Mathematical Model of TMA⁺ Diffusion and Prediction of Light-Dependent Subretinal Hydration in Chick Retina

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Purpose. To derive a mathematical model of TMA⁺ diffusion across the retina that can be used to estimate the amplitude and kinetics of the light-evoked increase in subretinal hydration and its effect on the concentration of other ions.

Methods. All experimental data were obtained in chick retina-pigment epithelium-choroid preparations as described in the accompanying paper.5

Results. Diffusional properties of the retina were derived from the time course of \([TMA⁺]_0\) in the subretinal space (SRS) after changes in the retinal perfusate. Then, the SRS volume changes underlying the light-induced \([TMA⁺]_0\) response can be derived using a mathematical model of TMA⁺ diffusion. Complete retinal depth series of light-evoked \([TMA⁺]_0\) responses could be simulated by producing a corresponding expansion of the SRS. Volume changes inferred from the diffusion model were 2.2 to 3.8 times larger and more prolonged than could be derived directly from \(\Delta[TMA⁺]_0\). The model predicted up to a 20% peak increase in subretinal-space hydration during illumination. The effects of this volume increase on subretinal K⁺ and Ca²⁺ were estimated. These predictions were supported by inhibiting the volume increase with DIDS, which blocks retinal pigment epithelium basal membrane Cl⁻ conductance.

Conclusions. The primary source of light-evoked changes in extracellular TMA⁺ concentration recorded throughout the retina is an increase in hydration (volume) of the subretinal space. The response spreads to the inner retina by diffusion. Effects of TMA⁺ diffusion lead to large underestimates of the underlying volume changes. The light-evoked volume change alters the composition of the subretinal space and light-induced responses of other ions. Invest Ophthalmol Vis Sci. 1994;35:2712–2724.

Recently, light-induced changes in concentration of the impermeant extracellular marker tetramethylammonium (TMA⁺) were detected in the frog¹⁻² and chick⁴⁻⁵ retinas. The decrease in TMA⁺ concentration ([TMA⁺]₀) was interpreted as originating from an increase in the volume (hydration) of the subretinal space (SRS) between photoreceptors and the retinal pigment epithelium (RPE) during illumination. [TMA⁺]₀ measurements may provide a good tool for assessing water movement across the retina and the RPE and, thus, for studying factors controlling retinal adhesion.

Changes in ionic concentrations produced by changes in extracellular space volume should be influenced by diffusion. A decrease in the SRS concentration of an extracellular space marker such as TMA⁺ creates a transretinal gradient of the marker. Because the gradient will dissipate due to diffusion, provided there is no transport of the marker, any local concentration changes should be transient, even if the SRS is maintained in the expanded–contracted state. In fact, the light-evoked TMA⁺ responses are transient, and their time constants are close to what is predicted by diffusion, about 50 to 100 seconds, taking into account retinal thickness and tortuosity of the retinal layers. That the TMA⁺ responses are largest and fastest in the SRS and become smaller and slower in the inner retinal layers is consistent with the idea that the volume changes are confined to the SRS and that the

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Model of TMA⁺ Diffusion

TMA⁺ response spreads to the proximal retina by diffusion.₃,₅ Thus it seems probable that during prolonged light stimulation, the magnitudes and kinetics of volume changes, as judged from TMA⁺ concentration measurements, are seriously distorted by TMA⁺ diffusion.

It is reasonable, therefore, to create a simple mathematical model of the TMA⁺ response, taking into consideration diffusion fluxes across the retina. The aim of the model is twofold: to determine whether diffusion can account for the characteristic depth profiles of the TMA⁺ response observed in the chick retina and to discover the kinetics of the volume changes underlying the response. Then the model can be used to make necessary corrections to changes in the concentration of other ions (for example, K⁺, Ca²⁺, Na⁺). A preliminary report of a portion of this work was previously presented.₆

METHODS

All experimental data were obtained from chick retina-RPE-choroid preparations as previously described.₄ All chicks were treated in accordance with the ARVO Resolution on the Use of Animals in Research. For model simulation, the data from the previous paper were used. Data on light-induced [Ca²⁺]₀ changes in the chick retina were obtained earlier by Gallemore and Steinberg (and unpublished observations, 1990).

The Model

The following are the basic postulates of the model:

1. [TMA⁺]₀ changes measured throughout the retina during illumination are solely due to the light-induced expansion of the subretinal space and to subsequent diffusion of TMA⁺.

2. The SRS is assumed to maintain a constant length, that is, the distance between the outer limiting membrane (OLM) and the apical surface of the RPE. It expands in the light by uniformly increasing in width due to the shrinkage of RPE processes, photoreceptor cells, or both. This means that light evokes changes in the extracellular volume fraction, α. (For a justification of these assumptions, see Discussion).

3. The extracellular volume fractions in the SRS (α) and within the proximal portion of the retina, that is, between the inner limiting membrane (ILM) and the OLM (α), may be different.

4. The apparent TMA⁺ diffusion constant, Dᵣ, within the proximal portion of the retina comprises only a fraction of that in the SRS (Dₛ) due to the tortuosity of the extracellular space and other possible diffusional restrictions.

5. To account for the abrupt changes in the amplitude of the light-dependent [TMA⁺]₀ decrease observed between about 75%-80% and 65% retinal depth, an additional diffusion barrier, Dₒ, is placed at the level of the OLM.

6. Diffusional flux of TMA⁺ across the RPE is neglected, and [TMA⁺]₀ at the retinal-vitreous border is maintained by the perfusion.

The retinal structure used in computations is summarized schematically in Figure 1.

