

AN ANALYSIS OF LIGHT-INDUCED ADMITTANCE CHANGES IN ROD OUTER SEGMENTS

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SUMMARY

1. Measurements were made of the time course and amplitude of the change in real part of admittance, ΔG , of a suspension of frog rod outer segments, following a flash of light bleaching about 1% of the rhodopsin content of the rods. The measurements, based on the use of a specially designed marginal oscillator, covered the frequency range between 500 Hz and 17 MHz.

2. The components of response, previously described for rods prepared by a method involving exposure to strongly hypertonic sucrose solutions, are present in similar form when rods are isolated and maintained in isotonic solutions made up with equi-osmotic concentrations of NaCl and sucrose or with Na_2SO_4 .

3. Component I, identified as a slowly developing positive ΔG apparent at very low frequencies, is frequency-independent up to the characteristic frequency of admittance for the suspension, f_Y (about 2 MHz for rods suspended in a solution having the conductivity of Ringer solution), but decreases at still higher frequencies.

4. Component II, identified as a rapidly developing positive ΔG which appears only above a critical frequency about 2.5 decades below f_Y , increases approximately logarithmically with frequency to reach a limiting amplitude in the region of f_Y .

5. The amplitude of component II, ΔG_{II} , measured in the region of f_Y , varies linearly with the conductivity of the suspending medium, G_o , under conditions in which the conductivity of the rod interior is also a linear function of the external conductivity. The relation for a flash bleaching 1% of the rhodopsin content of the dark-adapted rod is

$$\Delta G_{II} = 1.17 \times 10^{-4} G_o + 0.10 \mu\text{mho/cm.}$$

6. Measurements made on rods suspended in a low-conductivity solution, which has the effect of reducing the conductivity of the rod interior

to about one ninth its value for rods suspended in Ringer solution, reveal a decline in component II for frequencies above 8 MHz.

7. To explain the frequency dependence of component II and its dependence on conductivity, it is proposed that component II arises from a light-induced increase in conductance of the disk membranes which obstruct the longitudinal flow of current through the rod interior except at very high frequencies.

8. The disk-membrane conductance increase for rods suspended in a solution having the conductivity of Ringer solution is calculated to be 4.3×10^{-11} mho/rhodopsin molecule bleached, a value which is similar to what has been found for ionic channels operated by membrane potential change in the nerve membrane and by synaptic transmitter in the post-junctional membrane.

9. No component of response has been observed which could be reliably attributed to a surface membrane conductance decrease of the type observed in receptor cells in the retina.

INTRODUCTION

The primary aim of the work described in this paper was to detect light-induced changes in the passive electrical properties of rod outer segments, which might provide information about processes taking place close to the site of the rhodopsin molecules in the disks of the rod interior. In an earlier report (Falk & Fatt, 1968*b*) the response to flash illumination observed by alternating current measurements on packed suspensions of frog rods was resolved into several components on the basis of the time course and frequency dependence of the measured electrical parameter. Two of these (designated components I and II) are of special interest in that they occur only if the rhodopsin remains associated with the structure of the rod. They are further characterized in showing a saturation of response amplitude for a flash bleaching only a few percent of the rhodopsin content of the rods, with recovery of responsiveness to subsequent flashes requiring about 10 min.

A number of possible sites of origin of components I and II were suggested in 1968 and it was recognized that a decision between these possibilities would require the extension of measurements to higher frequencies than could be reached with the methods employed up to that time. In the present work the upper frequency limit for reliable measurements has been extended to the point where current flow through the interior of the rod is no longer restricted by the surface membrane capacitance for rods oriented either parallel or perpendicular to the applied electric field, and where the effect of the disk membrane capacitance may commence to be

significant for the passage of current in the longitudinal direction through the rod interior. An important deduction from the present observations is that component II provides a measure of a light-induced increase in ionic permeability of the disk membranes.

In addition to extending the frequency range of measurement, the present experiments have been concerned with rod suspensions maintained under isotonic conditions. Brief accounts of parts of this investigation have already been published (Falk & Fatt, 1972*a, b, c*).

METHODS

Preparation

Measurements were carried out on packed suspensions of randomly oriented rod outer segments detached from the retinas of frogs (*Rana temporaria*) which had been dark-adapted overnight. One animal provided sufficient material for an experimental run employing a conductivity cell of the type shown in Fig. 1. The dissection and subsequent stages in the preparation were carried out under dim red light. Retinas were placed in a vial containing 1–2 ml. of solution and the rod outer segments detached by gentle shaking. The retinas and any large tissue fragments were then removed by filtration through woven nickel gauze of 37 wires/cm followed by 170 wires/cm.

Preparation in isotonic solution. An impure suspension of rods in an isotonic modified Ringer solution, prepared by the above procedure, was transferred to the upper, cylindrical portion of the conductivity cell shown in Fig. 1. The rods were then separated from other particulate matter by low-speed centrifugation of the cell (50 *g* for 15 min). Examination of the cell at this stage, in a few trial runs to test the purification procedure, showed red blood cells together with fragments of retina of greater than cellular dimensions to be accumulated at the bottom of the shallow channel, occupying part of the space below the upper edge of the lower electrode (Fig. 1*b*). Rod outer segments, uncontaminated by other material, were packed on top of this, extending to a distance of a few millimetres beyond the upper electrode. The supernatant solution continued to hold in suspension small fragments of rods together with some melanin granules. It is assumed that these small particles fail to sediment during low-speed centrifugation owing to their Brownian motion, by which the tendency to sediment would be opposed by diffusion. In the usual procedure, the low-speed centrifugation was followed directly by centrifugation at 5200 *g* for 1–2 min. This resulted in firmer and more uniform packing of the rods; it also led to the accumulation of a layer of fine particles on top of the packed rod mass. With the conductivity cell packed with material in this way, the electrical measurements were confined to the layer of rods.

Preparation in hypertonic sucrose solutions. In some experiments separation of rods from other particulate matter was carried out in solutions in which the density was controlled by the addition of sucrose. The procedure was the same as was employed in earlier studies (Falk & Fatt, 1968*a* and *b*).

Solutions

Most experiments were done in a modified Ringer solution in which approximately half the NaCl was replaced by an osmotic equivalent of sucrose. This modification was based on observations which indicated that rod outer segments were slowly

penetrated by NaCl (over the course of several hours) when suspended in normal Ringer solution. Rods in normal Ringer solution slowly elongated and after a few hours underwent irreversible changes characteristic of hypotonic solutions. The presence or absence of Ca^{2+} (varied between 6 mM and very low concentrations obtained by addition of 2 mM-EDTA with no added Ca^{2+}) did not appear to affect this process significantly.

The slow changes indicative of penetration by the external solute did not take

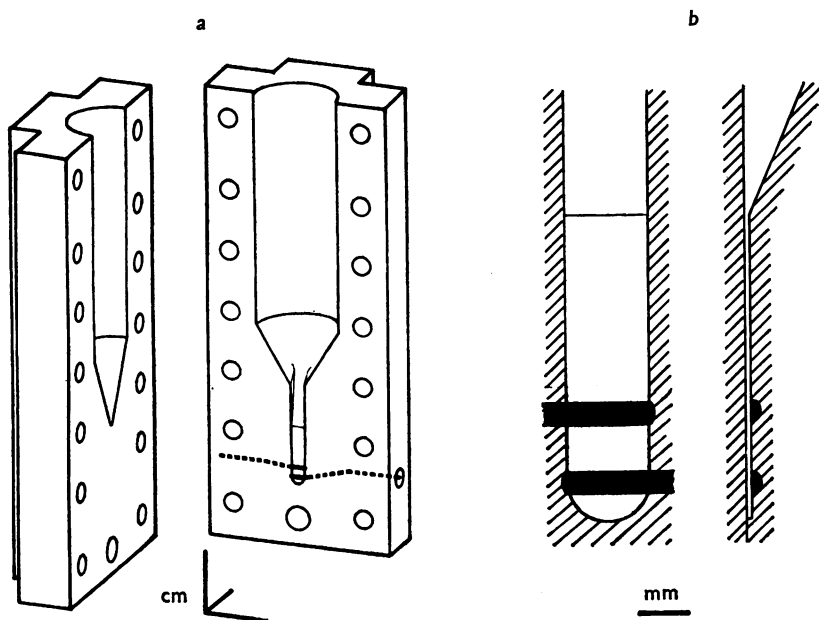


Fig. 1. Conductivity cell used for measurements on packed suspensions of rod outer segments. *a*, Perspective view of dismantled cell. In use, the two parts were held together by steel bolts passing through the holes along their edges. Platinum-wire leads are shown by heavy lines, which are dotted where leads pass through the insulating material of the cell. The major constructional material was a polyisopentene plastic (TPX, I.C.I.) which had desirable optical, electrical and mechanical properties. *b*, Orthogonal sectional views of the shallow channel into which the rods were packed by centrifugation. The exposed surfaces of platinum, constituting the electrodes, were flush with the walls of the channel and were coated with platinum grey from a solution of gelatin and PtCl_4 as described by Gesteland, Howland, Lettvin & Pitts (1959). Illumination of the packed rods was through the transparent wall of the channel, light passing from the side of the cell in which the electrodes were fixed, so that the electrode surface was not directly illuminated. The major feature in which the cell differed from that described previously (Falk & Fatt, 1968*a*) was that the electrodes were arranged horizontally, one above the other, with the upper electrode earthed so that a thermistor probe for temperature measurement could be introduced into the upper part of the cell without disturbing the electrical measurements. In a few experiments, involving the use of low-conductivity solutions, the width of the channel was increased from 1.6 to 4.5 mm.

place when NaCl was replaced by isosmotic Na_2SO_4 or of sucrose or when one half the NaCl was replaced by sucrose. Under these conditions rods could be maintained for several days without change in structure, provided some colloidal substance, either bovine serum albumin (BSA) or methylcellulose, was present in the medium at a concentration of about 0.5% or greater. Microscopic examination showed the surface of rods suspended in isosmotic sucrose or Na_2SO_4 solution to be crenated and the rods to be shorter in length than when freshly prepared in ordinary Ringer solution. These changes may be assumed to arise from Cl^- leaving the rod interior (accompanied by cations, presumably Na^+ and K^+). No volume change took place when rods were suspended in a Na_2SO_4 solution the osmolarity of which was 0.8 times that of Ringer solution.

The composition of the standard modified Ringer solution was as follows: 60 mM-NaCl, 100 mM sucrose, 3 mM phosphate, bovine serum albumin 0.01 g/ml. (BSA, crystalline), with an additional 7.5 mM- Na^+ to give pH 7.0. In some experiments a cationic, rather than anionic, buffer was used, the phosphate being replaced by either imidazole or Tris. In a number of experiments 1–2 mM-EDTA or EGTA was added to the solution in order to remove traces of metal ions capable of reacting with sulphhydryl groups. The use of these chelating agents was based on the observation that components I and II of the response to light are abolished by micromolar concentrations of copper ions which occur as a contaminant of serum albumins (G. Falk & P. Fatt, in preparation). Alternatively, in a few cases the specific copper-combining agent, 3-methyl-5-hydroxy-5-(D-arabino-tetrahydroxybutyl)-thiazolidine-2-thione (MHATT) (Corbett, 1966), was added in a concentration of 1 mM. In some experiments the modified Ringer solution contained various admixtures of ATP, Mg^{2+} , Ca^{2+} and/or K^+ , each in a concentration of 1–3 mM. None of these variations was found to have any specific action on the response to light described in this paper. In all cases the concentrations of Na^+ and Cl^- were adjusted to maintain a constant osmolarity and pH.

