The Kinetics of Monazomycin-induced 
Voltage-dependent Conductance

II. Theory and a Demonstration 
of a Form of Memory

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ABSTRACT The empirical differential equation that describes the kinetics of 
monazomycin-induced voltage-dependent conductance is derived using a stan-
dard chemical kinetic formulation and the assumption that monazomycin entry 
into and exit from the membrane is autocatalytic. The predicted form of gating 
currents is shown and numerical calculations for this process are made using a 
range of values for two unmeasured variables. A form of “memory” is then 
demonstrated, along with the ability of the theoretical equation to explain the 
nature of the memory.

INTRODUCTION

In the preceding paper (Muller et al., 1981, referred to as paper I) we showed 
that Eq. 1 is adequate to describe the trajectory of monazomycin-induced 
conductance (g) changes in response to changes of membrane potential (V)

\[
\frac{dg}{dt} = A_g \left[ 1 - \left( \frac{g}{g_*} \right)^B \right].
\]  

(1)

In this equation, A is the rate constant, \( g_* \) the steady-state conductance and 
B an empirical constant (B = 0.7). A and g are functions of membrane 
potential and monazomycin concentration ([Mon]) as specified by Eqs. 2 and 3

\[
A = A_* \left( \frac{[\text{Mon}]}{[\text{Mon}]_*} \right)^x e^{xqV/RT}, \tag{2}
\]

\[
g = g_* \left( \frac{[\text{Mon}]}{[\text{Mon}]_*} \right)^y e^{yqV/RT}, \tag{3}
\]

where \( A_* \) and \( g_* \) are, respectively, A and g in the presence of a reference
concentration of monazomycin ([Mon]₀) when V = 0; x and s are constants such that x ≈ 2.6 and s ≈ 5.8; and q, k, and T have their usual meanings.

Our goal here is to derive Eq. 1 from a molecular model. Once this is done, we will be in a position to specify the meaning of the various parameters in Eqs. 1-3. In addition, by making use of single channel conductance measurements, the derived analogue of Eq. 1 will allow us to get estimates of how much monazomycin is actually involved in conductance changes and also to get some idea of the time-course and magnitude of the gating current for this voltage-dependent system. We will then present the characteristics of the memory exhibited by this conductance and show that our theory (within the limits imposed by our assumptions) quantitatively accounts for this very interesting phenomenon.

MATERIALS AND METHODS

Our experimental procedures are described in paper I.

THEORY

A. Physical Model

The scheme we wish to present as the structural basis of monazomycin-induced conductance is the "insertion-aggregation" process (Mueller, 1979; see also Finkelstein and Holz [1972]; Muller and Finkelstein, [1972a]) with one major modification. Justification for applying this scheme to the poorly characterized monazomycin molecule is given in Heyer et al. (1976b).

We may imagine the monazomycin molecule as shown in Fig. 1. We take it to be rod-like and mostly nonpolar except for a positive charge at one end and a row of hydrophilic groups (hydroxyls) occupying perhaps one-quarter of the circumference of a cross section of the rod. Such a molecule is amphipathic in nature and would tend to form cylindrical "micelles." In aqueous solution the cylinder would have a nonpolar core; in the membrane the center of the cylinder would be polar. A channel would then be a micelle whose potential lumen is open and water-filled, and whose ends are anchored in the two aqueous phases. We imagine there are also nonconducting micelles of smaller molecularity spanning the membrane from interface to interface. The macroscopic conductance of the membrane thus depends on the total amount of monazomycin in the membrane and the position of the equilibrium between conducting and nonconducting forms. We assume that this equilibrium—the aggregation step—is independent of the transmembrane potential difference; the voltage dependence of the system presumably arises from the energy requirement for moving the positive charge of the monazomycin from the cis to the trans interface—the insertion reaction.

In this view, a conductance change is a voltage-driven change in the "partition coefficient" between solution and membrane. The channels are not

1 In this paper, we will use only two parameters to describe the voltage and concentration dependence of A and gₑ; that is, we take y = x and n = s. This simplifies the notation without obscuring anything of importance.
permanent structures but rather are statistical entities that form a membrane pool in relatively rapid equilibrium with the aqueous monazomycin (Muller and Finkelstein, 1972b). Thus, in a cycle from high to low to high conductance the membrane pool must be largely replaced. In fact, when there is a high steady-state conductance there is also a rapid turn-over of the spanned monazomycin population. The monazomycin that participates in the insertion reaction may come either directly from aqueous solution or it may first have to adsorb to the membrane surface; we have no basis for deciding between these alternatives.

If we were to stop our description here and proceed to formalize it, we would wind up with, as shown by Mueller (1979), a kinetic equation analogous

![Figure 1](image-url)
to the Hodgkin and Huxley (1952b) equation for the potassium gating system in nerve. In paper I we showed that this is not adequate for the monazomycin system. We also reasoned, from the exponential nature of the beginning of conductance increases and the dependence of the rate constant of the exponential on aqueous monazomycin concentration, that there had to be an autocatalytic step in the pathway to channel formation. When this autocatalysis is added to the insertion-aggregation sequence, the result is an equation identical in form to Eq. 1. The basis for the autocatalysis is by no means clear, but it is possible to imagine that spanned monazomycin disrupts the lipid matrix of the membrane so that the energy barrier for insertion of more antibiotic is reduced.

From the foregoing, it is evident that we will treat the insertion process as rate limiting; this must be true if the rate constant we measure from the exponential portion of conductance rises is indeed a reflection of the insertion process. This assumption is supported by analysis of conductance fluctuations around the steady state (Kolb, 1979; Moore and Neher, 1976). Kolb (1979) reports two detectable processes: one that is fast and voltage independent, the other slower and voltage dependent. Because we have already identified the insertion reaction as the source of the voltage dependence, it must also be the slower process. Later on, when we consider the memory, we will see that lateral diffusion of monazomycin can become rate-limiting when the conductance is very low.

**B. Model I**

We will deal with only three oligomeric monazomycin species called $M_\alpha$, $M_\beta$, and $M_\gamma$ where $\alpha$, $\beta$, and $\gamma$ are the molecularities. $M_\alpha$ is the (volume) concentration of the aqueous form which enters and leaves the membrane. $M_\beta$ is the (surface) concentration of nonconducting spanned monazomycin, and $M_\gamma$ is the (surface) concentration of conducting channels. Because it is known that there are several channel types (Muller and Andersen, manuscript in preparation) the molecularity ($\gamma$) of the channels is not expected to be an integer. Rather, $\gamma$ is some implicit average of the variants' molecularities, weighted by their relative frequency of occurrence. (As we have already justified the idea that $g$, the measured conductance, is proportional to $M_\gamma$, we imagine that the contribution of each channel type is also weighted by their unitary conductances.) Similarly, because we have no reason to demand that only one species can shuttle across the interface nor that only one nonconducting species must exist in the membrane, $\alpha$ and $\beta$ are also averages and, therefore, continuous quantities.

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2 Other, indirect experiments lead to the conclusion that at least during conductance decays the interfacial reaction limits the break-up of channels (R. U. Muller, unpublished observations).

3 The following derivation could equally well be carried out assuming that $M_\alpha$ is a surface concentration. It is in any case worth pointing out that in spite of the similarity of the chosen symbols, $M_\alpha$ is in a physically different state than $M_\beta$ or $M_\gamma$. 
The insertion reaction may now be written as:

$$M_a + M_b \xrightarrow{k_i(V)} \left( \frac{\alpha + \beta}{\beta} \right) M_b.$$  

(4)

Here, $k_i(V)$ is the rate constant for insertion of $M_a$ into the film, whereas $k_{-1}(V)$ is the rate constant for $M_a$ going from the film back into the cis solution. The autocatalysis has been included by adding the quantity $M_b$ to both sides of the reaction scheme. At equilibrium, the insertion process is given by:

$$\frac{(M_a)(M_b)}{(M_b)^{\alpha + \beta/\beta}} = \frac{k_{-1}(V)}{k_i(V)} = K_i(V),$$  

(5)

where $K_i(V)$ is the equilibrium constant for this reaction. Note that at equilibrium, it is possible to reduce the left-hand side of Eq. 5 by the factor $M_b$ without affecting $K_i(V)$. This is of course required if we are to refer to the action of $M_b$ as catalytic.

The aggregation reaction involves channel formation from nonconducting spanned $M_b$:

$$(M_b)^\gamma = K_2(M_a)^\beta.$$  

(6)

This equation includes only the equilibrium constant $K_2$, because we take the insertion reaction to be rate-limiting.