To compute the diffusion in the retina, we used the principles of treatment of complex media described by Nicholson and Phillips.₈ In the following equations, C stands for their (Cᵢ)ᵢ the marker concentration averaged over small extracellular space volume (that is, the quantity sensed by the ion-selective electrode), and D = Dᵣ/α² is an apparent diffusion constant (that is, the diffusion constant for the free solution modified by the tortuosity of the extracellular space). Vectors in equations are reduced to scalars for one-dimensional diffusion across the retina.

In accordance with the model's postulates, the diffusion equation for the proximal portion of the retina (between the ILM and the OLM) is:

\[
\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2} \tag{1}
\]

Here, t is time and x is the coordinate across the retina. (Here and throughout, C may stand for Cᵢ, Cₛ, and so on when it is clear which concentration is meant). The same equation, with the decreased apparent diffusion constant, Dₒ, was applied to the diffusion barrier at the OLM.

In the subretinal space, the concentration of an ion changes not only because of diffusion but also because of changes in volume fraction. The concentration is:

\[
C = \frac{1}{\alpha} \frac{\partial Q}{\partial x}
\]

where \( \partial Q \) is the quantity of an ion in the layer \( \partial x \) per unit area, and \( \alpha \) stands for \( \alpha \). Thus,

\[
\frac{\partial C}{\partial t} = -\frac{1}{\alpha} \frac{\partial Q}{\partial t} - \frac{1}{\alpha^2} \frac{\partial Q}{\partial x} \frac{\partial \alpha}{\partial t} \tag{2}
\]

The second term in (2) is \(-\frac{C}{\alpha} \frac{\partial \alpha}{\partial t} \), and the first term reflects the change of \( \partial Q \) due to the diffusion flux:

\[
\frac{\partial}{\partial t} \frac{\partial Q}{\partial x} = -\frac{\partial}{\partial x} \langle J(x,t) \rangle.
\]
FIGURE 1. Diagram of the retinal structure used in the model. A tortuous extracellular space between the inner and outer limiting membranes (ILM and OLM) is connected to the subretinal space (SRS) whose volume \( C_s(t) \) is time-dependent. \([TMA^+]_v\) in the vitreous is settled by perfusion; diffusion across the RPE is blocked. \( D_1, D_2, \) and \( D_3 \) are apparent diffusion constants for \( TMA^+ \) in different retinal regions. \( C(x,0) \) is the concentration in the retinal chamber, which is constant in light stimulation experiments and time-dependent in perfusion experiments; \( C_1 \) and \( C_2 \) are, respectively, the concentrations in the retina between the ILM and OLM, and the SRS. The thickness of the retinal layers used for computations may be different in different experiments and was determined from the electrode depth.

where \( \langle J(x, t) \rangle \) is the macroscopic diffusion flux per unit area (equation (A8) in 8). On the other hand, according to 8 (equation (8))

\[
\langle J(x, t) \rangle = -\alpha \cdot D_1 \cdot \frac{\partial C}{\partial x}.
\]

So, for the SRS:

\[
\frac{\partial C(x, t)}{\partial t} = D_1 \cdot \frac{\partial^2 C(x, t)}{\partial x^2} - \frac{C(x, t)}{\alpha} \frac{\partial \alpha}{\partial t}
\]

Equations (1) and (3) were solved by a simple version of the finite-difference method; the space integration step was 5 \( \mu \). Finite-difference equations for equation (3), together with the border conditions, are given in the Appendix (equations [5a], [11a]). To test the accuracy of our solutions, numerical computations were performed for several system configurations for which analytic solutions were available. These included instantaneous release of a diffusible substance at the RPE border of the SRS in a uniform, semi-infinite retina, and in the retina with an impermeable retinal-vitreous border; sudden shrinkage of a previously swollen SRS, with subsequent diffusion across the uniform retina with an impermeable inner border; diffusion through two layers (SRS and proximal retina) that have different diffusion constants when the concentration is maintained constant at the RPE border. In all cases, the difference between the analytic solution and the result obtained with the model was less than 1% during the first few seconds, and less than 0.1% after this time.

By solving equations (1) to (3), one can estimate the concentration changes \( C(t) \) at any depth \( x \) provided that the kinetics of the volume changes, \( \alpha(t) \), is known. We, however, are interested in estimating \( \alpha(t) \) from measured \( C(t) \). This can be accomplished by using the linear system approach. For a linear system, with zero starting conditions, the response \( C(t) \) to an arbitrary input \( \alpha(t) \) is given by the convolution integral

\[
C(t) = \int_0^t \alpha(\tau) \cdot h'(t - \tau) \cdot d\tau
\]

where \( h(t) \) is the system’s step response, that is the \( C(t) \) changes after a stepwise, unit-amplitude increase in \( \alpha \), and the dash means the time derivative. And, vice versa, with known \( C(t) \) and \( h(t) \), \( \alpha(t) \) can be found by the reverse procedure, deconvolution. In the particular case of the exponential step response, convolution-deconvolution computations can be performed easily; corresponding equations are given in the Appendix (equations [14a] to [16a]).

The equation (3) is nonlinear, so the linear approach can be used as an approximation only. To test the applicability of the method, we computed first, at a certain position in the SRS, the model response to a sudden increase in \( \alpha \) (Fig. 2A, thick line). For \( \Delta \alpha < 25\% \), concentration changes can be accurately approximated with a sum of two exponents, thus providing the step response (Fig. 2A, hyphenated line). Then we applied a more complex waveform of \( \alpha(t) \) that resembled real volume changes (Fig. 2B, thick solid line), and \( \Delta C(t) \) was computed again (Fig. 2B, thin line). The obtained concentration response was used to derive the underlying volume changes by deconvolution (Fig. 2B, hyphenated line). Comparison of the applied and derived \( \alpha(t) \) shows that the accuracy of the solution is good. A small discrepancy in the OFF-component is due to the nonlinearity that results, among other things, in the difference between the ON- and OFF step responses. Thus, for \( \Delta \alpha < 25\% \), the accuracy of the linear approach is satisfactory. Volume changes observed experimentally in the chick retina fell within this range (see Results).

