing (Intramedic Pe-20, I.D. 380 μ; Clay-Adams, Inc., Parsippany, N.J.) through which the suspension of outer segments flowed. The well was also connected to a syringe which contained the same incubating solution in which the outer segments were suspended. This syringe was used to drive the suspended outer segments from the loading well into the mixing chamber.

The test solution, with an osmotic pressure which usually differed from that of the incubating solution, was stored in a second syringe. This syringe injected the test solution into the mixing chamber. The plungers of both syringes were mounted so that they could be driven simultaneously, or independently, by a constant speed motor (Cole-Parmer Instrument Co., Chicago, Ill, Model 4420). The rate at which the plungers were driven was calibrated in terms of the time required for a solution front to advance from the mixing point to the exit point of the mixing chamber. Thus, by using different driving rates, the osmotic behavior of the outer segments could be studied as a function of time following an osmotic shock. We found that if both the test solution and the incubating solution were the same, mixing did not, itself, produce any volume changes.

The lengths and widths of the outer segments were measured directly from the Polaroid photographs (see Fig. 5) using a dissecting microscope with a calibrated reticle. For each experiment a photograph of a control group of outer segments was obtained by driving only the syringe containing the incubating solution. The dimensions of 20–40 of these control outer segments were arithmetically averaged. Photographs of 50–200 outer segments were then obtained at 3, 7, or 10 sec after the osmotic shock by driving both syringes. In the Results the dimensions are expressed in percentage units relative to the dimensions of the outer segments in the standard solution.

In making measurements we were careful to select unbroken rod outer segments. In the case of the frog we did not distinguish between green and red rods, but only 4% of the rods are green (Liebman and Entine, 1968).

In long-term observations under the phase microscope, we found that outer segments suspended in hyperosmotic KCl solutions began to elongate and undergo other structural changes some 1000 sec after their separation from the retina. Changes of the same nature and time-course, although not of the same extent, also occurred when outer segments were suspended in the standard solution. Similar postmortem degenerative changes have been discussed by other investigators (Robertson, 1966). In order to avoid such nonspecific degeneration, all our experiments were completed within 1000 sec after the outer segments were detached from the retina.

Light Exposure Two methods were used. In the first a single flash from an M-3 flashbulb ($\sim$30 msec duration) was presented through a filter combination which passed wavelengths between 560 and 750 nm (Wratten 23-A, Eastman Kodak Co., Rochester, N.Y.; Schott KG-3, and Edmund Scientific Co., Barrington, N.J., IR filters). We found that within our limits of resolution no volume changes were induced by flash illumination alone. The suspended outer segments were flash irradiated in the

loading well several minutes prior to the osmotic shock, and the control photograph for each experiment was obtained after this illumination.

In the second method, outer segments were continuously and uniformly illuminated in the mixing chamber starting 6 sec after they had been osmotically shocked. The radiation source in this case was a tungsten lamp the output of which was adjusted to a constant value by means of a light meter. This light was presented through a filter combination which passed wavelengths between 540 and 800 nm (Wratten 21, Eastman Kodak Co., and Corning No. 3965, Corning Glass Works, Corning, N.Y.).

Source Calibration Carefully calibrated neutral density filters (Wratten 96, Eastman Kodak Co.) were used to regulate the light exposure. Both light sources were calibrated in terms of their ability to bleach rhodopsin. The M-3 flash exposure was calibrated using suspensions of frog outer segments under conditions identical with those of the experiment. The tungsten lamp was calibrated using rhodopsin-digitonin solutions prepared from cattle eyes (Matthews et al., 1963). Frog and cattle rhodopsin have the same extinction coefficient (Bridges, 1970; Hubbard et al., 1959). The fraction of pigment bleached was measured in both preparations in the presence of 50 mM NH_2OH by measuring absorption spectra in a recording spectrophotometer. On the basis of this calibration procedure we estimate that our measurements of the fraction of pigment bleached in the outer segments are accurate to within ±20%.

Solutions All solutions were prepared in distilled, demineralized water with reagent grades salts and in every case were buffered to pH 7.4. The concentrations of nonisosmotic solutions are always expressed in units of isosmotic pressure (one unit, 1 Is, is 232 mosmoles/liter in the case of the frog, and 265 mosmoles/liter in the case of the rat). In general, test solutions were prepared by adding salts to the standard solution in order to produce osmotic pressures several times that of the standard (Table I). The concentrations required were determined from freezing point depression data (Weast, 1967). Some solutions were prepared with an osmotic pressure equal to that of the standard, but with different chemial compositions. These solutions were always tested on isolated outer segments against the standard, to insure that the different chemical components did not, of themselves, produce volume changes under isosmotic conditions.

RESULTS

Behavior in Potassium Chloride

In the dark, the volume of isolated outer segments is reduced in hyperosmotic KCl solutions (Fig. 2). We find that the outer segments shrink to a stable volume with a half-time of about 1 sec. They remain at this stable volume for at least 800 sec unless the osmotic pressure of the solution is changed, as shown by the double shock experiment in Fig. 2. One group of outer segments was incubated in the standard 1 Is solution and shocked with a 3.5 Is KCl solution (the result of mixing equal volumes of the 1 Is standard and the 6 Is solution C of Table I). A second group of outer segments was incubated in a 3.5 Is KCl solution, allowed to reach equilibrium, and then shocked a second time with

TABLE I
SOLUTIONS

	NaCl	KCl	$CaCl_2$	Tris	$MgCl_2$	Na_2SO_4	K_2SO_4	$NaNO_3$	KNO_3	$Ca(NO_3)_2$	Na-EDTA	mosmols	Is units
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*		
Rat standard	140	3.5	1.8	10	0.5	0	0	0	0	0	0	265	1
Frog Cl standard	115	2.5	2	10	0	0	0	0	0	0	0	232	1
Frog SO_4 standard	0	0	2	10	0	52	1.8	0	0	0	0	234	1
Frog NO_3 standard	0	0	0	10	0	0	0	115	2.5	2	0	226	1
Frog high Ca standard	91	2.5	10	10	0	0	0	0	0	0	0	232	1
Frog low Ca standard	115	2.5	0	10	0	0	0	0	0	0	0.5	230	1
A	855	3.5	1.8	10	0.5	0	0	0	0	0	0	1584	6
B	730	2.5	2	10	0	0	0	0	0	0	0	1362	6
C	115	627.5	2	10	0	0	0	0	0	0	0	1362	6
D	0	0	2	10	0	394	1.8	0	0	0	0	927	4
E	0	0	0	10	0	0	0	789	2.5	2	0	1356	6
F	706	2.5	10	10	0	0	0	0	0	0	0	1362	6
G	730	2.5	0	10	0	0	0	0	0	0	0.5	1360	6

either a 2.25 Is KCl solution or a 5.25 Is KCl solution. Under the second shock the outer segments swell or shrink to a new stable volume with the same time-course as the first shock. Furthermore, the final volume attained under the second shock (filled circles in Fig. 2) is identical to the stable volume reached by outer segments which have been brought to the same osmotic pressure by a single shock (solid line). This behavior is characteristic of an osmometer exposed to a hyperosmotic solution of an impermeable solute. Light exposures which bleached the visual pigment prior to, or during, the osmotic shock in KCl did not alter this behavior of the outer segments.

In Fig. 3 we present equilibrium lengths, widths squared, and volumes of outer segments shocked in the dark in various osmotic concentrations of KCl. The equilibrium values were all measured 4 sec after the osmotic shock. In addition, we determined the minimum dimensions attained by the outer segments by allowing them to dry slowly on the surface of a nonwettable resin (Sylgard 184, Corning Glass Works). Under these conditions the majority of the outer segments became flattened and difficult to measure, but a few remained cylindrical, as was verified from viewing them both from above and from the side. The values obtained are presented in Fig. 3 as the dimensions at infinite concentration. We find the dry volume to be 30 $\pm$ 2% of the normal volume. This value agrees reasonably well with the estimate of 37% calculated by Blaurock and Wilkins (1969) from index of refraction measurements and

with the estimate of 32% reported by Weale (1971) based on birefringence measurements.