We now define $M_\Sigma$ as the total amount of monazomycin in the membrane:

$$(M_\Sigma) = \beta(M_b) + \gamma(M_a).$$  

(7)

The rate of change of $M_\Sigma$ is, from Eq. 4,

$$\frac{d(M_\Sigma)}{dt} = \alpha[k_i(V)(M_a)(M_b) - k_{-1}(V)(M_b)^{\alpha + \beta/\beta}].$$  

(8)

The factor $\alpha$ arises from the fact that $\alpha$ monomers enter or leave the membrane for each $M_a$ which does so.

We now eliminate $M_b$ by substituting Eq. 6 in Eqs. 7 and 8 to give:

$$\frac{d(M_\Sigma)}{dt} = \alpha[k_i(V)(M_a)K_2^{\beta/\gamma}(M_a)^{\beta/\gamma} - k_{-1}(V)(K_2)^{\alpha + \beta/\gamma}(M_a)^{\alpha + \beta/\gamma}]$$  

$$= \beta K_2^{\beta/\gamma}(M_a)^{\beta/\gamma} + \gamma(M_a).$$  

(9)

(10)

Although we use Eqs. 9 and 10 to eliminate $M_\Sigma$, Eq. 9 is important in its own right since $d(M_\Sigma)/dt$ is proportional to the gating current of the monazomycin conductance. This follows because each monazomycin molecule which enters or leaves the membrane must have its positive charge move through the transmembrane potential difference. We will return to this topic in section entitled Gating Current.
Taking differentials in Eq. 9 yields:
\[ d(M_\gamma) = \left[ \frac{\beta^2}{\gamma} K_2^{1/\gamma}(M_\alpha)^{\beta/\gamma - 1} \right] d(M_\alpha). \] (11)

Substituting this value for \( d(M_\gamma) \) into Eq. 10 gives us our kinetic equation for \( M_\alpha \):
\[ \frac{d(M_\alpha)}{dt} = \frac{\alpha k_1(V)(M_\alpha) \left( K_2^{1/\gamma}(M_\alpha) - k^{-1}(V)(K_2)^{0/\gamma}(M_\alpha) \right)}{\gamma} + \left( 1 + \frac{1}{\gamma} \right) \gamma. \] (12)

We now proceed to show that a limiting form of Eq. 12 is equivalent to Eq. 1.

For Eq. 12 to conform to Eq. 1, it is necessary that the power to which \( M_\gamma \) is raised in the positive term be unity. This follows when we remember that if \( M_\gamma \) is very low but is increasing to a much higher final value after a voltage step, it grows in an exponential manner. Under these circumstances we require:
\[ \frac{d(M_\gamma)}{dt} \propto (M_\gamma). \] (13)

There are two conditions which satisfy this requirement. First, if \( \beta = \gamma \), Eq. 12 becomes:
\[ \frac{d(M_\alpha)}{dt} = \frac{\alpha k_1(V)(M_\alpha) K_2^{1/\gamma}(M_\alpha) - k^{-1}(V)(K_2)^{0/\gamma}(M_\alpha)}{\gamma} + \left( 1 + \frac{1}{\gamma} \right) \gamma. \] (14)

The assumption \( \beta = \gamma \) has the interpretation that any nonconducting oligomers are simply closed channels. This, we feel, puts unnecessary constraints on the system because we have no reason to believe the smaller oligomers cannot exist. Moreover, taking \( \beta = \gamma \) precludes the possibility of combining our model with that of Mueller (1979). This would be unfortunate because Mueller's model explains several observables which ours does not address (and vice versa). Accordingly, we will not analyze this case further.

The more interesting alternative is that the channels comprise a minor fraction of the total amount of monazomycin in the spanned state, that is, \( \gamma(M_\alpha) \ll \beta(M_\alpha) \). In this case, Eq. 10 becomes:
\[ (M_\gamma) = \beta K_2^{1/\gamma}(M_\alpha)^{\beta/\gamma}, \] (15)

and therefore,
\[ \frac{d(M_\gamma)}{dt} = \left[ \frac{\beta^2}{\gamma} K_2^{1/\gamma}(M_\alpha)^{\beta/\gamma - 1} \right] \frac{d(M_\alpha)}{dt}. \] (16)

Substituting the expression for \( \frac{d(M_\gamma)}{dt} \) in Eq. 16 into Eq. 9 gives:
\[ \frac{d(M_\gamma)}{dt} = \frac{\alpha \gamma}{\beta^2} \left[ k_1(V)(M_\alpha)(M_\alpha) - k^{-1}(V)(K_2)^{0/\gamma}(M_\alpha) \right]. \] (17)
Eq. 17 can be shown to have the same form as Eq. 1 as follows. First, we define a rate constant, $A'$, as:

$$A' = \frac{\alpha \gamma}{\beta^2} k_1(V) (M_a).$$

(18)

Next, at equilibrium, $d(M_a)/dt = 0$ and $(M_a) = (M_a^\gamma)$. Therefore,

$$k_{-1}(V) = k_1(V) (M_a) (M_a^\gamma)^{-\alpha/\gamma} (K_2)^{-\alpha/\beta}. $$

(19)

Substitution back into Eq. 17 gives:

$$\frac{d(M_a)}{dt} = \frac{\alpha \gamma}{\beta^2} k_1(V) (M_a) (M_a^\gamma) [1 - (M_a^\gamma)^{-\alpha/\gamma} (M_a)^{\alpha/\gamma}], $$

(20)

or, using Eq. 18,

$$\frac{d(M_a)}{dt} = A'(M_a) \left(1 - \left(\frac{M_a}{M_a^\gamma}\right)^{\alpha/\gamma}\right), $$

(21)

which has the same form as Eq. 1.

We now wish to check if $M_a^\gamma$ and $A'$ of Eq. 21 vary in the same way with membrane potential and monazomycin concentration as do $g_\gamma$ and $A$ of Eq. 1.

Eq. 19, the equilibrium condition, may be rewritten as:

$$(M_a^\gamma) = \left(\frac{k_1(V)}{k_{-1}(V)}\right)^{\gamma/\alpha} (M_a)^{\gamma/\alpha} K_1^{\gamma/\beta}. $$

(22)

Because $M_a$ is an aqueous oligomer of molecularity $\alpha$, its concentration should vary as:

$$(M_a) \propto ([\text{Mon}])^\alpha. $$

(23)

We then have:

$$(M_a^\gamma) \propto ([\text{Mon}])^\gamma, $$

(24)

which is part of the desired result.

From thermodynamics, we also know that the equilibrium constant for an $\alpha$-valent cation with access to both a reference ($V = 0$) region and a region at voltage $V$ is given by:

$$K_1(V) = \frac{k_{-1}(V)}{k_1(V)} \propto e^{eqV/kT}, $$

(25)

so that

$$(M_a^\gamma) \propto e^{eqV/kT}. $$

(26)

Relationships 24 and 26 together indicate that $M_a^\gamma$ varies with $V$ and $[\text{Mon}]$ in the fashion prescribed by Eq. 3, assuming that $s$ measures the molecularity of the channels, or in other words, that $s = \gamma$.

Inserting relationship 23 into Eq. 18 shows that $A'$ varies with $([\text{Mon}])^\gamma$, a power function as demanded by Eq. 2. Because the power of the voltage
dependence must be equal to that of the concentration dependence, we also have:

\[ A' \propto e^{aqV/kT}. \]  

(27)

Thus, Eq. 21 is equivalent to Eq. 1 if

\[ \alpha = x \quad (28a) \]

\[ B = \frac{\alpha}{\gamma} = \frac{x}{s}. \quad (28b) \]

Because \( x = 2.63 \), \( s = 5.76 \), and \( B = 0.7 \) (paper I), the second condition in Eq. 28a and b cannot be satisfied, and model I fails. Note, however, that the failure is not fundamental, because Eqs. 1 and 21 do have the same form and because all of the phenomena in these two papers are qualitatively reproduced even if we set \( B = 0.46 \) (\( = x/s \)).

C. Model II

Formally, we can resolve the difficulty by noting that Eq. 27 (via Eqs. 18 and 25) ascribes all of the voltage dependence of the insertion reaction to the forward factor rate constant \( k_1(V) \). Although this is not precluded by thermodynamics (Eq. 25), it is not required. Thus, the possibility is open that the parameter \( x \) does not measure the molecularity of \( M_a \), whereas the product \( sB \) does.

