# Voltage-Dependent Conductance Induced in Thin Lipid Membranes by Monazomycin

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ABSTRACT When present in micromolar amounts on one side of phospholipid bilayer membranes, monazomycin (a positively charged, polyene-like antibiotic) induces dramatic voltage-dependent conductance effects. Voltage clamp records are very similar in shape to those obtained from the potassium conductance system of the squid axon. The *steady-state* conductance is proportional to the 5th power of the monazomycin concentration and increases exponentially with positive voltage (monazomycin side positive); there is an *e*-fold change in conductance per 4–6 mv. The major current-carrying ions are univalent cations. For a lipid having no *net* charge, steady-state conductance increases linearly with KCl (or NaCl) concentration and is unaffected by $Ca^{++}$ or $Mg^{++}$. The *current-voltage* characteristic which is normally monotonic in symmetrical salt solutions is converted by a salt gradient to one with a negative slope-conductance region, although the *conductance-voltage* characteristic is unaffected. A membrane treated with both monazomycin and the polyene antibiotic nystatin (which alone creates anion-selective channels) displays bistability in the presence of a salt gradient. Thus monazomycin and nystatin channels can exist in parallel. We believe that many monazomycin monomers (within the membrane) cooperate to form a multimolecular conductance channel; the voltage control of conductance arises from the electric field driving monazomycin molecules at the membrane surface into the membrane and thus affecting the number of channels that are formed.

## INTRODUCTION

The "ionic theory" of membrane excitability has received general acceptance since the investigations of Hodgkin and Huxley (1952). This theory states that the electrical potential across the plasma membrane is a consequence of (*a*) the ion asymmetries between cytoplasm and surrounding medium and (*b*) the relative permeability of the membrane to these ions. In excitable cells the action potential results because the membrane permeabilities (or conductances) to several of these ions (in the squid giant axon these are $Na^+$ and $K^+$) are strongly voltage dependent. According to the ionic theory,

metabolism serves only to establish and maintain ionic asymmetries; given the asymmetries, the action potential arises from voltage-dependent conductance changes in the membrane *independent* of metabolism.

In the past 20 years, numerous investigators have confirmed the essentials of this theory for a wide variety of excitable cells. Although the particular ion conductances that are under voltage control vary with the type of cell, in all systems studied the basis for electrical excitability is the strong dependence of one or several ion conductances on membrane potential. This being the case, the central question concerning the mechanism of excitability becomes: what are the physicochemical events that occur in the membrane in response to changes in membrane potential? That is, how does an electric field alter the permeability of the membrane to a given ion?

A potentially fruitful approach to this problem was opened by Mueller and Rudin and their colleagues (1962) with their discovery of "excitability-inducing material" (EIM), a proteinaceous material of bacterial origin which interacts with thin ($<100$ A) lipid (or bilayer) membranes to produce a voltage-dependent conductance system that mimics in many respects the properties of biological excitable systems (Mueller and Rudin, 1968 *a*). Mueller and Rudin subsequently discovered that two antibiotics, alamethicin (1968 *b*) and monazomycin (1969), also induce dramatic voltage-dependent conductance changes in thin lipid membranes. Because of their composition and structure, thin lipid membranes have become the model par excellence for biological membranes; and although it cannot be claimed that EIM, alamethicin, or monazomycin is responsible for electrical excitability in plasma membranes, we feel that understanding the mechanisms operative in these model systems will be very useful in formulating ideas about comparable phenomena in biological membranes.

We have, therefore, examined in some detail the action of one of these agents, monazomycin. Monazomycin is an antibiotic of proposed empirical formula $C_{62}H_{119}O_{20}N$ produced by a *Streptomyces* (Akasaki et al., 1963). It contains numerous hydroxyl groups, one sugar residue, and one amino group which gives it a single positive charge over the pH range of our experiments (Mitscher et al., 1967). These chemical data as well as the source (*Streptomyces*) of the molecule suggest monazomycin to be related structurally to the polyene antibiotics, even though it contains no polyenic chromophore.

In this and the succeeding paper we are concerned primarily with the *steady-state* conductance-voltage characteristic of thin lipid membranes in the presence of micromolar amounts of monazomycin, and the dependence of this characteristic on concentrations of antibiotic, salt, and divalent cations. Here we present results obtained from membranes made with a lipid bearing no *net* charge; in the following paper we examine the effect of lipid charge on the monazomycin-induced response, and show that the effects of such

charge are explained by diffuse double layer theory. Throughout both papers we draw comparisons between this model system and the voltage-dependent conductances in nerve.

## MATERIALS AND METHODS

Membranes were formed at room temperature by the brush technique of Mueller et al. (1963) across a 1 $mm^2$ hole in a Teflon partition separating two Lucite chambers containing symmetrical unbuffered KCl or NaCl solutions (pH $\approx$ 5.6–6.8). After the membranes were completely black, a small volume from a stock aqueous solution of monazomycin (30–2000 $\mu$g/ml) was stirred into the front chamber to a concentration of 0.1–35 $\mu$g/ml; records were first taken about 15 min later. (It generally took this amount of time for the response to develop fully.) Ion concentrations were altered during the course of experiments by adding small amounts of concentrated solutions to either or both chambers. The monazomycin was a generous gift from Dr. H. Yonehara; aqueous solutions stored at 4°C were stable for over a year as judged by their effect on the membranes.

Unless otherwise stated, all membranes were formed from decane solutions of 1–1.5% bacterial phosphatidylethanolamine (PE) (purchased from Supelco, Inc., Bellefonte, Pa.); its reported purity was 98% and it contained 45% cyclopropane fatty acids and 47% $C_{16}$ fatty acid. In the absence of monazomycin, membranes had ohmic conductances of less than $5 \times 10^{-8}/\Omega\ cm^2$. In the pH range of our experiments, PE is a zwitterion with zero net charge.

The basic quantity of interest, the membrane conductance ($g$), is defined by:

$$g = \frac{I}{(V - \mathrm{EMF})} \tag{1}$$

where $I$ is the (noncapacitance) current flowing through the membrane, $V$ is the potential difference across the membrane, and EMF is the diffusion potential existing across the membrane in the presence of a salt gradient.[1] In most of the experiments reported in this paper, the concentrations of permeant ions in the two chambers are identical, so that EMF = 0 and equation 1 reduces to

$$g = \frac{I}{V}. \tag{1 a}$$

As defined by equations 1 and 1 *a*, $g$ is the *chord* conductance of the membrane and is, as we shall see, a function of voltage and time.

Experimentally, $g$ was measured either by applying a step of $V$ across the membrane and recording $I$ (that is, voltage clamping), or by applying a step of $I$ and recording $V$ (current clamping.) In the former case a single pair of calomel electrodes coupled to the solutions through saturated KCl junctions was used; voltage clamping was

[1] The potential of the rear chamber, in which there is *no* monazomycin, is defined as zero, so that $V$ is the potential of the front chamber with respect to the rear. Therefore, positive current flows from front to rear.

achieved by an operational amplifier which sensed the potential difference between the electrodes and maintained this potential difference at the level set by the command voltage. In the latter case, either a single pair of calomel electrodes with saturated KCl junctions was used, or two pairs of Ag/AgCl electrodes were used—one pair for passing current and the other pair for recording the resulting membrane potential. The response was displayed on an oscilloscope.

## RESULTS AND DISCUSSION

The results and discussion are presented in four sections: in section A we show voltage and current clamp responses of monazomycin-treated PE membranes. In section B we present the *steady-state* properties of the system. In section C we see how a negative slope region appears in the steady-state *I-V* characteristic when a diffusion potential exists across the membrane. In section D we discuss results bearing on the molecular basis of monazomycin action.