RESULTS

Selection of the Parameters of the Model

In the described form, the model contains four free parameters (three diffusion constants and the ratio of the volume fraction in the SRS to that in the proximal retina). This produces too much freedom in fitting and leads to ambiguity in the interpretation of the results. Thus, some of the parameters should be evaluated from independent experimental data.
To characterize the overall diffusion properties of the retina, we measured the [TMA⁺]₀ changes in the SRS after its sudden reduction in the retinal perfusate (Fig. 3A). The [TMA⁺]₀ curve for the SRS was markedly slower than the one for the retinal bath due, obviously, to diffusional delay. The time courses of [TMA⁺]₀ in the perfusate were approximated with an exponent (Fig. 3A, dots), and the exponent was used as the input $C₀(t)$ for the model to fit the [TMA⁺]₀ curves in the SRS. At this point, we assumed a uniform retina where $α/α_s = 1$ and $D_s = D_o = D = D_{\text{eff}}$, and we attempted to find this single diffusion constant, $D_{\text{eff}}$, that would describe the curves. $D_{\text{eff}}$ could be used then to compare curves between tissues and to obtain an average. Figure 4A (filled circles) shows the solution for the curve of Figure 3A, which provided a good fit. The mean value of $D_{\text{eff}}$ obtained in 17 measurements on 6 tissues was $(3.6 \pm 0.35) \times 10^{-6}$ cm²/sec (mean ± SD).

For comparison, similar experiments were made with changing [K⁺]₀ in the vitreal perfusate. These [K⁺]₀ changes in the SRS were significantly slower than [TMA⁺]₀ changes and never reached steady-state levels during 5-minute low [K⁺]₀ perfusion (Fig. 3B). This is thought to be due to cellular buffering mechanisms. No meaningful $D_{\text{eff}}$ for K⁺ could be found because the diffusion model provided a poor fit to the data (Fig. 4B).

It should be noted that both [TMA⁺]₀ and [K⁺]₀ dark, steady-state levels in the SRS differed from the concentration in the perfusate (Figs. 3A, 3B), thus exhibiting a transretinal gradient. The [TMA⁺]₀ gradient was relatively small, not exceeding 0.8 mM, and virtually disappeared when the concentration of the ion in the retinal perfusate was reduced. Depending both on the light conditions and the ion concentration in the perfusate, [K⁺]₀ behavior was more complex. The mechanism(s) creating the gradient is unknown and was not taken into account when considering diffusion. To fit the time course of [I⁻]₀ in the SRS with the diffusion model, the $C₀(t)$ curve was scaled to make the steady-state concentrations in the retinal perfusate equal to those in the SRS.

Because the retina is actually not uniform, it must be characterized by several diffusion constants appropriate for the different layers, as defined above. The time course of [TMA⁺]₀ in the SRS could be fitted with various combinations of these diffusion parameters. Such fits were sensitive to any changes of either of the diffusion parameters ($D_s$, $D_o$, $D$, or $α/α_s$), provided that all other of these values were fixed. However, large variations in any single parameter could be compensated by appropriate selection of others. One good fit with different $D$-values is presented in Figure 4A (open triangles). For this fit, two of the values, $D_s$ and $α/α_s$, were obtained from the literature. Thus, the TMA⁺ diffusion constant in cerebellar slices was found to correspond virtually to the value in free solution,¹ from 1.1 to 1.3 $\times 10^{-5}$ cm²/sec. Because the SRS is composed of almost straight channels and we are interested in the diffusion along these channels, the tortuosity factor, $λ$, in the SRS must be close to 1. We can assume, then, that $D_s = 1.2 \times 10^{-6}$ cm²/s, with possible small variations in special cases. As for the ratio of volume fractions in the inner retina and SRS, $α_s/α_s ≈ 1$. 

FIGURE 2. Using the deconvolution procedure to derive volume changes underlying the [TMA⁺]₀ response. In A, the model response at 76% retinal depth to a sudden 20% increase in the SRS volume fraction is shown by the thick line. The concentration decrease is plotted downward (hyphenated line). The solid line shows the approximation of the step response by a sum of two exponents. In B, the thin line represents the concentration response computed with the model when fed with an exponential volume change shown by the thick solid line. Then the AC curve was deconvoluted with the step response from A. The derived volume change is shown with the hyphenated line. Model parameters: $D_s = 1.2 \times 10^{-6}$ cm²/sec, $D_o = 4 \times 10^{-6}$ cm²/sec, $D = D_o/16$, $α/α_s = 1$. Volume changes: ON-phase, the exponent with the amplitude of 20% and time constant of 20 seconds; OFF-phase, the exponent of the opposite sign, time constant 30 seconds, superimposed on the tail of the ON-phase.
FIGURE 3. Changes in SRS [TMA\(^+\)], and [K\(^+\)], after reduction in the ion concentration in the retinal perfusate. In (A), retinal perfusate [TMA\(^+\)], was reduced from 5 to 2 mM for 10 minutes. The thin line shows the time course of the perfusate concentration, the thick line the change in the SRS. Dots show an exponential approximation of [TMA\(^+\)], changes in the retinal bath. Time constants for the front and tail exponents are 29 seconds. (B), same for [K\(^+\)], after reduction from 5 mM to 1 mM in the retinal bath for 5 minutes. Time constants of [K\(^+\)], changes are 32 seconds (front) and 38 seconds (tail).