In the experiments in which density-variation with sucrose was used, the solution in which the rods were detached from the retina contained 34 g sucrose/100 g solution (sp.gr. 1.14) and a variable concentration of NaCl (used to vary the conductivity). Other constituents such as BSA, buffer, etc., were similar to those used in the modified Ringer solution. The concentration of sucrose in the medium in which rods were suspended when in the conductivity cell was 0.8 M.

Temperature. The temperature of the laboratory was controlled by a thermostat attached to the preparation box. The temperature of the rod suspension was monitored by a small thermistor in contact with the fluid just above the narrow channel in the conductivity cell. The temperature ranged in different experiments between 16 and 21°C, but was constant in any given experiment to within 1°C.

Apparatus for measuring G and ΔG

Design of the marginal oscillator. Measurements of the change in the real part of admittance, ΔG , were made with a specially constructed instrument, based on the marginal oscillator of Pound & Knight (Knight, 1961). The important feature of this instrument (Fig. 2) is that reliable measurements, unaffected by stray capacitances, can be made at high frequencies. In its essentials, the instrument is an inductance-capacitance parallel-tuned oscillator, with the sample under examination placed in parallel with the resonating elements. The feed-back (which is positive) and amplification are so adjusted (by R_3 , R_4 and ZD) that the level of oscillation is made sensitive to the dissipation of power in the resonating circuit or any network connected across it. An increase in the real part of admittance of the sample thus brings about a decrease in level of oscillation. On the other hand, the level of oscilla-

switch, S) by the relation

$$\Delta G = \frac{R_2}{R_1(R_1 + R_2)}. \quad (1)$$

When the capacitances are taken into account, the admittance change is complex ($\Delta Y = \Delta G + j\Delta B$), its real and imaginary parts being given by

$$\Delta G = \frac{R_2}{R_1(R_1 + R_2)} \left[1 - \frac{\omega^2(R_1C_1 - R_2C_2)^2 R_1^2}{(R_1 + R_2)^2 + \omega^2(C_1 + C_2)^2 R_1^2 R_2^2} \right], \quad (2)$$

$$\Delta B = \omega \frac{C_1 C_2}{C_1 + C_2} + \omega \frac{(R_1 C_1 - R_2 C_2)^2}{(C_1 + C_2)[(R_1 + R_2)^2 + \omega^2(C_1 + C_2)^2 R_1^2 R_2^2]}, \quad (3)$$

where $\omega = 2\pi f$, ω being the frequency in radians/sec and f in Hz. It will be noted that provided

$$R_2 C_2 = R_1 C_1 \quad (4)$$

eqn. (2) becomes identical with eqn. (1) and eqn. (3) reduces to

$$\Delta B = \omega \frac{C_1 C_2}{C_1 + C_2}. \quad (5)$$

Thus, under the particular condition indicated by eqn. (4), ΔG introduced by the calibrator is independent of frequency. Moreover, since R_2 was never greater than $R_1/100$, one has to sufficient accuracy

$$\Delta G = \frac{R_2}{R_1^2}, \quad (6)$$

$$\Delta B = \omega C_1. \quad (7)$$

The capacitance C_1 arose entirely from stray capacitances and was in the region of 0.25 pF. To meet the condition of eqn. (4), a small fixed-value mica or polystyrene capacitor was placed in parallel with each resistor used for R_2 . The required value of C_2 was that which gave a maximum deflexion on the oscilloscope when the relay was operated. Small metal film resistors were used for R_1 and R_2 in order to minimize variation in their values with frequency. An evident source of error in the calibration procedure was that arising at high frequencies from an increase in the apparent conductance of the resistor, R_1 . This was detectable in measurements made with an r.f. bridge which showed G for the resistor R_1 to rise by 2% at 4 MHz and by 9.5% at 17 MHz, relative to its low-frequency value. Correction could be made for this. However, there were indications of another source of error, probably in the inductance of the leads connecting the conductivity cell to the calibrator and the latter to the resonating circuit.

As the ultimate check on the calibration, infra-red flashes were applied to a conductivity cell filled with a solution of electrolyte. The oscilloscope deflexion produced as a result of the temperature rise, due to the absorption of the radiation by water, was compared with the deflexion given by the calibrator over a range of frequencies extending up to 17 MHz. A correction for the calibrator was thus arrived at on the assumption that ΔG for the solution was the same at all frequencies. (The Debye-Falkenhagen effect would be expected to produce a slight increase in G and hence in ΔG , but only at higher frequencies than were employed.) The correction factors to be applied at frequencies above 4 MHz were found to be influenced to a small extent by the value of G . For this reason the conductivity of the electrolyte solution was matched to the value of G of the preparation measured at high frequencies.

Calibration of G . To evaluate G for the sample at different frequencies, metal oxide resistors were employed to substitute directly for the sample. This calibration was made at the end of the experiment. It depended on returning to the same settings of the instrument controls, substituting known (calibrating) resistors and interpolating between readings on the a.c. voltmeter (connected at terminal 3 in Fig. 2) which gave a direct reading of the level of oscillation.

Variation in the value of G for the resistors at high frequencies did not constitute a serious problem in that, at the highest frequencies used, calibrating resistors of less than 30 k Ω were required and any error, which could be allowed for, was less than 5%. A check on the procedure for calibration of G was to observe the expected constancy of G for a conductivity cell filled with electrolyte solution. The procedure was found to be satisfactory for all but the highest frequencies, above 13 MHz, when the estimated G was liable to be as much as 5% greater than the value obtained in measurements at lower frequencies.

All values of admittance are given in terms of a sample of unit geometry, G and ΔG having the dimensions mho/cm.

Time resolution. The low-pass filter, used to remove the a.c. carrier wave from the rectified signal transmitted to the oscilloscope, was usually set (by adjustment of C_3) so that a step of voltage applied at the input to the filter would be recorded at 90% of its final level in about 0.5 msec. As a consequence, removal of the carrier wave was incomplete for frequencies below about 750 kHz. For such frequencies the backing-off control was set so that the base line for observation of ΔG on the oscilloscope corresponded to the lower envelope of the rectified carrier wave when an increase in G produced an upward deflexion. (For frequencies of 140–750 kHz there was a broadening of the oscilloscope record as both the upper and lower limits of the wave were apparent.)

At frequencies below 10 kHz the factor limiting the time resolution of the recorded response was the effective Q of the resonating circuit, since any change in level of oscillation must involve a transient change in frequency. As can be seen in the records of Fig. 3 for the response to a step of ΔG produced by the calibrator, the distortion increases with lowering of the frequency of measurement to give a lag of about 2 msec for a 70% rise to the final level when the frequency was in the region of 1 kHz. The same distortion of the time course of response is apparent in the records, also shown in Fig. 3, obtained on illumination of a packed rod suspension with a flash of infra-red radiation.

Illumination. The light source was a small commercial xenon flash lamp modified by the use of zener diodes to regulate the voltage across its capacitor. It was housed in a mu-metal box with a 1.5 cm diameter hole for exit of the light. The flash had a duration between half-maximum points of 0.5 msec. The light was filtered and focussed through a shuttered aperture on to the conductivity cell held within a compartment of the box containing the marginal oscillator. For producing a visible-light response of the rods, the light was filtered through 2 cm thickness of 5% aqueous CuSO_4 solution, removing radiation of wave-length greater than 600 nm (30% transmittance point), plus 2 mm thickness of Chance OY8 glass, removing wave-lengths less than 465 nm. Neutral density filters of colloidal carbon were used to control the intensity. For infra-red illumination the CuSO_4 filter was replaced by 2 mm thickness of Schott RG10 glass, removing wave-lengths less than 710 nm.

The extent of bleaching of the packed rods by a given intensity flash was estimated as previously (Falk & Fatt, 1968*b*) on the basis of the decrement of response amplitude occurring on repeated exposure of a preparation to flashes under conditions in which the responses to individual flashes were proportional to flash intensity. Responses at different frequencies of measurement were obtained usually with light

stimuli which bleached no more than about 1% of the rhodopsin content of the rods. These were delivered at intervals of about 15 min. Under these conditions, the amplitude of response is approximately proportional to the amount of rhodopsin bleached.

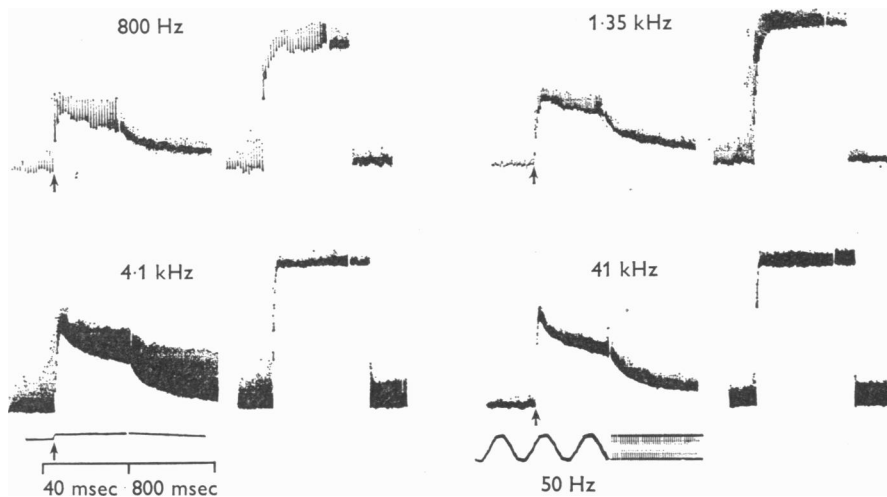


Fig. 3. Records of ΔG at different frequencies of measurement obtained in response to an infra-red flash applied to a packed suspension of rod outer segments and in response to operation of the calibrator. These records were obtained in the course of the experiment for which the response to a blue-green flash is illustrated in Fig. 4. From the fractional change in G obtained when the cell was filled with a standard electrolyte solution, the absorption of the infra-red flash was estimated to produce a temperature jump of about 0.04°C . The decay of ΔG subsequent to the flash is attributable to loss of heat by thermal conduction, mainly into the electrodes. The small step-like trace under the infra-red record at 4.1 kHz is the time integral of the flash obtained by means of a photo-transistor followed by an integrating circuit. Such records obtained on the second beam of the double-beam oscilloscope were used to give a precise indication of the timing of the flash. The timing of the flash is indicated on records by an arrow, the duration of the flash (less than 1 msec) being very short on the sweep speed employed (shown at a faster sweep in Fig. 10c of Falk & Fatt, 1968b). The difference in the time course of the early part of the records at different frequencies is due to the narrow band of the resonant circuit of the marginal oscillator when operating at low frequencies. The calibration pulse was obtained with $R_2 = 220 \Omega$, so that $\Delta G = 0.022 \mu\text{mho}$. With a cell constant of 33.7 cm^{-1} , the deflexion produced by operation of the calibrator is equivalent to a change in specific admittance of the sample of $0.74 \mu\text{mho/cm}$. Note that a split time base has been used for all recording, the later part of each trace being swept at $1/20$ the speed of the early part. A record of a 50 Hz sinusoidal signal obtained on this time base is shown below the response to the infra-red flash at 41 kHz.