Small osmotic gradients produce large changes in the length of the outer segments but their width and even the square of the width change only slightly. Thus, changes in volume and length occur nearly in parallel. We took advantage of this fact by restricting our subsequent experiments to small

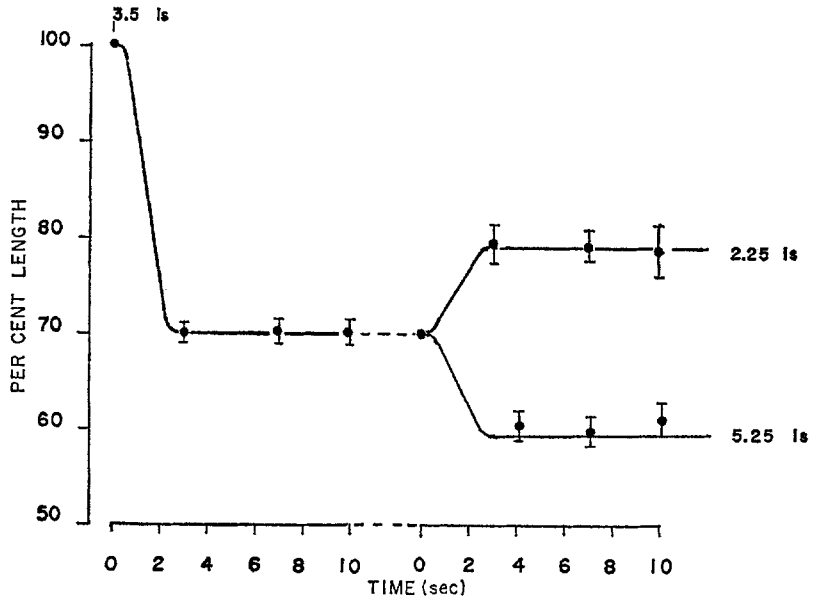

FIGURE 2. Double shock experiment in hyperosmotic KCl in the dark. Concentrations are expressed in isosmotic units. Under the conditions of the experiment, changes in length are equivalent to changes in volume (see text and Fig. 3). Solid lines show the stable volumes reached at 3 sec in control experiments in which the outer segments were shocked only once: 70 ± 1% for 3.5 Is, 79.5 ± 2% for 2.25 Is, and 61.1 ± 1% for 5.25 Is, mean ± SD.

osmotic gradients, in which case measurements of the length alone are sufficient to determine changes in volume.

If we assume that all the water contained in the outer segment is osmotically active it follows that the measured dry volume is equal to the osmotically inactive volume of the outer segment. The Boyle-van't Hoff equation for an ideal osmometer predicts a linear relation between volume and the reciprocal of concentration, with the intercept at infinite concentration yielding the osmotically inactive volume. The behavior of the outer segment in KCl does not follow this prediction, as can be seen in the right-hand diagram in Fig. 3—the outer segments neither shrink nor swell as much as would be expected from an ideal osmometer. We have adopted the working hypothesis that

deviation from ideal behavior is caused by a restoring force: when the outer segment shrinks, this restoring force adds to the "ideal" osmotic pressure of the solutes inside the outer segment, but when the outer segment swells the force subtracts from the "ideal" internal osmotic pressure. The origin of this

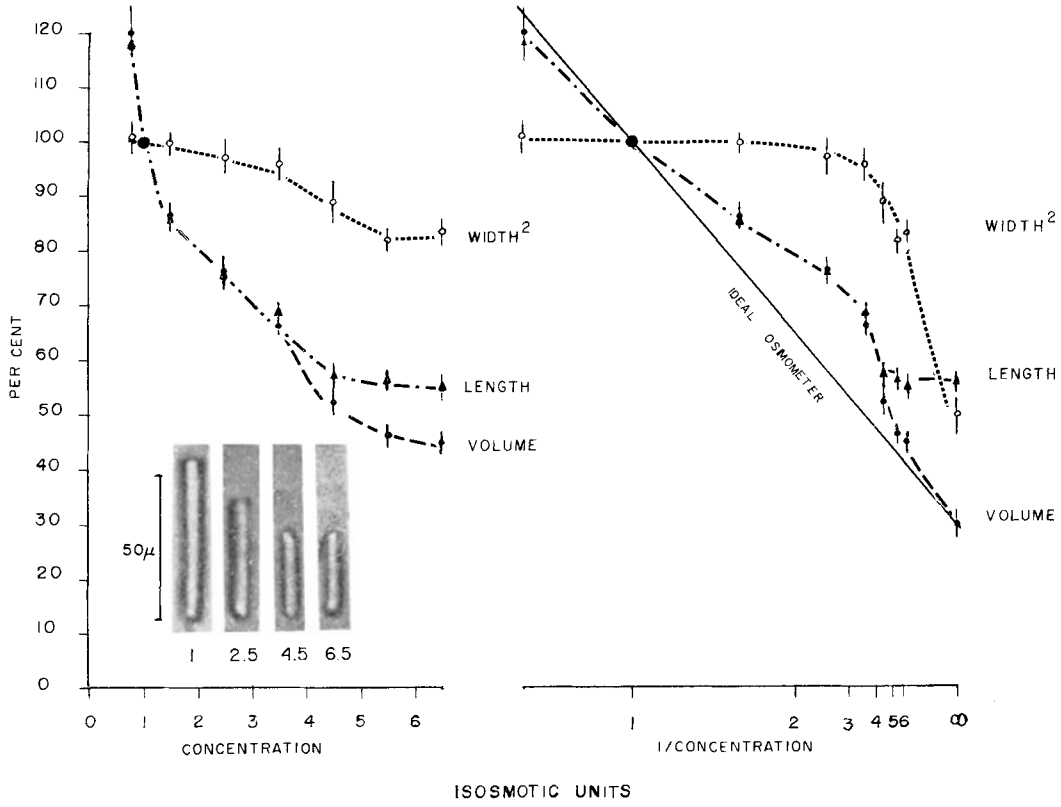

FIGURE 3. Changes in volume, length, and width squared as a function of KCl concentration in the dark. These dimensions were obtained 4 sec after mixing. The diagram to the right shows the same values as a function of the reciprocal of the concentration. The straight line indicates the behavior of an ideal osmometer with an osmotically inactive volume of 30%. The deviations from this line appear to arise from restoring forces which oppose any change from normal dimension: for small osmotic gradients, the width is held nearly constant by a relatively strong transverse restoring force, while changes in length are opposed by a relatively weak longitudinal restoring force.

restoring force is not yet known but some of its characteristics will be discussed below.

Behavior in Sodium Chloride

In the dark, outer segments shocked with hyperosmotic NaCl respond as shown in Fig. 4. At first the outer segments shrink with a time-course and to

an extent similar to that observed in equivalent KCl shocks. But then they recover in volume and return nearly to their starting value. This behavior is characteristic of an osmometer which contains an impermeant solute and which is shocked with a solution made hyperosmotic by adding a permeable

