# STEADY-STATE VOLTAGES, ION FLUXES, AND VOLUME REGULATION IN SYNCYTIAL TISSUES

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ABSTRACT Equations are developed that describe the steady-state relationships among ion fluxes, solute fluxes, water flow, voltage, concentration of solute, and hydrostatic pressure in a spherically symmetrical syncytial tissue. Each cell of the syncytium is assumed to have membrane channels for Na, K, and Cl, a membrane pump for Na/K, and some concentration of intracellular protein of net negative charge. However, the surface cells and inner cells of the tissue are assumed to have different distributions of membrane transport properties, hence there is a radial circulation of fluxes and a radial distribution of forces. Some reasonable approximations are made that allow analytic solutions of the nonlinear differential equations. These solutions are used to analyze data from the frog lens and are shown to account for the known steady-state properties of this tissue. Moreover, these solutions are used to make predictions on other steady-state properties, which have not been directly measured, and graphical results on the circulation of water, ions and solute through the frog lens are presented.

## INTRODUCTION

Many animal tissues are composed of cells that communicate through low-resistance gap junctions. Examples of such syncytial tissues in man are: the heart; the lens; smooth muscle; glial tissue; the liver; and many more including all epithelia. Some general properties of syncytial tissues are that their dimensions are large in comparison with the dimensions of a single cell whereas the space between cells is small. Moreover, there are often specialized regions of the tissue: the lens has a single layer of cuboidal epithelial cells covering its anterior surface whereas the mass of the lens is comprised of elongated fiberlike cells; in the heart the cells of the sinoatrial node, atrial muscle, atrioventricular node, ventricular muscle, Purkinje strands and papillary muscle all differ in some respects. Hence, there is not just unity but also diversity within a syncytium. Because of this diversity and because of the relative dimensions of syncytia, steady-state voltages, ion fluxes, and volume regulation can be more complex than in a single cell. Nonetheless, the same physical principles apply to either cells or tissues. We will therefore introduce the nomenclature and physical processes that we wish to characterize by first considering steady state in a single cell.

Our present understanding of electrochemical steady state in single cells has been lucidly described by Boyle and Conway (1941) (recently reviewed by Sten-Knudsen, 1978). The problem faced by all cells is that they contain negatively charged impermeant proteins, thus their cytoplasm cannot be in diffusional equilibrium with the extracellular milieu: electrical neutrality requires the intracellular chloride concentration must be less than that outside of the cell. The tendency for chloride to diffuse into the cell is countered by the existence of a negative resting voltage, which is maintained by a Na-K ATPase that actively extrudes sodium from the cell while accumulating potassium. The resting voltage and intracellular ion activities come to steady state when the flux of active transport just balances the passive electrodiffusion of Na and K. Moreover, if there are no active or exchange transport mechanisms for Cl, then the steady-state Nernst potential, $E_{Cl}$, will equal the resting voltage. Hence, the steady-state transmembrane flow of each of the three major permeable ions will satisfy

$$G_K(\psi_i - E_K) - 2I_p = 0, \quad (1)$$

$$G_{Na}(\psi_i - E_{Na}) + 3I_p = 0, \quad (2)$$

$$G_{Cl}(\psi_i - E_{Cl}) = 0, \quad (3)$$

where the above terms are defined in Fig. 1 and in the glossary at the beginning of the next section; moreover we have assumed the Na-K ATPase extrudes 3 Na ions for every 2 K ions pumped into the cell.

The conductances in Eqs. 1–3 depend on the population of channels present in the membrane (Rae and Levis, 1984; Rae, 1985). In general, the conductance contributed by a channel depends on the product of the open channel conductance times the probability of being open, each of which may depend on voltage, the concentration of permeant ions, the presence or absence of gating substances, hydrostatic pressure via deformation of the membrane and perhaps other factors (Auerbach and Sachs, 1984; Rae, 1985). The classical formulation of passive transmembrane ion flow as electrodiffusion (i.e. to use the Nernst

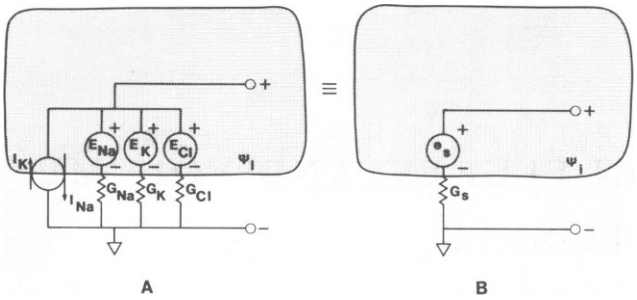


FIGURE 1 The equivalent circuit representation we use to describe electrochemical steady state across a membrane.

Plank equation as in Goldman, 1943) is clearly inconsistent with modern data on single channels, so we are left with the general but rather nonspecific relationships in Eqs. 1–3. However, patch-clamp techniques allow the characterization of channels in terms of reversal potential, open-channel conductance and probability of being open, thus the parameters in Eqs. 1–3 may, in principle, be specified by adding the parallel characteristics of each channel. Similarly, the pump current, $I_p$, depends on intracellular Na, extracellular K, and membrane potential, but since we do not know the details of this dependence, we leave it as an experimentally accessable parameter in the equations.

If the cell contains $A_i$ moles of negatively charged impermeant protein, then for intracellular electroneutrality

$$Cl_i + \bar{z}\, A_i/V_i = Na_i + K_i, \tag{4}$$

where $\bar{z}$ is the magnitude of the average valence of the impermenat anions and $V_i$ is the volume of the cell.

The last conservation law is that net fluid flow across the cell membrane must be zero. Namely,

$$0 = L_m[p_i - \sigma RT(Os_i - c_o)],$$
$$Os_i = Na_i + K_i + Cl_i + A_i/V_i. \tag{5}$$

If the steady-state volume of the cell, $V_i$, is such that the membrane is an uninflated sack, then $p_i = 0$ and we require osmotic balance

$$Na_i + K_i + Cl_i + A_i/V_i = c_o. \tag{6}$$

If there is some small hydrostatic pressure within the cell, then we need another equation to relate the compliance of the membrane, the cell volume and the pressure. Usually, cell membranes appear wrinkled as if the cell is not fully inflated, hence the usual assumption is that $p_i = 0$.

If the five parameters $\psi_i$, $E_K$, $E_{Na}$, $E_{Cl}$, and $V_i$ are considered the dependent variables of the problem (assuming $p_i = 0$), whereas $I_p$, $G_K$, $G_{Na}$, $G_{Cl}$, $A_i$, $\bar{z}$ and $c_o$ are considered the intrinsic or independent parameters, then Eqs. 1–5 can be solved numerically to obtain any of the five dependent variables in terms of the independent parameters (Jakobsson, 1980).

The flux equations Eqs. (1–3) can be combined to obtain a general relationship between the resting voltage and Nernst potentials for the major permeable ions.

$$\psi_i = \frac{G_K E_K + G_{Na} E_{Na} + G_{Cl} E_{Cl} - I_p}{G_S}, \tag{7}$$

where

$$G_S = G_K + G_{Na} + G_{Cl}. \tag{8}$$

We would like to have an analogous expression for resting voltage in syncytia.