### A. *The Voltage Clamp and Current Clamp Responses*

1. VOLTAGE CLAMP RESPONSE Fig. 1 *a* shows the current responses of a monazomycin-treated thin lipid membrane to a series of positive rectangular voltage pulses. Four features should be noted: (*a*) upon application of the voltage,[2] the current does not instantaneously achieve its final value but instead rises along an S-shaped curve to a steady-state value. When the voltage is returned to zero, the current immediately[2] drops to zero. This observation justified a major unstated assumption in defining $g$ by equation 1 *a*, namely, that there is no voltage-dependent EMF induced by the applied potential; i.e., the monazomycin-treated membrane is a dissipative element with no conservative component (except for the membrane capacitance). (*b*) The rate of current rise increases sharply with relatively small increments in the voltage steps. (*c*) The steady-state current is a supralinear function of voltage. (*d*) If negative voltages of the same magnitude are applied, no measured current flows; the conductance is that of an unmodified membrane ($\approx 10^{-8}/\Omega$ cm$^2$). Thus, monazomycin-treated membranes display enormous rectification. Note that the polarity of this rectification is consistent with the idea that the conductance changes because positively charged monazomycin is "driven" into or out of the membrane by the electric field.

Figs. 1 *b* and 1 *c* typify the current response observed when the polarity of the voltage is reversed from + to −. Note that the current immediately after potential reversal is of the same magnitude (but of opposite polarity) as that prior to reversal, i.e., the conductance does not change instantaneously. In contrast to the S-shaped rise of conductance, the decay to zero is exponential

[2] When the membrane potential is suddenly changed, the first electrical event that occurs is a surge of capacitive current. This event occurs too rapidly to be seen in Figs. 1 and 8. Any discussion of voltage clamp records in this report refers to events *after* the capacitive surge.

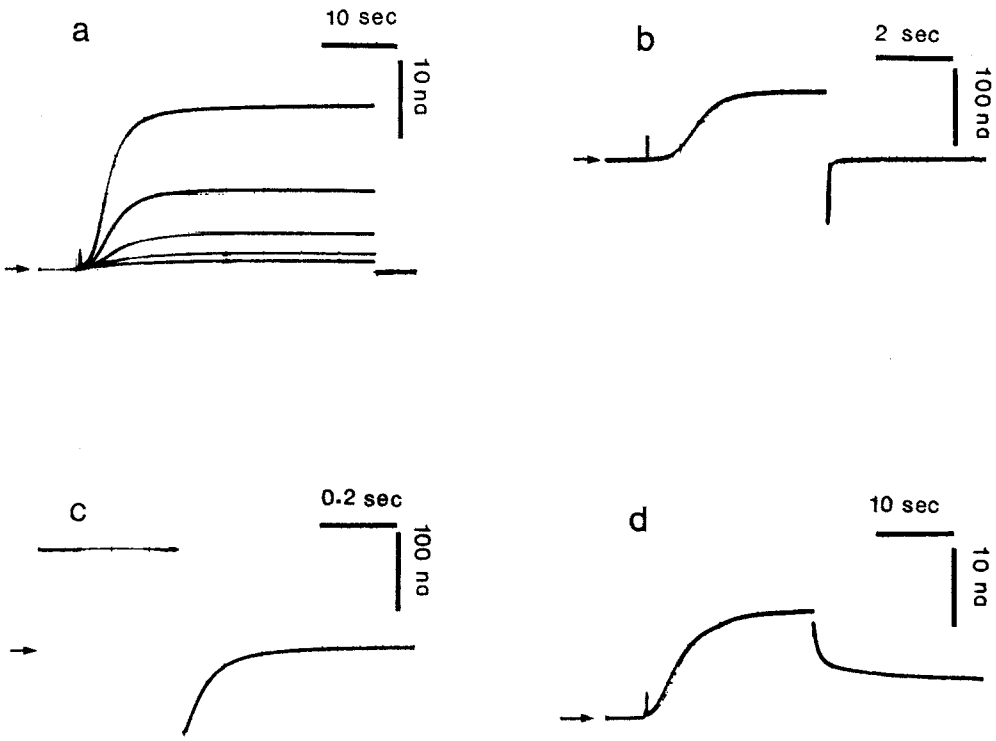


FIGURE 1. Voltage clamp responses of monazomycin-treated PE membranes. Membranes (area = 1 $mm^2$) were formed at room temperature in 0.1 M KCl; monazomycin was then added to the front chamber. The arrows designate the $I = 0$ level, and the vertical blips in (*a*), (*b*), and (*d*) mark the onset of stimulation. (*a*) Successive current responses to positive rectangular voltage pulses of 25, 29, 33, 37, and 41 mv. There is a 2 min interval between pulses. Monazomycin concentration = 33 μg/ml. (*b*) Record illustrating the difference in kinetics for turning on and turning off conductance. The initial stimulus is +60 mv; after the current reaches a steady state the stimulus is switched to −60 mv. Monazomycin concentration = 17 μg/ml. (*c*) Higher time resolution of the conductance turnoff. The current has already achieved a steady state value for a voltage of +65 mv, and the stimulus is then switched to −65 mv. Monazomycin concentration = 17 μg/ml. (*d*) Further illustration of difference in kinetics for conductance turnon and turnoff. The initial stimulus is +55 mv; after the current reaches a steady state, the voltage is reduced to +50 mv. Monazomycin concentration = 17 μg/ml.

and much faster. An exponential-like decay is also observed when the voltage is reduced from a higher to a lower positive value (Fig. 1 *d*).

The records of Fig. 1 are very similar to those obtained from the voltage-dependent potassium and (if we neglect inactivation) sodium conductances of the squid axon. The major difference is the time scale, here measured in seconds rather than in milliseconds; however, since the kinetics increase as the system is driven to higher conductances, this discrepancy is reduced at

conductances comparable to those of the squid axon ($10^{-3}/\Omega$ cm$^2$). The phenomenological similarity of monazomycin-treated thin lipid membranes and biological excitable membranes is a major reason for investigating this system. Clearly a detailed analysis of the kinetics should be made; however, we feel it is essential to understand the *steady-state* conductance-voltage relation first. The steady-state $g$-$V$ characteristic for a membrane separating symmetrical salt solutions is determined from data such as that in Fig. 1 *a* by dividing the steady-state current by the applied voltage.

2. CURRENT CLAMP RESPONSE Fig. 2 shows the voltage response of a monazomycin-treated thin lipid membrane to a rectangular pulse of positive current. The complicated transient results from interaction of the membrane's

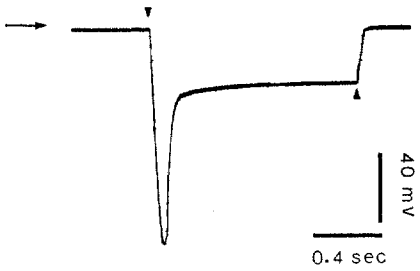


FIGURE 2. Current clamp record of monazomycin-treated PE membrane. The membrane (area = 1 mm$^2$) was formed at room temperature in 0.1 M KCl; monazomycin was then added to the front chamber to a concentration of 33 $\mu$g/ml. The horizontal arrow designates the $V = 0$ level; positive voltage is downward. At the first vertical arrowhead a positive step of current = 6 na is applied; at the second vertical arrowhead the current is returned to zero.

*real* capacitance with the *phenomenological* inductance arising from the monazomycin-induced, time-variant, voltage-dependent conductance. (For a clear discussion of this point, see Mauro, 1961.) A kinetic analysis of the conductance change is feasible only from voltage clamp data, which are not complicated by capacitance current and oscillation of the voltage (which controls the conductance). However, since the steady-state conductance depends only on the final maintained voltage and not on any preceding voltages, the steady-state $g$-$V$ characteristic can also be obtained from records such as Fig. 2 upon dividing the applied current by the steady-state voltage. (Generally, in both voltage and current clamp experiments, the $g$-$V$ characteristic was determined by continuously changing the independent variable in small increments and recording the steady-state value of the dependent variable, rather than by returning to $V = 0$ or $I = 0$ after determining each point.) The advantage of the current clamp technique was the ease of measuring very low conductances.