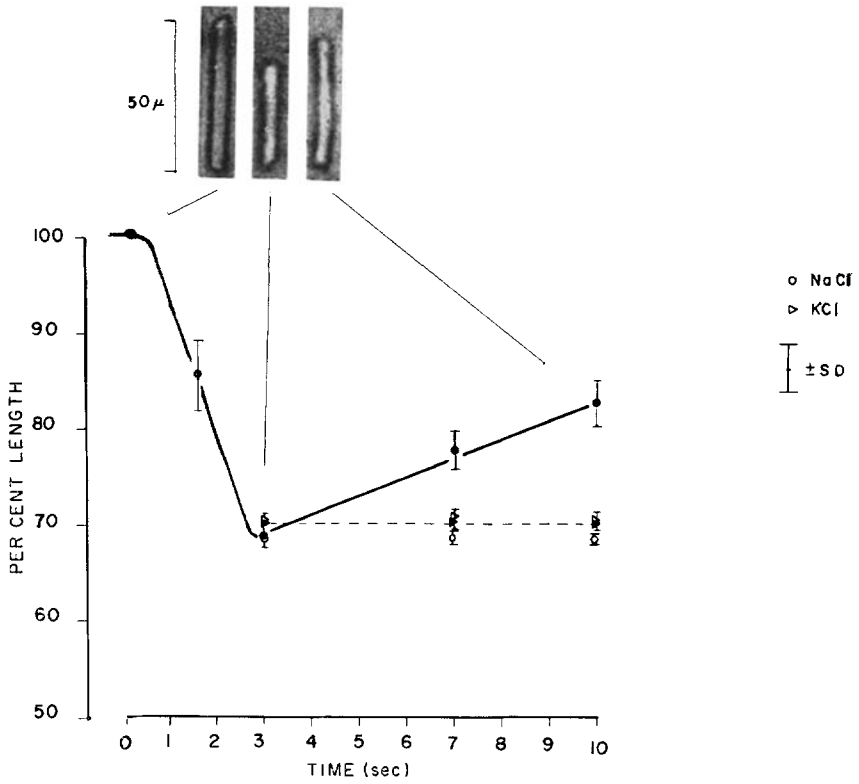

FIGURE 4. Osmotic behavior of outer segments following a 3.5 Is NaCl shock in the dark (●) or after illumination (○). The behavior following a KCl shock of the same magnitude is also presented, both in the dark (▶) and after illumination (▷). The solid line depicting the volume recovery following the NaCl shock in the dark is drawn with a slope of 1.9% normal volume/sec. The outer segments at the top of the illustration were selected to represent average dimensions at the times indicated. For the NaCl shock the volume in the dark at 3, 7, and 10 sec was 68.9 ± 1.7%, 77.8 ± 2.1%, and 82.7 ± 2.4%, respectively, and 68.5 ± 0.5%, 68.5 ± 0.5%, and 68 ± 1%, respectively, after illumination.

solute: the osmometer will shrink at first, but will then recover its starting volume as the permeable solute flows in and establishes osmotic balance with the outside solution. To confirm that outer segments respond this way to permeable solutes, a solution was made hyperosmotic by adding glycerol to the standard solution. On being shocked with this solution, outer segments shrank and then recovered in volume in a manner similar to their behavior

following NaCl shocks. Thus, outer segments appear permeable to both glycerol and NaCl. We have found that dark-adapted outer segments isolated from the retina of the rat also recover in volume following NaCl shocks.

Dark-adapted outer segments show a high selectivity of NaCl influx over KCl influx. Following an NaCl shock, frog outer segments recover in volume at a rate of about 2% of the normal volume per second. (We observe the same rate for rat outer segments.) This rate of volume recovery in NaCl compared to the absence of detectable volume recovery in KCl indicates the NaCl influx exceeds the KCl influx by at least two orders of magnitude.

To understand better the nature of the volume recovery process, outer segments were incubated in an isosmotic solution of pure NaCl. We observed no deviation from the normal dimensions for at least 1000 sec. If the deviation from ideal behavior observed in the KCl shocks arises only from nonideal behavior of impermeant solutes contained in the outer segment, then the outer segment would be expected to swell in a pure NaCl solution since the previous experiment shows that they are permeable to NaCl. (When outer segments recover their volume in NaCl, the internal and external osmotic pressure due to NaCl should be the same, leaving nothing to balance the residual osmotic pressure due to impermeant solutes within the outer segments. Hence, the outer segments could be expected to swell and perhaps to burst, much as erythrocytes swell in isosmotic solutions of pure permeants such as glycerol.) Since the outer segments do not swell in pure isosmotic NaCl, the restoring force, whatever its origin, must go to zero when outer segments approach their normal length. A restoring force with this characteristic could be expected to arise, for example, from long-range attractive and repulsive forces between membranes (Curtis, 1962), but not solely from the osmotic pressure of internal impermeant solutes.

The results of double shock experiments in NaCl (Fig. 6) show that the volume recovery is not the result of premature degeneration of the outer segments induced by NaCl. The response to the second NaCl shock is essentially the same as that to the first shock, despite the differences in both internal and external NaCl concentration. Indeed, the rate of volume recovery following both shocks is the same. This implies that NaCl influx is linearly proportional to the external NaCl concentration. On this basis we have calculated the Na influx in our standard solutions for frog and rat outer segments (Table II). The calculated influx in the rat is in close agreement with the value for dark current measured by Hagins et al. (1970) in the living retina.

Effects of Light on Na Influx

In contrast to the behavior of dark-adapted outer segments, when outer segments are flash illuminated prior to the hyperosmotic NaCl shock they behave indistinguishably from outer segments shocked with KCl—they shrink to a

stable volume and no detectable volume recovery ensues within the next 800 sec (Figs. 4 and 5). The action of light, therefore, is to prevent the entry of NaCl into the outer segment.

This effect of light could be specific for either Na or Cl, or perhaps for both

TABLE II

DARK Na INFLUX

	Frog	Rat
Dimension of rod outer segments		
Length, μ	50*	24‡
Width, μ	6	1.7
Volume, μ^3	1413	55.2
Rate of volume recovery with 2 mM Ca at 21°–23°C		
Per cent normal volume/sec	2	2
μ^3/rod sec	28.2	1.1
Na^+ ions/μ^3 standard solution	6.3×10^7	8.4×10^7
Na^+ influx, *Na^+/rod sec*	2.1×10^9	9×10^7
Rhodopsin molecules/rod	2×10^9§	7×10^7‖
Na^+ ions entering/rhodopsin molecule sec	~1	~1

* Liebman and Entine (1968).
‡ Hagins et al. (1970).
§ Hubbard (1954).
‖ Brown and Cone, unpublished observation.

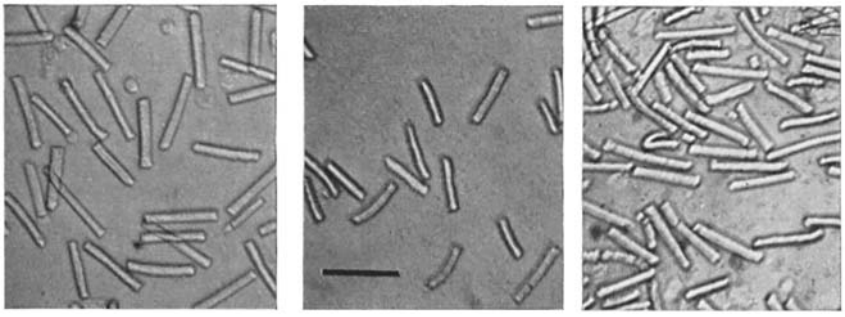

FIGURE 5. Typical fields of isolated outer segments. The left-hand photograph shows outer segments in the standard solution. The shrunken outer segments in the middle photograph were bleached prior to an NaCl hyperosmotic shock. The right-hand photograph shows dark-adapted outer segments after they recovered from a hyperosmotic NaCl shock. The calibration bar is 50 μ.

ions. Fig. 7 presents the response of outer segments shocked with 2.5 Is Na_2SO_4 solution (incubated in frog SO_4 standard and shocked by mixing with an equal volume of solution D of Table I). No volume recovery occurs in dark or light. Hence, the outer segments are impermeable to Na_2SO_4, yet they are permeable to NaCl. This result indicates that the volume recovery following a

hyperosmotic shock requires the presence of a permeable salt. That is, both anion and cation must be able to flow into the outer segment.