Volume regulation in the single cell is closely related to the extracellular concentration of chloride. Eqs. 4 and 6 can be combined to obtain

$$\frac{\bar{z} - 1}{2}\frac{A_i}{V_i} = \frac{1}{2}c_o - Cl_o\, e^{F\psi_i/RT}. \tag{9}$$

Thus, volume depends on extracellular osmolarity, extracellular chloride and membrane voltage.

If the potassium conductance is large, then $\psi_i$ will approximately equal $E_K$ over some range of values of $K_o$. In this situation the value of $\psi_i$ in Eq. 9 can be replaced with $E_K$ and the result is:

$$\frac{\bar{z} - 1}{2}\frac{A_i}{V_i} \approx \frac{1}{2}c_o - \frac{Cl_o K_o}{K_i}. \tag{10}$$

Volume will now depend upon the product $Cl_o K_o$. Such a system is referred to as Donnan equilibrium (Katz, 1966; Sten-Knudsen, 1978). However, a Donnan equilibrium is appropriate only if the above assumptions are valid.

A more general way of viewing the voltage Eq. 7 is that the membrane has a diffusion potential given by

$$E_S = (G_K E_K + G_{Na} E_{Na} + G_{Cl} E_{Cl})/G_S \tag{11}$$

and a metabolic potential of $I_p/G_S$, so the total diffusion-metabolic potential is

$$e_S = E_S - I_p/G_S. \tag{12}$$

If we have a single cell, then the physical voltage will equal the diffusion-metabolic potential of the membrane, but in a syncytium this may not be the situation.

It is often the case that the cells of a syncytium are not identical, either morphologically or electrically (see the previous paper by Mathias et al., 1985, or the review by Mathias and Rae, 1985, on electrical and structural properties of the lens). Moreover, syncytial tissues may include many hundreds of thousands of cells, accordingly the dimensions of the tissue are orders of magnitude greater than those for a single cell. If spatially separated populations of cells within a syncytium have different membrane properties, then ions will circulate between these populations and there may be current loops as well. Small currents, which flow over long distances, can produce significant standing voltages, particularly in the small

intercellular spaces of a tissue. Furthermore, if there is a net flux of ions, then water tends to follow (Mathias, 1985). Fluid movement is associated with gradients in hydrostatic pressure and with convection of solute, so steady state in a syncytial tissue may be much more complex than that for a single cell.

Even though no attempt has been made at solving the system of Eqs. 1–5, some useful relationships have been derived relating cellular volume and electrochemical steady state. The purpose of the analysis that follows is to derive similar relationships for a syncytial tissue and to provide some simple, analytical, approximate expressions describing voltage, hydrostatic pressure, and volume regulation in syncytia.

Definitions of the terms used in the analysis are presented in the Glossary below. The parameters are often subscripted to indicate a location or a particular solute, so the subscripts are defined in the subsequent list.

## GLOSSARY

| | |
|---|---|
| $a$ | tissue radius (cm) |
| $A_i$ | amount of intracellular impermeant protein (moles) |
| $c$ | concentration of mobile solute (moles/cm$^3$) |
| Cl | concentration of chloride (moles/cm$^3$) |
| $D$ | effective diffusion coefficient (cm/s) |
| $e$ | diffusion-metabolic potential of a membrane [see equation (22)] (volts) |
| $E$ | Nernst potential (volts) |
| $g$ | membrane conductance of an inner cell (S/cm$^2$) |
| $G$ | membrane conductance of a surface cell (S/cm$^2$) |
| $i_p$ | electrogenic current of active Na-K transport across inner cell membranes (amps/cm$^2$) |
| $I_p$ | electrogenic current of active Na-K transport across surface cell membranes (amps/cm$^2$) |
| $j$ | solute flux (moles/(cm$^2$ s)) |
| $k$ | electro-osmotic coefficient [see equation (30)] (cm$^2$/(s volt)) |
| K | potassium concentration (moles/cm$^3$) |
| Na | sodium concentration (moles/cm$^3$) |
| $Os$ | osmolarity (moles/cm$^3$) |
| $p$ | hydrostatic pressure (mmHg) |
| $r$ | radial location (cm) |
| $R$ | effective resistivity ($\Omega$ – cm) |
| $S_m/V_T$ | surface of membrane per unit volume of tissue (cm$^{-1}$) |
| $u$ | water flow velocity (cm/s) |
| $V_e/V_T$ | volume of extracellular space within the tissue per unit volume of tissue |
| $\bar{z}$ | average amount of net negative charge on the impermeant proteins $A_i$ |
| $\Delta e$ | difference between the cation and anion diffusion-metabolic potential [see Eq. 26)] (volts) |
| $\Delta g$ | difference between cation and anion conductance for the inner cell membranes [see Eq. 26] (S/cm$^2$) |
| $\Delta G$ | difference between cation and anion conductance for the surface cell membranes [see Eq. 26] (S/cm$^2$) |
| $\lambda$ | length constant for extracellular current flow [see Eq. 31] (cm) |
| $\eta$ | effective viscous resistance to water flow (cm$^2$/(s mmHg)) |
| $\rho$ | average excess mobile charge density (coul/cm$^3$) |
| $\sigma$ | reflection coefficient |
| $\tau$ | tortuosity factor |
| $\psi$ | voltage (volts) |

### Subscripts

| | |
|---|---|
| i, e, o | intracellular, extracellular but within the tissue, outside of the tissue |
| Na, K, Cl | pertaining to sodium, potassium, chloride |
| m | inner cell membrane |
| S | surface cell membrane |

## DERIVATION OF EQUATIONS

For simplicity, the derivation will consider a spherically symmetrical syncytium; however, this assumption is not essential and the differential equations can be applied in general if one replaces the operator $(1/r^2)(d/dr)(r^2 \cdot)$ with the divergence operator and $d/dr(\cdot)$ with the gradient operator. The derived equations represent conservation laws at each point within the tissue, and these must be satisfied regardless of inhomogeneities and anisotropies in the structure or nonlinearities in the electrical properties of the membranes. In subsequent sections, where approximate analytic solutions are derived, we must make explicit restrictive assumptions on symmetry, uniformity, and linearity. However, the differential equations of this section provide the framework to compute numerically the steady state of the tissue at whatever level of complexity that one can specify the local properties.