With this background we now turn to the presentation and analysis of the steady-state data.

### B. *Steady-State Properties of Monazomycin-Treated Membranes Formed from a Lipid with No Net Charge (PE)*

1. THE CONDUCTANCE-VOLTAGE ($g$-V) CHARACTERISTIC Two salient features emerge from a semilog plot of steady-state conductance vs. membrane potential (Fig. 3): (*a*) the logarithm of the conductance is a *linear* function of membrane potential, and (*b*) the slope of the line is very steep, i.e., conductance increases markedly for a few millivolts increase in potential. (For the particular membrane of Fig. 3 conductance changes *e*-fold every 5.7 mv.) We have found that the logarithm of steady-state conductance is linear with membrane potential over a wide range of experimental conditions. Deviations occur only at very low conductances ($<10^{-9}/\Omega$), where the conductance of the unmodified membrane is a significant fraction of the total, or at very high conductances ($>10^{-5}/\Omega$), where more than 1% of the potential is dropped across the electrodes and access resistance.[3]

We can write the dependence of $g$ on $V$ in the form

$$g \propto \exp(nqV/kT) \tag{2}$$

where $q$ is the charge on the electron, $k$ is the Boltzmann constant, $T$ is the temperature in degrees Kelvin, and $n$ is a constant. (We write the exponential dependence of $g$ on $V$ in this form, because equation 2 can be derived simply by assuming that the number of open [conducting] and closed [nonconducting] channels is governed by the Boltzmann distribution; see section D part 3.) The average value of $n$ with PE membranes is about 4.4, with almost all values falling between 4 and 5.5. Since $kT/q = 25.6$ mv at 25°C, this means that the conductance changes *e*-fold about every 6 mv. Such a steep exponential dependence of conductance on voltage is observed for the sodium and potassium conductances of nerve (e.g., Hodgkin and Huxley, 1952).

2. THE EFFECT OF MONAZOMYCIN CONCENTRATION ON THE $g$-V CHARACTERISTIC A set of parallel lines is generated when log $g$ vs. $V$ is determined

[3] In the voltage clamp experiments, the electrodes' resistance and the access resistance (i.e., the resistance of the 1 mm$^2$ hole in the absence of a membrane) are in series with the membrane. In 0.1 M KCl this combined series resistance was about $2 \times 10^4\ \Omega$. Thus, if the membrane resistance falls below $10^6\ \Omega$, a small percentage of the applied voltage does not appear across the membrane. Although an error of 5 mv out of 100 may seem trivial, this will produce significant deviation from linearity in the log $g$ vs. $V$ function because of the steep dependence of $g$ on $V$. In the current clamp experiments with separate stimulating and recording electrodes, only the access resistance is in series with the membrane. In 0.1 M KCl this is about $10^3\ \Omega$. Thus, the range over which the linear relation of log $g$ vs. $V$ is observed is extended to higher membrane conductances. In summary, we attribute any observed deviation from a simple exponential dependence of conductance on voltage to failure of all the recorded potential to appear across the membrane at high membrane conductances.

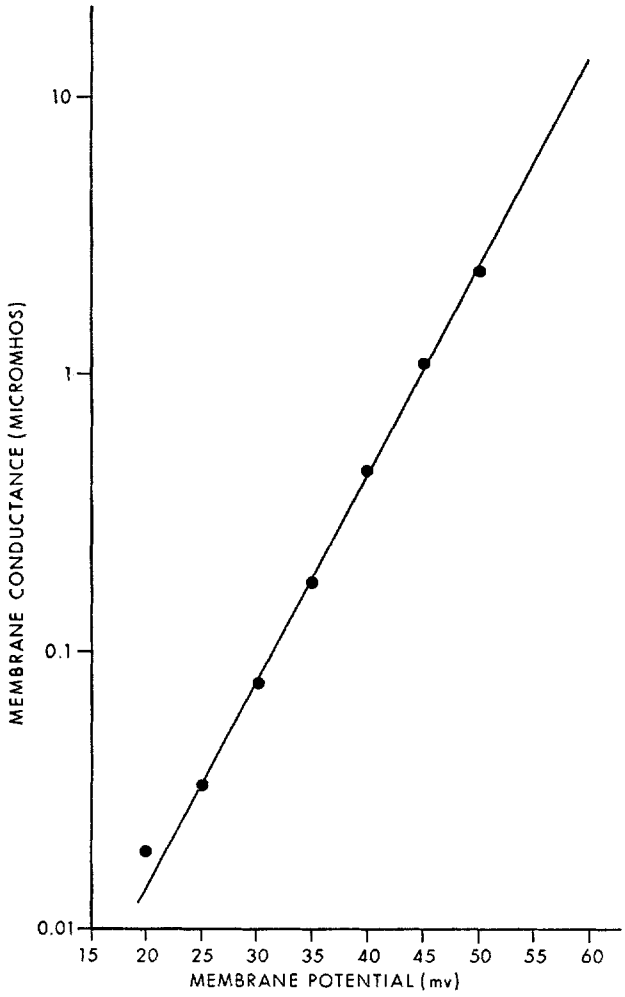


FIGURE 3. Steady-state $g$-$V$ characteristic of a monazomycin-treated PE membrane. The membrane was formed at room temperature in 0.1 M KCl; monazomycin was then added to the front chamber and the $g$-$V$ characteristic determined about 15 min later. Slope of line = $e$-fold conductance change per 5.7 mv; monazomycin concentration = 33 $\mu$g/ml; membrane area = 1 mm$^2$.

at different monazomycin concentrations (Fig. 4). At a given voltage, doubling the the monazomycin concentration increases membrane conductance about 30-fold, ie.

$$g \propto (\text{monazomycin})^s \qquad \text{(at a given } V\text{)} \quad (3)$$

where $s$ is a constant equal to about 5.

At this point we raise an issue that will concern us for the remainder of this

paper and throughout the next. In looking at Fig. 4, we can say (as implied above) that increases in monazomycin concentration shift the $g$-$V$ characteristic upwards along the conductance axis. We can equally well say, however, that they shift the characteristic to the left along the voltage axis. Formally, the results are the same no matter which view is chosen, but the implied physical picture of events on or in the membrane is quite different. Our present understanding of the mechanism of action of monoazomycin does

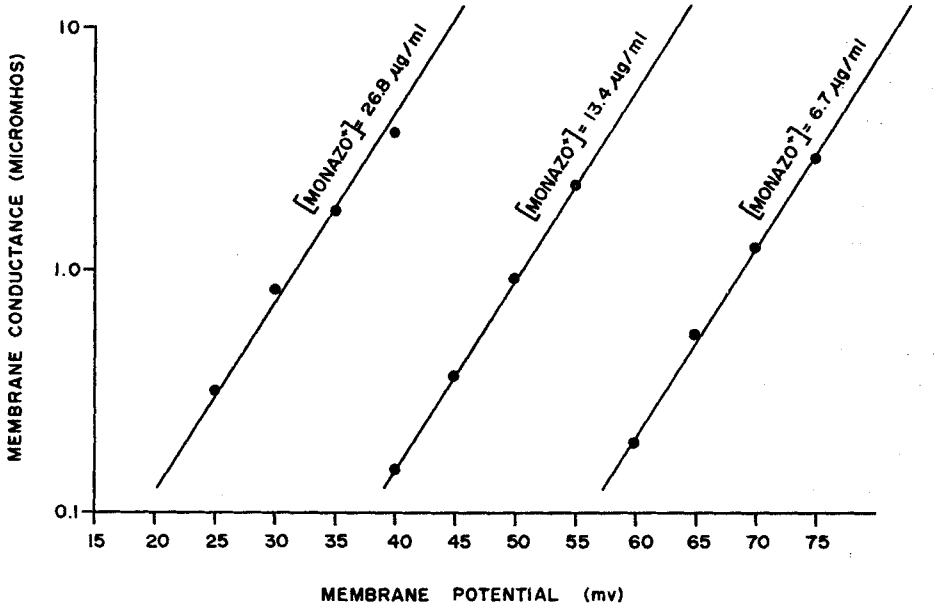


FIGURE 4. Effect of monazomycin concentration on the steady-state $g$-$V$ characteristic of a PE membrane. The membrane (area = 1 mm$^2$) was formed at room temperature in 0.1 M KCl. Monazomycin was then added to the front chamber to achieve the concentrations indicated in the figure. After each increment of monazomycin concentration, approximately 15 min were required for the membrane to achieve a stable $g$-$V$ characteristic. Slope of lines = $e$-fold conductance change per 5.5 mv.