Let us define a parameter \( \Delta \) such that \( x = a\Delta \).

\[ \text{Then, the insertion reaction scheme (Eq. 4) may be rewritten as:} \]

\[ \Delta M_a + M_\beta \xrightleftharpoons[k_{-1}(V)]{k_1(V)} \left( \frac{\alpha + \beta}{\gamma} \right) M_\beta - (1 - \Delta) M_a, \]

(29)

which preserves the overall stochiometry of the original. The corresponding equilibrium equation is:

\[ \frac{(M_\beta)^{\alpha + \beta}}{(M_a)^{1 - \alpha - \beta}} = \frac{k_{-1}(V)}{k_1(V) \alpha}. \]

(30)

If we now follow the steps used to get to Eq. 21, we obtain an expression of the same form, the only difference being that \( A' \) is now given by:

\[ A' \propto k_1(V) (M_\beta)^\Delta = k_1(V) ([\text{Mon}]). \]

(31)

Of course, \( k_1(V) \) is now:

\[ k_1(V) \propto e^{a\Delta qV/kT} = e^{aqV/kT}, \]

(32)

and \( k_{-1}(V) \) varies as:

\[ k_{-1}(V) \propto e^{-(1 - \Delta)aqV/kT} = e^{(x - a)qV/kT}. \]

(33)

\[ ^4 \text{This complexity is necessary if, at the end of this derivation (Eqs. 34-36), we want to obtain the measured voltage and concentration dependencies of } A'. \text{We do to just assert the conditions expressed in Eqs. 32 and 33, the exponent for the monazomycin concentration term would remain a.} \]
The theoretical equation set that matches Eqs. 1-3 is:

\[
\frac{d(M_\gamma)}{dt} = A'(M_\gamma) \left[ 1 - \left( \frac{M_\gamma}{M_\gamma^*} \right)^{a/\gamma} \right]
\]

(34)

\[A' = A'_\gamma \left( \frac{[\text{Mon}]}{[\text{Mon}]_*} \right)^{a\Delta} e^{a\Delta qV/kT}
\]

(35)

\[(M_\gamma^*) = (M_\gamma^*) \left( \frac{[\text{Mon}]}{[\text{Mon}]_*} \right)^{\gamma} e^{\gamma qV/kT},
\]

(36)

with:

\[s = \gamma; \quad s = 5.76 \]

(37)

\[\alpha = B\gamma; \quad B = 0.7, \quad \alpha = 4.03 \]

(38)

\[\Delta = \frac{\alpha}{x}; \quad x = 2.63, \quad \Delta = 0.65. \]

(39)

Also, as is now evident,

\[A = A' \]

(40)

and,

\[\frac{(M_\gamma^*)}{(M_\gamma^*)} = \frac{g_\gamma}{g_*}.\]

(41)

There remains the problem of justifying the reaction scheme expressed in Eq. 29. Pragmatically, it (and the parameter \(\Delta\)) provides a convenient way of divorcing the rate constant \(k_1(V)\) from the molecularity of \(M_\gamma\) while preserving the relative simplicity of the derivation of Eq. 21. Furthermore, the incorporation of \(\Delta\) into our scheme allows a simple explanation of an otherwise confusing finding reported in paper I. There we showed that although the basic shape of conductance increases remained the same at very high membrane potentials, the dependence of \(A\) on \(V\) became weaker. This is now comprehensible if we imagine that \(\Delta\) decreases at high membrane potentials.

On the other hand, finding a suitable molecular interpretation for \(\Delta\) and for the occurrence of a concentration term with a negative sign is much more difficult. We will consider this issue in general terms in the discussion, and present a specific molecular model that gives rise to Eqs. 34-36 in Appendix I.

**GATING CURRENT**

Our main purpose in this section is to describe the form of the gating current during conductance changes, as predicted by our theory. As we pointed out above (Theory, part B), the flux of monazomycin between the \(cis\) solution and the spanned state—the time derivative of \(M_\Sigma\)—is proportional to the gating current. This follows because a single positive charge crosses the
membrane each time a monomer goes into the spanned state from the cis solution.

We already have an expression for $M_x$ in terms of $M_y$ (Eq. 9), so the gating current is in principle calculable. Unfortunately, the calculation involves the use of two unknown quantities, $\beta$ and $K_2^{1/\gamma}$. Thus, the best we can do at present is to see what the gating current should look like with various combinations of $\beta$ and $K_2^{1/\gamma}$.

We will restrict our guesses for $\beta$ to the range $5 \geq \beta \geq 2$. We choose 5 as an upper limit in accordance with the argument presented when we dismissed the limiting form of Eq. 12 for $\beta = \gamma$. For the lower limit, it seems reasonable to assert that dimers can exist in the spanned state, whereas monomers cannot. If our picture of the monazomycin molecule (Fig. 1) is even approximately correct, an unassociated monomer would have its row of highly polar groups directly in contact with the hydrocarbon membrane interior; it requires at least two spanned monomers in association for their polar regions to be mutually shielded from the lipid environment. We will show our predictions for the gating current at the limits of the stated range and at $\beta = 3.5$, its midpoint.

Getting rational values for $K_2^{1/\gamma}$ is somewhat more complicated. In arriving at our kinetic equation, we showed that the initial exponential growth of $g$ required that nonconducting oligomers be the predominant form in the membrane (Theory, part C). For Eq. 12 to reduce to Eq. 34, it is necessary that:

$$\frac{\beta^2}{\gamma} K_2^{1/\gamma}(M_y)^{\beta/\gamma - 1} \gg \gamma$$

(42)

at least for values of $M_y$ that correspond to conductances up to $g \approx 10^{-6} \Omega^{-1}$ the highest we looked at. Actually, as it seems that conductance stops growing with membrane potential only when $g \approx 10^{-2} \Omega^{-1}$ (Mueller, 1979), we can imagine that condition 42 holds up to $10^{-4} \Omega^{-1}$. A number for $K_2^{1/\gamma}$ may now be found for each selected $\beta$ by solving the complete differential equation with $g_\infty = 10^{-4} \Omega^{-1}$ while varying $K_2^{1/\gamma}$ until the solution is just distinguishable from ones in which $K_2^{1/\gamma}$ is arbitrarily large.

To do the actual computations, we need the constant of proportionality between $g$ and $M_y$. Based on measurements by Muller and Andersen (1981) we will take the unitary channel conductance ($g_{\text{chan}}$) as $5 \times 10^{-12} \Omega^{-1}$. (This is $\sim 10\%$ too high, but is a nice, round number.) Then,

$$(M_y) = (4.23 \times 10^{-5})g,$$

(43)

where the units of $(M_y)$ are mol·dm$^{-2}$ and $g$ is the conductance of one of our films (area = $7.85 \times 10^{-5}$ dm$^2$) in reciprocal ohms (mho). At $10^{-4} \Omega^{-1}$, $(M_y) = 4.23 \times 10^{-13}$ mol·dm$^{-2}$. By way of orientation, the surface concentration of

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5 This number is taken from the saturation level shown by Mueller (1979), taking the negative surface potential of phosphatidylglycerol membranes (and the consequent increase of conductance per channel) into account.

6 Heyer et al. (1976b) observed steady-state conductances of this magnitude that still varied exponentially with $V$ using phosphatidylglycerol-cholesterol films.
phospholipid in our phosphatidylglycerol films is $\sim 3.3 \times 10^{-8}$ mol·dm$^{-2}$ (Muller and Finkelstein, 1972 b).

In Table I, we list the value of $K_2^{1/\gamma}$ for each selected $\beta$, found as just outlined. Also presented are the magnitude of $(M_2)$ for $g = 10^{-4}$ $\Omega^{-1}$ and $g = 10^{-6}$ $\Omega^{-1}$. It is important to note that the largest number for $(M_2)$ at $10^{-4}$

| $\beta$  | $K_2^{1/\gamma}$ | $(M_2) (g = 10^{-6} \Omega^{-1})$ | $(M_2) (g = 10^{-4} \Omega^{-1})$ |
|--------|-----------------|---------------------------------|---------------------------------|
| 2.0    | 6x10^{-8}       | 1.2x10^{-10}                    | 6.1x10^{-10}                    |
| 3.5    | 6x10^{-9}       | 3.9x10^{-11}                    | 6.4x10^{-10}                    |
| 5.0    | 7.5             | 1.3x10^{-11}                    | 6.8x10^{-10}                    |

The associated values of $M_2$ (ml·dm$^{-2}$) at $g = 1 \times 10^{-10}$ $\Omega^{-1}$ and $1 \times 10^{-4} \Omega^{-1}$ are also shown.

![Figure 2](image)

**Figure 2.** The S-shaped curve is a calculated conductance increase from $g_0 = 1 \times 10^{-10}$ $\Omega^{-1}$ to $g_\infty = 1 \times 10^{-6}$, with $A = 5 \times 10^{-1}$ s$^{-1}$ and $B = 0.7$, no scale is shown. The three peaked curves are calculated gating fluxes, any of which could give rise to the conductance change, given proper values of $\beta K_2^{1/\gamma}$. Going from the largest to the smallest, the pairs of $\beta$ and $K_2^{1/\gamma}$ were: 2.0, 6 x 10$^{-8}$; 3.5, 6 x 10$^{-9}$; 5.0, 7.5. The peaks of the three gating fluxes are: 7.6 x 10$^{-11}$, 3.0 x 10$^{-11}$, and 1.1 x 10$^{-11}$ mol·dm$^{-2}$·s$^{-1}$. For membranes 0.8-mm$^2$ in area, the peak gating currents are 5.8 x 10$^{-10}$, 2.3 x 10$^{-10}$, and 8.3 x 10$^{-11}$ A. $\Omega^{-1}$ is $\sim 7 \times 10^{-10}$ mol·dm$^{-2}$; this is only $\sim 2\%$ of the surface concentration of phospholipid and seems to us to be not unrealistically high.