RESULTS

Response of rods suspended in isotonic solutions

Time course and frequency dependence of components I and II. Records of the time course of ΔG following illumination by a brief flash are shown in Fig. 4. The records were obtained for different frequencies extending from 800 Hz to 17 MHz. A change in the time course of the recorded response with frequency of measurement is apparent. The fraction of the response completed up to 30 msec following the flash (the approximate time of the break in the trace where the sweep speed is decreased abruptly) is seen to increase progressively with frequency over the range 800 Hz–4.3 MHz. This is accounted for by an increase in amplitude of the early part of the response, while the later part (from 30 msec following the flash to about 5 sec later) changes only slightly. Above 2.6 MHz and extending

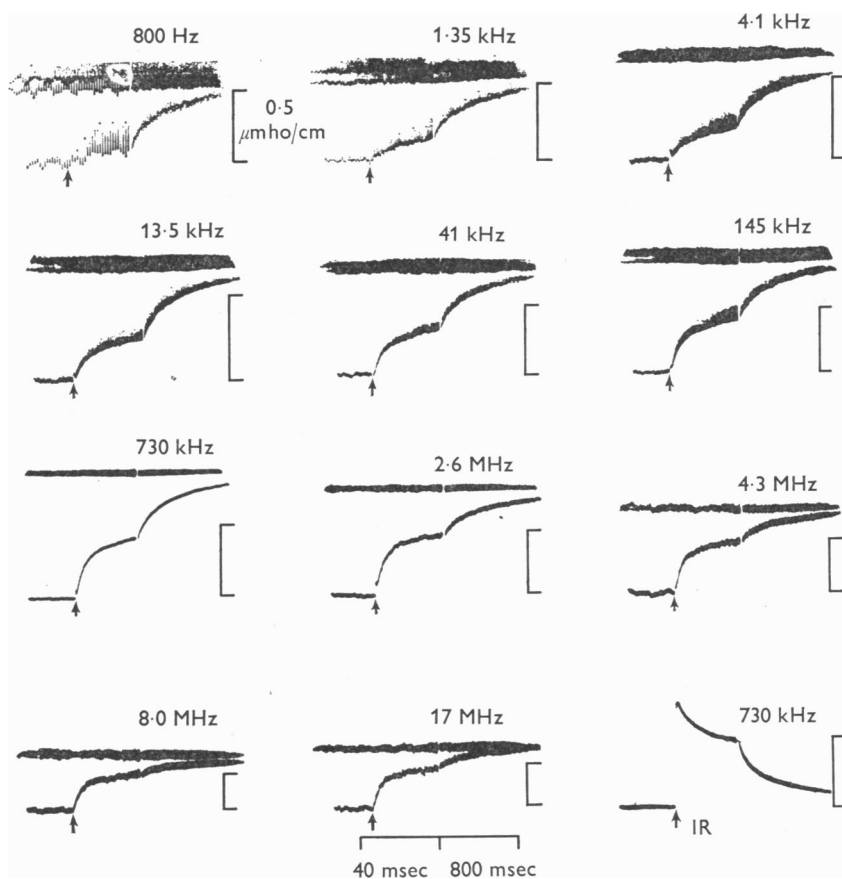


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to the upper limit of measurement at 17 MHz, there is a diminution of the later part of the response. This variation of time course with frequency of measurement is a strong indication that the response is a composite, made up of contributions from different components, each having a distinctive time course and frequency dependence. The observations are in agreement with previous work (Falk & Fatt, 1968*b*), which was, however, more restrictive in that no information could then be obtained about the time course of response above 300 kHz, and determinations of amplitude were unreliable above 60 kHz.

In Fig. 5 the recorded response amplitude (ΔG) is plotted as a function of frequency of measurement at a number of different times following the flash. The curves drawn to fit the plotted data show the response to be of constant amplitude below about 1 kHz. The present observations do not compel the conclusion that there exists such a limiting behaviour at low frequencies; evidence for it comes from the previously reported measurements on changes in both the real and imaginary parts of admittance (Falk & Fatt, 1968*b*). These showed clearly that the value of ΔG reached about 5 sec following the flash was independent of frequency for frequencies

Legend for Figure 4.

Fig. 4. Records of change in real part of admittance (ΔG) in response to a flash of blue-green light (or infra-red for the lower right record). The order in which records were obtained at different frequencies was staggered to minimize the apparent effect on frequency dependence should any progressive change occur during the course of the experiment. Each record was obtained in response to a separate flash, the flashes being delivered at intervals of 12–16 min. Each record consists of 3 sweeps commencing at intervals of 2.5 sec and utilizing a split time base. The flash was delivered at the time indicated by the arrow on the first sweep. In some of the records this was accompanied by an electrical artifact. The subsequent upward deflexion of the trace represents an increase in G , continued on the following sweeps. (These nearly coincided for records at frequencies of 730 kHz and above.) The fuzzy appearance at low frequencies is a consequence of incomplete filtering of the alternating current used in the measurements. At such frequencies, the record consists of the lower envelope of the remaining a.c. signal. The same time scale was used for all records. The vertical scale (ΔG) was different for each record: the calibration bars in each case represent the same change in real part of the specific admittance of the sample (0.5 $\mu\text{mho/cm}$). The flash applied to the sample had an intensity sufficient to bleach 1.2% of the rhodopsin. The calibration bars for the different records in this and succeeding figures have been adjusted to allow for the progressive decline in response amplitude due to diminution of the rhodopsin content of the rods during the course of the experiment. The rods were prepared under isotonic conditions. The solution bathing the rods had the following composition (in mM): 69 Na⁺, 100 sucrose, 6 phosphate, 50 Cl⁻, 2 EDTA plus 0.02 gBSA/ml.; pH 7.0. Temperature 18.4–18.9° C.

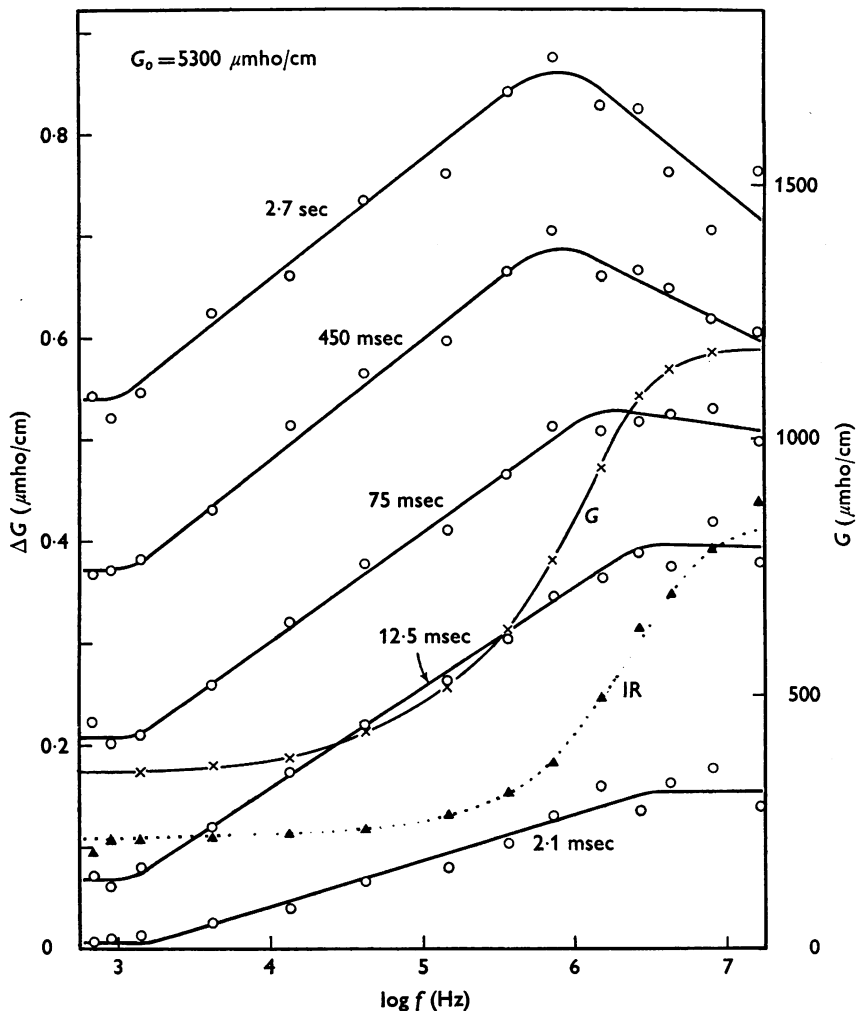


Fig. 5. Plots of G and ΔG against log frequency for the experiment illustrated in Figs. 3 and 4. Circles give ΔG (displacement of G from its initial value) obtained at different times following a flash bleaching 1.2% of the initial rhodopsin content of the rods. The minimum time for which data are given (2.1 msec) has been determined by the presence of an electrical artifact at shorter times, produced by the operation of the flash lamp. Little further change in G took place beyond 2.7 sec (uppermost curve for ΔG) which could be distinguished from slow, spontaneous drift occurring in the dark. Triangles give ΔG measured 12.5 msec following an infra-red flash. Note that the scale for this curve (IR) is not the same scale as for ΔG in response to a blue-green flash. The latter should be multiplied by a factor of 3 to represent the IR scale. Crosses give G measured about half way through the series of observations of ΔG .

below about 1 kHz and extending down to the lowest frequency of measurement at 15 Hz.

Between 2 kHz and about 1 MHz the response amplitude, measured at any specified time following the flash from 2.1 msec onward, is seen to increase linearly with the logarithm of frequency. The steepness of this relation increases with time following the flash to reach nearly its final, limiting value by 12.5 msec. Beyond this time and extending for a few seconds, the response amplitude in the frequency range below 1 MHz is displaced uniformly to larger values.

The frequency dependence and time course of ΔG up to 1 MHz can, in accordance with the scheme previously adopted, be interpreted on the assumption that the response consists of two principal components: component I, which has a slow time course of development and an amplitude that is frequency-independent over the frequency range considered here (i.e. up to 1 MHz), and component II, which develops more rapidly and for which ΔG varies logarithmically with frequency. The present experimental findings as plotted in Fig. 5 show the response amplitude as continuing to increase logarithmically with frequency up to about 2 MHz.