If the effect of light is to specifically block Na entry, then light should block the entry of any Na salt where the anion is permeant. Conversely, if light specifically blocks Cl entry, then light should not affect the influx of a permeant Na salt where the anion is not Cl. Fig. 7 shows that it is the influx of Na and not Cl which is controlled by light, since outer segments shocked with

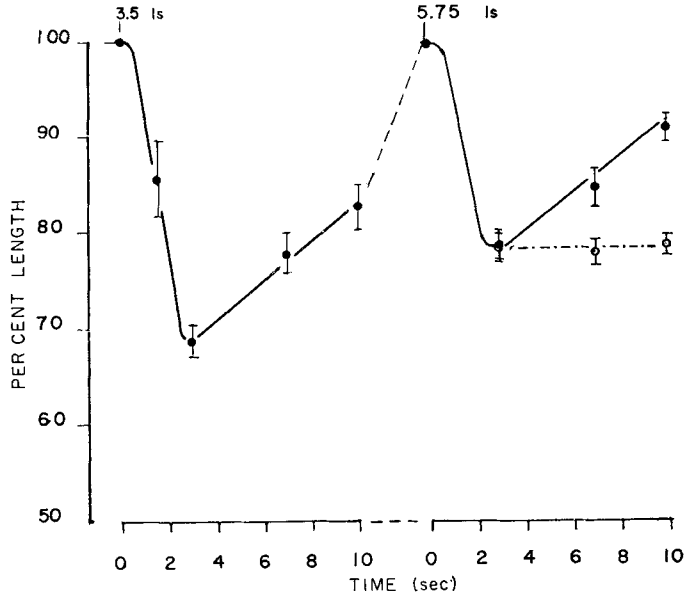

FIGURE 6. Double shock experiment in hyperosmotic NaCl. The response in the dark of one group of outer segments to a 3.5 Is NaCl shock is shown on the left. A second group of dark-adapted outer segments was allowed to recover following a 3.5 Is NaCl shock, and was then shocked a second time with a 5.75 Is solution. The response is shown to the right (●). The second shock produces a minimum volume of 78.9 ± 1.3% which recovers to 91.1 ± 1.4% at 10 sec. Outer segments which were flash illuminated after the first shock do not recover in volume after the second (○). The solid lines drawn through the recovery phases have the same slope of 2% normal volume/sec.

hyperosmotic solutions that contained NO_3 as the only anion behaved the same as if they had been shocked with solutions of Cl salts. In particular, dark-adapted outer segments recover their volume in $NaNO_3$ at a rate similar to that in NaCl, and this recovery is inhibited by flash illumination prior to the osmotic shock. Thus, light must control the influx of Na.

Mechanisms of the Light-Dependent Reduction in Na Influx

Light could block the influx of Na by at least three mechanisms: (*a*) light could activate an Na pump which expels Na as it enters, making the outer

segment appear impermeable to Na, (*b*) light could abolish the restoring force needed to drive the outer segment back to its normal length, and (*c*) light could increase the passive resistance to Na influx in the outer segment. The first mechanism seems unlikely since the standard solution contains no metabolite or appropriate energy source and also since the presence of a light-sensitive Na-K–activated ATPase in outer segments is doubtful (Bownds and Gaide-Huguenin, 1970; Frank et al., 1971). However, endogenous ATP sources may be present in frog outer segments, so we have investigated the effects of 5 ×

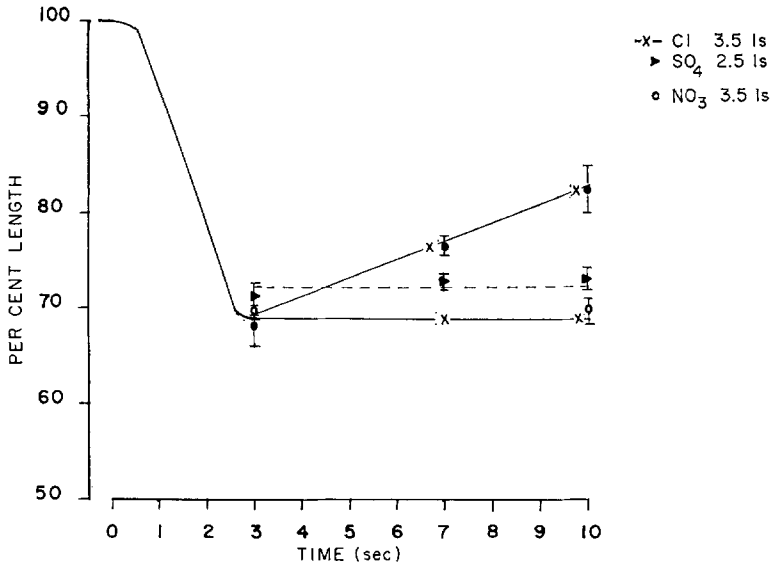

FIGURE 7. Osmotic behavior in Cl-substituted hyperosmotic solutions of Na salts. Outer segments shocked with 3.5 Is $NaNO_3$ (dark, ●; light, ○) behave indistinguishably from those shocked with NaCl (–x–). In the dark the volume recovers at a rate of 2% normal volume/sec in both salts, and the minimum volume reached is 68.9 ± 1.7% in NaCl and 67.7 ± 2.6% in $NaNO_3$. The SO_4 anion is apparently impermeable, and no volume recovery occurs in the dark (▸). A smaller shock, 2.5 Is, was used for clarity in the figure.

10^{-3} M KCN, which inhibits oxidative coupled metabolic activity (although outer segments are not known to contain mitochondria) and 10^{-5} M ouabain, which is well known to inhibit active Na transport. If the lack of volume recovery following a hyperosmotic NaCl shock is the result of active transport which is initiated by light but inhibited by these poisons, then the volume should recover following illumination in the presence of the inhibitors. This is not the case as Fig. 8 shows. Indeed the behavior in dark and light is the same in the presence or in the absence of these inhibitors.

If the effect of light is to abolish the restoring force, then after illumination no volume recovery should occur following a shock in a solution made hyperos-

motic with any permeable solute whatsoever. We have used two permeable solutes to test this prediction, glycerol and ammonium acetate. We find that both dark-adapted and illuminated outer segments recover their volume following hyperosmotic shocks with these solutes. Thus the restoring force is not abolished by illumination. The most likely mechanism by which light blocks Na influx appears therefore to be an increase in Na^+ resistance.

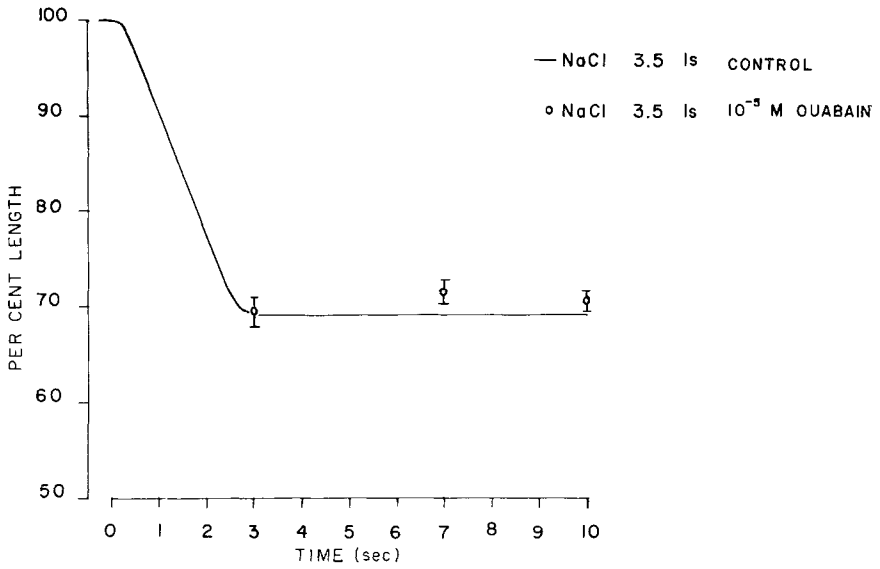

FIGURE 8. Effect of ouabain on the behavior of outer segments flash illuminated prior to a 3.5 Is NaCl shock. Ouabain, 10^{-5} M, was present in both incubating and test solutions. Outer segments were incubated for 5 min prior to the osmotic shock. A stable volume was reached and the behavior in the presence of the inhibitor (O) is not different from the behavior in its absence as shown by the solid line taken from Fig. 4.

Rectification in the Na flux

We have shown that Na can flow into the outer segment and that K cannot, but so far we have not considered the efflux of either ion. For example, although Na^+ flows into the outer segment under a concentration gradient, it may not flow out when the gradient is reversed. Such rectification of the flux is in fact suggested by the observations presented in Fig. 6: the volume recovery occurs at a rate which is independent of both the internal and external NaCl concentrations.