not allow us to choose between these alternatives in the case of shifts produced by changes in monazomycin concentration. Changes in the ionic compositions of the bathing solutions can also produce parallel displacements of the $g$-$V$ characteristic. In these cases, we feel that we understand the underlying mechanisms well enough to make a clear choice between translation along the voltage axis and translation along the conductance axis.

If sufficient monazomycin is added to the system, the membrane achieves a significant conductance at $V = 0$. In this case relation 2 continues to hold even for negative values of $V$, until we approach the conductance of unmodified membrane. Generally, relation 2 is valid only in the first quadrant

merely because we usually work with monazomycin concentrations such that membrane conductance is significant ($>10^{-9}/\Omega$) only at potentials $\geq +20$ mv. Thus, there is nothing "special" about the action of monazomycin in the absence of a potential difference ($V = 0$), nor does the functional dependence of $g$ on $V$ change at $V = 0$.

In commenting on the dependence of conductance on voltage, we could compare this model system with axonal membranes. No such comparison can be made, however, concerning the dependence of conductance on monazomycin concentration, since at present it is not possible to vary the concentration of the molecules responsible for the potassium or sodium conductance systems. The model membrane permits variation of a parameter not accessible for investigation in biological membranes.[4]

3. THE IONIC COMPOSITION OF THE BATHING SOLUTIONS AND THE $g$-V CHARACTERISTIC The conductance of a monazomycin-treated PE membrane at a given voltage increases linearly with symmetrical increases of uni-univalent salt concentration (Figs. 5 *a* and 5 *b*). (Since our interpretation is that increased salt concentration enhances the conductance per site without affecting the number of sites at a given voltage, we consider the $g$-$V$ characteristic to be shifted along the conductance axis.) For the relationship to approximate linearity, a small concentration of divalent cation ($\approx 10^{-3}$ M) must be present. The reason for this stricture will become clear in the succeeding paper, where experiments using negatively charged phosphatidylglycerol (PG) membranes are presented.[5]

If divalent cations are added to KCl (or NaCl) solutions bathing PE membranes, conductance at a given voltage is slightly reduced. Again, the reason is discussed in the following paper. Suffice it to say that the effect of divalent cations on PE membranes is negligible compared with the effects on PG membranes.

4. ION SELECTIVITY If sufficient monazomycin is added so that membrane conductance is significant at $I = 0$, then the ion selectivity of the monazomcyin-induced conductance elements can be determined from the EMF's developed by ion gradients across the membrane. The slope of the line in Fig. 6 is 51 mv for a 10-fold ratio of KCl activity,[6] with the more dilute side positive with respect to the more concentrated side. Thus, the membrane is much more permeable to monovalent cations than to mono-

[4] It is not clear whether this is good or bad.

[5] Here we may note that even without divalent cation present, the conductance of monazomycin-treated PE membranes *increases* as the KCl concentration is raised, although the increase is less than linear. This is in sharp contrast to the drastic *depression* of conductance in PG membranes with increases of KCl concentration.

[6] Mueller and Rudin (1969) report somewhat poorer selectivity.

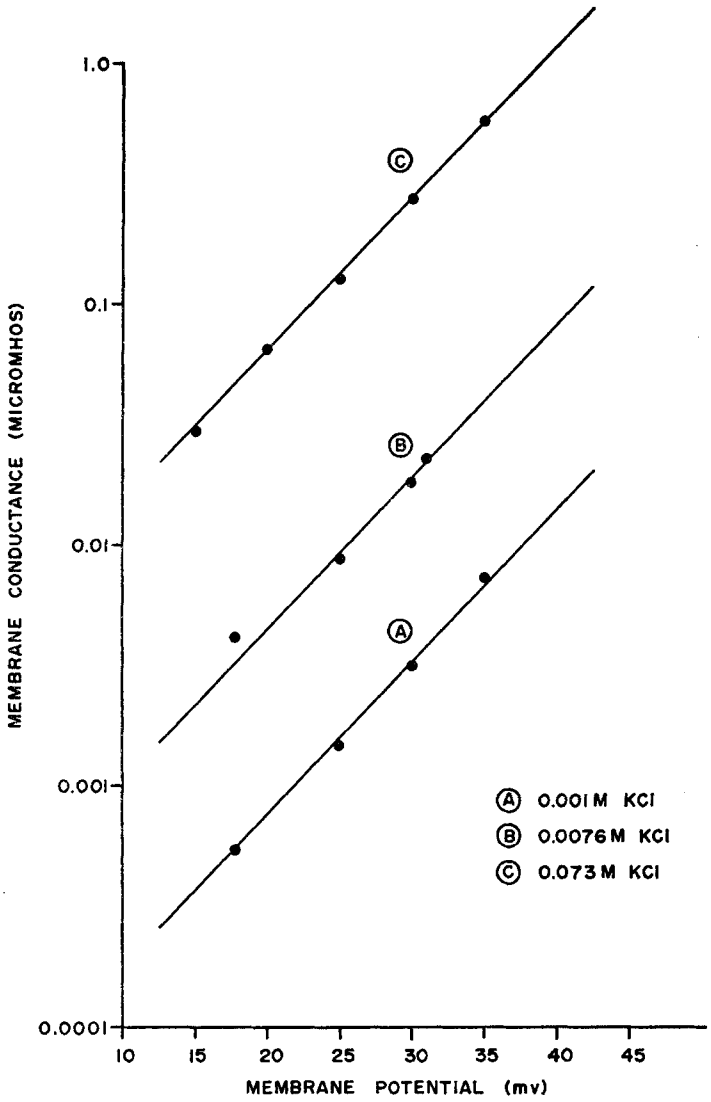


FIGURE 5 *a*. The effect of KCl concentration on the steady-state *g-V* characteristic of a monazomycin-treated PE membrane. The membrane was formed at room temperature in a solution containing $10^{-3}$ M KCl and $10^{-2}$ M $MgSO_4$. Monazomycin was added to the front chamber and, after approximately 15 min, curve A was obtained. The KCl concentration was then increased on both sides to the values indicated for curves B and C. It required only about 1 min (the time to stir in the KCl solutions) for the new *g-V* characteristic to be established. Slope of lines = *e*-fold conductance change per 7.0 mv; monazomycin concentration = 33 $\mu$g/ml; membrane area = 1 $mm^2$.

valent anions.[7] Trivial bi-ionic potentials indicate, however, that the membranes do not discriminate significantly between $K^+$ and $Na^+$. In this respect the monazomycin-induced conductance elements differ from the voltage-dependent conductances in nerve, which are selective for either $Na^+$ or $K^+$. Finally, the membrane is virtually impermeable to divalent cations. When