For the shorter times of 2.1–12.5 msec following the flash, the response amplitude is constant at frequencies above 1 MHz. For the longer times of 450 msec–2.7 sec, a different behaviour is noticeable: the response reaches a maximum at about 1 MHz from whence it declines to the extent of about one quarter of the low-frequency (1 kHz) amplitude by the upper frequency limit of the experiment (17 MHz). To account for the behaviour observed at frequencies above 1 MHz, it is sufficient to assume that component II does not continue to increase with frequency above about 2 MHz, but is frequency-independent over at least a decade of frequencies in this region. On the other hand, component I is assumed to be frequency-independent up to about 1 MHz and to decline at higher frequencies. Further information on the behaviour of component I at high frequencies will be presented in the following paper (Falk & Fatt, 1973).

*Relation between the frequency dependence of the response
and the dispersion of admittance*

It is of interest to relate the position (on the frequency scale) of any transition in the frequency dependence of ΔG and the characteristic frequency f_Y , obtained as the frequency at which G is at the mean of its limiting low- and high-frequency values (or as the frequency at which G increases most steeply with $\log f$). A plot of values of G , from measurements made during the course of observation of the response to a flash, is included in Fig. 5. As has been found in most experiments, the curve for G vs. $\log f$ is not quite a regular sigmoid (i.e. a curve with twofold rota-

tional symmetry). The degree of uncertainty that this introduces in deciding upon the position of f_Y is, however, small, and one may in the present experiment assign to f_Y a value of 1.0 MHz. It is to be noted that the frequency at which component I commences to decline on the above analysis of response corresponds closely with f_Y .

An important feature of component II is its low-frequency cut-off, in the region of 2 kHz, nearly 3 decades below f_Y . The theory of the low-frequency cut-off (which will be designated f_{IIU}) is given in the Discussion in relation to the proposal that component II has its origin in an increase in the longitudinal conductance of the rod interior. At high frequencies the approach of component II to a limiting value occurs in the region of f_Y .

ΔG resulting from an infra-red flash

The amplitude of the response to an infra-red flash may be used as a test, albeit a somewhat indirect one, of the consistency of measurements at different frequencies. The aim in applying this test was to reveal any serious failure of the calibration for ΔG . From general theoretical considerations it may be shown that for any network composed of resistors and capacitors arranged in any way (including networks with distributed elements), if there occurs a small proportional variation η in all the resistive elements, this will be manifest at the terminals of the network as a change in the real part of admittance given, as a function of frequency, by

$$\Delta G(f) = \left(G(f) - \frac{1}{2.3} \frac{dG(f)}{d \log f} \right) \eta. \quad (8)$$

The amplitude of the response to an infra-red flash is plotted against log frequency in Fig. 5 (curve labelled IR). In agreement with eqn. (8), the rise in ΔG with frequency lags behind the rise in G with frequency.

Response of rods suspended in hypertonic sucrose solution

The resolution into components, carried out on the response of rods suspended in isotonic solutions, is consistent with the analysis previously made (Falk & Fatt, 1968*b*). The latter was based on measurements on rods separated from other retinal constituents by flotation in solutions made strongly hypertonic with sucrose. It was therefore of interest to compare the response of rods in an isotonic solution with that obtained in a hypertonic sucrose solution over the extended frequency range at present employed.

The response of rods suspended in a hypertonic sucrose solution is shown in Fig. 6. Comparison with Fig. 4 shows that the major features of the response are similar for rods in hypertonic sucrose as for those in isotonic solution of comparable conductivity. The final amplitude of response (ΔG measured a few seconds after the flash) is plotted as a function of frequency in Fig. 7*a*.

Variation of component II with conductivity

In an earlier report on light-induced admittance changes in rod suspensions purified by the sucrose density-variation method, component II was described as having a constant amplitude (for a given small amount of rhodopsin bleached) over a range of conductivities of the suspending medium extending from the lowest conductivity employed (about

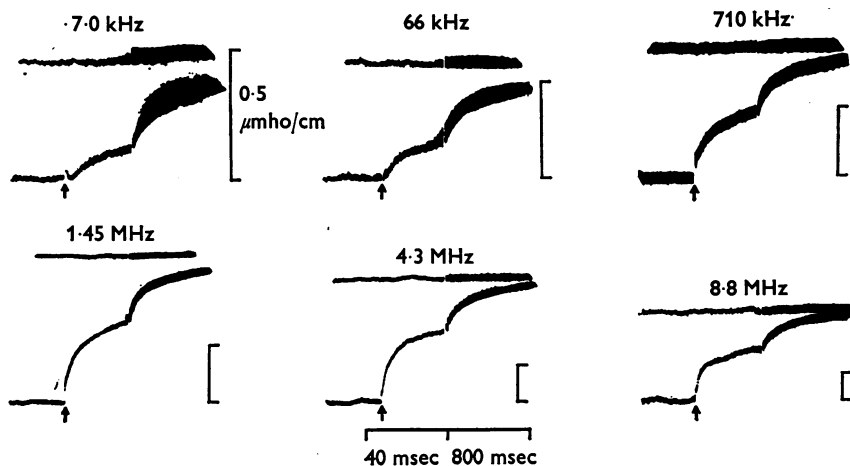


Fig. 6. Records showing time course of ΔG following application of a blue-green flash (bleaching 1% rhodopsin) to a packed suspension of rods prepared with the use of hypertonic sucrose solutions for purification. The conditions of stimulation and the manner of display of the records were similar to those used for Fig. 4. The rods were shaken off the retina in a solution having the composition (in mM) 170 Na^+ , 16.5 K^+ , 2 Mg^{2+} , 5 ATP, 10 phosphate, 154 Cl^- together with sucrose 0.385 g/ml., 0.01 gBSA/ml., pH 6.8. The sucrose content of the medium was reduced to 0.25 g/ml. before the rods were sedimented in the conductivity cell. There is no reason to suspect from other experiments that the inclusion of Mg^{2+} and ATP had any particular influence on the response. Plots of G and ΔG against log frequency are given in Fig. 7a for this experiment.

500 $\mu\text{mho/cm}$ as determined by the presence of buffer plus protein) up to that obtained by the addition of 30 mM-NaCl (about 2300 $\mu\text{mho/cm}$). At higher conductivities obtained on the addition of 100–300 mM-NaCl the amplitude of component II was observed to increase. In the earlier analysis of measurements, ΔG corresponding to component II was taken as the difference in response amplitude measured at a frequency of 60 kHz from that measured at low frequencies where ΔG was frequency-independent. The reason for using this analysis was that 60 kHz was the highest frequency at which reliable measurements could be made with the measuring

apparatus then available. It was, nevertheless, noted that, over the range of conductivities examined, the frequency at which the logarithmic rise of ΔG with frequency commenced (i.e. f_{IIY}) increased with the conductivity of the suspending medium (G_o), in an approximately proportional relationship.

In view of the interest that attaches to the effect of conductivity in relation to possible mechanisms of origin of the response, a re-examination of this question seemed desirable, using the marginal oscillator to obtain measurements of ΔG at the higher frequencies where component II appears to reach a limiting value. From previous work (Falk & Fatt, 1968*a*) it is known that the conductivity of the interior of rods, prepared by density variation with sucrose, varies linearly with the external conductivity G_o and that it displays an appreciable, though relatively small value for the intercept at zero G_o , due presumably to the presence of indiffusible ionized groups inside the rod. Suspensions of rods were prepared by this method, with NaCl added in different concentrations to give a wide range of values of G_o . Fig. 7 shows plots of ΔG against $\log f$ for two values of G_o . A feature of such experiments is that both f_{IIY} and the frequency at which ΔG reaches a limiting or maximum value are observed to vary nearly in proportion to G_o (except when G_o was reduced below about 900 $\mu\text{mho/cm}$, when the two frequencies tended to reach fixed values of about 600 Hz and 200 kHz respectively). The slope of the linear variation of ΔG against $\log f$ varied in a similar manner with G_o . Fig. 7 also includes plots of G against $\log f$ for the two rod suspensions. It will be seen that the characteristic frequency, f_Y , increased with G_o , as did the difference between the limiting values of G at high and at low frequencies. This is in agreement with the idea that the internal conductivity varied with the conductivity of the medium.

Fig. 8 gives a plot of the frequency-dependent part of the response (corresponding to component II and designated ΔG_{II}) against G_o . The value of ΔG_{II} has been derived for each experiment as the difference between the final level of ΔG obtained in records of the response at, or slightly above, f_Y (where ΔG as a function of frequency has reached a maximum or limiting value) and that obtained at low frequencies below which it is assumed that there would be no further frequency dependence. This method of estimating ΔG_{II} corresponds to a natural limiting value and would be expected to reveal the magnitude of the change in conductance of the resistive element, which change underlies the response. The data presented in Fig. 8 show clearly that the amplitude of response, referable to component II, varies linearly with G_o . The regression line, fitted by the method of least squares, gives the relation

$$\Delta G_{II} = 1.17 \times 10^{-4} G_o + 0.10 \mu\text{mho/cm}.$$

The divergence of the present result from that previously reported can be attributed entirely to the fact that in the earlier work the value of ΔG_{II} was obtained from measurements at a fixed frequency of 60 kHz. It is now evident that the change in steepness of the dependence of ΔG on

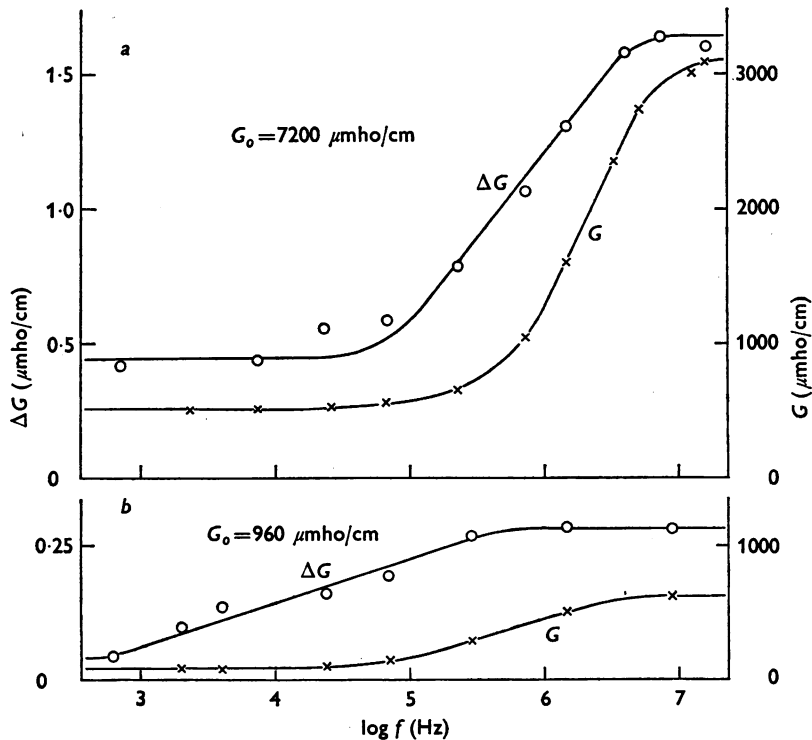


Fig. 7. Plots of G and ΔG against log frequency for rods in hypertonic sucrose solutions of varying conductivity, G_0 . ΔG was measured 3 sec following a flash bleaching 1% of the initial rhodopsin content of the rods. *a*, from the same experiment as Fig. 6 in which records of the time course of ΔG are shown. The conductivity of the suspending medium in the experiment of *a* is about 8 times that for the experiment in *b*. The composition of the solution in *a* is given in the legend to Fig. 6. The solution in which the rods were suspended when in the conductivity cell for the experiment in *b* is as follows: 26 mM- Na^+ , 8.3 mM- K^+ , 6 mM- Mg^{2+} , 10 mM-ATP, 5 mM phosphate, sucrose 0.25 g/ml., 0.01 gBSA/ml., pH 6.9.