### Conservation Equations

The derivation of conservation laws for each ion is similar to the derivation of syncytial current flow presented in Eisenberg et al. (1981) and Mathias (1983). Consider a small cuboid of tissue, which contains one or more cells and intercellular space. In spherical coordinates, the volume of the cuboid can be expressed as $\Delta V(r) = r^2 \Delta\phi \Delta\theta \Delta r$; the radial face of the cuboid at $r + \Delta r/2$ has area $\Delta S(r + \Delta r/2) = (r + \Delta r/2)^2 \Delta\phi\Delta\theta$ and at $r - \Delta r/2$, $\Delta S(r - \Delta r/2) = (r - \Delta r/2)^2 \Delta\phi\Delta\theta$. The total amount of an ion $X$ = Na, K, or Cl crossing the membranes contained in the small cuboid is given by $\Delta V (S_m/V_T) j_{m,X}$, where $j_{m,X}$ is the membrane flux in moles/(cm$^2$ s). The amount of radial intracellular flow of ion X crossing the face of the cuboid at $r - \Delta r/2$ is $\Delta S(r - \Delta r/2) j_{i,X}(r - \Delta r/2)$ and at $r + \Delta r/2$ it is $\Delta S(r + \Delta r/2) j_{i,X}(r + \Delta r/2)$, where $j_{i,X}$ is the radial intracellular flux in moles/(cm$^2$ s). If we assume there are no angular components of flux, then the change in radial flow must equal the membrane flow. Namely,

$$\left(r + \frac{\Delta r}{2}\right)^2 j_{i,X}\left(r + \frac{\Delta r}{2}\right) - \left(r - \frac{\Delta r}{2}\right)^2 j_{i,X}\left(r - \frac{\Delta r}{2}\right) = -r^2 \Delta r \frac{S_m}{V_T} j_{m,X}(r).$$

If we assume that $\Delta r$ is small relative to spatial gradients in flux, then this tends to the radial divergence operator in spherical coordinates

$$\frac{1}{r^2}\frac{d}{dr}[r^2 j_{i,X}(r)] = -\frac{S_m}{V_T} j_{m,X}(r).$$

The ions lost from the intracellular compartment must be gained by the extracellular compartment within the cuboid, hence by similar reasoning we can write

$$\frac{1}{r^2}\frac{d}{dr}[r^2 j_{e,X}(r)] = +\frac{S_m}{V_T} j_{m,X}(r).$$

Lastly, we must relate the radial and membrane fluxes to the forces which drive them. At any radial location, $r$, there will be a transmembrane driving force as is specified in Eqs. 1–3, except that the voltage in the intercellular space may not be zero. Thus, in Eq. 13 the extracellular voltage, $\psi_e$, modifies the electrochemical driving force. The radial flux can be driven by convection, diffusion, and conduction. The convected flux is the product of the fluid flow velocity, $u$, and the concentration of ion X at location $r$. The flux due to diffusion and conduction is assumed to be described by the Nernst Plank equation (Sten-Knudsen, 1978). The diffusion coefficient, $D_e$, is an effective parameter that depends on the volume fraction of extracellular space, $V_e/V_T$, the tortuosity, $\tau$, of the clefts and the actual diffusion coefficient for the ion X (Mathias, 1983). Similarly, $D_i$ depends on gap junctional properties as well as the diffusional properties of the cytoplasm. Though the diffusion coefficients differ slightly for different ions, no correction for these differences is included in the subsequent equations.

$$\frac{1}{r^2}\frac{d}{dr}r^2\left[X_e u_e - D_e\left(\frac{dX_e}{dr} + X_e\frac{FZ}{RT}\frac{d\psi_e}{dr}\right)\right]$$
$$= +\frac{1}{FZ}\frac{S_m}{V_T} g_X[\psi_i - \psi_e - E_X + i_X/g_X];$$
$$\frac{1}{r^2}\frac{d}{dr}r^2\left[X_i u_i - D_i\left(\frac{dX_i}{dr} + X_i\frac{FZ}{RT}\frac{d\psi_i}{dr}\right)\right]$$
$$= -\frac{1}{FZ}\frac{S_m}{V_T} g_X[\psi_i - \psi_e - E_X + i_X/g_X]. \quad (13)$$

At the outer surface of the tissue the intracellular fluxes up to the membrane must equal the fluxes across the membrane, the extracellular potential is assumed to equal the bath voltage and the concentration of any extracellular ion is assumed to equal that in the bath.

$$X_i u_i - D_i\left[\frac{dX_i}{dr} + X_i\frac{FZ}{RT}\frac{d\psi_i}{dr}\right] = \frac{1}{F}G_X[\psi_i - E_X + I_X/G_X],$$
$$\psi_e = 0, \quad X_e = X_o, \quad \text{on } r = a. \quad (14)$$

For approximate electrical neutrality, we require

$$Na_e + K_e - Cl_e = \rho_e/F,$$

and

$$Na_i + K_i - Cl_i = \rho_i/F. \quad (15)$$

$\rho_e$ is the small amount of excess mobile positive charge that is present in narrow intercellular spaces owing to the fixed negative charges on the outer surface of cell membranes. A significant amount of fluid can be moved by electro-osmosis because of the presence of $\rho_e$ (McLaughlin and Mathias, 1985), but in regard to electroneutrality, $\rho_e$ is negligible in comparison to total sodium-chloride. In the intracellular compartment, $\rho_i$ represents the excess of $K_i^+$ and $Na_i^+$ over $Cl_i^-$ due to the presence of large, negatively charged proteins, $A_i$. These proteins are generally too large to pass through gap junctions, hence they will not contribute to diffusion, conduction or convection in the same manner as the small ions. We will therefore assume that in regard to Eq. 15 the excess mobile charges are approximately given by

$$\rho_e/F \approx 0; \quad \rho_i/F \approx \bar{z}A_i/V_i; \quad (16)$$

accordingly, the concentrations of total mobile solute[1] are assumed to be

$$c_e = Na_e + K_e + Cl_e;$$
$$c_i = Na_i + K_i + Cl_i. \quad (17)$$

The osmolarity of the extracellular and intracellular spaces is given by

$$Os_e = c_e,$$
$$Os_i = c_i + A_i/V_i. \quad (18)$$

## Voltage Equations

Given the assumptions in Eqs. 15–17, if the cation fluxes (Eq. 13) are subtracted from the anion fluxes (Eq. 13), one obtains two equations and boundary conditions on ionic current flow.

$$\frac{1}{r^2}\frac{d}{dr}(r^2 i_e) = +\frac{S_m}{V_T} g_m[\psi_i - \psi_e - e_m],$$
$$\frac{1}{r^2}\frac{d}{dr}(r^2 i_i) = -\frac{S_m}{V_T} g_m[\psi_i - \psi_e - e_m], \quad (19)$$

where

$$i_e = -\frac{1}{R_e}\frac{d\psi_e}{dr},$$
$$i_i = -\frac{1}{R_i}\frac{d\psi_i}{dr} + \rho_i u_i, \quad (20)$$

and on the boundary

$$i_i = G_s(\psi_i - e_s), \quad \psi_e = 0, \quad \text{on } r = a. \quad (21)$$

[1] It is possible that the large intracellular proteins are indeed able to move from cell to cell but with a very small diffusion coefficient. For example, there could be periodic cell fusions (Macsai et al., 1984, report such fusions between lens cells), or there could be protein breakdown and movement of amino acid chains, then reassembly of protein.