1.7 can be derived from the resistivity measurements of Ogden and Ito\(^{10}\) on pigeon and chick eyecups. A lower SRS volume fraction, compared to that in the inner retina, also follows from Newman's\(^{11}\) analysis in frog. This, however, contradicts electrical data on the frog eyecup\(^{12}\) that demonstrated a larger extracellular volume in the SRS. As a compromise, we chose for modeling \(\alpha_i = \alpha_e\). For comparison, some computations were also performed with the SRS volume fraction different from that in the proximal retina (see Discussion).

Two other important parameters of retinal structure are the apparent diffusion constants in the inner retina and at the presumed barrier at the OLM. Once \(D_i\) and \(\alpha_i/\alpha_e\) were fixed, \(D_o\) and \(D_{ol}\) values could be found by fitting both the perfusion data (as in Fig. 4A)

FIGURE 4. Modeling the diffusion of TMA\(^+\) and K\(^+\) across the retina. (A), fitting the front of the SRS [TMA\(^+\)], changes shown in Figure 2A with two different diffusion models. The line represents the experimental record. Filled circles correspond to the uniform retinal structure with effective diffusion constant \(D_{eff} = 3.65 \cdot 10^{-6} \text{ cm}^2/\text{sec}\). Open triangles correspond to \(D_i = 12 \cdot 10^{-6} \text{ cm}^2/\text{sec}, D_{ol}/(1.72)^2, D_o = D_i/16,\) and \(\alpha_i/\alpha_e = 1\). (B), same for [K\(^+\)],. Nonuniform retinal structure as in (A), but \(D_i = 0.82 \cdot 10^{-6} \text{ cm}^2/\text{sec}\) (triangles) and \(2.2 \cdot 10^{-6} \text{ cm}^2/\text{sec}\) (circles).
and the characteristic amplitude depth profile of the light response (Figs. 5, 6). This provided a virtually unique estimate for $D_0$ and $D_i$ (see Results).

The assumption of RPE impermeability for TMA$^+$ (see postulate 6 of the model above) was tested in experiments with complete removal of TMA$^+$ from the choroidal perfusate, keeping it normal in the retinal bath. This led to a slow (time constant 100 to 200 seconds) decrease in $[\text{TMA}^+]_o$ in the SRS that did not exceed 1% (data not shown). Thus, though the RPE is not completely TMA$^+$ impermeable, the diffusion flux across it could be neglected for our purposes.

The Model

The mean value of $D_{av}$ obtained with $[\text{TMA}^+]_o$ changes in the retinal perfusate was used to choose possible combinations of local diffusion properties of the retina when fitting light responses. Nonuniformity of retinal structure revealed itself in a specific depth profile of the response (Fig. 5A). The experimental data show the sudden decline in response amplitude that occurred between 76% and 64% retinal depth; this special depth dependence was also observed in other experiments (Fig. 6). To account for this change, a diffusion barrier $D_o$ was placed at 75% retinal depth; the width of the barrier was equal to a single integration step, that is, 5 $\mu$m. For various combinations of $D_i$ and $D_o$ yielding the same $D_{cl}$ (as judged by fitting the perfusion data), the step response was computed. Then volume changes underlying the $[\text{TMA}^+]_o$ response in the SRS were derived and used as an input $a_i(t)$ for the model to compute the expected responses at different retinal depths. The pair of values consistent with both the depth profiles and average perfusion data was found to be $D_i = 4 \times 10^{-6} \text{ cm}^2/\text{sec}$ and $D_o = D_i/16$.

FIGURE 5. Modeling the series of $[\text{TMA}^+]_o$ responses at different retinal depths. (A) The concentration response recorded from the SRS at 76% retinal depth was used to derive underlying volume changes (shown in B). The volume response was then fed to the model input, and the expected concentration response was computed and converted into $\Delta V_{\text{TMA}^+}$. Computed responses (thick lines) are superimposed on those recorded experimentally (thin lines). (B) Comparison of volume changes deduced directly from the $[\text{TMA}^+]_o$ response (thick line) with those derived with the diffusion model (thin, noisy curve). The derived response can be well approximated with two exponents, with the time constant of 21 seconds for front and 17 seconds for tail, and the amplitude 17% (hyphenated line). Parameters of the model as in Figure 2. Model responses in A and B are Gaussian-filtered. SD = 1 second.

FIGURE 6. Comparison of experimental and computed amplitude depth profiles of the $[\text{TMA}^+]_o$ response. Hyphenated line through circles represents the amplitudes of the $V_{\text{TMA}^+}$ decrease taken from Figure 5A. Hyphenated line through triangles was taken from another similar experiment. Thick line shows the computed profile taken from Figure 5A. Data were normalized at 76% to 78% retinal depth.
Figure 5A shows the final solution of the model for a selected typical depth profile from one experiment where light-evoked [TMA\(^+\)]\(_o\) changes had been recorded in response to 60 sec of illumination (experimental data from\(^5\)). The calculated light-evoked changes in [TMA\(^+\)]\(_o\) (thick lines) are superimposed over those that were actually recorded (thin lines).

Notably, volume changes derived from the response at 76% depth resulted in a good fit not only at 76% but at different retinal depths, with respect to both amplitudes and time courses (Fig. 5A). The maximum amplitudes of the responses of Figure 5A have been plotted in Figure 6 (filled circles) vs. retinal depth, along with the results from a second experiment (triangles), while the solid line shows the computed response. The agreement between experimental and computed depth profiles is quite good, except for the last point at the vitreal-retinal border (8% depth), which may be at least partly explained by an electrical artifact.