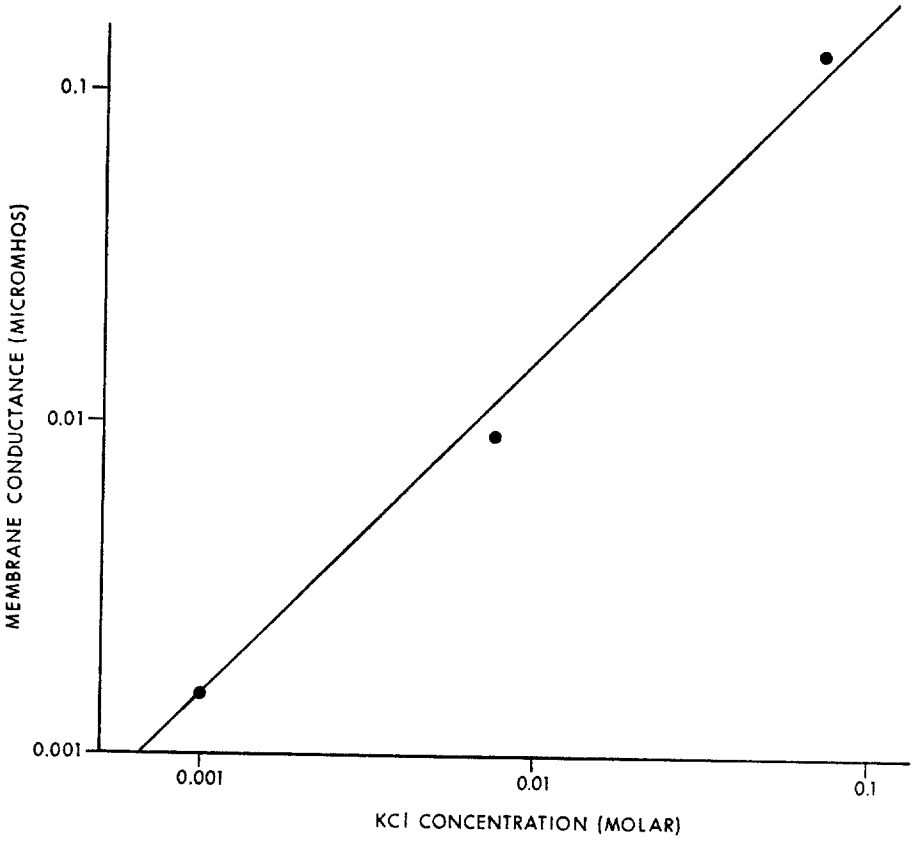


FIGURE 5 *b*. Log-log plot of steady-state conductance (at a given voltage) vs. [KCl] for a monazomycin-treated PE membrane. The data points are taken from Fig. 5 *a* for a membrane potential of 20 mv. The line drawn in the figure has a slope of 1.

$CaCl_2$ gradients are established in the absence of monovalent cations, anionic potentials develop. As expected, conductance is much smaller when no monovalent cation is available to carry current across the membrane.

5. SUMMARY OF THE STEADY-STATE RESULTS WITH PE MEMBRANES Combining relations 2 and 3 with the results in the two previous sections, we can summarize the dependence of steady-state conductance on voltage, monazo-

[7] This is seemingly paradoxical, in that monazomycin is positively charged. A similar situation was noted for several positively charged polyene antibiotics (Cass et al., 1970).

mycin concentration, and salt concentration by the expression

$$g \propto [K^+][\text{monazomycin}]^s \exp(nqV/kT) \quad (4)$$

where $s \approx 5$ and $n \approx 4.4$. Divalent cations have little or no effect on the conductance of PE-treated membranes.

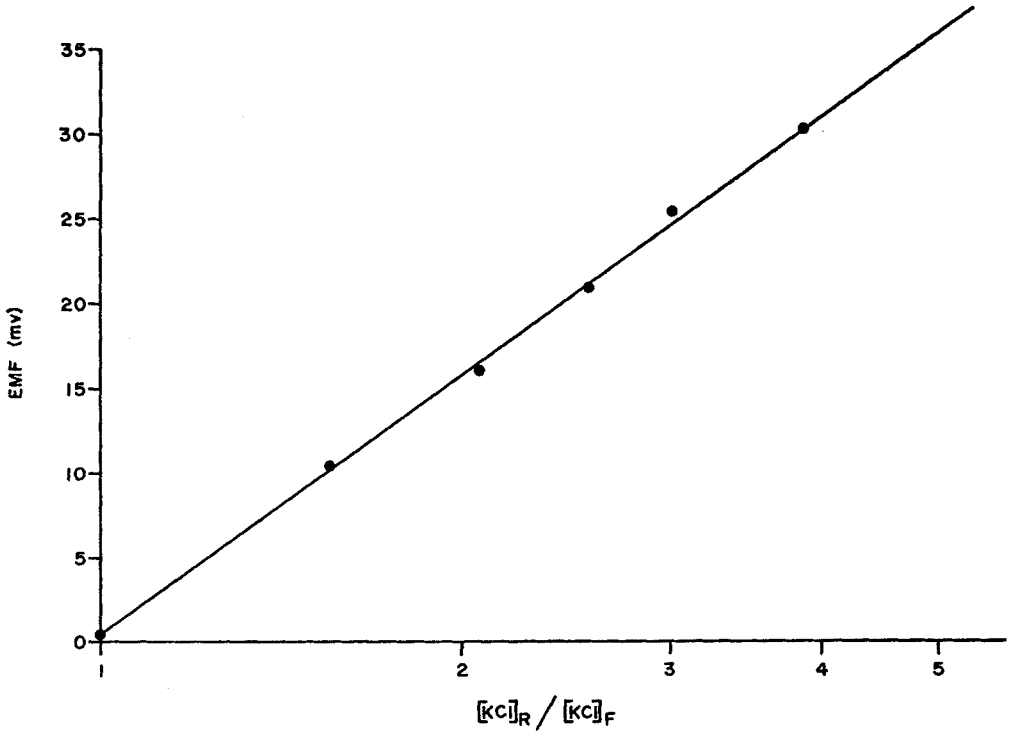


FIGURE 6. Membrane EMF vs. ratio of KCl activity for a monazomycin-treated PE membrane. Slope of line = 51 mv per 10-fold ratio of activity. The membrane was formed at room temperature in 0.1 M KCl; monazomycin was then added to the front compartment. The KCl activity in the front chamber ($[KCl]_F$) remained that of 0.1 M KCl; the activity in the rear compartment ($[KCl]_R$) was increased by additions of small volumes of concentrated KCl. The potential of the rear chamber is defined as zero. Monazomycin concentration = 33 μg/ml.

### C. *Negative Slope Conductance*

The existence in an *I-V* characteristic of a region with negative slope conductance ($dI/dV < 0$) is dear to neurophysiologists. In this section we examine its occurrence in monazomycin-treated membranes and illustrate how it can be induced by a diffusion EMF.

If a region of negative slope conductance exists, then at some voltage $dI/dV = 0$. For a PE membrane[8] treated with monazomycin, we can write

[8] We shall see in the succeeding paper why we restrict our treatment to PE membranes.

from relation 2

$$g = C \exp (nqV/kT) \qquad (2')$$

where $C$ is a constant. Substituting this into equation 1 we have

$$I = (V - \text{EMF})g = C(V - \text{EMF}) \exp (nqV/kT). \qquad (5)$$

Taking $dI/dV$ and setting it equal to zero we obtain:

$$V^* = \text{EMF} - \frac{kT}{nq} \qquad (6)$$

where $V^*$ is the potential at which $dI/dV = 0$. The negative slope region extends from $V = -\infty$ (where $I = 0$) to $V^*$.