The parameters in the above equations are defined by

$$R_e^{-1} = D_e \frac{F^2}{RT} c_e;$$

$$R_i^{-1} = D_i \frac{F^2}{RT} c_i;$$

$$G_S = G_{Na} + G_K + G_{Cl};$$

$$g_m = g_{Na} + g_K + g_{Cl};$$

$$e_S = (G_{Na}E_{Na} + G_K E_K + G_{Cl}E_{Cl} - I_P)/G_S;$$

$$e_m = (g_{Na}E_{Na} + g_K E_K + g_{Cl}E_{Cl} - i_P)/g_m. \quad (22)$$

## Solute Flux Equations

If the cation and anion fluxes (Eq. 13) are added together, we obtain two solute flow equations and boundary conditions

$$\frac{1}{r^2}\frac{d}{dr}(r^2 j_e) = +\frac{1}{F}\frac{S_m}{V_T}[\Delta g_m(\psi_i - \psi_e) - g_m \Delta e_m];$$

$$\frac{1}{r^2}\frac{d}{dr}(r^2 j_i) = -\frac{1}{F}\frac{S_m}{V_T}[\Delta g_m(\psi_i - \psi_e) - g_m \Delta e_m]; \quad (23)$$

where

$$j_e = c_e u_e - D_e \frac{dc_e}{dr};$$

$$j_i = c_i u_i - D_i \frac{dc_i}{dr} - D_i \frac{\rho_i}{RT}\frac{d\psi_u}{dr}; \quad (24)$$

and on the boundary

$$c_e = c_o, \quad j_i = \Delta G_S \psi_i - G_S \Delta e_S, \quad \text{on } r = a. \quad (25)$$

The parameters appearing in the above equations are defined by

$$\Delta G_S = G_{Na} + G_K - G_{Cl};$$

$$\Delta g_m = g_{Na} + g_K - g_{Cl};$$

$$\Delta e_S = (G_{Na}E_{Na} + G_K E_K - I_P - G_{Cl}E_{Cl})/G_S;$$

$$\Delta e_m = (g_{Na}E_{Na} + g_K E_K - i_p - g_{Cl}E_{Cl})/g_m. \quad (26)$$

One can see that the electrically neutral flux is very much dependent on voltage and is determined by those same factors which determine ion flux. The difference is just that the anion current is subtracted from the cation current when one computes the electrically neutral solute flux.

## Fluid Flow Equations

In order to complete the specification of the problem, the fluid flow velocities $u_{i,e}$ must satisfy conservation laws and the velocities must be related to the potential functions for the flows. Mathias (1985) shows that fluid flow in long narrow intercellular spaces is laminar and therefore is described by Poiseuille's equation; McLaughlin and Mathias (1985) extend this analysis to include electro-osmosis. The expressions which describe the flow of water along intercellular spaces in Eqs. 27–29 are derived in those papers.

The potential function for intracellular fluid flow in Eq. 28 is written as a weighted sum of all of the forces. There is a large excess of mobile positive charge in the intracellular compartment (see Eq. 16), thus one expects electro-osmosis to be an important factor in fluid movement. However intracellular water flow will usually move from cell to cell through gap junctions, so electro-osmosis will be the Schmid type (Schmid, 1950, discussed in Hill, 1975) rather than the type derived by McLaughlin and Mathias (1985) for extracellular flow. Moreover, fluid movement through gap junctions may be in some respects like transmembrane flow, thus osmosis may play some role and a term proportional to concentration gradients is included in Eq. 28. The water flow equations are

$$\frac{1}{r^2}\frac{d}{dr}(r^2 u_e) = +\frac{S_m}{V_T} L_m[p_e - p_i + \sigma_m RT(Os_i - Os_e)];$$

$$\frac{1}{r^2}\frac{d}{dr}(r^2 u_i) = -\frac{S_m}{V_T} L_m[p_e - p_i + \sigma_m RT(Os_i - Os_e)]; \quad (27)$$

where

$$u_e = -\frac{1}{\eta_e}\frac{dp_e}{dr} - k_e \frac{d\psi_e}{dr};$$

$$u_i = -\frac{1}{\eta_i}\left[\frac{dp_i}{dr} + \sigma_i RT \frac{dc_i}{dr}\right] - k_i \frac{d\psi_i}{dr}; \quad (28)$$

and on the boundary[2]

$$p_e = 0, \quad u_i = -L_S[p_i - \sigma_s RT(Os_i - c_o)], \quad \text{on } r = a. \quad (29)$$

The coefficients for the intercellular hydrostatic and electro-osmotic gradients are derived in Mathias (1985) and McLaughlin and Mathias (1985) respectively

$$\eta_e = \frac{12\eta}{w^2 \tau\, V_e/V_T};$$

$$k_e = -\frac{\epsilon_o \epsilon_r \zeta}{\eta}\tau \frac{V_e}{V_T}; \quad (30)$$

where: $\eta$ is the viscosity of water; $w$ is the width of the intercellular space; $\epsilon_o$ is the permittivity of free space; $\epsilon_r$ is the dielectric constant of water; $\zeta$ is the potential at the membrane-fluid interface due to fixed negative charges on the membrane (note $k_e$ is generally positive since $\zeta$ is

[2]This expression is only approximately correct. Mathias (1985), Fig. 4, shows there must be a local variation in osmolarity within each surface cell in order to provide the working osmotic gradient needed to drive the transmembrane flow. The above boundary condition is appropriate to the order zero approximation in the perturbation expansion.

generally negative); and other parameters are defined in the Glossary. The coefficients for the intracellular potential function are essentially phenomenological in nature and we need experimental data to provide an estimate of their values. That is, flow through one connexon of a gap junction is likely to differ from laminar flow through a pipe, hence we cannot utilize classical physics to estimate the relationship between forces and flows, rather, we need to measure the relationship.

## APPROXIMATE SOLUTIONS

The differential equations just derived involve radially varying coefficients and nonlinearities, so their solution cannot be represented analytically except in approximate form. However, an approximate analytical representation can provide a great deal of insight, moreover it often suggests experiments, and it generally assists in the interpretation of data. This section will therefore focus on analytical approximations, even though there is an accompanying contraction in the scope of the results.

The approximations needed to render the equations soluble have been mostly worked out in previous publications, (Eisenberg et al., 1979; Mathias, 1985; McLaughlin and Mathias, 1985). They are: (*a*) intracellular gradients in voltage, concentration, and hydrostatic pressure are much smaller than their extracellular counterparts, accordingly, these intracellular parameters can, to a first approximation, be considered spatially uniform; (*b*) cell membranes typically have a large water permeability, such that a small transmembrane concentration difference will produce a large transmembrane fluid movement, hence the resulting water flow convects away solute and keeps steady state transmembrane osmotic differences quite small in comparison with total solute concentration; (*c*) the values of Nernst potentials are approximately spatially uniform; (*d*) the membrane conductances are approximately spatially uniform.

The last two assumptions are probably the least general, thus, before using the following results as a quantitatively accurate description of a particular tissue, one should consider the validity of approximations *c* and *d*. (See the section on "Validity of Assumptions" in regard to the lens.)

### Solution of the Voltage Equations

To obtain expressions relating steady-state voltages with the Nernst potentials, the rate of active transport and the membrane selectivity, we integrate the flux equations over all the membranes of the syncytium and, for steady state, we require that this integral must be zero. The generic flux integral is

$$0 = 4\pi a^2 G_X[\psi_i - E_X + I_X/G_X] + 4\pi \frac{S_m}{V_T} g_X \cdot \int_0^a [\psi_i - \psi_e(r) - E_X + i_X/g_X] r^2 dr, \quad (31)$$

where $E_X$, $g_X$, $i_X$, and $\psi_i$ are assumed to be radially independent and we need to determine an expression for $\psi_e(r)$.