log f would nearly compensate for the shift in the position of f_{II} as G_0 was varied. Accordingly, over a considerable range of conductivities the amplitude of ΔG_{II} , measured by the previous method, would appear constant. Even by the present analysis, the amplitude of component II is not proportional to the conductivity of the medium. In previous work on the properties of rod suspensions prepared in sucrose solutions, the linear

relation of internal to external conductivity was found to have a slope of 0.50 with a limiting internal conductivity of $280 \mu\text{mho/cm}$ at zero G_o (Falk & Fatt, 1968*a*). Assuming this relation to hold in the present experiments and ΔG_{II} to vary proportionally with the internal conductivity, then one

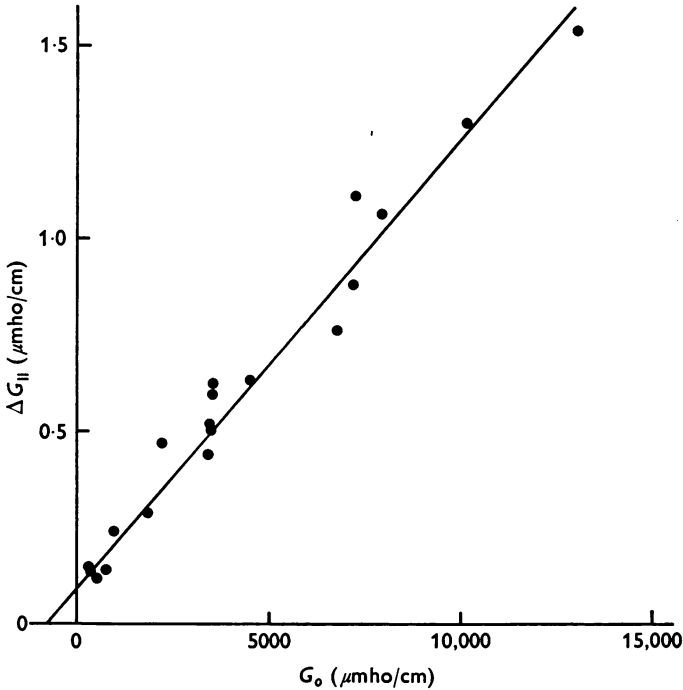


Fig. 8. The dependence of the amplitude of component II (ΔG_{II}) on conductivity of the suspending medium (G_o). The rods were purified by density-variation with sucrose; the conductivity was varied with NaCl, the electrolyte concentration being kept constant in any experiment during the isolation procedure and subsequent measurements. The solutions were buffered to pH 7, the buffer in most, but not all experiments, being phosphate (3–6 mM). In all experiments, but for the lowest values of G_o , the flash bleached 1% of the rhodopsin content of the dark-adapted rod. At the lowest values of G_o , flashes bleaching 1.5% were used, but the values given are adjusted to correspond to 1% bleaching. A factor of 1/1.45, rather than 1/1.5, was applied to take into account some non-linearity in the response-intensity relation. The text describes the way in which ΔG_{II} was arrived at. All values of ΔG_{II} have been corrected for bleaching by prior flashes.

would predict that a line relating ΔG_{II} to G_o would intercept the G_o -axis at $-560 \mu\text{mho/cm}$. The line fitted to the data in Fig. 8 intercepts the axis at $-870 \mu\text{mho/cm}$, which may be taken to be in reasonable agreement, although one cannot press the comparison in view of the scatter in the data for both types of measurement.

High-frequency behaviour of ΔG for solutions of low-conductivity

As will be shown in the Discussion, the low-frequency cut-off of component II can be explained on the basis of a small light-induced change in conductance of the rod interior for current flowing in the longitudinal direction, with the frequency dependence of this current, and hence of the recorded ΔG , being determined by the surface membrane capacitance. It is further suggested that the underlying conductance change may take place across the disk membranes. It therefore becomes of interest to examine the behaviour of component II at high frequencies in the expectation of observing a high-frequency cut-off due to the capacitance of the disk membranes.

The range of frequencies in which a frequency dependence of admittance parameters occurs, due to any particular capacitance element, will vary directly with the conductance through which the element discharges. Since in the present experiments the upper frequency limit of measurement was set by the available apparatus, effort was directed to decreasing the conductance in series with the disk membranes by effecting a reduction of the ionic conductivity of the spaces inside the rod. Since the lower limit to the value of internal conductivity obtained on reduction of G_0 was found to be considerably lower when rods were subjected to hypertonic sucrose solution, as in the procedure for purification of the rod suspension by density variation with sucrose, than when they were maintained in isotonic solution, the procedure involving hypertonic sucrose solutions was followed in the present experiments. For each experiment, rods were separated from the retinas of 6 frogs. Following dilution of the suspension (the sucrose concentration being reduced from 0.37 to 0.24 g/ml.), the rods were sedimented in the conductivity cell in a selective manner by prolonged low-speed centrifugation. The procedure followed in packing the rods in the conductivity cell was thus the same as that used for rods prepared in isotonic solution. As a large excess of rods was available, this procedure led to a fairly uniform population of undamaged rods occupying the space between the electrodes. Owing to limitation on the sensitivity of the marginal oscillator (in terms of its ability to detect an absolute change in G at the terminals of the instrument), and the need to restrict the amount of rhodopsin bleached to no more than about 2% per flash, it was necessary to use a modified conductivity cell. This was similar to that used in other experiments (shown in Fig. 1) except that the shallow channel running vertically and extending between the electrodes was widened from 1.6 to 4.5 mm, the cell constant being thereby altered.

Records of the response to a flash bleaching 1.5% of the rhodopsin content of a packed rod suspension are shown in Fig. 9. In this experiment

a mixed imidazole-phosphate buffer was used in order to avoid the appearance of any change in G due to an uptake of H^+ (Radding & Wald, 1956; Falk & Fatt, 1966), the condition of the rod suspension being such that any increase in conductivity due to an increased ionization of phos-

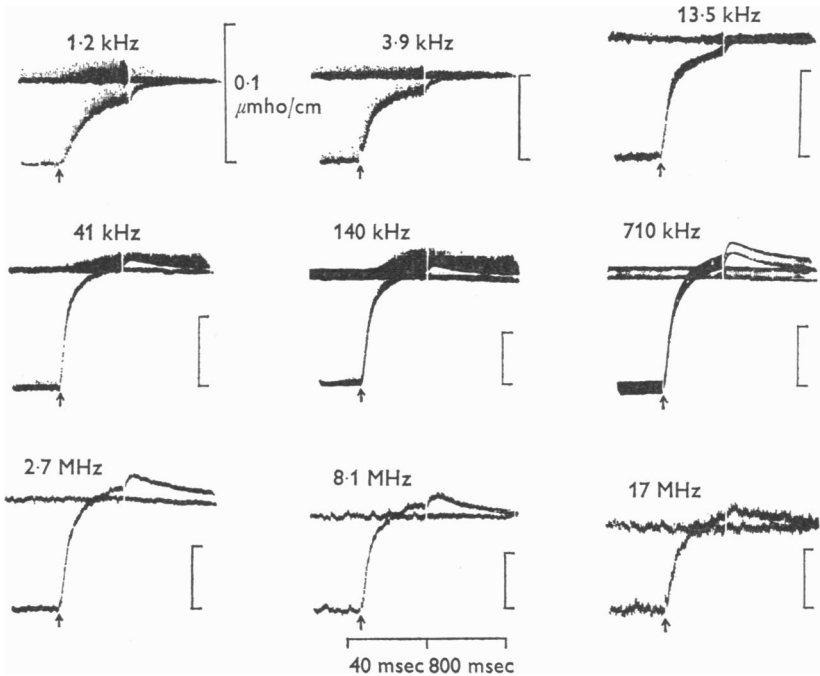


Fig. 9. Records of ΔG obtained in response to a flash bleaching 1.5% of the initial rhodopsin content for rods suspended in a solution of low conductivity. The frequency of the applied a.c. used in the measurement is given for each record. The amplitude of ΔG recorded 100 msec after the flash as well as the initial G on which ΔG is superimposed are plotted against log frequency in Fig. 10. The rod suspension was purified by differential sedimentation on changing the density of the suspending medium with sucrose (sucrose concentration reduced from 0.37 to final 0.24 g/ml.). In addition to sucrose, the suspending medium contained 1 mM imidazole, 1 mM phosphate, 4.7 mM- Na^+ , 1.5 mM- Cl^- , 0.01 gBSA/ml., pH 7.0. Conductivity of the suspending medium was 401 $\mu\text{mho/cm}$, temperature 18.8° C.

phate would be nearly balanced by a decrease due to a decreased ionization of imidazole (Falk & Fatt, 1968*b*).

A feature of the records obtained for rods in low-conductivity solutions is that both components I and II appear faster than was usually found for rods in high-conductivity solutions. It seems probable that this was a consequence of the low ionic strength of the solution. Possibly related to the more rapid time course of component II, there was observed to be a

final stage of decay in the response at high frequencies, with a maximum in ΔG occurring about 100 msec following the flash. Both the rapid time course of development of the response and the partial decay of component II were confirmed in two other experiments carried out under conditions similar to those of the experiment illustrated.