It appears from equation 6 that there will always exist a region with negative slope conductance, even when the membrane separates identical salt solutions (EMF = 0). Equation 6—and equation 2′ from which it was derived—are not quite correct, however, because we neglected to include the parallel conductance of the unmodified membrane. If this is added to the right-hand side of equation 2′, then we find that there will not necessarily be a negative slope region when EMF = 0, unless $C$ is sufficiently large compared to unmodified membrane conductance. This means we must add sufficient monazomycin to make the membrane conductance at $V = 0$ large compared to that of unmodified membrane. In general we do not employ such large concentrations of monazomycin; consequently, we see only rectification in symmetrical solutions. We can convert this rectifying $I$-$V$ characteristic into one containing a negative slope region simply by applying a KCl gradient to generate a large positive EMF. At $V = \text{EMF}$, $I$ will be zero, but the conductance will be high, thus insuring a region of negative slope, with $V^*$ given by equation 6.

Fig. 7 *a* demonstrates that a simple monotonic curve, obtained when the membrane separates symmetrical KCl solutions, is converted to one with a negative slope region by the application of a KCl gradient, and then reconverted to a monotonic curve by restoration of the KCl symmetry. Despite the qualitative difference in the $I$-$V$ characteristics with and without the KCl gradient, the $g$-$V$ characteristic is virtually unchanged (Fig. 7 *b*). (The small shift results from the larger conductance per site with increased concentration of current carrier ($K^+$).) Thus, the drastic change in $I$-$V$ characteristic is an analytical consequence of introducing a positive EMF into the system *without any significant alteration of the g-V characteristic* of the membrane.[9]

[9] This point is generally recognized by electrophysiologists and is specifically verbalized by Mueller and Rudin (1968 *a*) in their discussion of voltage-dependent conductances in thin lipid membranes.

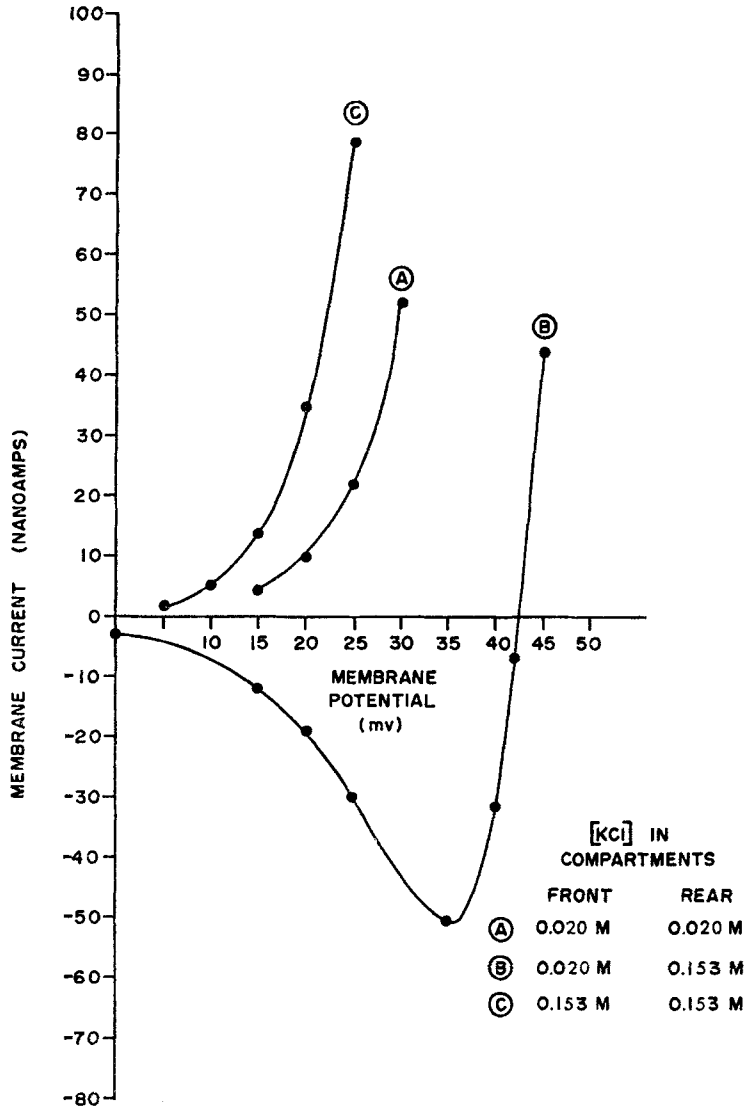


FIGURE 7 *a*. Steady-state *I-V* characteristics of a monazomycin-treated PE membrane: development of a negative slope-conductance region by the addition of a salt gradient. The membrane was formed at room temperature in 0.02 M KCl; monazomycin was then added to the front chamber and approximately 15 min later the *I-V* characteristic labeled A was obtained. A KCl gradient was established by raising [KCl] to 0.153 M in the rear chamber. This gave rise to a diffusion EMF of 42 mv. The *I-V* characteristic labeled B was taken approximately 1 min after the gradient was established. The gradient (along with the EMF) was then abolished by raising [KCl] in the front to 0.153 M. The *I-V* characteristic labeled C was taken approximately 1 min after the gradient was abolished. Monazomycin concentration = 33 $\mu$g/ml; membrane area = 1 mm$^2$.

This last point is an important one. Implicit in substituting equation 2′ into equation 1 is that the monazomycin-induced conductance is a unique function of $V$ regardless of whether $V$ arises from an $IR$ drop, a diffusion EMF, or a combination of the two; i.e., the conductance at a given $V$ is independent of EMF. This is (approximately) true for a PE membrane (Fig. 7 *b*). This is

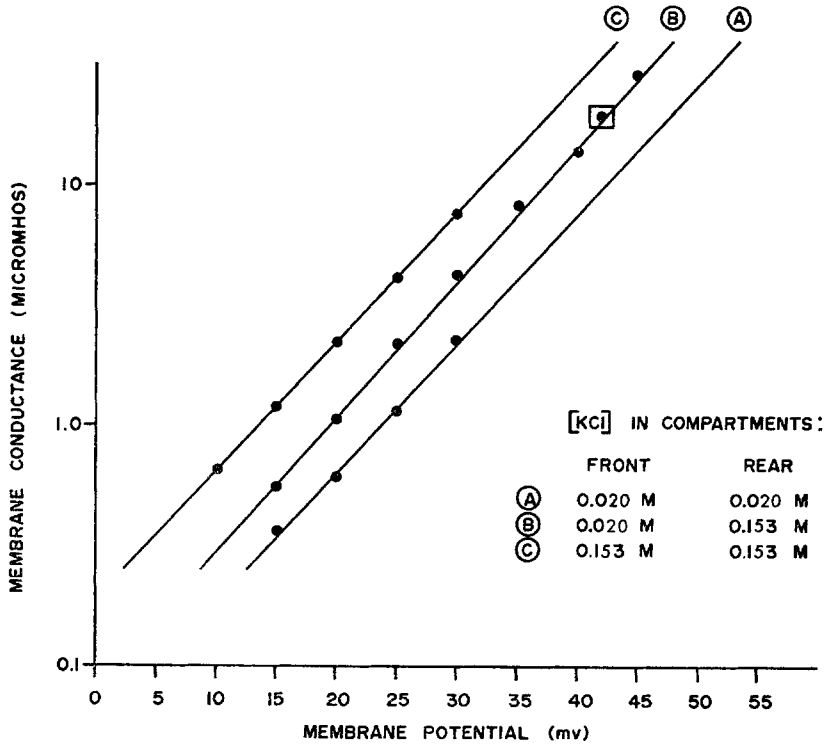


FIGURE 7 *b*. The steady-state $g$-$V$ characteristics of a monazomycin-treated PE membrane in the absence (A and C) and presence (B) of a diffusion EMF created by a salt gradient. The $g$-$V$ characteristics have been calculated from the corresponding $I$-$V$ characteristics in Fig. 7 *a* by equation 1 *a* (for curves A and C) and by equation 1 (for curve B) with EMF = 42 mv. (The point marked by the square was determined for $V$ = EMF = 42 mv by dividing by 42 mv, the "instantaneous" current that was obtained when the potential was shifted from 42 mv to zero. This procedure was necessary, since at $V$ = EMF, $I$ = 0 and equation 1 is indeterminate. It is gratifying that the point falls on line B.) Slope of lines = $e$-fold conductance change per 8 mv.