Consider an osmometer with an internal restoring pressure F in the presence of a permeable solute C, with internal concentration C_i and external concentration C_e. If the permeability of water is much greater than the permeability of C, then at all times the internal and external pressures will be essentially in

balance:

$$F + RTC_i = RTC_e .$$

The internal restoring pressure which exists in addition to the ideal internal osmotic pressure can be expressed in isosmotic units; $f = F/RT$, hence:

$$f + C_i = C_e . \tag{1}$$

The net flux of the permeable solute into the osmometer is

$$\phi_c = \frac{dv}{dt} C_i . \tag{2}$$

For diffusion under a concentration gradient, the net flux will be

$$\phi_c = P_{\text{in}} A C_e - P_{\text{out}} A C_i , \tag{3}$$

where A is the area across which the flux occurs and P is the effective permeability of the osmometer to species C. P_{in} is for influx, and P_{out} is for efflux. Consider two cases:

	Rectification	Nonrectification
	$P_{\text{in}} \gg P_{\text{out}}$	$P_{\text{in}} = P_{\text{out}} = P$
From equation 3		
	$\phi_c = P_{\text{in}} A C_e$ (4)	$\phi_c = PA(C_e - C_i)$.
Substitute equation 2 in equation 4		
	$\frac{dv}{dt} = P_{\text{in}} A \frac{C_e}{C_i}$ (5)	$\frac{dv}{dt} = PA \frac{C_e - C_i}{C_i}$.
Substitute equation 1 in equation 5		
	$\frac{dv}{dt} = P_{\text{in}} A \frac{C_e}{C_e - f}$	$\frac{dv}{dt} = PA \frac{f}{C_e - f}$.
For $C_e \gg f$,		
	$\frac{dv}{dt} \simeq P_{\text{in}} A$ (6)	$\frac{dv}{dt} \simeq \frac{PAf}{C_e}$.

Thus, in the absence of rectification the rate of volume recovery should be inversely proportional to the external concentration of C, but if rectification occurs the rate is independent of concentration, which is what we observe. Interestingly, the rate of volume recovery in this case depends only on $P_{\text{in}}A$.

We have not yet fully characterized the rectification properties of the outer segment but we can report one experiment which confirms that Na flux is rectified. If internal NaCl flows out in response to a concentration gradient, outer segments incubated in the standard NaCl solution should shrink when shocked with an isosmotic solution containing only impermeant solutes and no Na. We have tested this prediction by shocking outer segments with Na-free solutions made isosmotic with KCl or with melezitose, an impermeant trisaccharide (mol wt 504). No shrinkage occurs in either case for at least 900 sec following the shock. Hence, over this time period NaCl efflux is negligible. NaCl influx, by contrast, requires only a few seconds.

Under our conditions, therefore, isolated outer segments have a negligibly small Na efflux compared to the Na influx. In the living retina the outer segment can also be expected to have a negligibly small Na efflux, since both the existing negative membrane potential (Tomita, 1970) and the expected concentration gradient should favor Na influx. This suggests that the values of Na influx measured by our technique (Table II) and that of the dark current measured by extracellular electrodes (Hagins et al., 1970) reflect, in both cases, the same Na permeability of the outer segment.

Photon Dependence of Membrane Resistance Increase

The photon sensitivity of the resistance increase to Na influx has been studied by flash irradiation 100–1000 sec prior to the hyperosmotic NaCl shock and also by steady illumination delivered during the volume recovery phase after the osmotic shock. In both cases the rate of volume recovery is reduced in proportion to the light flux of the exposure. If the outer segments are illuminated before the osmotic shock (Fig. 9) the rate of recovery is halved when 2×10^6 rhodopsin molecules/rod are bleached, and the magnitude of the resistance increase is independent of the time interval between the light exposure and the osmotic shock.

When the outer segments are irradiated during the recovery phase we find there is a transient reduction in recovery rate with much lower light exposures—the recovery rate is halved when only 60–90 rhodopsin molecules/rod are bleached per second (Fig. 10). The time-course of this resistance increase has not been carefully studied, but following a dim flash it lasts approximately 1 sec.

The reciprocal of the volume recovery rate can be functionally defined as a measure of the resistance of the outer segment to Na entry. By comparing the rate of recovery in the dark to the rate following different light exposures it is possible to express the light-sensitive Na resistance in units of dark resistance:

$$r \equiv \frac{1}{dv/dt}$$

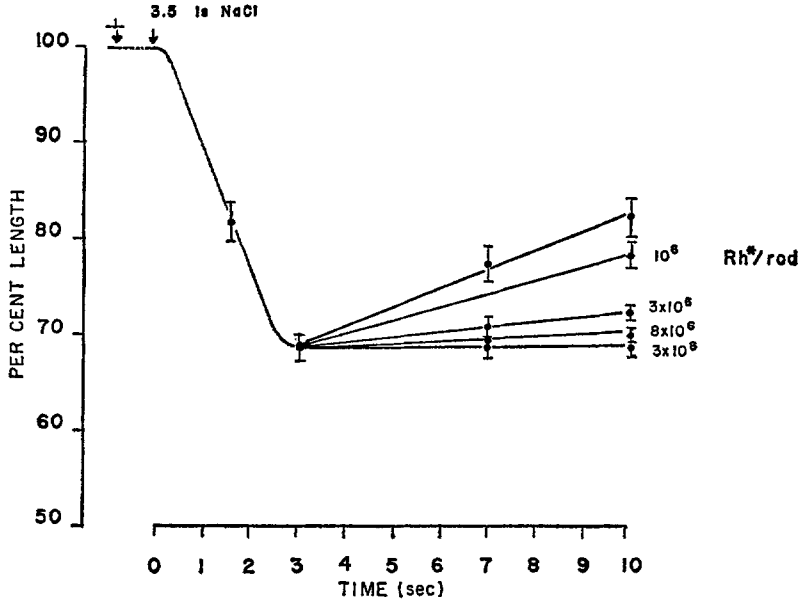

FIGURE 9. Afterimage resistance; the long-lasting effect of light on the recovery rate following a 3.5 Is NaCl shock. The outer segments were flash illuminated in the loading well 100–1000 sec prior to the osmotic shock. The numbers of rhodopsin molecules bleached per rod by the flash are indicated.

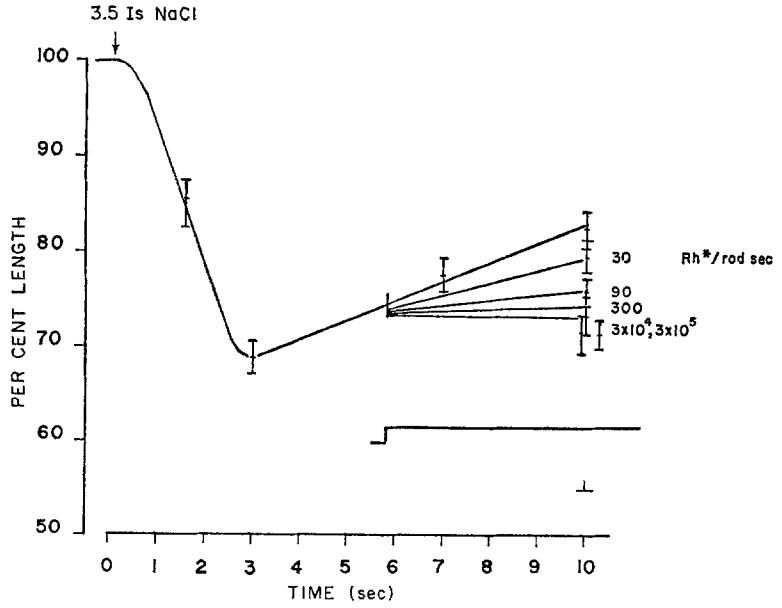

FIGURE 10. Photoresistance; effect of dim, continuous illumination started 6 sec after the osmotic shock with a 3.5 Is NaCl solution. The recovery rates for different numbers of rhodopsin molecules bleached per rod second are presented. Mean ± SD.