In Fig. 2, we show gating currents $(I_g)$, calculated using the numbers from $7$ Calculating $(M_2)$ at $g = 10^{-2}$ $\Omega^{-1}$ reveals that $(M_2)$ for $\beta = 3.5$ and 5 grows very rapidly, so that it becomes 30% of the phospholipid concentration for $\beta = 3.5$ and is 110% at $\beta = 5$. It is still only $\sim 10\%$ for $\beta = 2$. 
Table I, on the same time axis as the conductance response from \( g_0 = 1 \times 10^{-10} \Omega^{-1} \) to \( 1 \times 10^{-6} \Omega^{-1} \). It is evident that \( I_g \) will have a pronounced peak, regardless of what \( \beta \) and \( K_1^{1/\gamma} \) turn out to be. It is also interesting that the time of occurrence of the peak gating current is very insensitive to the choice of \( \beta \) and \( K_2^{1/\gamma} \), even though the maximum \( I_g \) is \(~6.5\) fold higher when \( \beta = 2 \) than when \( \beta = 5 \).

Fig. 3 shows the computed gating currents that flow during a conductance decrease; note the difference in time scales between Figs. 2 and 3. As we would expect from the model, the maximum of \( I_g \) is now at \( t = 0 \), even though the decay of \( I_g \) is certainly not a first-order decay. Furthermore, the magnitudes of the maximums are much greater than those calculated for conductance increases with corresponding values of \( \beta \) and \( K_1^{1/\gamma} \). This is true even though we used a value of \( A \) 40-fold lower for the curves in Fig. 3 than for those in Fig. 2.

In light of the strictly theoretical nature of the preceding discourse, it seems gratuitous to say that real measurements of \((M_x)\) and \(I_g\) would be desirable. With regard to the latter, our theory is at least consistent with our inability to find any asymmetry of the capacitance current after voltage steps from \( V = 0 \) to equal positive and negative levels (R. U. Muller, unpublished observa-
On the other hand, we have seen negative current tails when steps were made to $V = 0$ from positive potentials after a steady-state conductance had been reached. Because these experiments were done in symmetrical salt, the ionic current should have been zero. Although we are not yet confident that these tails represent the gating current, they are not unreasonably large and, encouragingly, are not present when the experiment is repeated using the carrier valinomycin. In any case; given the success of our model in other areas, we feel that it will be most profitable to look for the gating current during conductance decreases.

Finally, if the offset gating current really is as large and fast as our theory predicts, we can provide an explanation of a phenomenon reported by Mueller (1979). In Fig. 1c of his paper, conductance decreases from an initial high level to a wide range of lower levels are seen to be nonmonotonic; after an initial ohmic current jump, the current goes through a maximum before relaxing toward the steady-state. The maximum is most pronounced for intermediate-sized negative voltage steps.

Qualitatively, we can ascribe the transient conductance increase to an upward perturbation of the juxtamembrane monazomycin concentration caused by the rapid efflux after a negative voltage step. The smallness of the maximum for small negative-going voltage steps would then be due to the relatively low amount of monazomycin that leaves the membrane. The disappearance of the maximum for large negative voltage steps would reflect that the potential can be set low enough that essentially no monazomycin will tend to enter the membrane, no matter how high the aqueous concentration may temporarily become.

**INACTIVATION**

Heyer et al. (1976b) demonstrated that the conductance responses we have been discussing can be altered to resemble those of the sodium gating system of squid giant axons in that they exhibit inactivation. (Inactivation is said to occur when a conductance increase at constant potential goes through a maximum before the steady-state is reached.) This "depletion" inactivation, which is seen at high conductance when the aqueous monazomycin concentration is low enough, depends on the existence of a monazomycin flux through the membrane into the trans solution. When the flux is great enough, the monazomycin concentration at the cis solution-membrane interface falls and the conductance comes down from its maximum.

The magnitude of the steady-state flux was shown by Heyer et al. (1976b) to be proportional to the steady-state conductance. Here we will assume that the proportionality holds at all times so that a new term must be included in Eq. 9, which now becomes:

$$\frac{dM_o}{dt} = \alpha k_1(V) (M_o)^{\Delta + \gamma} (M_v)^{\beta / \gamma}$$

$$- \alpha k_{-1}(V)(M_o)^{-(1-\Delta)} (K_2)^{\beta / \gamma} (M_v)^{\gamma} - \gamma k_3(M_v), \quad (44)$$

where $k_3$ is the rate constant for the depletion process.
We will show that the rate of monazomycin removal from the membrane due to the transmembrane flux is very small compared with the rate at which it returns to the cis solution. In other words, we want to prove that the back flux in the insertion reaction is the major determinant of the steady-state level of $(M_z)$.

The total flux of monazomycin into the membrane $(\Phi_{in})$ may be calculated from:

$$\Phi_{in} = \frac{\beta^2}{\gamma} AK^{1/\gamma}(M_z)^{\beta/\gamma}. \quad (45)$$

Steady-state values of $\Phi_{in}$ for our three $\beta$-$K^{1/\gamma}$ combinations are shown in Table II. The smallest of these, for $\beta = 5$ and $g_{en} = 1 \times 10^{-6} \Omega^{-1}$, is $5.7 \times 10^{-12}$ mol·s$^{-1}$·dm$^{-2}$. The depletion flux at the same steady-state conductance is $4.7 \times 10^{-14}$ mol·s$^{-1}$·dm$^{-2}$, < 1% of $\Phi_{in}$. Thus, our earlier equilibrium treatment of this system is not much in error. In the near future we hope to report on the results of calculations in which the kinetic equation (modified for the transmembrane flux) is coupled to the diffusion equation for monazomycin in the cis unstirred layer; such calculations should reproduce the actual time-course of the inactivation.

### Table II

| $\beta$  | $\Phi_{in}$ (mol·dm$^{-2}$·s$^{-1}$) |
|----------|-------------------------------------|
| 2.0      | $2.1 \times 10^{-11}$               |
| 3.5      | $1.2 \times 10^{-11}$               |
| 5.0      | $5.7 \times 10^{-12}$               |

Steady-state values of $\Phi_{in}$ (mol·dm$^{-2}$·s$^{-1}$) associated with the three values of $\beta$ used in the gating current and inactivation calculations.

**Memory**

What we refer to as a memory in the monazomycin system can be demonstrated with a simple experimental protocol. Once a steady-state conductance is reached at a positive potential $(V_{on})$, the voltage is switched to a more negative potential $(V_{off})$; we often used $V = 0$ for a certain amount of time $(t_{off})$. The original potential is then reimposed, and the amount of time it takes the conductance to get to one-half of the steady-state level $(t_{1/2})$ is measured. This procedure is then repeated for various $t_{off}$. In Fig. 4, we plot $t_{1/2}$ as a function of log $t_{off}$; we see that the speed of the onset kinetics, as measured by $t_{1/2}$, decreases continuously over the range $3 \leq t_{off} \leq 1,800$ s.

By "memory," we therefore mean that from a knowledge of the kinetics just after $t = 0$ (the instant of the voltage step), it is possible to determine the time that has passed since the previous voltage step was turned off. The membrane "remembers," in a detectable way, when it was last stimulated.

Except for the point at $t = 3,600$ s, the data are quite typical of this kind of experiment. There is generally a good linear relationship between $t_{1/2}$ and log
Figure 4. Demonstration of the memory exhibited by monazomycin-modified membranes. The data points were obtained as described in the text. $V_{on} = 45$ mV, $V_{off} = 0$ mV. The mean steady-state conductance for the plotted points whose $t_{1/2}$ was measured was $\sim 2.7 \times 10^{-7}$ $\Omega^{-1}$. The monazomycin concentration was $2.0 \times 10^{-6}$ g/ml. The line drawn from $t_{off} = 1$ to $100$ s is the same line which passes through the theoretically calculated points in Fig. 5. The line drawn from $t_{off} = 100$ to $2,000$ s is fitted by eye.

t_{off} \leq 100$ s. For larger values of $t_{off}$, the curve generally accelerates upwards, sometimes as shown, sometimes even more steeply. The point at 3,600 s is anomalous because it is the only one we have for off-times that long. Thus, we are not sure if the flattening between 1,800 and 3,600 s is real. We will return to this point shortly.