*not* true for a PG membrane; in the absence of divalent cation, a positive EMF shifts the $g$-$V$ characteristic to the right (see succeeding paper).

### D. *Observations Bearing on the Mechanism of Monazomycin Action*

1. DYNAMIC EQUILIBRIUM BETWEEN MONAZOMYCIN ON THE MEMBRANE AND IN SOLUTION Replacement of the aqueous solution containing monazomycin with a similar solution lacking monazomycin causes a rapid fall of

conductance to the low, voltage-independent value of unmodified membrane within the 3 min required to change solutions. Thus, monazomycin associated with the membrane is in rapid equilibrium with free monazomycin in solution. Since rapid washout of monazomycin occurs both in the absence and in the presence of an applied positive potential difference, the electric field does *not* stabilize existing conduction sites when the monazomycin reservoir in the bulk aqueous phase is removed.

It is interesting that despite the insolubility of monazomycin in hydrocarbon, small amounts can cross the membrane. If the membrane is driven into a very high conductance state by a large positive voltage, then upon reversal of the voltage sign, the current declines exponentially (as in Fig. 1 *b*) but then increases again (Fig. 8). This "reverse turn-on" of membrane

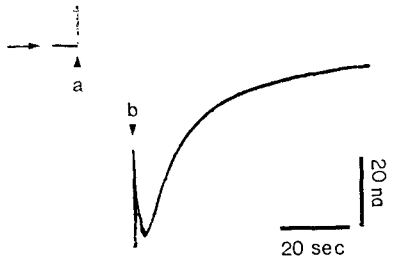


FIGURE 8. Demonstration of "reverse turn-on" of conductance following strong stimulation. The membrane was formed at room temperature in 0.01 M KCl; monazomycin was then added to the front chamber. The horizontal arrow designates the $I = 0$ level. At *a* the membrane was stimulated with +115 mv; a huge positive current (off the scale in the record) developed. At *b* the stimulus was reversed to −115 mv. Initially a huge negative current (off the scale in the record) obtained which decayed rapidly; before reaching zero, however, the negative current increased and then slowly decayed back toward zero, which it still has not reached in the record. In this experiment a membrane formed from a decane solution containing 0.5% PG and 0.5% cholesterol was used, since such membranes are better able to withstand large voltages and very high conductances. Monazomycin concentration = 1 μg/ml; membrane area = 1 $mm^2$.

conductance is very much smaller than the conductance achieved with the positive voltage, and is transient. We believe that small amounts of monazomycin have been transported through the membrane and have accumulated in the unstirred layer at the rear water-membrane interface during the preceding strong positive voltage stimulus. The accumulated monazomycin is then available to produce a conductance increase by the usual mechanism, when the stimulating polarity is reversed. Further support for this contention comes from the observation that the magnitude of the reverse turn-on response increases with the duration of positive voltage stimulus. The transient nature of the response results from diffusion of monazomycin away from the rear interface into the bulk solution.

2. A MONAZOMYCIN-NYSTATIN BISTABLE SYSTEM We feel that most of the area of a monazomycin-treated membrane is unmodified even at relatively high conductances. This point of view allows us to predict responses for a membrane treated both with nystatin, a known pore former (Holz and Finkelstein, 1970), and with monazomycin. In nystatin alone, the membrane (i.e., its nystatin channels) is anion selective, and its conductance is *not* voltage dependent. A gradient of KCl—rear concentration greater than front—creates a negative "resting" potential. If monazomycin is subsequently added to the front chamber, this negative potential will keep the monazomycin system turned off, and the membrane will remain at this potential. If only monazomycin were present, the same KCl gradient would produce a positive membrane potential that would turn on the monazomycin conductance. Clearly, then, if sufficient monazomycin is present such that the conductance of the monazomycin system at this positive potential is much larger than the conductance of the nystatin system, *and*, if indeed, these two systems can act independently (i.e., there is room on the membrane for both), then there must be two stable potentials at either of which the combined system can reside. The equivalent circuit is shown in Fig. 9. Thus, a membrane treated with appropriate amounts of nystatin and monazomycin should, in the presence of a KCl gradient, be bistable. This expectation has been realized (Fig. 10), and we infer that a bilayer treated with both nystatin and monazomycin is a *mosaic* structure. The voltage-invariant nystatin channels coexist in parallel with the voltage-dependent monazomycin-induced conduction sites, and both act *independently*.

3. A FORMAL MODEL FOR THE MONAZOMYCIN SYSTEM Let:

$p$ = number of open (conducting) channels,

$r$ = number of closed (nonconducting) channels,

$N \equiv p + r$ = total number of channels,

$g$ = conductance of a single open channel,

$f \equiv p/N$ = fraction of channels open,

$g_m = fgN$ = total membrane conductance (The conductance of a closed channel and of unmodified membrane is taken to be zero.).

Assume that there is a difference in (chemical) energy, $E_1$, between an opened and a closed channel and that it requires $n$ monazomycin molecules (each of charge $+1$) to produce a channel. Then from the Boltmann distribution we can write

$$r = A \tag{7 a}$$

$$p = A \exp \frac{E_1 + nqV}{kT} \tag{7 b}$$

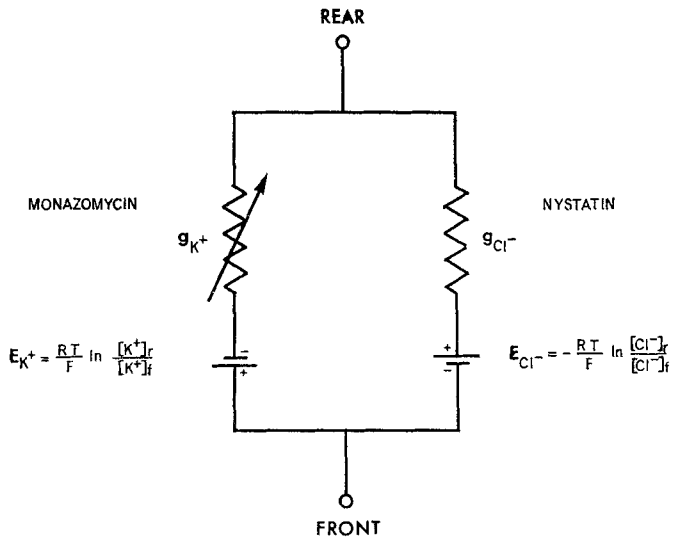


FIGURE 9. Equivalent circuit for a membrane treated with both nystatin and monazomycin. Note that the nystatin-induced conductance ($g_{Cl}$) is constant, whereas the monazomycin-induced conductance ($g_K$) is voltage dependent.

$$f = \frac{p}{p + r} = \frac{\exp \frac{E_1 + nqV}{kT}}{1 + \exp \frac{E_1 + nqV}{kT}}.$$

Then

$$g_m = fgN = gN \frac{\exp \frac{E_1 + nqV}{kT}}{1 + \exp \frac{E_1 + nqV}{kT}}. \tag{8}$$

For small[10] values of $V$(i.e., $E_1 + nqV \ll 0$),

$$g_m = gN \exp \frac{E_1 + nqV}{kT},$$

and combining the unknown constants ($g$, $N$, and $E_1$) we have, finally,

$$g_m = C \exp (nqV/kT) \tag{9}$$

which we observe experimentally.

[10] In the monazomycin system, $E_1$ has a large negative value; ie., at zero voltage most of the channels are closed.