Figure 5B shows the subretinal volume changes underlying the responses of Figure 5A. The amplitude of \(\Delta\alpha\), is 2.8 times larger than can be inferred directly from the [TMA\(^+\)]\(_o\) curve, and the volume response is more prolonged showing no relaxation during illumination. Volume changes can be approximated with two exponents, one for the front and another for the tail of the response (Fig. 5B, hyphenated line), that imply a steady-state volume increase in light. Indeed, the derivation of volume changes produced by 300-second stimuli sometimes yields a curve that exponentially rises to a stable level maintained during illumination; turning the light off results in the relaxation to the dark level (Fig. 7A). In some retinas, however, after returning to baseline during a 300-second stimulus, [TMA\(^+\)]\(_o\), then rose above the dark level. In that case, the derived volume changes were transient, with slow relaxation to the dark level during illumination and with a prominent OFF-component after cessation of light (Fig. 7B).

The amplitude of volume changes in different retinal preparations varied from 14% to 20% (16.7 ± 2.2, mean and SD, six retinas); it also could change with condition of the preparation. The ratio of \(\Delta\alpha\), to \(\Delta[TMA^+]\), was from 2.2 to 3.8 (3.0 ± .5, six retinas), depending mainly on the rate of the \([TMA^+]\), response. This is understandable because diffusion processes essentially high-pass filter the concentration response at the site of volume change.

Light-Evoked Changes in [Ion\(_o\)]

SRS volume changes detected by \(\Delta[TMA^+]\), should also influence the concentration of all other ions, thus distorting the pattern of their light-induced movements. Indeed, recently Huang and Karwoski\(^8\) showed that the recorded decrease in [Ca\(^{2+}\)]\(_o\), in the SRS of the frog retina during prolonged illumination is converted into an increase (that is, release from photoreceptors) if the correction for the [TMA\(^+\)]\(_o\) changes is made. Because, as shown in the present work, real volume change is significantly greater than can be inferred directly from [TMA\(^+\)]\(_o\), recordings, its effect on the ionic concentrations could be even more dramatic.

Thus, we are interested in estimating the effect of the increase in SRS volume on ionic concentrations in the SRS, that is, to understand original ionic movements from recordings distorted by volume changes. In these experiments, we recorded light-evoked sub-
FIGURE 8. Correction of light-evoked subretinal [K⁺]₀ and [Ca²⁺]₀ responses for the volume change using the light-evoked [TMA⁺]₀ response as the basis for the correction (see text for method). (A) 60-second stimulus and (B) 300-second stimulus. Thin lines, original records; thick lines, as corrected. (To amplify the [Ca²⁺]₀ response, which was normally small, we lowered [Ca²⁺]₀ in the perfusate to 0.18 mM.)

retinal changes in [TMA⁺]₀, [K⁺]₀, and [Ca²⁺]₀, in the same retina. To predict the effect of the volume change on Δ[Ion]₀, one approach is to use the estimate of the volume obtained from the model and apply this to the light-evoked change in [Ion]₀. This, however, does not take into account diffusion of the ion. Another approach is to assume that the candidate ion and TMA⁺ are similarly affected by diffusion and simply use the light-evoked [TMA⁺]₀ change to correct the light-evoked [Ion]₀ response, as has already been done. A problem here is that the assumption of similar diffusion is not necessarily correct, which is obvious from a comparison of curves for [TMA⁺]₀ and [K⁺]₀ (Figs. 3A, 3B). Neither approach, it should be

FIGURE 9. Correction of light-evoked subretinal [K⁺]₀ and [Ca²⁺]₀ responses for the volume change using the changes inferred from the model. The volume changes displayed at the top in (A) 60-second and (B) 300-second stimulus traces were derived from the [TMA⁺]₀ curves of Figures 8A and 8B.
Thus, for potassium, the corrected concentration (that ion-sensitive electrode is and \( \alpha_r \) is the relative volume. The potential of the
get

\[ C = \frac{Q}{\alpha_0 \alpha_r(t)}. \]

Here \( \alpha_0 \) is the SRS volume fraction in the dark, and \( \alpha_r(0) \) is the relative volume. The potential of the

\[ V_{TMA} = S_{TMA} \cdot \ln(Q) = S_{TMA} \cdot (\ln(Q) - \ln(\alpha_0) - \ln(\alpha_r)) \]

where \( S_{TMA} \) is the electrode slope per e-fold change in TMA\(^+\) concentration. Removing constant values we get

\[ \alpha_t = \exp \left( - \frac{\Delta V_{TMA}}{S_{TMA}} \right) \] (5)

Thus, for potassium, the corrected concentration (that is, the concentration that would be observed if there were no volume changes and no diffusion), is

\[ [K^+]_n = [K^+]_r \cdot \alpha_t. \]

Then, the corrected potassium voltage response is

\[ \Delta V_{K^+} = S_K \cdot \ln([K^+]_r) = \Delta V_K - \frac{S_K}{S_{TMA}} \cdot \Delta V_{TMA}. \]

Because the electrode slopes for K\(^+\) and TMA\(^+\) are virtually the same, it simply means that the \( V_{TMA} \) response should be subtracted from the \( V_K \) response to obtain the corrected record. Similarly, the correction of \( V_{Ca^{2+}} \) responses (see below) can be made using \( S_{Ca^{2+}} \), the slope of the \( Ca^{2+} \)-sensitive electrode. The same correction could be applied if \( TMA^+ \) and the ion in question were influenced by diffusion in an identical manner.

Figures 8A and 8B show the results of correcting the \( V_K \) responses directly with the \( V_{TMA} \) response. Original \( V_K \) responses are shown in the middle traces by thin lines, whereas thick lines are the corrected curves. The major effect is to produce a more transient \( V_K \) response after light onset and offset.