The curve in Fig. 5 was calculated by first determining how $g$ declined from $g_0 = 3.0 \times 10^{-7}$ $\Omega^{-1}$ to $g_\infty = 1.9 \times 10^{-10}$ $\Omega^{-1}$ with $A = 0.01$ s\(^{-1}\). $t_{off}$ is represented by the time elapsed from $t = 0$. We then used $g (t_{off})$ for selected values of $t_{off}$ as $g_0$ for a new set of solutions with $g_\infty = 3.0 \times 10^{-7}$ $\Omega^{-1}$ and $A = 0.32$ s\(^{-1}\). Finally, we took $t_{1/2}$ from each such solution to get the curve.

The correspondence between the data in Fig. 4 and the theoretical curve is very close for $t_{off} \leq 100$ s. For longer times, however, the theoretical curve approaches a horizontal asymptote as the experimental relationship accelerates upwards, although, as noted before, the experimental curve also becomes horizontal between 1,800 $< t_{off} \leq 3,600$ s.

The reason for the horizontal asymptote of the theoretical curve lies in the continuous nature of our model. We are implicitly treating the concentrations of nonconducting oligomers and of the channels as space-averages. Because $g$ at $V_{off}$ smoothly approaches $g_\infty$ in a decelerating fashion, the value of $g_0$ we use to get $t_{1/2}$ at $V_{on}$ itself reaches a minimum. Obviously then, solutions for the conductance rise (and therefore $t_{1/2}$) must begin to differ less and less.

In the real case, when $g_0$ for the conductance rise is very small, the remaining
monazomycin molecules in the membrane must be much further apart than at high $g$. Then, remembering that insertion is autocatalytic, we are forced to picture the membrane as inhomogeneous. The conductance increase should proceed "normally" near the remaining molecules, but will not take place at all in the regions between them until lateral diffusion in the plane of the film begins to generate an even spatial distribution of monazomycin. The upward acceleration of the data points thus represents the increased amount of time required to get to the steady-state when lateral diffusion times begin to exceed the time constant of the conductance increase of the continuous approximation.

Ultimately, we expect $t_{1/2}$ to reach a maximum, and thereafter to become independent of $t_{off}$; this will occur when $g_m$ is reached at the more negative potential, regardless of whether more than one process determines the time course of approach to $g_m$ at the higher potential. However, if $g_m$ at the more negative potential is low enough, or goes to zero, so that there is no monazomycin remaining in the membrane, $t_{1/2}$ will become a stochastic variable. The random fluctuations of $t_{1/2}$ around some mean will then reflect the statistics of the very-low-probability, noncatalyzed insertion of monazomycin that must exist (paper I). Thus, our uncertainty about the mean of the point at $t_{off} = 3,600$ s.

It is worthwhile to point out that these memory experiments are difficult to do because they require the steady-state conductance at the selected positive potential to be stable within $\sim 10\%$ over a period of several hours. Often, this criterion was not met, with the result that the variation of $t_{1/2}$ with $t_{off}$ became partially confounded with changes in the kinetics directly attributable to

![Figure 5](image-url)

**Figure 5.** The relationship between $t_{1/2}$ and $t_{off}$ as calculated from Eq. 1. The parameters used were: $g_m^{46 \text{ mV}} = g_0^{46 \text{ mV}} = 3 \times 10^{-7} \text{ \Omega}^{-1}$, $g_m^{0 \text{ mV}} = 1.9 \times 10^{-10} \text{ \Omega}^{-1}$, $A^{46 \text{ mV}} = 0.322$, $A^{0 \text{ mV}} = 0.01$, $B = 0.7$. Further description in text. The dots in this figure merely indicate the specific $t_{off} = t_{1/2}$ points at which calculations were done; they are not data points.
changes of $g_m$. Nevertheless, the main effect was still apparent even with conductance changes (at constant potential) of $\geq 50\%$. The membrane used for the experiment of Fig. 6 showed a slow oscillation of $g_m$ at $+85$ mV from $5.3 \times 10^{-8} \Omega^{-1}$ to $8.0 \times 10^{-8} \Omega^{-1}$ and back down to $5 \times 10^{-8} \Omega^{-1}$ over $\sim 1.8$ h. Two sets of $t_{1/2}$ measurements were taken, both with ascending values of $t_{\text{off}}$, so the experiment was replicated as $g_m$ increased and then fell. Although the effect of the $g_m$ oscillation is evident in Fig. 6, the shapes of both sets of data are substantially the same.

![Graph](image)

**Figure 6.** Persistence of the relationship between $t_{1/2}$ and $t_{\text{off}}$ in the presence of slow shifts of $g_m$ at constant $V_{\text{on}}$. The lower line (dots) was obtained first, as $g_m$ rose from $5.3 \times 10^{-8}$ to $8.2 \times 10^{-8} \Omega^{-1}$; the points were taken with the shortest value of $t_{\text{off}}$ first. The upper line (crosses) was obtained later, as $g_m$ fell from $8.2 \times 10^{-8}$ to $5.1 \times 10^{-8} \Omega^{-1}$, again with the shortest value of $t_{\text{off}}$ first. $V_{\text{on}}$ was $85$ mV, $V_{\text{off}}$ was $0$ mV. The monazomycin concentration was $3 \times 10^{-6} \text{g.m}^{-1}$.

With this in mind, we show in Fig. 7 the effect on $t_{1/2}$ of varying $V_{\text{off}}$. In this experiment, $t_{1/2}$ increased at constant $t_{\text{off}}$ as $V_{\text{off}}$ was made more negative, causing an approximately parallel upward shift of the curves. Although this was generally the pattern, the effect of varying $V_{\text{off}}$ is weak enough that conductance drifts could cause the curves at different $V_{\text{off}}$ to cross.

The data in Fig. 8 are from an experiment in which $V_{\text{off}}$ was always $0$ mV and $V_{\text{on}}$ was varied. Here, the decreasing slope of the $t_{1/2}$-$t_{\text{off}}$ characteristic with $V_{\text{on}}$ is clear; this change in slope reflects the faster kinetics at higher membrane potential.

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In this figure, $t_{1/2}$ was measured at the point $g_{1/2} = (g_m - g_0)/2$ rather than at $g_{1/2} = g_m/2$. The reason for this is that for short $t_{\text{off}}$ the first way of calculating $t_{1/2}$ gave longer values at high $V_{\text{on}}$ because $g(t_{\text{off}} = 3 \text{ s})$ is a very big fraction of $g_m$ for low $V_{\text{on}}$. 
DISCUSSION

A. Limitations of the Model

The description we have provided of the kinetic behavior of monazomycin-induced conductance contains defects of several kinds. Two of these are experimental in nature; the other two are theoretical.

The first sort of problem concerns the restrictions we imposed on the range of our experimental variables. Our model seems to be quantitatively accurate for membrane potentials such that \(-50 \leq V \leq 100\) mV, and for monazomycin concentrations such that \(0.25 \leq [\text{Mon}] \leq 9.0 \times 10^{-6}\) g/ml. Taken at face value, these limits would allow for a 10-fold conductance variation with 60
50
40
30
20
10
0
-10
-20
-30
-40
V_{off} = -60 \text{mV}
V_{off} = -50 \text{mV}
V_{off} = -40 \text{mV}
V_{off} = -30 \text{mV}
V_{off} = -20 \text{mV}
V_{off} = -10 \text{mV}
V_{off} = 0 \text{mV}

FIGURE 7. The increase of \(t_{1/2}\) at constant \(t_{off}\) as \(V_{off}\) is made more negative. The values of \(V_{off}\) are shown on the graph. \(V_{on} = 59\) mV; \(g_{\infty}\) was \(\approx 2.1 \times 10^{-1}\) \(\Omega^{-1}\). The monazomycin concentration was \(2.0 \times 10^{-6}\) g.ml\(^{-1}\).

monazomycin concentration (at fixed \(V\)) and a 10\(^{14}\)-fold variation with membrane potential (at fixed [Mon]). Because our conductance measurements were held between \(10^{-10} \leq g \leq 10^{-6}\) mho, it is evident that we investigated only certain combinations of \(V\) and [Mon], and it is also evident that other combinations should be tried. Of interest would be measurements made at much higher conductance,\(^9\) aimed at seeing how the system saturates with respect to \(g_{\infty}\) and \(A\) at the same time. It would also be useful to look more closely at changes in the strength of the concentration and voltage dependences of \(g_{\infty}\) and \(A\) in the presence of low monazomycin concentration at high applied potentials (Mueller, 1979; and R. U. Muller, unpublished observations).

\(^9\)This could be done with a four-electrode voltage clamp. The same thing could also be effectively accomplished by lowering the conductance per channel, and our real interest lies in how the system behaves at high \(M_{\infty}\) rather than in the process of ion transport per se.
Because these are the conditions best suited for observing single channels, such data would be particularly helpful in establishing the link between unitary conductance events and the behavior of multichannel membranes.