$$\frac{\phi_d}{\phi_l} = \frac{(dv/dt)_d C_i}{(dv/dt)_l C_i}$$

$$r_l = \frac{\phi_d}{\phi_l} r_d = \frac{(dv/dt)_d}{(dv/dt)_l} r_d .$$

The observations shown in Figs. 9 and 10 were analyzed by this method and the results are presented in Fig. 11. It can be seen in this figure that the light-

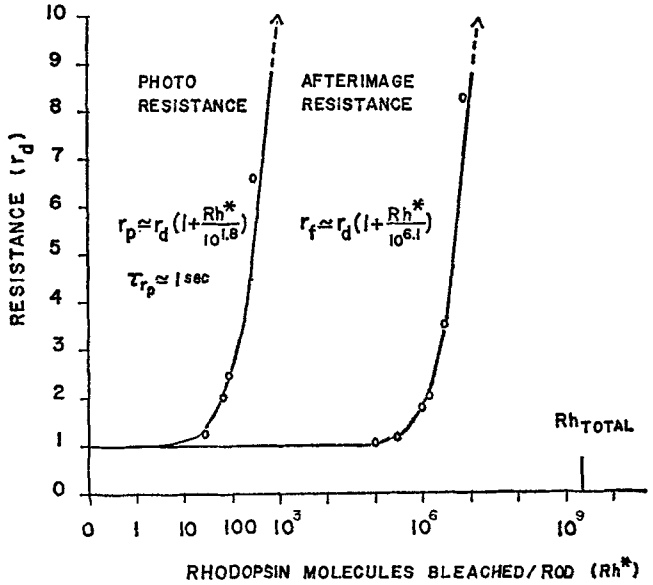

FIGURE 11. Light dependence of the Na resistance. Light exposures are expressed as the number of rhodopsin molecules bleached per rod, while resistance is expressed in units of resistance to Na influx in completely dark-adapted outer segments. The lines represent the function shown. Both the photoresistance and the afterimage resistance increase linearly with light exposure. To compare the magnitude of the resistances, the light exposure is presented on a logarithmic scale.

dependent resistance is linearly proportional to the number of rhodopsin molecules bleached:

$$r_l = r_d\left(1 + \frac{Rh^*}{a}\right)$$

where Rh^* is the number of bleached rhodopsin molecules per outer segment and a is the number of molecules needed to make the light resistance equal to twice the dark resistance. When expressed in terms of conductance this relationship is

$$\frac{1}{r_l} = \frac{1}{r_d}\left(\frac{1}{1 + \frac{Rh^*}{a}}\right).$$

This is the same function that Baylor and Fuortes (1970) inferred from their intracellularly recorded data. This suggests that both the osmotic and the electrophysiological methods characterize the same light-dependent Na^+ permeability. Moreover, the osmotic method indicates that excitation of a single rhodopsin molecule briefly reduces the Na influx by 1–2%, preventing some 10^7 Na ions from flowing into the outer segment. This is essentially the same photon response that has been found in the living rat retina (see Table III). We have termed the transient response "photoresistance," in part to distinguish it from the stable resistance increase produced by more intense light exposures. We have termed the stable increase "afterimage resistance." Afterimages depend directly on the photochemistry of the visual pigment (Brindley, 1959) and they decay in parallel with the regeneration of rhodopsin (Barlow and Sparrock, 1964; Rushton, 1961). In isolated outer segments rhodopsin

TABLE III

LIGHT DEPENDENCE OF Na RESISTANCE

	Frog	Rat
Photoresistance (r_p)		
Rh^*/rod sec at $r_p=2r_d$‡	75	
Total Na^+ blocked/Rh^*	1.3×10^7	0.5×10^7§
Afterimage resistance (r_f)		
Rh^*/rod at $r_f=2r_d$	1.2×10^6	

‡ Rh^* is number of bleached rhodopsin molecules and r_d is the resistance to Na^+ influx in the dark.

§ From Hagins et al. (1970), assuming Na^+ is the major charge carrier of dark current and that quantum efficiency of bleaching is 0.5.

does not regenerate, and this may account for the stability of the afterimage resistance.

Effects of Ca

Recently, Yoshikami and Hagins (1971) have suggested that in rat rods extracellular Ca has an effect equivalent to that of light. Effects of Ca on the influx of NaCl under hyperosmotic shocks are presented in Fig. 12. We find in the frog that no volume recovery occurs in Ca concentrations five times that of the standard, whereas in low Ca concentration, brought about with the help of ethylenediaminetetraacetate (EDTA), recovery occurs at a slightly higher rate than under the standard conditions. These results show that the "dark" influx of NaCl is affected by external Ca, but they are difficult to quantitate because of a second phenomenon: Ca also affects the extent of volume reduction both in NaCl and KCl shocks. Ca thus appears to increase the restoring force in the outer segment.

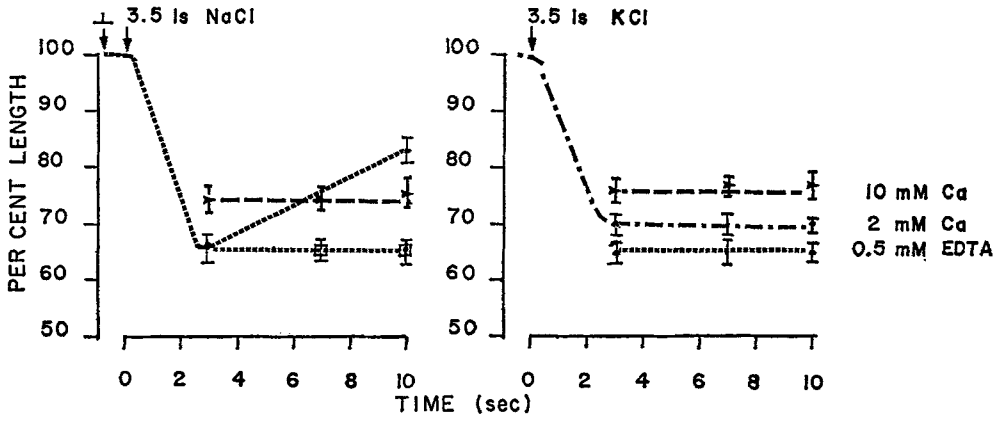

FIGURE 12. Effects of Ca. Two effects can be observed: in 10 mM Ca solutions (– – –) the outer segments do not shrink as much as in the standard 2 mM solution (–·–·–), and in the dark the volume does not recover following the NaCl shock. In Ca-free solutions (0.5 mM EDTA) the outer segments shrink more than in the standard (——), and following the NaCl shock in the dark the volume recovers slightly more rapidly than in the standard (2.4% instead of 2% normal volume/sec). The light-dependent Na resistance is still observed in this solution (–□–).

DISCUSSION

A satisfactory understanding of the Na and K permeabilities of isolated outer segments is difficult to achieve from previously published literature—not only are there different methodological approaches to the problem, but results are contradictory.

Etingof et al. (1964) and Bonting and Bangham (1967) have used flame photometry to measure the concentrations of Na and K in the supernatant or in the pellet of centrifuged suspensions of purified outer segments. They both agree that Na and K slowly leak from the outer segments in darkness, but while Etingof et al. maintain that light increases the leakage of both ions, Bonting and Bangham report an increase in K outflux and a decrease in Na outflux (which they interpret as an Na influx). However, the long period of time involved and the disruptive manipulation of outer segments in these experiments raise questions as to the physiological implications of the results.

Important postmortem degenerative changes occur shortly after separation of the outer segments from the retina. Changes in the structure and birefringence of isolated outer segments have been discussed by other authors (Sidman, 1957; Robertson, 1966). In addition, Harosi (1971) has found with the use of microspectrophotometry that although some conversion of retinaldehyde to vitamin A occurs in freshly isolated outer segments, none occurs in outer segments separated from the retina for longer than an hour.