Light-evoked changes in subretinal \([Ca^{2+}]_n\) have been recorded previously in chick preparations.\(^7\) Figures 8A and 8B (bottom traces, thin lines) show the light-evoked \( V_{Ca^{2+}} \) responses to 60-second and 300-second stimuli. \( V_{Ca^{2+}} \) increases slowly after the onset of illumination, peaks at about 30 seconds, and then drops slowly to approach the dark level. There is a large undershoot of \( V_{Ca^{2+}} \) after the offset of illumination. Figures 8A and 8B (bottom traces, thick lines) show the light-evoked \( V_{Ca^{2+}} \) responses after subtracting \( V_{TMA} \). The major change is a substantial enhancement of the increase in \( V_{Ca^{2+}} \) after light onset, as well as in the undershoot after light offset.

In the alternative approach—correcting for the volume changes estimated by the model—\([K^+]_o\) and \([Ca^{2+}]_o\) were multiplied by the \( \alpha_0 \) derived from the model. Figures 9A and 9B show these corrections for 60-second and 300-second stimuli, respectively. The correction for calcium using the model closely resembled the correction of Figure 8 for \% \([TMA^+]_o\) the changes in \( Ca^{2+} \) being enhanced. For \( K^+ \), the volume corrections also predict a more transient decrease after light onset and a more transient overshoot after light offset. It differs from the \% \([TMA^+]_o\) correction of Figure 8, however, in predicting that \( K^+ \) would rise substantially above the dark level during illumination. Note that the corrected curves of Figure 9 essentially do not give imaginary concentrations but, rather, a real value that is a total amount of the ion in the subretinal space. They can be used to estimate the direction of ionic movements in different phases of the light response.

Inhibition of the Volume Increase with DIDS

We have shown elsewhere that it is possible to block the light-evoked \([TMA^+]_o\) decrease substantially and, therefore, the underlying volume increase with 4,4'-dithiostilbene-2,2'-disulfonate (DIDS).\(^5\) DIDS has been shown to block the chloride conductance of the basolateral membrane of chick RPE, which leads to suppression of the transepithelial fluxes of \( Cl^- \) and of water.\(^13\)\^-17\) By perfusing the choroidal side of the retina-RPE-choroid preparation with 50 \( \mu \)M DIDS, we were able to decrease the light-evoked \([TMA^+]_o\) response by 70% to 80%.\(^5\) By using DIDS, it should be possible to observe light-evoked changes in \([Ion]_o\) in the absence of most of the volume change.

The results of such experiments are shown in Figures 10 and 11. During perfusion with DIDS, light-evoked changes in \([Ca^{2+}]_o\), (Fig. 10) were progressively enhanced for both the on- and off-phases of the response to 60 seconds of illumination. After returning to the control perfusate (Fig. 10, Recovery), there was almost complete recovery. The \([Ca^{2+}]_o\) responses during DIDS well resembled those predicted in Figures 8 and 9 for responses in the absence of volume changes. Similar results were obtained in a total of three experiments.

The results for potassium were more varied, and findings of two experiments are shown in Figure 11.
Model of TMA+ Diffusion

The validity of the model depends, of course, on the values chosen for the apparent diffusion constants and volume fractions of different retinal layers. There is less freedom in selection of these values. Thus, with lower $D_o$, higher $D_u$ and $D_i$ are necessary to yield the same $D_{tot}$. This leads to more uniform diffusion properties and, hence, to a smoother depth profile. So the best fit to the experimental data was achieved with the tortuosity factor in the SRS, $\lambda = 1$, and $D_u = 12 \cdot 10^{-6}$ cm$^2$/sec, as in free solution. Relatively free diffusion of TMA+ in the SRS agrees with its behavior in other tissues\textsuperscript{8,18,19} and with its use as an extracellular marker whose diffusion is only restricted by geometric factors. This is in contrast to the behavior of K+, whose diffusion is drastically modified by cellular buffering and/or other factors (compare Figs. 3, 4A, 4B).

$D_i = 4 \cdot 10^{-6}$ cm$^2$/sec corresponds to a tortuosity factor $\lambda = 1.73$ that is within the range 1.55 to 2 found for neural tissues of similar morphology\textsuperscript{8,12,19-22}. Important was the value for $D_o$, the diffusion barrier at the OLM, which was dictated by a sudden drop in response amplitude between 75% and 65% retinal depth (Figs. 5, 6). This local diffusion barrier could not be substituted by a lower $D_i$ that resulted in a smoother depth profile. In general, a 20% change in either diffusion constant led to intolerable disagreement with the experimental depth profiles, both in the amplitude

![Diagram](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933404/)

**FIGURE 10.** Effect on $V_{Ca^{2+}}$ of perfusing the choroidal side of the preparation with 50 $\mu$M DIDS. Light-evoked responses were recorded before (control), during, and after (recovery) DIDS perfusion.

In Figure 11A, the light-evoked $[K^+]_o$ response became more transient at 15 minutes in DIDS and resembled the response predicted in Figure 8A. In the other experiment (Fig. 11B), a similar change occurred at 45 minutes in DIDS. In both experiments, the amplitude of the light-evoked $[K^+]_o$ decrease was reduced by DIDS, and recovery was incomplete after return to the control solution. Similar results were obtained in a total of six experiments.

**DISCUSSION**

**Validity of the Model**

We have shown that a relatively simple model that takes into account the diffusion of TMA+ in the retina can account for the depth profile of light-evoked [TMA+]$_o$ responses. This model predicts a light-evoked volume change that is much greater than the % change in [TMA+]$_o$, and it also shows how light-evoked changes in [Ion]$_o$ are affected by the volume changes.