The second problem involves the use of unknown quantities—$\beta$ and $K_2$—in gating current and flux calculations, or to put it more directly, our lack of actual gating current measurements. Our model makes predictions with regard to the shape of the gating current (Fig. 2) and the relationship between $M_z$ and $M_y$, as in Eq. 10. Taking logarithms in this equation gives:

$$\log(M_z) = \frac{\beta}{\gamma} \log(M_y) + \log(\beta K_z^{1/\gamma}). \quad (10a)$$

Thus, the relationship between $\log(M_z)$ and $\log(M_y)$ should be linear (in the range of $M_y$ such that the approximation that leads to Eq. 34 holds). Assuming this turns out true and knowing $\gamma$, the magnitude of $\beta$ can be obtained from the slope of the line. The product $\beta K_z^{1/\gamma}$ can be found by setting $(M_y) = 1$ so that $K_2$ is obtained also. In principle, of course, $\beta$ and $K_2$ can be estimated from any method that measures $M_z$ when $M_y$ is known. One possibility is to use a fluorescent monazomycin derivative, but this would probably prove difficult because of the high water solubility of monazomycin. A second method would be to add a known amount of single-walled vesicles and to measure the conductance decrease due to the reduced monazomycin concent-

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10 In Eq. 10a we are implicitly referring the surface concentrations $M_z$ and $M_y$ to a standard value of 1 mol·dm$^{-2}$. Because the highest $M_y$'s we deal with are $\approx 10^{-14}$ mol·dm$^{-2}$, a lot of extrapolation is necessary.
tration. This is easy to do physically, but many assumptions about the state of the vesicles would be necessary.

Finally, there are difficulties in the formalism itself, two of which we will analyze. First, there is the "fix" we used in Eqs. 29-41: the introduction of the parameter \( \Delta \). This was done to allow for voltage dependence of the contribution of both \( k_1(V) \) and \( k_{-1}(V) \) to the voltage dependence of the equilibrium constant of the insertion reaction, \( K_1(V) \).

Formally, the appearance of \( \Delta \) is certainly allowed and, in a sense, is required. \( K_1(V) \) was defined as:

\[
K_1(V) = \frac{k_{-1}(V)}{k_1(V)}.
\]

Because the entering (and exiting) species is an \( \alpha \)-valent cation, in the absence of other significant energy terms, \( K_1(V) = K_1^0 e^{-\alpha qV/kT} \) where \( K_1^0 = K_1(V = 0) \). This makes it evident that \( k_1(V) \) and \( k_{-1}(V) \) must both be exponential functions of the voltage:

\[
k_1(V) = k_1^0 e^{\Delta \alpha qV/kT}
\]

\[
k_{-1}(V) = k_{-1}^0 e^{\rho \alpha qV/kT}.
\]

Since

\[
\frac{k_{-1}^0}{k_1^0} = K_1^0,
\]

we have, from Eq. 5,

\[
e^{\rho \alpha qV/kT} = e^{-\Delta \alpha qV/kT}.
\]

Taking logarithms and solving for \( \rho \) shows that \( \rho = -(1 - \Delta) \). Thus, including \( \Delta \) is simply an identity from the thermodynamic point of view. Kinetically, however, \( \Delta \) must be used if \( k_{-1}(V) \) is to vary with voltage.

Splitting the voltage dependence of the insertion reaction in this way is equivalent to including a negative term in \( M_\alpha \) on the right side of Eq. 29, the reaction scheme. Such a negative term means that the species involved is acting as an inhibitor. In other words, \( M_\alpha \) participates in a "normal," mass-action fashion as far as insertion is concerned but acts in addition to reduce the rate at which monazomycin removal goes on.

Although there may be a molecular model that directly corresponds to the proposed reaction scheme, we have not been able to find it. Nevertheless the final kinetic picture (Eqs. 34-36) can be shown to arise from a model which breaks up the insertion reaction into two steps. We present this as Appendix I for two reasons. First, it is appropriate to do so after we discuss the "serial-aggregation" scheme of Mueller (1979), to which it bears a relatively strong resemblance. Second, the new model does not have the simplicity of our original. Its peculiarities are such that we would rather treat it as a proof that the kinetic equations have a physical embodiment rather than as a definitive picture.
The second difficulty with the formalism lies in its failure at very low conductance. We have already seen (section entitled Memory) that the model makes incorrect predictions for the dependence of $t_{1/2}$ on $t_{off}$ in memory experiments. As we noted before, after a positive voltage step from a low conductance state the formation of new channels will initially be confined to loci right around the few monazomycin molecules that were still in the membrane. Our model has no provision for describing the time it takes for lateral diffusion of oligomers to fill in the spaces between the original seeds, and therefore must produce incorrect results when lateral diffusion times become significant compared with $1/A$. In addition, our omission of the effects of lateral diffusion must also cause calculations of the time-course of conductance decays toward low levels to be erroneous; if monazomycin exit is indeed autocatalytic, large intramolecular distances will slow the real process compared with calculations based on a spatially averaged concentration.

Neglect of intermolecular distance is not the only cause of inaccuracies at low conductance. Our continuous model always supplies a non-zero number for the total monazomycin concentration in the film and therefore always predicts a non-zero conductance. The problem here is not that conductances lower than $g_{chan}$ can arise. Fractional values of $g_{chan}$ are perfectly acceptable as time averages for states in which the probability of one open channel is low. Rather, with a discrete model, it is possible for all of the monazomycin to leave the membrane. Then the conductance will drop to zero and stay there until such time as a molecule enters via the noncatalyzed pathway discussed in paper I. Events in which the single channel activity suddenly ceases are relatively common (Muller and Andersen, manuscript in preparation); these are outside the discourse of our present model.

For the reasons just given and others which will be included in Muller and Andersen (manuscript in preparation), we feel that it will be worthwhile to develop the stochastic analogue of our kinetic scheme. The result of such an analysis would be especially interesting if lateral diffusion was treated explicitly, as opposed to taking the space average.

**B. Comparison with the Serial-Aggregation Scheme**

Baumann and Mueller (1974) and Mueller (1979) have proposed a kinetic scheme for monazomycin-induced conductance whose basis is also coupled insertion and aggregation reactions. It differs from ours in two critical ways. First, the insertion reaction is taken to be a first-order reaction. (Monomers are assumed to be the entering species, but this need not be made a crucial part of the picture.) Second, the rate constants for aggregation of two monomers to form a dimer, a dimer and a monomer to form a trimer, etc., and the rate constant for each possible disaggregation step are specified. In oxidized cholesterol membranes modified by excitability-inducing material (EIM), membrane potential controls the fraction of time a channel spends in the open state (Eherenstein et al., 1970). Thus, in principle, the (time average) conductance of a film with just one channel can take on any value between $g = 0$ and $g = g_{chan}$. In addition, differences in the conductance of the various oligomers may be included. Mueller (1979) set the conductance of monomers and dimers to zero and that of higher oligomers (up to the sixmer) to be all equal.
other words, the aggregation reactions are not considered to be near equilibrium during conductance changes.

The power of this picture derives from the heterogeneity of the several rate constants. By suitably adjusting the rate constants for (nonconducting) dimers in particular, Mueller (1979) has shown that a very interesting form of inactivation is possible. The basis of this process is a rapid generation of conducting oligomers followed by a slower relaxation to a state in which dimers predominate. In similar fashion, other cases of nonmonotonic approach to the steady-state, which are experimentally observable, can be predicted.

In contrast, our model is based on a single first-order differential equation and therefore cannot generate anything but monotonic approaches to the steady-state unless additional process are considered. We think that depletion inactivation will occur with our scheme if monoazomycin diffusion through the cis unstirred layer is included and have tentatively suggested that transient monoazomycin concentration increases may be responsible for the short-lived conductance increases that are sometimes seen with negative-going voltage steps. Presumably, other post hoc additions to the autocatalytic model could explain other phenomena. Nevertheless, it seems most reasonable to us to ultimately combine the two schemes. Despite demonstrated successes, we cannot ignore the subtleties of aggregation indefinitely.

We have not investigated the possibility that serial-aggregation per se can predict the exponential growth of $g$ with positive voltage steps, the far from first-order conductance decays, or the memory. On the face of it, this seems unlikely, given that all of the proposed reactions are of a first-order nature, but only a real analysis will tell. In any case, the best discrimination between the two schemes in their present forms will be provided by gating current measurements, because serial-aggregation demands a first-order entry process, whereas autocatalysis predicts a clear-cut maximum significantly after the voltage step.