The expression for $\psi_e(r)$ can be computed from the equations describing extracellular ion flow (Eqs. 19–21), where $e_m$ is determined from Eq. 22 and the flux integrals (Eq. 31). The solution is

$$\psi_e(r) = (\psi_i - e_m)\left(1 - \frac{a \sinh r/\lambda}{r \sinh a/\lambda}\right), \quad (32)$$

where the length constant $\lambda$ is defined by

$$\frac{1}{\lambda^2} = R_e \frac{S_m}{V_T} g_m. \quad (33)$$

Substituting Eq. 32 into the conservation Eq. 31 for X = Na, K, Cl leads to three conservation laws that are the syncytial analogs of Eqs. 1–3 for a single cell

$$G_{Na}(\psi_i - E_{Na}) + \frac{a}{3}\frac{S_m}{V_T} g_{Na}(e_m - E_{Na}) + g_e \frac{g_{Na}}{g_m}(\psi_i - e_m) = -3\left(I_P + \frac{a}{3}\frac{S_m}{V_T} i_p\right), \quad (34)$$

$$G_K(\psi_i - E_K) + \frac{a}{3}\frac{S_m}{V_T} g_K(e_m - E_K) + g_e \frac{g_K}{g_m}(\psi_i - e_m) = 2\left(I_P + \frac{a}{3}\frac{S_m}{V_T} i_p\right), \quad (35)$$

$$G_{Cl}(\psi_i - E_{Cl}) + \frac{a}{3}\frac{S_m}{V_T} g_{Cl}(e_m - E_{Cl}) + g_e \frac{g_{Cl}}{g_m}(\psi_i - e_m) = 0, \quad (36)$$

where $g_e$ is the input conductance to the cablelike structure of intercellular clefts:

$$g_e = \frac{1}{\lambda R_e}\left(\coth a/\lambda - \frac{1}{a/\lambda}\right). \quad (37)$$

Moreover, the three conservation laws (Eqs. 34–36) can be combined to obtain a general relationship between the intracellular voltage and the major permeant ions

$$\psi_i = \frac{G_S e_S + g_e e_m}{G_S + g_e}. \quad (38)$$

This result is the syncytial analog of Eq. 7 for a single cell. The total input conductance per unit area of surface of our syncytial sphere is given by $g_e + G_S$, and the weight on the inner membrane diffusion-metabolic potential, $e_m$, is simply the fraction of the total input conductance that describes current flow into the intercellular spaces.

### Volume Regulation

If we assume the dependent variables of Eqs. 34–36 are $\psi_i$, $E_{Na}$, $E_K$, and $E_{Cl}$, the above flux equations provide three

equations in four unknowns. A fourth condition is obtained by combining Eqs. 15 and 16 for intracellular electrical neutrality. However, Eq. 16 introduces yet another dependent variable: $V_i$ = the volume of the intracellular space. In our analysis of a single cell, we invoked osmotic balance as the final equation needed to specify the problem, but for a syncytium the situation may be somewhat different.

Eqs. 27–29 allow for a net transmembrane flow of fluid at any given spatial location, so the solution of these equations must be investigated before one can safely predict the final equation. Eq. 27 relates hydrostatic and osmotic gradients in the intracellular and extracellular spaces. The extracellular equation can be written in the following form

$$\lambda_w^2/a^2\left[\frac{1}{\bar{r}^2}\frac{d}{d\bar{r}}\left(\bar{r}^2\left[\frac{dp_e}{d\bar{r}}+\eta_e k_e\frac{d\psi_e}{d\bar{r}}\right]\right)\right] = p_e - p_i + RT(Os_i - Os_e), \quad (39)$$

where we assume $\sigma_m \approx 1.0$ and

$$\bar{r} = r/a$$

$$\frac{1}{\lambda_w^2} = \eta_e \frac{S_m}{V_T} L_m.$$

Mathias (1985) used the above equation as the starting point for a perturbation expansion and analysis of water transport by epithelia. The perturbation depended on the smallness of the dimensionless parameter, $\lambda_w^2/a^2$. For a syncytium, the radius, $a$, is much larger than the thickness of an epithelium, hence we expect $\lambda_w^2/a^2$ will generally be a small parameter. For example, if we use the value of $L_m \simeq 5 \times 10^{-9}$ (cm/s)/mmHg (Spring, 1983), then for a small frog lens we can calculate (see Table I) that $\lambda_w^2/a^2 \simeq 5 \times 10^{-4}$. We will therefore assume $\lambda_w^2/a^2 \simeq 0$, whereupon Eq. 39 simplifies to[3]

$$0 = p_e - p_i + RT(Os_i - Os_e). \quad (40)$$

Eq. 40 shows that transmembrane hydrostatic gradients $p_i - p_e$ will be balanced by transmembrane osmotic pressure $RT(Os_i - Os_e)$.

Eqs. 27 and 29 can be combined to formulate an integral constraint on transmembrane fluid flow.

$$0 = 4\pi\frac{S_m}{V_T}L_m\int_0^a r^2\{p_e(r) - p_i + RT[Os_i - Os_e(r)]\}\,dr + 4\pi a^2 L_s\{p_i - RT[Os_i - Os_e(a)]\}.$$

On substitution of Eq. 40 into the above integral constraint, we find

$$p_i = RT(Os_i - c_o) \quad (41)$$

which is the same result we obtained for a single cell. Substituting Eq. 41 into Eq. 40 yields the "balanced gradient" result (Mathias, 1985).

$$p_e(r) = RT[Os_e(r) - c_o]. \quad (42)$$

Thus, Eq. 40 requires a balance in transmembrane gradients but in order to achieve such a balance, we must have the even stronger constraint that the net intracellular pressure is zero (Eq. 41) and the net extracellular pressure is zero (Eq. 42).

As was found for a single cell, Eq. 41 implies the intracellular osmolarity does not equal the osmolarity of the bath whenever there is an intracellular hydrostatic pressure. If $p_i$ is indeed different from zero, we need another equation to relate $p_i$, $V_i$, and the compliance of the tissue.

In regard to the extracellular Eq. 42, we must have some hydrostatic pressure in the clefts if there is any water flow. The value of $RT$ scales osmotic gradients to hydrostatic pressure: $RT = 20$ mmHg/mM. Tiny osmotic gradients, in a balanced gradient system, balance large hydrostatic pressures (large in regards to animal cells), hence gradients in osmolarity are expected to be quite small and these results are the basis of assumption *b* at the beginning of this section.

We now have established the syncytial analogs of all of the relationships that could be obtained for a single cell. However, more interesting things are going on in the syncytium, since there can be circulating water flow.

## Solution of the Water and Solute Flux Equations

Water flow is driven by the transmembrane flux of solute. Very small transmembrane osmotic gradients are sufficient to move significant amounts of water from the cells and into the extracellular space (Mathias, 1985). The water flow velocity can therefore be determined by integrating the extracellular solute flux equations (Eqs. 23 and 24), but first consider the relative values of convected solute vs. diffusion of total solute.

Eq. 42 allows one to express concentration gradients in terms of hydrostatic gradients. If both the diffusion and convection components of extracellular solute flux are compared in units of hydrostatic pressure, it is straightforward to assess which is dominant. The result of such a comparison is that diffusional flux is <2% of convected flux.[4] We can therefore neglect the diffusion term in the extracellular solute flux equation (Eq. 23), assume total

[3]This approximation can be valid only if it is compatible with the boundary conditions. In order to obtain a self consistent set of problems one usually resorts to the formal framework of a perturbation expansion, however because such an expansion has been performed for an analogous problem (Mathias, 1985), we will take some short cuts. In particular, the boundary condition (Eq. 29) on intracellular water flow is consistent with the above approximation if the values of intracellular pressure, voltage and osmolarity are approximately spatially uniform, and the membrane reflection coefficients are near unity.