Plots of the frequency dependence of ΔG at 100 msec following the flash are given in Fig. 10 for the experiment for which records are shown

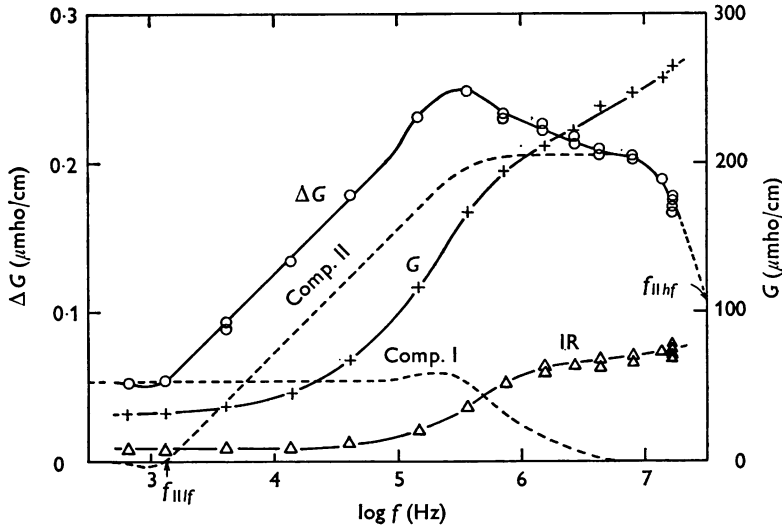


Fig. 10. Plots of G and ΔG against log frequency in the experiment for which records are shown in Fig. 9. Circles: amplitude of response measured at 100 msec following a flash bleaching 1.5% of the initial rhodopsin content of rods. Triangles: change evoked by a flash of infra-red illumination producing a small temperature rise. Crosses: G of the rod suspension on which the response to light is superimposed. N.B. the 1000-fold difference in scales for G and ΔG . Interrupted curves show the composition of the response to light resolved into components I and II.

in Fig. 9. The mid-point on the S-shaped portion of the curve for G against $\log f$ in Fig. 10 indicates the characteristic frequency, f_Y (dependent on the surface membrane capacitance of rods oriented mainly perpendicular to the applied field), to be about 200 kHz. This is lower by a factor of 0.11 than what was obtained for rods suspended in a solution having a conductivity similar to that of Ringer solution. It is in accordance with the lowering, by a similar factor, of the conductivity of the internal spaces of the rods, as can be determined in an approximate manner from the extent of the dispersion in G covered by the S-shaped portion of the curve extending between 20 kHz and 2 MHz. (The continuing rise of G above 2 MHz is considered below.)

As is characteristic of component II, the rise in ΔG shown in Fig. 10 begins several decades below f_Y , at 1 kHz. The rise is nearly linear with $\log f$ up to about 100 kHz. The curve for ΔG reaches a peak at about 300 kHz and with increasing frequency of measurement, declines from this peak amplitude to reach a steady level at about 4 MHz. This behaviour is adequately explained by the attainment of a steady level in component II at about 0.5 MHz combined with a fall in component I between 0.2 and 4 MHz. The extent of the fall in ΔG is seen to agree with the amplitude of component I inferred from the value of ΔG at and below 1 kHz.

Above 8 MHz and extending to the highest frequency of measurement, there is seen to occur a further decline in response amplitude. The possibility of detecting this decline within the frequency limit of 17 MHz imposed by the apparatus was critically dependent on the conductivity of the solution used for preparing the rod suspension. No decline was detected when the conductivity was increased by a factor of 2 above that for the experiment illustrated. On the other hand there was no noticeable lowering of the frequencies at which the decline occurred, when the conductivity was lowered still further. It appeared, however, from the dispersion of G that this did not produce a significant further decrease in the internal conductivity of the rods. It seems reasonable to attribute the decline of response amplitude at the highest frequencies of measurement to component II. The suggested resolution of the response into components is indicated by the dashed lines in Fig. 10.

An additional characteristic of rod suspensions prepared with low-conductivity solutions is a continued rise in G at the highest frequencies of measurement. It seems probable that the two phenomena observed in this region, the fall of ΔG and the rise of G , are related, both being consequent on the passage of current in the longitudinal direction through the capacitance of the disk membranes.

Surface membrane conductance change

Investigations carried out in recent years, involving the use of intracellular micro-electrodes for current application and voltage recording, have revealed that the receptor cells of the vertebrate retina undergo a decrease in surface membrane conductance in response to light (Toyoda, Nosaki & Tomita, 1969; Baylor & Fuortes, 1970). Examination of the spatial distribution of current in the extracellular space of the all-rod retina of the rat has succeeded in showing that the change occurs in the outer segments (Hagins, Penn & Yoshikami, 1970).

One may inquire into the possibility of such a change in surface membrane conductance being detected by the present method of admittance

measurement on suspensions of rods. The maximum decrease in membrane conductance observed with micro-electrodes amounts to about 2 mmho/cm² (calculated from a change of inside-to-outside resistance from 15 MΩ in the dark to 20 MΩ in the light for a rod cell having an outer-segment surface membrane area of 1000 μm²). For a packed suspension of randomly oriented rods having anisotropic interiors (the effective conductivity of the interior being much lower in the longitudinal than in the transverse direction for the frequencies under consideration), the predicted change in specific admittance, ΔG , recorded at low frequencies will be given with sufficient accuracy by the product of the membrane conductance change and the radius of the rod. A membrane conductance decrease of 2 mmho/cm² would thus yield a value of ΔG of $-0.6 \mu\text{mho/cm}$. An admittance change of this magnitude would be readily detected by the present method of measurement.

The experiments on suspensions of rods failed to reveal a response that could be unambiguously attributed to a decrease in surface membrane conductance. Such a change would appear as a frequency-dependent ΔG , constant at low frequencies, but declining steeply at high frequencies to vanish at the characteristic frequency f_Y (see Fig. 20 in Falk & Fatt, 1968*b*, or Fig. 8 in Falk & Fatt, 1973). Saturation of response amplitude would be expected to occur with flash intensities bleaching a very small proportion of rhodopsin molecules ($< 0.001\%$). A negative-going ΔG has been observed and will be described in the following paper (Falk & Fatt, 1973), but it does not have the requisite properties since it is frequency-independent and varies in proportion to the rhodopsin bleached even for large bleaches.

In a few experiments on rods prepared under isotonic conditions, a very slow negative ΔG was detected which was saturated for a flash bleaching less than 0.1% of the rhodopsin content of the rods. This response developed with a latency of 2–3 sec, reached 50% of its final amplitude at about 10 sec and was effectively completed by 25 sec from the flash. No reversal of the negative change in G was discernible during the succeeding 100 sec. The greatest response amplitude was $-0.4 \mu\text{mho/cm}$, recorded at low frequencies (1.4–360 kHz). The response was undetectable at frequencies above f_Y . However, insufficient information was obtained to establish the precise form of the frequency dependence in the region of f_Y , upon which a decision as to the site of origin of the response must depend.

In an effort to make the chemical condition of the isolated rods approach more closely to what is expected to obtain when the rods are attached to the retina, several experiments were carried out in which ATP, Mg²⁺ and K⁺ (alternatively with and without EGTA) were added to the suspending

medium. These experiments failed to yield a response having the characteristics expected for a surface membrane conductance decrease. There is a strong possibility, however, that the ATP in the suspending medium would not penetrate the surface membrane of rods.

DISCUSSION

In the following paper (Falk & Fatt, 1973) methods will be described whereby rod suspensions such as those used in the experiments of this paper could be fractionated to yield a population of rods giving component I alone (i.e. free of component II). By such means the characteristics of component I, especially its decline at high frequencies, could be examined in greater detail, and a discussion of the origin of this component will therefore be deferred. The present discussion will be confined to consideration of the mechanism of origin of component II.

A primary feature of component II, which must be explained by any theory of its origin, is its frequency-dependence whereby, with increasing frequency, ΔG makes its appearance at a sharply defined frequency (f_{IIY}) about 2.5 decades below the characteristic frequency for the major dispersion of admittance (f_Y). In a previous paper (Falk & Fatt, 1968*b*) the possibility was considered that this component of response might involve a frequency-dependent admittance element distinct from the surface membrane capacitance. On reconsideration it appears that the same frequency-dependent element could determine both f_{IIY} and f_Y . What is required is that there should be a large difference in the resistive elements in series with the capacitance of the surface membrane. Such a difference would apply if f_Y were effectively determined by conduction through rods oriented with their axes perpendicular to the electric field applied to the suspension, while f_{IIY} was determined by conduction through rods oriented with their axes parallel to the field. A change in conduction in the interior of the rods perpendicular to the field will be observable only at frequencies above f_Y , whereas, owing to shape and to anisotropy in the properties of the rod interior, a change in conduction through rods parallel with the field will be observable at much lower frequencies.

Theory of the low-frequency cut-off of component II

Rods oriented with their axes parallel to the electric field may be treated as cables of finite length, open circuited at both ends (Fig. 11, circuit 2). This type of circuit problem has been solved previously in relation to the transverse tubular system in striated muscle (Fatt, 1964, eqn. (28*a*); see also the more general solution given by Cole, 1968, pp. 70-72). Making use of the result previously obtained, one has for the

admittance measured with external electrodes in contact with the ends of a single isolated rod,

$$y = \frac{r_o + r_l}{r_o l} \left[r_l + r_o \frac{\tanh W}{W} \right]^{-1} \tag{9}$$

with $W = \sqrt{[j\omega c_m(r_o + r_l)]} \frac{1}{2}l$, where l is the length of the rod and r_o , r_l and c_m are respectively the external longitudinal resistance, the internal longitudinal resistance and the surface membrane capacitance, all referred to

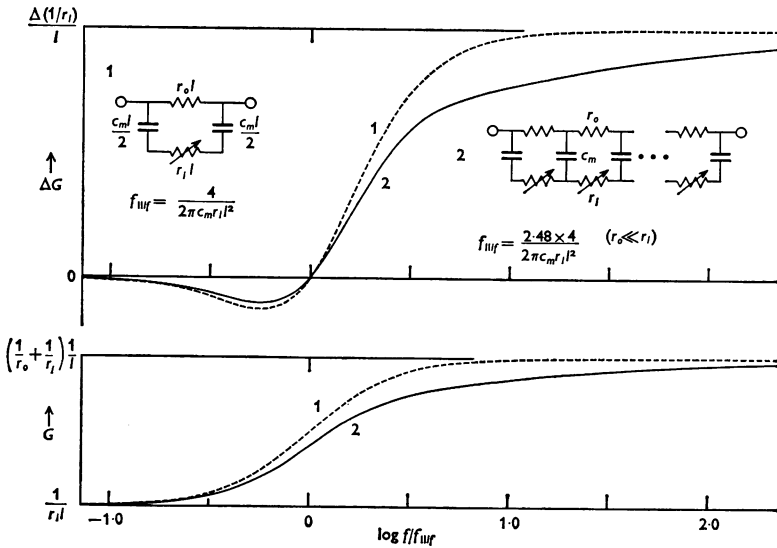


Fig. 11. Theoretical curves showing the frequency dependence of G and ΔG for two models with variable resistive elements: (1) composed of lumped elements, (2) composed of distributed elements. The behaviour of the latter model is obtained from eqns. (11) and (13). The frequency f_{11U} has been defined for each of these models so that ΔG will pass through zero when $f = f_{11U}$.

unit length of rod. The contribution to a suspension of such rods made by conduction through them will be

$$Y = n \left(y l - \frac{1}{r_o} \right) = n \frac{1 - \frac{\tanh W}{W}}{r_l + r_o \frac{\tanh W}{W}}, \tag{10}$$

where n is the number of rods in the suspension per unit area of the plane perpendicular to the field. For the case in which $r_o \ll r_l$, eqn. (10) reduces to

$$Y = \frac{n}{r_l} \left(1 - \frac{\tanh W}{W} \right) \tag{11}$$

with $W = \sqrt{(j\omega c_m r_l)} \frac{1}{2}l$. The response to a small change in the longitudinal

conduction of the rod interior ($1/r_i$) will be given by

$$\Delta Y = \frac{dY}{d(1/r_i)} \Delta(1/r_i) \quad (12)$$

from which, on carrying out the indicated differentiation, one obtains

$$\Delta Y = n \left(1 + \frac{1}{2} \operatorname{sech}^2 W - \frac{3}{2W} \tanh W \right) \Delta(1/r_i). \quad (13)$$

Curves describing the frequency dependence of G and ΔG , obtained from eqns. (11) and (13) as the real parts of Y and ΔY are shown in Fig. 11. The frequency is normalized to

$$f_{\text{II}f} = 2.48 \left[2\pi c_m r_i \left(\frac{l}{2} \right)^2 \right]^{-1}. \quad (14)$$

The normalization of frequency is such that ΔG is constrained to pass through zero at the frequency $f_{\text{II}f}$ in going from the region of small negative values to rapidly increasing positive values. For a rod in the shape of a circular cylinder, eqn. (14) can be re-written as

$$f_{\text{II}f} = \frac{2.48 G_i a}{\pi C_m l^2}, \quad (15)$$

where G_i is the effective conductivity of the rod interior in the longitudinal direction, C_m is the surface membrane capacitance per unit area and a is the radius of the rod.