![Diagram](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933404/)

**FIGURE 11.** Effect on $V_{K^+}$ of perfusing the choroidal side of the preparation with 50 $\mu$M DIDS. Data are shown for two separate tissues, A and B. Light-evoked responses were recorded before (control), during, and after (recovery) DIDS perfusion.
and the time course of the responses in the inner retina (provided, of course, that the set of values yielded the same $D_{mn}$). At the same time, these variations of the parameters led to few percent changes in the derived volume response. This rigidity of the solution is, of course, due to the necessity to satisfy, during fitting, both the results of the perfusion experiments (Figs. 3A, 4A) and the observed depth profile (Fig. 5). These experiments characterize the overall diffusion properties of the retina and the nonuniformity of the retinal structure, thus strictly limiting freedom of fitting. Once $D_n$, $D_h$, $D_{mn}$, and $\alpha/\alpha_s$ were fixed, the system step response can be determined, and the volume changes can be unambiguously derived by the deconvolution procedure.

Most important for establishing the amplitude of volume changes was the ratio of the extracellular volume fractions in the proximal retina and SRS, $\alpha/\alpha_s$. Thus, instead of $\alpha_s/\alpha_s = 1$, if $\alpha_s/\alpha_s = 1.7$ had been selected, which follows from the resistivity measurements, then the volume response would be 20% larger. Effects of changing $\alpha/\alpha_s$ on the $D_{mn}$ and depth profile can be easily compensated by small changes in other model parameters. In sum, the model itself does not provide a reliable clue for choosing the volume fractions, and the value of $1$ selected for the ratio was a reasonable compromise between various indirect estimates. Evidently, direct experimental measurements of this important parameter will be necessary.

### Sustained Versus Transient Volume Changes

In some experiments, the sustained, single-exponential volume changes were sufficient to describe the entire $[\text{TMA}^+]_o$ response (Figs. 5, 7A). In others, transient, slowly relaxing volume effects were observed (Fig. 7B). A good indication of the transient character of volume changes is seen in a $[\text{TMA}^+]_o$ response, with one obtained from resistivity measurements, then the volume response would be 20% larger. Effects of changing $\alpha/\alpha_s$ on the $D_{mn}$ and depth profile can be easily compensated by small changes in other model parameters. In sum, the model itself does not provide a reliable clue for choosing the volume fractions, and the value of $1$ selected for the ratio was a reasonable compromise between various indirect estimates. Evidently, direct experimental measurements of this important parameter will be necessary.

### Mode of Volume Changes

To simplify computations, it was assumed that the subretinal space expands in light by uniformly increasing in width because of the shrinkage of RPE apical processes and, perhaps, the contraction of photoreceptors. This may not be completely valid; photoreceptors and RPE processes may also change in length. Because the time to establish diffusion equilibrium within the SRS (less than 50 $\mu$ long) should be about 1 second, the difference between various modes of volume changes is insignificant after this time.

### [TMA$^+$]$_o$ Response and the SRS Volume Change

Perhaps one of the most important conclusions from modeling is that both the kinetics and the amplitude of the SRS volume change, as judged from the light-evoked $[\text{TMA}^+]_o$ response, are strongly affected by $\text{TMA}^+$ diffusion. In particular, $\text{TMA}^+$ diffusion accounts for the return to the dark level during prolonged illumination and for the overshoot after light offset. Light-induced volume changes underlying the $[\text{TMA}^+]_o$ response well may be an essentially sustained, monophasic deviation from the dark level (Fig. 7A).

It is worth noting that the diffusion of $\text{TMA}^+$ that returns its concentration to the dark level makes the $[\text{TMA}^+]_o$ curve approach the time derivative of the volume changes. This means that the amplitude of $\Delta[\text{TMA}^+]_o$ depends not only on the amplitude of $\Delta\alpha$, but on its rate as well. Thus, factors that influence the time course of volume effects will also influence the magnitude of the $[\text{TMA}^+]_o$ response.

The modeled volume changes are significantly larger than any that would have been directly inferred from $[\text{TMA}^+]_o$ responses had not the effect of diffusion been taken into account (2.2 to 3.8 times larger than the changes in $[\text{TMA}^+]_o$) (Fig. 7). Large volume changes should significantly affect the light-induced shifts in concentration of other ions in the SRS. This effect may qualitatively change the interpretation of many ionic measurements by converting, for example, an apparent light-induced ion uptake into release, as already shown by Huang and Karwoski for $[\text{Ca}^{2+}]_\text{i}$ in the frog retina. The correction of the observed $\Delta[\text{Ion}]_\text{i}$, by $\Delta[\text{TMA}^+]_o$ (Fig. 8) predicted the (imaginary) light-evoked ionic responses that would be observed if there were no volume changes and if the ion in question diffused like $\text{TMA}^+$. Experiments with blocking the volume response with DIDS (Figs. 10, 11) partly model this situation. As indicated earlier, the curves corrected with the modeled volume changes (Fig. 9) actually show a real value, namely the total amount of the ion in the extracellular space. Such records cannot be observed experimentally, even in the absence of volume effects, because changed ionic concentrations will result in changed active and passive ionic fluxes. However, these records can be used for estimating net ionic fluxes into the SRS in normal conditions.

### Key Words

subretinal space, $\text{TMA}^+$, photoreceptor, RPE, extracellular volume

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APPENDIX

Finite-Difference Equations

Finite-difference equations for computer solution can be obtained as follows:

Quantity of an extracellular marker within the extracellular space at nth space integration step at time t, per unit area of the retinal layer, is

\[ Q_n(t) = C_n(t) \cdot \alpha(t) \cdot \Delta x \]  \hspace{1cm} (1a)

Here, as in the text, \( C_n(t) \) is the concentration in the microscopic sense, \( \alpha(t) \) is the extracellular volume fraction, and \( \Delta x \) is the space integration interval. Net inward diffusion flux (per unit retinal area) is

\[ F_n(t) = \alpha(t) \cdot D \cdot (\frac{C_n - C_{n-1}}{\Delta x}) \]  \hspace{1cm} (2a)