[4]This result applies to total solute and not to a particular ion, such as potassium, which is in low concentration in the extracellular space.

solute concentration is approximately radially uniform, and integrate to obtain the water flow velocity

$$c_o u_e(r) = -\frac{1}{F}\frac{S_m}{V_T} \cdot \left[(g_m \Delta e_m - \Delta g_m e_m)\frac{r}{3} + \Delta g_m \lambda^2 \frac{d\psi_e(r)}{dr}\right], \quad (43)$$

where we have combined Eqs. 19 and 23 before performing the integration, and the constant of integration was taken to be zero in order for the flow to remain finite at $r = 0$.

If we substitute Eq. 28 into Eq. 43 and integrate once more, the extracellular hydrostatic pressure can be derived

$$p_e(r) = -\frac{\eta_e}{c_o F}\frac{S_m}{V_T}(g_m \Delta e_m - \Delta g_m e_m)\left(\frac{a^2 - r^2}{6}\right) + \left(\frac{\eta_e \lambda^2}{F c_o}\frac{S_m}{V_T}\Delta g_m - k_e \eta_e\right)\psi_e(r). \quad (44)$$

This equation indicates that there may be significant hydrostatic gradients within the intercellular space of a syncytium and small osmotic gradients.

## Summary

All of these results can be summarized as follows. In syncytial tissues, the values of Nernst potentials, resting voltage and intracellular volume are determined from five equations: three equations requiring conservation of Na, K and Cl, given in Eqs. 34–36; one equation requiring intracellular electrical neutrality, given in Eq. 15; and one equation requiring osmotic balance (assuming $p_i = 0$), given in Eq. 41. These five equations have direct analogs in the analysis of a single cell, Eqs. 1–5. However, because of the spatial localization of transport properties, a syncytium may also have a circulating flux of solute, a circulating flow of water, standing extracellular voltage gradients and significant extracellular hydrostatic gradients. The transmembrane solute flux is determined from the Nernst potentials and membrane voltage by subtracting the anion current from the cation current; the water flow is determined by the amount of solute that crosses the inner cell membranes and must be convected along the intercellular spaces; the standing extracellular voltage gradients are determined by the circulating ionic current and the effective resistivity of the extracellular clefts; the hydrostatic pressure is determined by the hydraulic resistance of the intercellular spaces in connection with the total amount of water flow less the amount of water moved by electroosmosis.

## GRAPHICAL RESULTS FOR THE FROG LENS

We do not presently have good estimates for all of the lens parameter values in the previous equations, so this section is not a quantitatively accurate description of the lens. Rather, these results are an example of the complex steady state that can exist in syncytia. We believe the lens is in a similar steady state.

Table I lists the parameter values used in the numerical calculations. The parameters in the left column were measured experimentally by Mathias, et al., (1979). The paper by Mathias, et al., (1985), which accompanies this report, suggests that most of the lens K-permeability lies on surface membranes whereas inner-fiber membranes are predominantly Na and Cl selective. Moreover, Neville et al. (1978) have shown that most of the electrogenic active transport in the lens occurs near the surface. The values of active transport and conductance listed in Table I are extrapolations of these approximate separations of properties to the idealized situation where the transport and selectivities are entirely localized. The idealized localization of selectivity, in connection with the measured values of total conductance and intracellular resting voltage, were substituted into the three ion flux balance equations (Eqs. 34–36), and values of $E_{Na}$, $E_K$, $E_{Cl}$, and $I_P$ were calculated such that a true steady state exists. These calculated values are reported in Table I, but they are essentially the same as values that have been reported elsewhere from experiment (Paterson, 1972; Mathias and Rae, 1985). The values of $\eta_e$ and $k_e$ are computed using Eq. 30 in connection with the structural parameters listed in Table I. We assumed the zeta potential is $-15$ mV, which is similar to values for other membranes where it has been measured (McLaughlin and Mathias, 1985). The cleft width $w$ is estimated by

$$\frac{V_e/V_T}{S_m/V_T} = \frac{w}{2}; \quad (45)$$

the value of $\tau V_e/V_T$ is calculated from the ratio of measured effective cleft resistivity, $R_e$, to the resistivity of Ringer's solution (~90 Ω cm). The value of $V_e/V_T$ and $S_m/V_T$ are estimated in Mathias et al. (1979).

Lastly, we assume that intracellular osmolarity equals that of the bath (Eq. 6), hence intracellular hydrostatic pressure in Eq. 41 is assumed to be zero. The lens is surrounded by a tough elastic capsule and if one punctures this capsule, the cytoplasm usually oozes out as if there is indeed some small steady-state intracellular hydrostatic

TABLE I

| Measured | Estimated/Calculated | |
|---|---|---|
| $\psi_0 = -68$ mV | $I_p = 2.3$ μa/cm² | $S_m/V_T = 6{,}000$ cm$^{-1}$ |
| $a = 0.16$ cm | $i_p = 0$ | $V_e/V_T = 0.012$ |
| $g_m = 0.44$ μS/cm² | $E_{Na} = 40$ mV | $\tau = 0.16$ |
| $G_s = 0.21$ mS/cm² | $E_K = -90$ mV | $\eta_e = 2.4 \times 10^9$ mmHg s/cm² |
| $\lambda = 0.09$ cm | $E_{Cl} = -58$ mV | $k_e = 3.3 \times 10^{-7}$ cm²/(volt s) |
| $R_e = 47$ KΩ cm | $A_i/V_i = 78$ mM | $g_{Na} = g_{Cl} = 1/2\, g_m$ |
| $K_0 = 3$ mM | $\bar{z} = 1.5$ | $G_K = G_S$ |
| $Na_0 = 107$ mM | $e_m = -9$ mV | $\Delta e_m = 49$ mV |
| $Cl_0 = 110$ mM | $e_S = -101$ mV | $\Delta e_s = -101$ mV |

pressure. However, the osmotic pressure of a 50 $\mu$M transmembrane concentration gradient will balance 1 mmHg of hydrostatic pressure, so the assumption of osmotic balance should be accurate.

## Resting Voltages and Ionic Current

Fig. 2 *A* illustrates the expected approximate distribution of steady-state voltages within the lens. The intracellular resting voltage is assumed to be approximately spatially uniform.[5] The large spatial variation in $\psi_e(r)$ is due to two factors. First, the intercellular spaces of the lens are long, narrow, and tortuous, hence they impose a large resistance to ionic current flow. Second, the selectivity of the inner-cell membranes is quite different from that of surface cells, so the steady-state intracellular voltage is much more negative than the diffusion potential, $e_m$, of the inner-cell membranes. A large driving force for current flow therefore exists across the inner fiber-cell membranes and this current must circulate through the high-resistance extracellular clefts.