In this model $n$ is the number of monazomycin molecules needed to form a channel and should equal $s$ in relation 3. Experimentally, this equality is approximately obeyed, at least for PE and PG membranes. Despite this agreement with theory, we do not believe that $n$ (or $s$) represents the molecularity of the channel, since it appears that these numbers can vary enormously depending on the lipid; e.g., Mueller and Rudin (1969) report values of 24

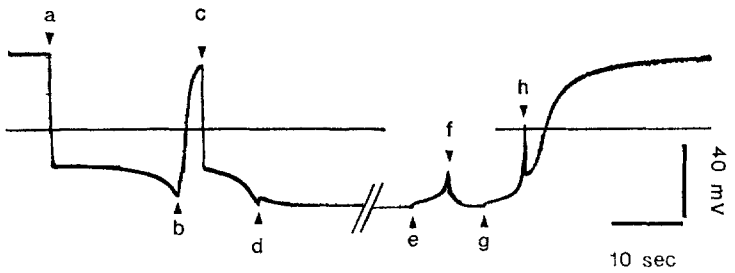


FIGURE 10. Bistable property of a membrane treated with both nystatin and monazomycin. Membrane was formed at room temperature in a solution containing 0.01 M KCl and 0.001 M $MgSO_4$. Nystatin was added to both chambers to a concentration of 5 $\mu$g/ml. After approximately 20 min the membrane reached a steady conductance of about $10^{-7}/\Omega$. At this point the KCl concentration was raised in the rear chamber to 0.077 M. This produced a membrane EMF of −41 mv (front negative with respect to rear). Monazomycin was then added to the front chamber to a concentration of 3 $\mu$g/ml. This produced no significant change in the EMF. After approximately 15 min, the above record was obtained. (In the record the horizontal line is $V = 0$; above the line are negative potentials and below the line are positive potentials.) At *a*, a positive step of current = 3.75 na was applied and at *b* it was removed. The potential returned toward the original −41 mv. At *c*, the current was again applied, and at *d* it was removed. This time the potential continued increasing and achieved a new stable state of +41 mv. At *e*, a negative step of current = −4.05 na was applied and at *f* it was removed. The potential returned to +41 mv. At *g* the current was again applied and at *h* it was removed. This time the potential continued to decrease and flipped back to the original state of −41 mv. Note the much smaller *IR* drops at *e* and *g* compared with that at *a*, thus demonstrating that the conductance is much higher at +41 mv (where the monazomycin conductance is turned on) than at −41 mv (where only the nystatin conductance is significant). The membrane was formed from a decane solution containing 0.5% PG and 0.5% cholesterol. Membrane area = 1 $mm^2$.

with some lipids. (It is also impossible to infer the molecularity of nystatin and amphotericin B pores from the dependence of conductance on antibiotic concentration [Finkelstein and Holz, 1972].) All that we can infer from the large values of $s$ and $n$ is that *many* monazomycin molecules interact to form one conduction site.

4. CARRIER VS. CHANNEL FORMER Cooperative interaction among many monazomycin molecules is the only simple way of explaining the S-shaped rise of current after application of a voltage step and the striking dependence

of conductance on voltage and monazomycin concentration.[11] This strongly suggests that monazomycin functions as a channel former rather than as a carrier. Furthermore, monazomycin is quite insoluble in hydrocarbon, whereas a carrier (e.g., valinomycin) must be soluble in the hydrocarbon interior of the membrane. In addition, we have never seen a diffusion potential across a membrane with equal concentrations of KCl (or NaCl) on both sides, even though monazomycin is present on only one side. If monazomycin-cation complex were the current carrier in the membrane, we should expect (if the boundary processes are not rate limiting) a diffusion potential under these circumstances.[12]

5. HOW DOES MEMBRANE POTENTIAL CONTROL THE NUMBER OF CONDUCTING CHANNELS? We suggest that the number of conducting channels is completely determined by the concentration of monomeric monazomycin in (or on) the membrane. In the steady state, a voltage-*independent* equilibrium exists between monazomycin monomers and $(\text{monazomycin})_x$, where $x$ is the number of molecules needed to form a channel. The dependence of conductance on voltage would thus arise from voltage dependence of intramembrane monomer concentration. This mechanism differs from one in which the electric field affects the conformation of an already existing channel and converts it from one conductance state to another; the latter may be applicable to EIM. Our hypothesis is supported by the observation that monazomycin channels are not stabilized by membrane potential. Conductance rapidly returns to the very low value characteristic of unmodified membrane when monazomycin is removed from the aqueous phase, whether or not a positive potential difference exists across the membrane during this time.[13] If the function of the electric field were to somehow hold monazomycin molecules together in a conducting configuration, one might expect that the conductance would remain high, until the potential was returned to zero.

[11] Interestingly, there does *not* appear to be interaction between monazomycin molecules from opposite sides of the membrane, in contrast to the behavior of nystatin and amphotericin B (Cass et al., 1970). Thus, the action of monazomycin on one side of the membrane is not enhanced by addition of monazomycin to the other side. Of course, the *I-V* characteristic is symmetrical with equal concentrations on the two sides.

[12] One would also expect a diffusion potential if a carrier such as valinomycin was introduced into only one compartment. None appears, however, because the uncomplexed antibiotic is very permeant. Since valinomycin traverses the membrane much more rapidly than it diffuses through the unstirred aqueous layers, its concentration is essentially the same at both water-membrane interfaces. This explanation cannot be invoked for the highly impermeant monazomycin. (We know it is impermeant, because we observe tremendous rectification.)

[13] Because it required 3 min to remove monazomycin from solution (see section D part 1), our time resolution is no better than about 1.5 min. In the following paper, however, we argue that for a membrane having a negative surface charge, the interfacial monazomycin concentration is reduced upon addition of divalent cation to the solution containing monazomycin. If this is correct, we can resolve the effect of changing interfacial monazomycin concentration in less than 15 sec. At this time resolution there is still no indication that a positive potential difference stabilizes channels.

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## REFERENCES

AKASAKI, K., K. KARASAWA, M. WATANABE, H. YONEHARA, and H. UMEZAWA. 1963. Monazomycin, a new antibiotic produced by a Streptomyces. *J. Antibiot. (Tokyo) Ser. A.* **16**:127.

CASS, A., A. FINKELSTEIN, and V. KRESPI. 1970. The ion permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56**:100.

FINKELSTEIN, A., and R. HOLZ. 1972. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *In* Membranes—A Series of Advances. G. Eisenman, editor. Marcel Dekker, Inc., New York. **2**. In press.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* **117**:500.

HOLZ, R., and A. FINKELSTEIN. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nysatin and amphotericin B. *J. Gen. Physiol.* **56**:125.

MAURO, A. 1961. Anomalous impedance. A phenomenological property of time-variant resistance. *Biophys. J.* **1**:353.

MITSCHER, L. A., A. J. SHAY, and N. BOHENOS. 1967. LL-A491, a monazomycin-like antibiotic. *Appl. Microbiol.* **15**:1002.

MUELLER, P., and D. O. RUDIN. 1968 *a*. Resting and action potentials in experimental bimolecular lipid membranes. *J. Theor. Biol.* **18**:222.

MUELLER, P., and D. O. RUDIN. 1968 *b*. Action potentials induced in bimolecular lipid membranes. *Nature (Lond.).* **217**:713.

MUELLER, P., and D. O. RUDIN. 1969. Translocators in bimolecular lipid membranes: their role in dissipative and conservative bioenergy transductions. *Curr. Top. Bioenerg.* **3**:157.

MUELLER, P., D. O. RUDIN, H. TI TIEN, and W. C. WESCOTT. 1962. Reconstitution of excitable cell membrane structure *in vitro*. *Circulation.* **26**:1167.

MUELLER, P., D. O. RUDIN, H. TI TIEN, and W. C. WESCOTT. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**:534.