C. Implications for Biological Voltage-dependent Conductances

It is well known that the original Hodgkin and Huxley (1952b) scheme for the potassium gating system gives solutions that rise too quickly when their parameter $n$ is raised to the fourth power. If $x$ is the power, setting $x \geq 6$ or generates better fits when the holding potential is near the undisturbed resting potential, but if the axon is hyperpolarized before being depolarized, it is necessary to let $x \approx 25$ to get enough "delay" (Cole and Moore, 1960). As has

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13 It is possible to see a form of inactivation whose properties (as far as they have been investigated) are commensurate with Mueller's proposal. In particular, the steady-state conductance continues to grow exponentially with voltage, even when inactivation is present. This means that the peak conductance grows faster than exponentially (R. U. Muller, unpublished observations). In depletion inactivation (Heyer et al. 1976b) and long-chain quaternary ammonium-based inactivation (Heyer et al. 1976a) the voltage dependence of $g$ weakens as inactivation appears, and the peak conductance never reaches the level of $g_e$, which would have been expected for the applied potential had inactivation not occurred.
been pointed out (e.g., see Begenisch [1979]), such large powers for \( n \) make the interpretation that a potassium channel consists of \( x \) independent subunits untenable.

Because increased duration of the initial horizontal limb of a potassium conductance increase is an automatic consequence of hyperpolarization in our model (corresponding to a decrease of \( g_0 \)) we checked to see if our kinetic equation could reproduce the potassium data of Hodgkin and Huxley (1952b). Plotting \( \log g_K \) vs. \( t \) for the largest steps in Fig. 3 of Hodgkin and Huxley (1952b) shows that the first decade of conductance change can be quite well described as a growth regime. For the 109-mV step of that figure the time constant (defined as \( 1/A \)) is \( \sim 1.9 \times 10^{-4} \) s, as compared with the value for \( \tau \) of \( 1.05 \times 10^{-3} \) s in the Hodgkin and Huxley (1952b) scheme. Taking \( 1/A = 1.9 \times 10^{-4} \) s and extrapolating back to \( t = 0 \) sets \( g_0 = 5.6 \times 10^{-5} \Omega^{-1} \text{cm}^{-2} \).

With the measured level of \( g_m \) (20.7 \( \times 10^{-3} \Omega^{-1} \text{cm}^{-2} \)) we used our empirical equation (Eq. 1) and varied \( B \) to look for a fit. None of the solutions thus obtained were even as good as the one published by Hodgkin and Huxley (1952b). In particular, if \( B \) was large enough to make the solution conform to the first four data points, the subsequent rise to the steady-state is much faster than the real system. Even using our full kinetic equation (Eq. 12) and varying \( \beta \) and \( K_{1/2} \) to ridiculous extremes was of no avail.

Although this is somewhat disappointing, it is not unexpected because we derived Eq. 12 assuming an infinite pool of potential channel-formers, an absurdity in the case of system with membrane-bound channels. It is probably worth the effort to derive an analogue of Eq. 12 (with autocatalysis) taking the depletion of the \( M_\alpha \) form into account as more and more monomers (subunits) go into the \( M_\beta \) form. This is certainly an appropriate modification and is in the right direction to decelerate the conductance growth more rapidly without affecting the initial exponential rise.

In another sense, our failure to reproduce the potassium kinetics with Eq. 12 is a nice result. It proves that our scheme is not so general as to allow for any sort of S-shaped conductance growth.

An additional comment about the potassium conductance is in order. The issue of whether or not conductance changes from different starting potentials to the same final level superimpose is not the same as deciding if cooperativity among subunits exists. As Hill and Chen (1971) pointed out, superimposition is possible with cooperative or independent subunit movement, so long as the reaction path goes only through equilibrium states. To reinforce this, we note that it is hard to imagine a more cooperative scheme than ours; nevertheless, that the time derivative of the conductance depends only on the conductance requires superimposition.

The fact that superimposition does not hold for the monazomycin system

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14 This very low value for \( g_K \) is not without precedent. Hill and Chen (1971) report that the Hodgkin and Huxley (1952b) scheme can do very well if it is assumed that the values of \( g_K \) at short times are artifactual in the sense they are due to some conductance other than \( g_K \) itself so that \( g_K \) may be taken as smaller than the \( 2.4 \times 10^{-4} \Omega^{-1} \text{cm}^{-2} \) used in the original work.
for short off-times (see paper I, Results: section H) thus requires an explanation other than cooperativity. The most likely one is serial aggregation; our best guess at this moment is that the faster than expected (from superimposition) rise of \( g \) is a nascent form of the type of inactivation we discussed above.

With regard to the possibility of cooperativity within the sodium gating system of squid giant axon, the stubborn persistence of the finding that the maximum gating current occurs significantly after the moment of a positive-going step (e.g., see Armstrong and Bezanilla [1977]) comes to mind. Also suggestive is the observation by Hodgkin and Huxley (1952 a) that the initial decay of the sodium conductance is too rapid for a first-order process.

**D. Implications for Biological Memory**

At present, most neuroscientists agree that the locus of memory is the synapse—the functional connection between neurons. More specifically, changes in the efficacy of transmission across synapses are thought to be the cellular bases of changes in the responses of an organism as a function of experience.

It seems to us that the existence of a monazomycin-like molecule within the nervous system could provide a molecular-level explanation of how variations in the use of a synapse can lead to variations in the magnitude of effect that a presynaptic cell exerts on a postsynaptic partner. By "monazomycin-like" we mean that the molecule should induce a voltage-dependent conductance with kinetics that are describable by our autocatalytic scheme; details such as the sign of the voltage dependence, the particular ions that are permeant, and the time-scale of the effects can all be treated as parameters to be varied. The arguments we will present here are strictly qualitative; numerical calculations on appropriate model neurons are now being worked on.

The most general way of stating the hypothesis is to say that a monazomycin-like conductance can act as an amplifier (with positive or negative feedback) of the postsynaptic conductance triggered by the chemical transmitter. Imagine that the "monazomycin" is located within the postsynaptic cytoplasm, that its voltage dependence has the same sign as the actual antibiotic, and that it is indiscriminately permeant to univalent cations or selective for sodium. Such an arrangement would tend to augment the magnitude of the depolarization of an excitatory postsynaptic potential (EPSP) in a frequency-dependent way; the result would be a synapse that shows sensitization. On the other hand, by "changing" to potassium selectivity, this parallel conductance would tend to decrease the size of an EPSP; such a synapse would exhibit habituation.

It is also possible to build associative synapses, in which the change in efficacy depends on the activity of other inputs to the postsynaptic cell as well as that of the synapse under consideration. Here we imagine that the "monazomycin" is released from the presynaptic terminal along with the transmitter substance, so that it is presented (in significant quantities) only to the postsynaptic terminal (the subsynaptic membrane). This arrangement allows several independently modifiable synapses to be present on the same neuron.
We now take advantage of the strong voltage dependence of the conductance by placing the synapse on a distal dendrite, which we assume to be electrically inexcitable. The subsynaptic membrane will then be subjected to an EPSP of amplitude $V_1$ and an attenuated action potential of amplitude $V_2$. Temporal overlap of an EPSP and an action potential would then generate a voltage change of maximum size $V_1 + V_2$.

Occurrence of either the EPSP or action potential alone would not generate much, if any, parallel conductance, the former due to the relatively small size of $V_1$, the later because no "monazomycin" is present except during synaptic action and also because $V_2$ itself might not be great enough. On the other hand, one or more "simultaneous" pairings of EPSP and action potential would cause monazomycinoid to enter the membrane so that subsequent EPSP would be accompanied by a parallel voltage-dependent conductance increase, in which case we would say that synaptic transmission efficacy had increased. If the EPSP-plus-monazomycinoid conductance change remained below threshold for the action potential, the augmentation of the EPSP would decay if no further pairings happened. By contrast, if the combined conductance became capable of evoking a spike, the process would be self-perpetuating, so long as the input pathway was activated with sufficient frequency.

Another, quite different sort of role for a monazomycin-like memory molecule is suggested by the work of Kandel and his colleagues on aplysia. They have shown (e.g., see Kandel [1979]) that habituation of the gill withdrawal reflex is a result of a use-dependent decrease of presynaptic calcium conductance, which in turn causes the quantal output of the terminal to decrease. Such an effect is consistent with modulations of a calcium-permeant, monazomycin-type gating system. This suggestion per se is not very thrilling, as a host of other possibilities exist to explain the decreased calcium conductance. What is of direct interest is that a quantitative description of the time-course and magnitude of the suggested calcium modulating system is available; our proposal can be tested in detail.

Finally, we note that holding mechanisms of the type suggested must be considered under the rubric of short-term, labile memory; all of the changes involved are reversible in the thermodynamic sense of the word. What is intriguing is that the proposed mechanisms will show "forgetting" without any further assumptions; mere disuse is sufficient. Moreover standard manipulations that cause loss of short-term memory, such as electro-convulsive shocks, would also be expected to play havoc with the kind of holding mechanisms postulated. A search for monazomycin-like molecules of neural origin might be quite profitable.