One unusual feature of this steady-state voltage distribution is illustrated in Fig. 2 *B*, where the physical voltage across inner membranes, $\psi_i - \psi_e$, is graphed as a function of radial location. The Nernst potential for chloride, $E_{Cl}$, is substantially more positive than the intracellular voltage one records with a microelectrode (Duncan, 1970; Paterson and Eck, 1971; Guerschanik et al., 1977), but if one calculates the real transmembrane voltage, then $E_{Cl}$ is positive to the transmembrane voltage in the cortex and negative to the transmembrane voltage in the nucleus. The chloride transmembrane current therefore changes direction in accordance with the arrows in Fig. 2 *B*. We have assumed there are no active or exchange transport mechanisms for chloride so the total current entering the cells in the cortex just balances the total current leaving the cells in the nucleus and we attain steady state. It is commonly assumed that if the Nernst potential for an ion differs from the intracellular voltage, then that ion must be actively transported. Fig. 2 *B* illustrates that in a syncytial tissue one cannot make such an assumption, since $E_{Cl}$ seems to be out of electrochemical equilibrium with $\psi_i$, yet it is indeed in passive steady state with $\psi_i - \psi_e$.

There are two major components of ion flux along the intercellular spaces of a syncytial tissue: (*a*) ionic current flow, which is driven by radial voltage gradients; (*b*) convected ion flux, which is driven by radial hydrostatic pressure gradients as well as electro-osmosis. Although convection and conduction will both move significant amounts of ions along the intercellular clefts, it is the transmembrane electrochemical gradients that move the ions into the intercellular spaces. The values of Nernst potentials in Table I were calculated in accordance with

[5]In actuality, there are small radial gradients in the intracellular voltage in the frog lens. These are measured and analyzed in Mathias and Rae (1985).

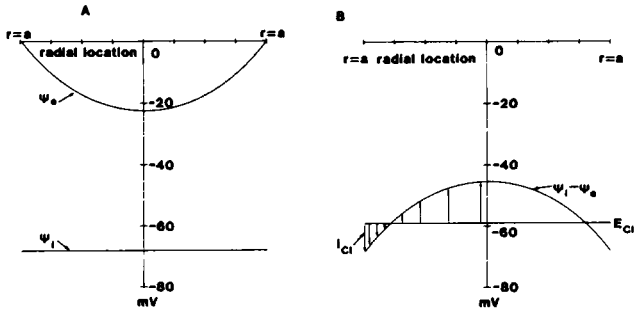


FIGURE 2 (*A*) The predicted radial distribution of steady state extracellular voltage in the frog lens, assuming $\psi_i$ is approximately spatially uniform (Mathias and Rae, 1985, calculate the first order spatial variation in $\psi_i$). (*B*) The relationship between the Nernst potential for chloride and the physical voltage across inner cell membranes. We assume chloride is not actively transported, yet there is a circulating chloride flux which crosses the membranes as indicated by the arrows.

Eq. 35 such that each transmembrane ion flux balances. This is illustrated in Fig. 3 *A* and *B*, where transmembrane ionic currents for Cl and Na are graphed.

Fig. 3 *A* illustrates the total transmembrane chloride current in a unit radial distance, given by $4\pi r^2(S_m/V_T)g_{Cl}(\psi_i - \psi_e - E_{Cl})$. The flux goes to zero at the center of the lens because the amount of membrane per unit length ($4\pi r^2 S_m/V_T$) goes to zero. The chloride transmembrane current leaves cells in the nucleus, crosses zero at $r \approx 0.77a$ and enters cells in the cortex, because the sign of $\psi_i - \psi_e - E_{Cl}$ changes from positive to negative at this value of $r$. The integral of the current is zero, so there is no net flow of chloride from the lens.

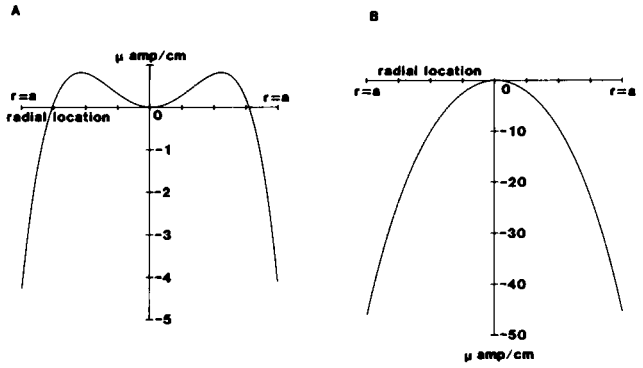


FIGURE 3 The radial distribution of ion flows across the inner cell membranes. (*A*) The transmembrane chloride current (note that flux has the opposite directionality). The chloride current is from intracellular to extracellular across the membranes of cells located at $0 \le r \le 0.77\ a$. The current changes direction at $r = 0.77\ a$ and flows from extracellular to intracellular across the membranes of cells located at $0.77\ a \le r \le a$. The change in direction is because the physical transmembrane potential, $\psi_i - \psi_e$, crosses the diffusion (Nernst) potential, $E_{Cl}$, at $r = 0.77\ a$ (see Fig. 2). The integral of the chloride current over $r$ is zero since we assume no chloride flows across the surface membranes. (*B*) The transmembrane sodium current. This current is from extracellular to intracellular at all radial locations since the physical transmembrane voltage is always significantly negative to $E_{Na}$. The integral of this current over $r$ represents the current of active sodium transport across the surface membranes.

Fig. 3 *B* illustrates the transmembrane sodium current per unit radial length. The passive sodium current is everywhere inward so there is a net flow of sodium into the intercellular clefts of the lens. The integral of the curve in Fig. 3 *B* is equal to the sodium current being actively transported out of the cells at the surface: $[4\pi a^2(3I_p)]$. We have assumed the Na-K ATPase extrudes 3 Na for 2 K being taken in, hence the net Na extrusion is proportional to $3I_p$. Once again, the net flux from the lens is zero since the passive current into the extracellular clefts equals the active current out of the intracellular space.

We have assumed there is no potassium permeability on the inner cell membranes so there will be no potassium flux along the intercellular clefts. Mass balance for potassium requires $0 = 4\pi a^2(2I_p) + 4\pi a^2 G_K(\psi_0 - E_K)$, in accordance with Eq. 35.

## Hydrostatic Pressure, Fluid Movement and Solute Fluxes

The net flux of osmotically active solute is proportional to the cation current minus the anion current, thus, owing to the circulation of sodium, there is a large solute flux and we expect significant water movement. The amount of water that follows the sodium flux must flow along the intercellular spaces. The voltage gradients along the intercellular spaces are in the right direction to move the fluid by electro-osmosis, however, if the inner membranes have a typical zeta potential, electro-osmosis will not be sufficient to move all of the water. A hydrostatic pressure must develop such that intercellular spaces of the nucleus are significantly below the pressure in the bath.

Fig. 4 *A* illustrates the distribution of hydrostatic pressure along the intercellular spaces. The largest negative going pressure is the total force needed to move the fluid flux along the intercellular clefts. Because we assume electro-osmosis moves a significant component of the fluid, the actual magnitude of pressure is reduced: the amount of the reduction is illustrated by the positive going curve in Fig. 4 *A*.

Fig. 4 *B* illustrates the radial fluid flow, $4\pi r^2 u_e(r)$ (cm$^3$/s), described by Eq. 44. The fluid flow has two components: one driven by electro-osmosis, marked $u_{eo}$, and the other driven by hydrostatic pressure, marked $u_{hp}$. The larger the flow $u_{eo}$, the less the total hydrostatic pressure in Fig. 4 *A*.