D is the apparent diffusion constant for the corresponding retinal layer. Then

\[ \Delta Q_n(t) = F_n(t) \cdot \Delta t \]  \hspace{1cm} (3a)

Thus, the concentration at time \( t + \Delta t \) is

\[ C_n(t + \Delta t) = \frac{Q_n(t) + \Delta Q_n}{\alpha(t + \Delta t) \cdot \Delta x} \]  \hspace{1cm} (4a)

Finally, from (1a) to (4a) we get:

\[ \frac{\Delta C_n}{\Delta t} = \frac{D}{\Delta x^2} \cdot (C_{n-1} - 2C_n + C_{n+1}) \cdot \frac{\alpha(t)}{\alpha(t + \Delta t)} \]

\[ - \frac{C_n \cdot \alpha(t + \Delta t) - \alpha(t)}{\alpha(t + \Delta t) \cdot \Delta t} \]  \hspace{1cm} (5a)

With \( \Delta t, \Delta x \rightarrow 0 \) this yields equation (3) in the text.

To obtain the equation for the border between two layers with different diffusion constants, \( D_a, D_b \) and different volume fractions, \( \alpha_a, \alpha_b, \) the method described by Crank \(^9\) (pp 204–205) was used. The boundary conditions to be satisfied at the interface, at the point \( n_i \), are

\[ C_n = C_s; \quad \alpha_a \cdot D_a \cdot \frac{\partial C_n}{\partial x} = \alpha_b \cdot D_b \cdot \frac{\partial C_n}{\partial x} = -F \]  \hspace{1cm} (6a)

where \( F = F(C_n, t) \) denotes the flux across the boundary. The medium \( a \) lies to the left and the medium \( b \) to the right of the boundary. By imaging the medium \( a \) to be extended one step, \( \Delta x \), to the right of \( n_i \), for the point on the interface we have

\[ \frac{\partial C_n}{\partial t} = D_a \cdot \frac{C_{n+1} - 2C_n + C_{n-1}}{(\Delta x)^2} \]  \hspace{1cm} (7a)

Eliminating the fictitious concentration \( C_{n+1} \) from (7a), (8a), and using (6a) yields

\[ \frac{\partial C_n}{\partial t} = \frac{2D_a}{\Delta x} \cdot (\frac{C_{n-1} - C_n}{\partial x}) \cdot \frac{F}{D_a \cdot \alpha_a} \]  \hspace{1cm} (9a)

Similarly, by extending the medium \( b \) one step to the left of \( n_i \), we find

\[ \frac{\partial C_n}{\partial t} = \frac{2D_b}{\Delta x} \cdot (\frac{C_{n-1} - C_n}{\partial x}) \cdot \frac{F}{D_b \cdot \alpha_b} \]  \hspace{1cm} (10a)

Finally, eliminating \( F \) from (9a) and (10a), we get

\[ \frac{\partial C_n}{\partial t} = \frac{2}{(\Delta x)^2} \cdot D_a \cdot \alpha_a \cdot (C_{n+1} - C_n) - D_b \cdot \alpha_b (C_n - C_{n-1}) \]  \hspace{1cm} (11a)

Convolution and Deconvolution With an Exponential Step Response

Let the system step response be a single exponent with the time constant \( T \), \( h(t) = \exp(-t/T) \). The input signal, \( f(t) \), digitized at \( \Delta t \) intervals, is represented by a series of values, \( f_{n-1}, f_n, f_{n+1}, \) and so on, with linear interpolation between them. Then the output at the \( n+1 \)-th time point is the output at the \( n \)-th point, decayed along the exponent, plus the output to \( f(t)\cdot f_n \) that is,

\[ V_{n+1} = V_n \cdot \exp(-\Delta t/T) + \int_{n \cdot \Delta t}^{(n+1) \cdot \Delta t} h'(t - \tau) \cdot f(\tau) \cdot d\tau \]  \hspace{1cm} (12a)

\[ h'(\tau) = \delta(\tau) - \frac{1}{T} \cdot \exp(-\tau/T) \]  \hspace{1cm} (13a)

where \( \delta(\tau) \) is the unit impulse function. Computing the integral we get

\[ V_{n+1} = V_n \cdot k_1 + (f_{n+1} - f_n) \cdot k_2 \]  \hspace{1cm} (14a)

where \( k_1 = \exp(-\Delta t/T), k_2 = T \cdot (1 - k_1/\Delta t) \). Conversely, the deconvolution is

\[ f_{n+1} = V_{n+1}/k_2 - V_n \cdot k_1/k_2 + f_n \]  \hspace{1cm} (15a)
If the step response is a sum of several exponents,
\[ h(t) = a_1 \cdot \exp(-t/T_1) + a_2 \cdot \exp(-t/T_2) + \ldots, \]
the convolution can be computed simply as a sum of outputs of each exponent, that is, \( V_{n+1} = a_1 \cdot V_{n+1}^1 + a_2 \cdot V_{n+1}^2 + \ldots, \) and \( V_{n+1}^m \) are given by (14a). (Here superscripts are, of course, indices, not powers). For deconvolution, it can be rewritten as
\[ V_{n+1} = a_1 \cdot V_n^1 \cdot k_1^1 + a_2 \cdot V_n^2 \cdot k_1^2 + \ldots + \Delta V \]
\[ \Delta V = (a_1 \cdot k_2^1 + a_2 \cdot k_2^2 + \ldots) \cdot (f_{n+1} - f_n) \] (16a).
Thus, starting with \( V_0 = V_0^1 = V_0^2 = \ldots = 0 \) and \( f_0 = 0 \), at each time step one can find first \( \Delta V \), then \( f_{n+1} - f_n \) and finally \( V_{n+1}^1, V_{n+1}^2, \ldots \) for the next step.

References