Bowmaker (1970) reports that outer segments obtained by gently shaking

frog retinas show a continuous Na loss in the dark over a course of several hours. His results appear to indicate an initial rapid efflux of Na. However, the dilution procedure he describes, but does not consider in the analysis of his results, can well account for this rapid initial loss of Na in his observations. On the other hand, his results clearly indicate a slow Na efflux which occurs at a rate of about 10^6 Na ions/outer segment second. This slow efflux is consistent with the rectification of Na flux we observe, since it is about 1000-fold smaller than the rate of Na influx we have observed. Bowmaker also reports that both light and ouabain inhibit the slow efflux of Na.

The osmotic behavior of outer segments has recently been studied by several investigators. Brierley et al. (1968) have shown that outer segments, even though fragmented, are impermeable to K influx. This is consistent with our results, but it raises an important question: Does the osmotic response of an outer segment reveal the permeability characteristics of the plasma membrane, the disc membrane, or both? Two reports have appeared (Heller et al., 1971; Cohen, 1971) which deal with this problem. These reports pursue purely morphological arguments, and their results seem equivocal. In Cohen's study of the response of intact cells to hypotonic shocks, the discs do not appear to swell as expected when the cell interior has become hypotonic, as evidenced by the ballooning of the outer segment. In contrast, Clark and Branton (1968) report that when the intact cell is incubated in hypotonic media the discs swell, but the outer segments do not balloon. Heller and his collaborators argue that osmotic responses of fragmented outer segments are indicative of the permeability of the disc membrane, yet hypotonic shocks of such fragments produce a much greater increase in the space between the discs than in the space enclosed by the discs as seen in their electron micrographs. Moreover the osmotically active volume in their pellets is greater than the volume enclosed by the discs. This suggests that the properties of the plasma membrane may also be involved in the response of fragments of outer segments.

Under our conditions we think that the osmotic technique characterizes the same membrane permeabilities which have been studied by electrophysiological techniques. The osmotically determined rate of Na entry in the dark in rat outer segments (Table I) agrees well with the dark current reported by Hagins et al. (1970) in their elegant microelectrode investigation of the living retina. The agreement is further improved if a temperature correction is considered since our experiments were conducted at 21°–23°C, in contrast to 31°–33°C in the experiments of Hagins et al. In addition, as pointed out in Table III, the magnitudes of the photoresponses observed with both techniques are essentially the same. Finally, the light dependence of the Na resistance increase measured osmotically is best fit by the same function used by Baylor and Fuortes (1970) to fit their intracellularly recorded data.

A Model of the Photoreceptor

We have developed a model for the rod photoreceptor which can account for the characteristics that we and others have found and which incorporates important elements of previously published proposals by Sillman et al. (1969) and by Baylor and O'Bryan (1971). The model rests upon two assumptions: (*a*) that an Na-K exchange pump is located near the junction between the inner and outer segments and (*b*) that the K permeability of the receptor is located primarily in the synaptic area.

The interior of the receptor is nearly isopotential: the voltage gradient produced by the dark current flowing intracellularly through the cytoplasmic resistance is apparently small compared with the resting membrane potential of 20–30 mv (Hagins et al., 1970; Murakami and Pak, 1970). The value of the resting membrane potential is distant from the equilibrium potentials of both Na and K (Toyoda et al., 1970), and the receptor has Na and K batteries of the usual polarity (Sillman et al., 1969). Since we have shown that the outer segment is permeable to Na influx but not to K influx, the outer segment may be the site of the Na battery but not of the K battery. The K battery appears, therefore, to be located in an area of the receptor membrane other than the outer segment. This can explain the KCl-supported osmotic swelling of intact receptors observed by Cohen (1971). (The non–outer segment membrane area which is permeable to K may also be permeable to Na, and this question needs further research, but the existence of such Na permeability does not affect the qualitative characteristics of the model.)

We assume the existence of an Na-K exchange pump located near the junction between the inner and outer segments, as is suggested by the observations of Penn and Hagins (1969). This pump could be electrogenic and thus responsible for the "active component" of current recently proposed by Zuckerman (1971). In our model, for simplicity, we assume the pump is nonelectrogenic.

As a result of the above conditions, in the dark Na flows into the outer segment tending to drive the membrane potential towards the Na equilibrium potential. But this equilibrium potential is not reached since the Na-K pump will take Na out again, thus producing the steady Na current described by Penn and Hagins (1969) and Yoshikami and Hagins (1970).

The K which the exchange pump takes in will flow out of the receptor through the K-permeable membrane and thus tend to drive the membrane potential towards the K equilibrium potential. This will produce a second loop of current if the K-permeable membrane is located towards the synaptic end of the receptor (Fig. 13). If such a current loop exists, positive charge will flow continuously from the synaptic end to the outer segment in the extra-

cellular space and in the reverse direction intracellularly. However, the dominant charge carrier will be Na in the outer segment region and K in the cell body region.

We suggest that the synapse itself may be permeable to K, for in this case the proposed K current could play an important role in the synaptic activity of the receptor. Indeed, Nelson (1971) has found that in the Necturus retina horizontal cells and hyperpolarizing bipolar cells do not show an accessible

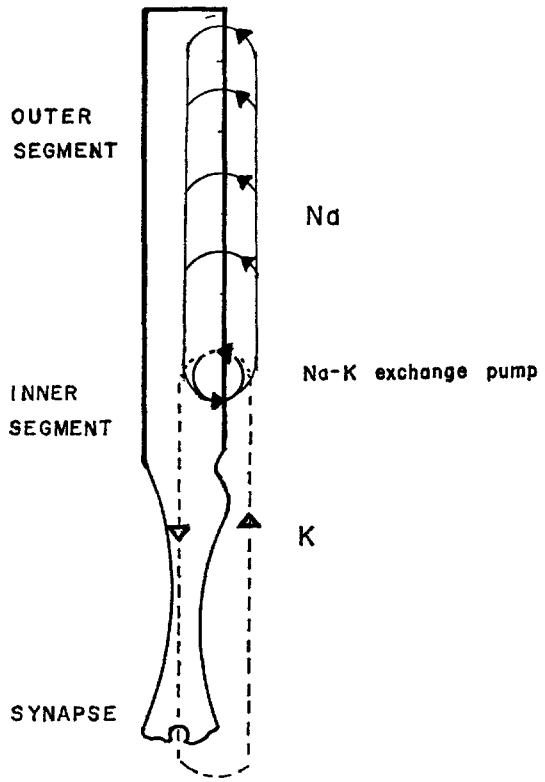

FIGURE 13. Proposed model for the rod photoreceptor.

reversal potential, and he has suggested that these cells may be driven by changes in the ionic surround of their synaptic regions. Such changes could be brought about by light-dependent variations in the K current through the invaginated synaptic contact between the photoreceptor and the second-order neuron in the retina.

During illumination, the Na current will be decreased by the reduction in Na permeability and the cell will hyperpolarize since the membrane potential will become dominated by the K permeability. The hyperpolarizing shift towards the K equilibrium potential will diminish the driving force on the K current, which will thus be reduced. Therefore the light-dependent decrease

in Na current will in turn reduce the proposed K current and this could modify the ionic composition of the small volume enclosed by the invaginated synapse.

The model is consistent with the loss of photoresponses in ouabain-poisoned retinas (Frank and Goldsmith, 1967; Sillman et al., 1969). After ouabain inhibits the Na-K pump, Na and K will continue to flow until the membrane potential approaches zero, thus abolishing both the radial current and the photoresponses. Inhibition of the Na-K pump can also explain the disappearance of Na dark current in the presence of cyanide (Penn and Hagins, 1969) which, we have found, does not by itself affect the Na influx or its dependence on light.

The direct effect of Ca on the Na resistance of the isolated outer segment strengthens the proposal of Yoshikami and Hagins (1971) that Ca mediates the effect of light on the Na permeabitity of the outer segment. Cone (1972) has recently shown that rhodopsin undergoes rapid rotational diffusion in the receptor membrane, and has suggested that rhodopsin may be a light-activated diffusional carrier which could excite the receptor by transporting Ca.