In this case the voltage dependence of the conductance must be the reverse of the actual case; that is, more channels are formed when the potential of the trans (intracellular) solution becomes more positive with respect to that of the cis (extracellular) solution.

The idea that monazomycin could be released from presynaptic terminals may be extended to include the idea that a transmitter may act as part or all of its associated conductance pathway instead of simply acting as a trigger; such a conductance might or might not exhibit significant voltage dependence. A good candidate for a molecule that could act in this way is substance $P$, because it is a rather large and complex species.
APPENDIX I

We prove here that if the insertion reaction is decomposed into two steps, it is possible to obtain the correct kinetic scheme without the need to use a negative concentration term. It is worthwhile pointing out that the idea of inhibition nonetheless comes into play.

The two parts of the insertion reaction may be written as:

\[ M_{a_1} + M_{\beta} \xrightleftharpoons[k_{-1}(V)]{k_1(V)} \tilde{M} \]  
(A 1)

and

\[ M_{a_2} + \tilde{M} \xrightleftharpoons[k_{2}(V)]{k_2(V)} M_{\beta}. \]  
(A 2)

\( \alpha_1 \) and \( \alpha_2 \) are the molecularities of two aqueous oligomers such that \( \alpha_1 + \alpha_2 = \alpha \). \( \tilde{M} \) is an intermediate spanned species which we choose not to write as \( M_{(a_1 + \beta)} \) because we preclude its direct incorporation into channels. Reaction A 1 is rate-limiting; reaction A 2 is assumed to be at equilibrium as expressed by use of the equilibrium constant \( [K_2(V)] \) only. As should be evident, \( \alpha_1 \) takes the place of the product \( \Delta \alpha \) and is numerically equal to the parameter \( \alpha \).

The aggregation reaction is unchanged:

\[ M_{\beta} \xrightleftharpoons[\gamma]{K_2(V)} M_{\gamma}. \]  
(A 3)

As before, we will assume that \( M_{\beta} \) comprises essentially all of the monazomycin in the membrane. The concentrations \( \tilde{M} \) and \( M_{\gamma} \) will be made to approach zero by approximately picking \( K_2(V) \) and \( K_1(V) \).

We may express the rates of change of the spanned species as:

\[ \frac{d(\tilde{M})}{dt} = \Phi_1 - \Phi_2 \]  
(A 4)

\[ \frac{d(M_{\beta})}{dt} = -\Phi_1 + \left( \frac{\alpha + \beta}{\beta} \right) \Phi_2 - \Phi_3 \]  
(A 5)

\[ \frac{d(M_{\gamma})}{dt} = \Phi_3. \]  
(A 6)

The \( \Phi \)'s are the net fluxes that contribute to the three species from reactions A 1–3. An explicit expression is immediately available for \( \Phi_1 \) because reaction A 1 is rate-limiting:

\[ \Phi_1 = k_1(V)(M_{a_1})(M_{\beta}) - k_{-1}(V)(\tilde{M}). \]  
(A 7)

By contrast, only trivial expressions for \( \Phi_2 \) and \( \Phi_3 \) can be written because reactions A 2 and A 3 are assumed to be at equilibrium. From reactions A 2 and A 3, we do, however, have the relationships:

\[ K_1(V)(M_{a_2})(\tilde{M}) = (M_{\beta})^{\frac{\alpha + \beta}{\beta}} \]  
(A 8)
and

\[ (M_\beta) = K_2^{1/\gamma} (M_\gamma)^{\beta/\gamma}. \]  

(A 9)

Accordingly, we look for a quantity \( (M_s) \) whose time derivative is a linear function of only \( \Phi_1 \):

\[ (M_s) = (\alpha + \beta) \tilde{M} + \beta M_\beta + \gamma M_\gamma. \]  

(A 10)

\( (M_s) \) is closely related to \( (M_2) \) as defined in Eq. 5. It is not, however, the total amount of monazomycin in the film because \( \alpha + \beta \) is not the molecularity of \( \tilde{M} \). This is because monazomycin can enter the film via reaction A 2, which is at equilibrium. This distinction of course will be lost when we take the approximation \( (M_s) \approx (M_\beta) \).

The differential equation for \( (M_s) \) is:

\[
\frac{d(M_s)}{dt} = \alpha \Phi_1 = \alpha [k_1(V)(M_{a_1})(M_\beta) - k_{-1}(V)(\tilde{M})].
\]  

(A 11)

If we now express \( (\tilde{M}) \) and \( (M_s) \) in terms of \( (M_\beta) \) using Eqs. A 8 and A 9, and combine Eqs. A 10 and A 11 we get:

\[
\frac{d}{dt} \left[ (\alpha + \beta) \left\{ \frac{(M_\beta)^{\beta}}{K_1(V)M_{a_2}} \right\} + \beta (M_\beta) + \frac{\gamma (M_\gamma)}{(M_\gamma)} \right] = \alpha \left[ k_1(V)(M_{a_1})(M_\beta) - \frac{k_{-1}(V)}{K_1(V)} \left\{ \frac{(M_\beta)^{\beta}}{(M_{a_2})} \right\} \right].
\]  

(A 12)

We now take the approximation \( (M_s) \approx (M_\beta) \) by first defining:

\[ \tilde{k}_{-1} = \frac{k_{-1}(V)}{K_1(V)}. \]  

(A 13)

We let \( k_{-1}(V) \) and \( K_1(V) \) grow without limit, holding their ratio constant, and also let \( K_2 \) approach zero. Then,

\[
\frac{d(M_\beta)}{dt} = \alpha(M_\beta) \left( k_1(V)(M_{a_1}) - \tilde{k}_{-1}(V) \frac{(M_\beta)^{\alpha/\beta}}{(M_{a_2})} \right).
\]  

(A 14)

We now change our variable to \( (M_s) \) using Eq. 9 and

\[
\frac{d(M_s)}{dt} = \frac{\beta}{\gamma} \frac{d(M_\beta)}{dt} = \frac{\beta}{\gamma} \frac{d(M_\gamma)}{dt},
\]  

(A 15)

so that

\[
\frac{d(M_s)}{dt} = \frac{\alpha \gamma}{\beta^2} (M_s)(k_1(V)(M_{a_1}) - \tilde{k}_{-1}(V) \left[ \frac{(M_\beta)^{\alpha/\beta}}{K_2^{\beta/\gamma}(M_{a_2})} \right])
\]  

(A 16)

or

\[
\frac{d(M_s)}{dt} = A(M_s) \left[ 1 - \left( \frac{M_\gamma}{M_s} \right)^B \right],
\]  

(A 17)
with

\[ A = \frac{\alpha \gamma}{\beta^2} k_1(V) (M_{a_1}) \]

(A 18)

\[ B = \alpha / \gamma \]

(A 19)

\[ (M_{a_1}) = \frac{k_1(V)}{k_{-1}(V)} \]

(A 20)

The following steps are given without comment to show that \( A \) and \( M_{a_1} \) have the right forms:

\[ \frac{k_1(V)}{k_{-1}(V)} = K_{O} e^{aqV/kT}; \quad K_{1}(V) = K_{Q} e^{aqV/kT}; \quad \tilde{K}_1(V) = K_{Q1} e^{aqV/kT} \]

\[ (M_{a_1}) = \lambda_1([\text{Mon}])^a; \quad (M_{a_2}) = \lambda_2([\text{Mon}])^a \]

\[ (M_{a_1})(M_{a_2}) = \lambda([\text{Mon}])^a \]

with \( \lambda = \lambda_1\lambda_2 \).

Thus,

\[ (M_{a_1}) = K_{Q}^{2/\gamma} \lambda^{\gamma/\alpha} (K_{Q1}^{2/\gamma} \lambda^{\gamma/\alpha})^{\gamma/\alpha} ([\text{Mon}])^{\gamma q V/kT}. \]  (A 21)

If

\[ k_{-1}(V) = k_{Q1} \quad \text{and} \quad k_1(V) = k_{Q} e^{aqV/kT}, \]  (A 22)

then

\[ A = \frac{\alpha \gamma}{\beta^2} \lambda_1 k_{Q} e^{aqV/kT} ([\text{Mon}])^{\alpha}. \]  (A 23)

With regard to the inhibition of monazomycin removal from the membrane by aqueous monazomycin, we point out the occurrence of \((M_{a_1})\) in the denominator of the negative term of Eq. A 16. Physically, this corresponds to the fact that reaction A 2 constitutes a sink for \((M)\), which serves as the intermediate for monazomycin removal in reaction A 1.

Finally, we point out the marked resemblance that this scheme bears to the serial-aggregation scheme; we feel this appendix is sufficient to show that autocatalysis and serial-aggregation are not mutually exclusive.

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