Calculation of the longitudinal conductivity from $f_{\text{II}f}$

The position of the low-frequency cut-off can be used to obtain an estimate of the longitudinal internal conductivity. Taking a value for C_m of $1 \mu\text{F}/\text{cm}^2$, for a $3 \mu\text{m}$ and for l $50 \mu\text{m}$, one may proceed to calculate G_i from the observed cut-off frequency which, for rods suspended in a solution of conductivity similar to that of Ringer solution, was about 2 kHz. Thus, G_i is found to have a value of about $200 \mu\text{mho}/\text{cm}$, which is about $1/50$ the conductivity of Ringer solution. A lowering of the effective longitudinal conductivity by a factor of $1/50$ is in reasonable accord with the electron microscopic picture of transverse sections of rods fixed *in situ* (i.e. while attached to the retina). These show approximately this fraction of the cross-sectional area of the rod interior to be unobstructed by disk membranes, gaps occurring at the incisures and at the perimeter of the disks between the disk edges and the surface membrane.

The question arises whether conduction through the interior of rods oriented parallel to the applied electric field will make any noticeable contribution to G of the suspension. An estimate of this contribution may be made by means of eqn. (18) in the theory of suspensions treated in

Appendix A. Using the values of $200 \mu\text{mho/cm}$ for G_l , 12 for x (the shape factor) and 0.27 for p' (the volume fraction occupied by rods oriented with their axes parallel to the field), one thus obtains a value of $60 \mu\text{mho/cm}$. A low-frequency deviation of the impedance or admittance locus in the complex-plane plot was observed previously (Falk & Fatt, 1968*a*), indicative of a conductance path in parallel with the one determining the principal dispersion. Conduction through rods oriented parallel with the field would appear to be of sufficient magnitude to account for the low-frequency deviation.

A value of $200 \mu\text{mho/cm}$ for the internal longitudinal conductivity may be compared with the conductivity, G_t , of the rod interior measured in the transverse direction. As determined in this and in a previous paper (Falk & Fatt, 1968*a*), G_t for rods suspended in a solution of conductivity similar to that of Ringer solution has a value of about $5000 \mu\text{mho/cm}$. This reduction in internal conductivity to about one half of that of the suspending medium is consistent with the reduction in the volume of aqueous space due to the membranes constituting the disks.

The characteristic frequency, f_Y , for conduction through rods oriented with their axes perpendicular to the applied field will depend on G_t in the following way:

$$f_Y = \left[2\pi \frac{C_m a}{G_t} \right]^{-1}, \quad (16)$$

where C_m and a are as defined in eqn. (15). For the values of C_m and a as given above, and for G_t of $5000 \mu\text{mho/cm}$, f_Y is about 2.7 MHz. One can thus account for a 1000-fold difference between f_{IIU} and f_Y .

Factors in rod geometry producing a distribution in f_{IIU}

One of the striking features of component II is the wide range of frequencies over which ΔG increases approximately linearly with log frequency. The variation of ΔG with frequency for the cable model of the rod is plotted in Fig. 11 and may be compared with the curve (drawn as a dashed line) describing the behaviour of a lumped network (circuit 1), consisting of a variable resistive element with a fixed capacitive element in series. Although the cable model introduces a greater spread in the rise of ΔG with frequency, it falls far short of accounting for the wide range of frequencies over which the observed ΔG for the rod suspension increases.

In a previous attempt to account for this discrepancy (Falk & Fatt, 1972*c*) the suggestion was made that a distribution in f_{IIU} might be produced by variation in the orientation of individual rods relative to the direction of the applied electric field. On reconsideration of the problem, it now appears that this possibility must be rejected on general theoretical

grounds. This follows from the circumstance that, for an obliquely oriented rod, the applied field can be treated as the resultant of two superimposed fields, one directed parallel to the rod axis and the other perpendicular to it. Provided the rods have linear electrical properties, an assembly of whatever orientation must always display a behaviour capable of being reproduced by a combination of rods oriented parallel to the field with others oriented perpendicular to it. Thus there can be no spread of the frequency dependence of G or ΔG arising from random orientation.

One is left with no alternative but to suppose that the wide frequency range for the increase of ΔG , reflecting a distribution of f_{III} , is the result of a variation in the properties of different rods composing the population. A variation in rod length from 50 to 30 μm would produce a three-fold spread in f_{III} . In addition to this one must assume there occurs a variation in the fraction of cross-sectional area unobstructed by disk membranes.

*A change in conductance of disk membranes vs. a change
within incisures*

It has been supposed that the value of G_t is effectively determined by conduction within the incisures and at the periphery of the disk stack, with the contribution made by ionic conductance of the disk membranes being negligibly small. One may now consider whether, even though G_t is determined in this way, the change, ΔG_t , might be caused by an increase in conductance of the disk membranes rather than in the space unobstructed by the disks. Taking into account the dimensions of the structures forming this unobstructed space, one may regard conduction within such structures as being effectively two-dimensional. Furthermore, from consideration of the over-all geometry of the rod, it is clear that these conducting sheets may be regarded as generated from line segments *parallel* to the rod axis. Assuming that all directions radial to the rod axis are equivalent, one may infer that isotropic conduction in these sheets will give rise to anisotropic conduction through the volume of the rod, such that conduction in the direction parallel to the rod axis will be twice that for conduction in any direction perpendicular to the axis.

Now, for an ensemble of randomly oriented rods in a uniform electric field, the electrical behaviour will be exactly the same as would be given if one third of the rods were oriented parallel to the applied field and two thirds perpendicular to the field. The net result of anisotropic conduction through the volume of the rod and random orientation of rods is that equal contributions to the measured conductance will be made by conduction in the longitudinal direction through the rods as in the transverse direction.

Hence, if component II did arise from an increase in conductance of the unobstructed space, the plateau in ΔG for this component, reached above f_Y , should include equal contributions from changes in G_t and G_l . There would, in this situation, be an increase in component II above f_Y equal to that occurring below f_Y . In conflict with this prediction, no such increase was found experimentally. One is thus led to reject the idea of component II arising in the incisures or at the disk-stack perimeter and to consider instead a conductance change across the disk membrane.

The high-frequency cut-off of component II

Having inferred a probable origin of component II on the basis of its low-frequency cut-off, one is led to consider the high-frequency behaviour which would follow. It may be reasoned in a general way that, if the response arises from a change in conductance of the disk membranes, then as the frequency is raised, the recorded ΔG should eventually fall to zero owing to the shunting effect of the capacitance of these membranes. The frequency at which the shunting effect is exerted will be dependent on the conductance through which the capacitance discharges.

An elaboration of the cable model, taking into account the capacitance of the disk membranes, is treated in Appendix B. As derived there (eqn. (28)), the position of the high-frequency cut-off of ΔG (where $\Delta G = 0$) will be given approximately by

$$f_{II hf} = \frac{1}{2\pi C_d s \left(\frac{1}{G_k} + \frac{1}{G'_o} \right)},$$

where C_d is the capacitance per unit area of disk membrane, s is the average repeat distance between disk membranes, G_k is the specific conductance of the rod interior, which applies for current flowing in the longitudinal direction and which is in series with the capacitance of the disk membranes, and G'_o is the conductance of the space external to the rods (those oriented parallel to the field), scaled so as to apply to a unit cube of rod. A reasonable estimate may be made, taking C_d to be $1 \mu\text{F}/\text{cm}^2$, s 15 nm, and G_k and G'_o about 15 and 6 mmho/cm respectively for rods in Ringer solution. The estimate of G'_o is made on the assumption that for frequencies in the region of $f_{II hf}$, this conductance would be effectively determined by the passage of current through the interior of rods oriented perpendicular to the applied field, neighbouring those oriented parallel to the field. One thus arrives at a value for $f_{II hf}$ of 450 MHz.

For a model having these parameters it is clear that no appreciable decline with frequency can be expected up to the highest frequency accessible by the present method (about 17 MHz). It was in an effort to

bring the predicted high frequency decline into the range of observations that experiments were carried out on rods purified in sucrose and suspended in solutions of low-conductivity. By this means the conductance of the interior of rods for current flowing in the transverse direction (as inferred from the measurements of G), was reduced by a factor of about one ninth from that obtaining for rods in Ringer solution. It is expected that G_k and G'_0 would both be reduced by this factor, resulting in a lowering of $f_{\text{II}M}$ to about 50 MHz. The observed decline in ΔG as the frequency was raised above 10 MHz is in agreement with this prediction, and thus provides support for the view that component II originates in a conductance increase of the disk membranes.

Calculations of ΔG_i from ΔG for the suspension

For rods suspended in a solution having a conductivity similar to that of Ringer solution, the magnitude of component II reached at frequencies near f_Y amounted to a ΔG of about $0.5 \mu\text{mho/cm}$ for a flash bleaching 1% of the rhodopsin content of the rods. Applying eqn. (19) of Appendix A and making similar assumptions as used in calculating G_i from G of the suspension, one finds that the observed ΔG corresponds to a value for ΔG_i of $1.5 \mu\text{mho/cm}$. For a much brighter flash, producing a saturating response, ΔG_i would be about $10 \mu\text{mho/cm}$, corresponding to a 5% increase of G_i .

The magnitude of the change in the disk membrane properties deducible from ΔG_i

In order that the longitudinal conductivity of the rod interior should increase by $1.5 \mu\text{mho/cm}$ (in response to 1% bleaching of rhodopsin), the disk membranes having an average of repeat distance of 15 nm must undergo a membrane conductance increase of 1.0 mho/cm^2 . This value of membrane conductance change is an order of magnitude greater than the change found to occur in electrically excitable cell membranes during an action potential. It is, on the other hand, the same as the change estimated to occur in the post-junctional membrane of striated muscle during the course of neuromuscular transmission (Katz, 1958).