Note Added in Proof Recent observations (Korenbrot, Brown, and Cone, manuscript in preparation) in which freeze-etch techniques have been applied to outer segments isolated under conditions similar to those described here show that the discs remain essentially collapsed (in accord with the observations of Clark and Branton, 1968), and that the length changes we observe arise almost entirely from changes in the volume between discs. These results are consistent with the possibility that the light-regulated Na influx occurs through the plasma membrane. In this case equation 6 indicates that the permeability of the plasma membrane for Na influx is $P_{in} = \frac{dV/dt}{A} = 2.6 \times 10^{-6}$ cm/sec, a value comparable to the cation permeability of several excitable membranes (Hurblut, W. P. 1970. *In* Membranes and Ion Transport. E. E. Bittar, editor. Interscience Publishers Inc., New York. Hodgkin, A. L., and P. Horowics. 1959. *J. Physiol. (Lond.).* **148**:127). Na channels have recently been found to have a resistance of about 10^{10} ohms/channel in a variety of membranes (Hille, B. 1970. *Biophys. Soc. Annu. Meet. Abstr.* **10**:182a). If the Na channels in the plasma membrane of the outer segment are assumed to have a similar resistance, then, taking the driving force to be equivalent to about 10–40 mv, the Na influx we observe in the frog can be accounted for by 100–1000 Na channels/outer segment.

We wish to thank D. C. Petersen and E. Z. Szuts for helpful discussions.
This research was supported in part by a grant from the National Eye Institute, United States Public Health Service.

Received for publication 30 July 1971.

REFERENCES

ARDEN, G. B., and W. ERNST. 1970. The effect of ions on the photoresponse of pigeon cones. *J. Physiol. (Lond.).* **211**:311.

BARLOW, H. B., and J. M. B. SPARROCK. 1964. The role of afterimages in dark adaptation. *Science* (*Wash. D.C.*). **144**:1309.

BAYLOR, D. A., and M. G. F. FUORTES. 1970. Electrical responses of single cones in the retina of the turtle. *J. Physiol.* (*Lond.*). **207**:77.

BAYLOR, D. A., and P. M. O'BRYAN. 1971. Electrical signaling in vertebrate photoreceptors. *Fed. Proc.* **30**:79.

BLAUROCK, A. E., and M. H. F. WILKINS. 1969. Structure of frog photoreceptor membranes. *Nature* (*Lond.*). **223**:906.

BONTING, S. L., and A. D. BANGHAM. 1967. On the biochemical mechanism of the visual process. *Exp. Eye Res.* **6**:400.

BORTOFF, A. 1964. Localization of slow potential responses in the necturus. *Vision Res.* **4**:627.

BOWMAKER, J. K. 1970. Sodium movements from frog rod outer segments. *Vision Res.* **10**:601.

BOWNDS, D., and A. C. GAIDE-HUGUENIN. 1970. Rhodopsin content of frog photoreceptor outer segment. *Nature* (*Lond.*). **225**:870.

BRIDGES, C. D. B. 1970. Molar absorbance coefficient of rhodopsin. *Nature* (*Lond.*). **227**:1258.

BRIERLEY, G. P., D. FLEISCHMAN, S. D. HUGHES, G. R. HUNTER, and D. G. MCCONNELL. 1968. On the permeability of isolated bovine retinal outer segment fragments. *Biochim. Biophys. Acta.* **163**:117.

BRINDLEY, G. S. 1959. The discrimination of afterimages. *J. Physiol.* (*Lond.*). **147**:194.

CLARK, A., and D. BRANTON. 1968. Fracture faces in frozen outer segments from the guinea pig retina. *Z. Zellforsch. Mikrosk. Anat.* **91**:586.

COHEN, A. I. 1971. Electron microscope observations on form changes in photoreceptor outer segments and their saccules in response to osmotic stress. *J. Cell Biol.* **48**:547.

CONE, R. A. 1972. Rotational diffusion of rhodopsin in the visual receptor membrane. *Nature* (*New Biol.*) (*Lond.*). **236**:39.

CURTIS, S. A. G. 1962. Cell contact and adhesion. *Biol. Rev.* **37**:82.

ETINGOF, R. N., S. A. SHULKOYUKOV, and V. G. LEONT'EV. 1964. Escape of Na and K ions from the outer segments of the photoreceptor of the retina under the influence of illumination and Vitamin A. *Dokl. Akad. Nauk SSSR.* **156**:979.

FRANK, R. N., H. D. CAVANAGH, and K. R. KENYON. 1971. Studies on adenosine triphosphatase activity and rhodopsin in purified cattle rod outer segment preparation. Spring meeting, Association for Research in Vision and Ophthalmology. Sarasota, Florida.

FRANK, R. N., and T. H. GOLDSMITH. 1967. Effect of cardiac glycosides on electrical activity in the isolated retina of the frog. *J. Gen. Physiol.* **50**:1585.

HAGINS, W. A., R. D. PENN, and S. YOSHIKAMI. 1970. Dark current and photocurrent in retinal rods. *Biophys. J.* **10**:380.

HAROSI, F. 1971. Frog rhodopsin in situ: orientational and spectral changes in the chromophore of isolated retinal rod cells. Ph.D. Thesis. Johns Hopkins University, Baltimore, Md.

HELLER, J., T. J. OSTWALD, and K. BOK. 1971. The osmotic behavior of rod photoreceptor outer segment discs. *J. Cell Biol.* **48**:633.

HUBBARD, R. 1954. The molecular weight of rhodopsin and the nature of the rhodopsin-digitonin complex. *J. Gen. Physiol.* **37**:381.

HUBBARD, R., P. K. BROWN, and A. KROPF. 1959. Action of light on visual pigments. *Nature* (*Lond.*). **183**:442.

LIEBMAN, P. A., and G. ENTINE. 1968. Visual pigments of frog and tadpole (Rana pipiens). *Vision Res.* **8**:761.

MATTHEWS, R. G., R. HUBBARD, P. K. BROWN, and G. WALD. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* **47**:215.

MURAKAMI, M., and W. L. PAK. 1970. Intracellularly recorded early receptor potential of the vertebrate photoreceptor. *Vision Res.* **10**:965.

NELSON, R. 1971. Electrical properties of retinal neurons. Ph.D. Thesis. Johns Hopkins University, Baltimore, Md.

PENN, R. D., and W. A. HAGINS. 1969. Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature* (*Lond.*). **223**:201.

ROBERTSON, J. D. 1966. Granula-fibrillar and globular substructure in unit membranes. *Ann. N. Y. Acad. Sci.* **137**:421.

RUSHTON, W. A. H. 1961. Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision. *J. Physiol. (Lond.).* **156**:193.

SIDMAN, R. L. 1957. The structure and concentration of solids in photoreceptor cells studied by refractometry and interference microscopy. *J. Biophys. Biochem. Cytol.* **3**:15.

SILLMAN, A. J., H. ITO, and T. TOMITA. 1969. Studies on the mass receptor potential of the isolated frog retina. II. On the basis of the ionic mechanism. *Vision Res.* **9**:1443.

TOMITA, T. 1970. Electrical activity of vertebrate photoreceptors. *Quart. Rev. Biophys.* **3**:179.

TOYODA, J., H. HASHIMOTO, H. ANNO, and T. TOMITA. 1970. The rod response in frog as studied by intracellular recording. *Vision Res.* **10**:1093.

TOYODA, J., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of Necturus and Gekko. *Vision Res.* **9**:453.

WEALE, R. A. 1971. On the birefringence of rods and cones. *Pfluegers Arch. Eur. J. Physiol.* **329**:244.

WEAST, R. C. 1967. Handbook of Chemistry and Physics. Chemical Rubber Company, Cleveland, Ohio.

YOSHIKAMI, S., and W. A. HAGINS. 1970. Ionic basis of dark current and photocurrent of retinal rods. *Biophys. Soc. Annu. Meet. Abstr.* **10**:60a.

YOSHIKAMI, S., and W. A. HAGINS. 1971. Light, calcium and the photocurrent of rods and cones. *Biophys. Soc. Annu. Meet. Abstr.* **11**:47a.

ZUCKERMAN, R. 1971. Mechanisms of photoreceptor current generation in light and darkness. *Nature (Lond.).* **234**:29.