A more revealing way of looking at the change in properties of the disk membrane is to calculate the increment of conductance across the membrane that would be produced by the bleaching of a single rhodopsin molecule. For a disk-membrane repeat distance of 15 nm and a rhodopsin concentration within the disk stack of 2.5 mM, the area density of rhodopsin molecules within the disk membrane is calculated to be $2.3 \times 10^{12} \text{ cm}^{-2}$. For the bleaching of 1% of these molecules to give a conductance increase of 1.0 mho/cm^2 , it is required that a single rhodopsin molecule produce a

conductance increment of 4.3×10^{-11} mho. It is noteworthy that this value is similar to what has been estimated for the individual Na^+ -channel operating in the generation of the nerve action potential (Hille, 1970). The calculated value is also similar to the conductance increment produced in the post-synaptic membrane by the activation of a single receptor molecule by synaptic transmitter (Katz & Miledi, 1971).

A change in electrical properties of the disk membrane, *per se*, cannot produce the signal transmitted by the rod in view of the fact that the membranes of most individual disks are separated from one another and from the surface membrane. It would be relevant in this connexion to carry the calculations further to evaluate the flux of ions occurring across the disk membrane for the bleaching of a single rhodopsin molecule. On the assumption that there is no electrochemical potential difference across the membrane for those ions which contribute to the membrane conductance increment, the flux of univalent ions in each direction can be obtained by multiplication of the conductance by $N_0 RT/F^2$ (the symbols here having their usual connotation). One thus arrives at the result that the bleaching of a single rhodopsin molecule produces a channel through which ions can move at the rate of $6 \times 10^6 \text{ sec}^{-1}$. At present there is no evidence for any ionic selectivity of the channels and it is possible that substances other than ions could also move through them. One can envisage the release of an intracellular transmitter from within the disks, taking place over a period of time of some tens of milliseconds following a flash and giving a large amplification in terms of the number of molecules released per rhodopsin molecule bleached.

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APPENDIX A

Relation between the admittance parameters of a suspension and the properties of its constituent rods

The response to a light flash obtained in admittance measurements on a suspension of rods has been considered to arise from a change in the conductivity of the rod interior for current flowing in the longitudinal direction. From conditions of symmetry (the electric field existing in one out of the three dimensions of space) the suspension of randomly oriented rods may be treated as if the response arose in one third of the rods present, this fraction being made up of rods oriented with their axes parallel to

the applied electric field and occupying a reduced volume fraction $p' = p/3$. One may make use of Maxwell's (1891, art. 314) theory for a dilute suspension of spheres, generalized by the introduction of the form factor x , so as to be applicable to particles of any shape (Fricke, 1924; Velick & Gorin, 1940). (It is to be noted that x depends not only on the shape and orientation of the particles, but also on the ratio of internal to external conductivity.) For the conductivity of the suspension, this gives

$$G = G_o \frac{x(1-p') G_o + (1+xp') G_i}{(x+p') G_o + (1-p') G_i}, \quad (17)$$

where G_o is the conductivity of the suspending medium and G_i is the longitudinal conductivity of the rod interior.

Considering G as a function of G_i and assuming for the present problem that $G_i \ll G_o$ (a condition which allows x to be treated as independent of G_i/G_o), one obtains

$$G(G_i) - G(0) = \left(\frac{x+1}{x+p'} \right)^2 p' G_i. \quad (18)$$

This gives the contribution to the conductivity of the suspension made by the rods oriented parallel to the field, i.e. the increase in the measured conductivity over what it would be if the rods were non-conductors. The change in conductivity of the suspension produced by a change in the conductivity of the rod interior can then be written as

$$\Delta G = \left(\frac{x+1}{x+p'} \right)^2 p' \Delta G_i. \quad (19)$$

In the case of random orientation, the projection of the length of rods in the direction of the field is, on the average, one half their length. Taking this into account for the value of x , one may, as an approximation, regard rods as prolate spheroids with an axial ratio of about 4. The value of x for such spheroids is 12. (The effect of axial ratio is, in any event, small. With the value of p' of 0.27, variation of the axial ratio from 2 to infinity causes the factor $[(x+1)/(x+p')]^2$ to range from 1.31 to 1.00.)

APPENDIX B

An extended cable model for the rod yielding a high-frequency cut-off of the response

Considering the case of rods oriented parallel to the applied field, one may elaborate the model described on page 209 by taking the element of the path in the interior of the rod to be made up of a parallel combination of a variable conductance G_i and a capacitance $C_a s$, this combination having in series with it a conductance G_k (circuit diagram of Fig. 12). Here C_a

represents the capacitance per unit area of disk membrane and s the average repeat distance of disk membranes. All three quantities $C_d s$, G_l and G_k are thus taken to refer to a unit cube of rod. The conductance of the external path G'_o is also scaled to be that accompanying a unit cube of rod.

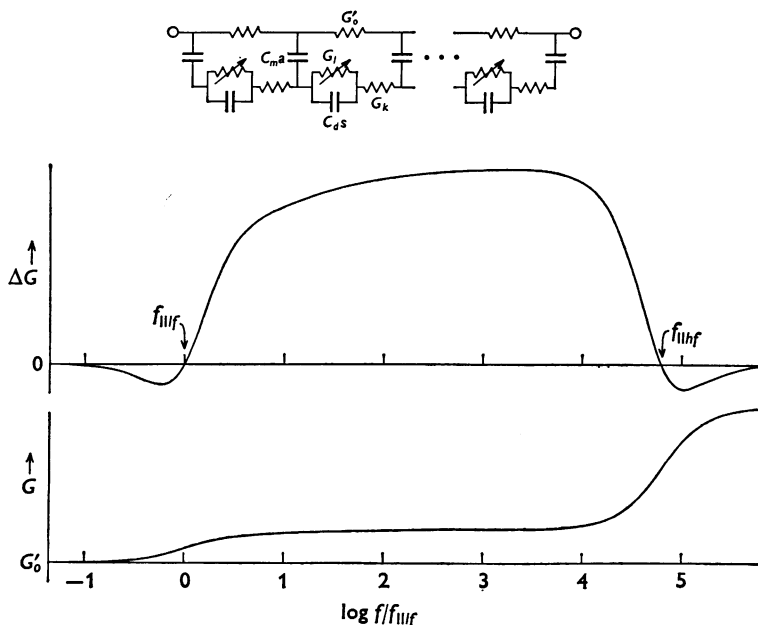


Fig. 12. Theoretical curves showing the frequency dependence of G and ΔG for a model with distributed elements, including a variable conductance G_l as shown at the top of the Figure. The behaviour of the model is obtained from eqns. (25) and (26). The curves shown are computed for $f_{III f} = 600 f_{III f}$, where the characteristic frequencies are as defined in eqns. (27) and (28). It can be shown that the height of the initial step of the curve for G will be close to $[(1/G_k) + (1/G_l)]^{-1}$, while the plateau for ΔG will approximate $[1 + (G_l/G_k)]^{-2} \Delta G$.

Generalizing eqn. (9), one may write for the admittance between the ends of a rod, as measured with external electrodes,

$$y = \frac{r_o + z_l}{r_o} \left[z_l + r_o \frac{\tanh W}{W} \right]^{-1} \tag{20}$$

with

$$W = \sqrt{[j\omega y_m (z_l + r_o)]} \frac{1}{2} l,$$

where z_l is the impedance per unit length along the interior of the rod, y_m is the admittance of unit length between the inside and outside, and

l is the length of the rod. Hence the contribution to the specific admittance of the suspension made by the rods will be

$$Y = n \left(yl - \frac{1}{r_o} \right) = n \frac{1 - \frac{\tanh W}{W}}{z_l + r_o \frac{\tanh W}{W}}, \quad (21)$$

where n is the number of rods per unit area of suspension, perpendicular to the applied field.

For the present model one has

$$z_l = \left(\frac{1}{j\omega C_d s + G_l} + \frac{1}{G_k} \right) \frac{1}{\pi a^2}, \quad (22)$$

$$y_m = j\omega C_m 2\pi a, \quad (23)$$

$$r_o = \frac{1}{G'_o \pi a^2}, \quad (24)$$

where a is the radius of the rod. Substituting these expressions for z_l , y_m , r_o into eqn. (21) and then obtaining the response by differentiation, according to the relationship $\Delta Y = (dY/dG_l) \Delta G_l$, one arrives at the following set of expressions for Y and ΔY in terms of the specific properties of the rods:

$$Y = \frac{n\pi a^2 \left(1 - \frac{\tanh W}{W} \right)}{\frac{1}{j\omega C_d s + G_l} + \frac{1}{G_k} + \frac{1}{G'_o} \frac{\tanh W}{W}}, \quad (25)$$

$$\Delta Y = \frac{n\pi a^2 [1 + \frac{1}{2} \operatorname{sech}^2 W - (3/2W) \tanh W] \Delta G_l}{\left[1 + (j\omega C_d s + G_l) \left(\frac{1}{G_k} + \frac{1}{G'_o} \frac{\tanh W}{W} \right) \right]^2}, \quad (26)$$

where

$$W = \sqrt{\left[\frac{j\omega C_m a}{2} \left(\frac{1}{j\omega C_d s + G_l} + \frac{1}{G_k} + \frac{1}{G'_o} \right) \right]} \frac{l}{a}.$$

From knowledge of the distances involved and from the expectation that capacitances of membranes will not differ greatly, it is clear that

$$C_d s \ll C_m l^2 / 2a.$$

The frequency dependence of Y and ΔY given by eqns. (25) and (26) can then be described conveniently in terms of two characteristic frequencies which are separately determined by the capacitances C_m and C_d ; thus

$$f_{\text{Hf}} = \frac{2 \cdot 48}{2\pi} \frac{2a}{\left(\frac{1}{G_l} + \frac{1}{G_k} + \frac{1}{G'_o} \right) C_m l^2} \approx \frac{2 \cdot 48}{2\pi} \frac{2a G_l}{C_m l^2}, \quad (27)$$

$$f_{\text{II}M} = \frac{1}{2\pi} \frac{G_l + \left(\frac{1}{G_k} + \frac{1}{G'_o}\right)^{-1}}{C_d s} \approx \frac{1}{2\pi} \frac{1}{C_d s \left(\frac{1}{G_k} + \frac{1}{G'_o}\right)}. \quad (28)$$

The approximate relations depend on the condition that $G_l \ll G_k, G'_o$.

Plots of $G = \text{Re} [Y]$ and $\Delta G = \text{Re} [\Delta Y]$ are shown in Fig. 12. As the frequency increases, the general behaviour of G is to rise in two stages, while that of ΔG is to rise and then fall. The lower-frequency transition follows closely the behaviour for the simple cable model shown in Fig. 11, while the higher-frequency transition follows the behaviour of the model for lumped elements, consisting of a variable conductance shunted by a capacitance and this combination in series with a fixed conductance (Fig. 20 in Falk in Fatt, 1968*b*).

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