Fig. 5 illustrates the radial flux of Na and Cl along the intercellular spaces. These fluxes were computed assuming diffusion is negligible and assuming that radial concentration gradients are small (see Fig. 6 and related discussion for an estimate of how small), hence

$$j_{Na} = \left[\mathrm{Na}_o u_e(r) - \frac{\mathrm{Na}_o}{Fc_o}\frac{1}{R_e}\frac{d\psi_e(r)}{dr}\right]4\pi r^2;$$

$$j_{Cl} = \left[\mathrm{Cl}_o u_e(r) + \frac{\mathrm{Cl}_o}{Fc_o}\frac{1}{R_e}\frac{d\psi_e(r)}{dr}\right]4\pi r^2. \qquad (46)$$

The first component on the right hand side of Eq. 46 is the convected flux. From Fig. 4 we see that fluid moves from surface to center along the intercellular spaces, so both Na and Cl are carried inward by convection. In Fig. 5 *A* for chloride flux, the large negative going curve represents convection of chloride towards the center of the lens. However, because the chloride ion is negatively charged, it is conducted up the voltage gradient, outward along the intercellular spaces. The large positive flux in Fig. 5 *A* therefore represents the conduction of chloride towards the surface of the lens. The net chloride flux is the sum of

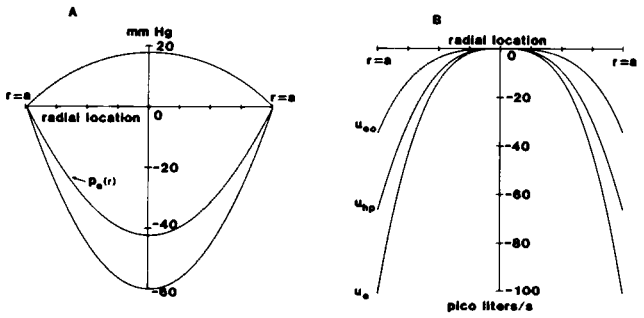


FIGURE 4 Hydrostatic pressure and fluid movement within the intercellular clefts of the frog lens. (*A*) The largest negative pressure represents the total driving force needed to produce the fluid flow, $u_e$. The actual hydrostatic pressure, $p_e$, is less negative owing to a component of fluid moved by electro-osmosis. The estimated reduction in pressure due to electro-osmosis is illustrated by the positive going curve. (*B*) The radial fluid flow along the intercellular clefts. A negative flow indicates fluid is moving inward, towards the lens center, along the clefts. The total flow, $u_e$, consists of two components: $u_{hp}$ is driven by hydrostatic pressure; $u_{eo}$ is driven by electro-osmosis.

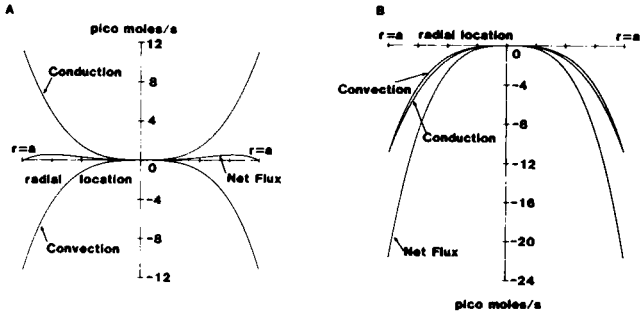


FIGURE 5 Radial ion fluxes along the intercellular clefts of the lens. A positive flux indicates the solute is moving radially outward, from lens center to surface, along the clefts. (*A*) The net chloride flux is the sum of the chloride convected inward by the fluid flow (see Fig. 4 *B*) and the outward conduction of the negatively charged chloride up the radial voltage gradient (see Fig. 2). The net flux is also the scaled integral of the transmembrane current illustrated in Fig. 3 *A*. Note that the radial flux goes to zero at the lens surface, $r = a$, hence there is no net movement of chloride into or out of the extracellular clefts of the lens. (*B*) The net sodium flux is the sum of inward convection and inward conduction; it is also the scaled integral of the transmembrane current illustrated in Fig. 3 *B*. At the surface, $r = a$, some 22 p moles/s of sodium is flowing into the extracellular clefts. This is balanced by the active transport rate, $4\pi a^2(3\,I_p/F)$, of sodium out of the lens across surface cell membranes.

inward convection and outward conduction: It is the relatively small flux in Fig. 5 *A*, which is zero at the center and goes to zero at the surface. We therefore see that chloride is indeed being conserved, since there is no flux at the surface. Moreover, this flux represents the integral of the transmembrane chloride current illustrated in Fig 3 *A*.

Sodium is positively charged, so it will be conducted inward along the negative voltage profile in the intercellular clefts. Consequently, conduction and convection of sodium add to produce the large negative flux illustrated in Fig. 5 *B*. At the surface of the lens, we see there is some 22 p mol of sodium entering the intercellular spaces every second. If we calculate the sodium being actively transported, given by $4\pi a^2(3I_p/F)$, we find this is precisely the amount of sodium being extruded by the surface cells every second.

## Validity of Assumptions

Given the large amount of fluid movement along the intercellular clefts, one wonders about the validity of neglecting transmembrane osmotic gradients. However, the amount of fluid being circulated by the lens is (per unit area of outer surface) much less than that typically transported by leaky epithelia, such as the renal proximal tubule, whereas the total surface area of the membranes lining the intercellular spaces of the lens is thousands of times greater than the area of lateral membranes in the proximal tubule (once again per unit area of outer surface). Thus, owing to the enormous area of inner membrane, the water flow per unit area of inner membrane is rather small, accordingly the working osmotic gradients should be negligible. The second kind of osmotic gradient is that which balances hydrostatic pressure (see Mathias, 1985, or Eq. 42). In Fig. 4 *A* we calculate there is approximately −42 mmHg of hydrostatic pressure in the extracellular space at the center of the lens, and this corresponds to an osmotic change of ~2 mM, or 1% of $c_o$. We will assume this is negligible. Lastly, we can have concentration gradients in K at the expense of Na, such that $Na_e + K_e = c_o/2$. Such concentration gradients will have no osmotic effect but they might lead to significant diffusion or affect the values of $g_m$ and $e_m$. In fact, if we wish to calculate extracellular potassium flux, such gradients are indeed important and diffusion cannot be neglected.

The flux of potassium is given by

$$j_K = K_e(r)u_e(r) - \frac{K_e(r)}{Fc_o}\frac{1}{R_e}\frac{d\psi_e(r)}{dr} - D_e\frac{dK_e(r)}{dr}. \quad (47)$$

We have assumed there is no potassium permeability or active transport on the inner membranes, so the radial flux of potassium must be zero everywhere. Setting $j_K = 0$ yields

$$D_e\frac{dK_e}{dr} = K_e\left[u_e - \frac{1}{Fc_o}\frac{1}{R_e}\frac{d\psi_e}{dr}\right]. \quad (48)$$

Both of the terms in brackets in Eq. 48 are large and negative; that is, both convection and conduction tend to move potassium inward along the intercellular spaces. We therefore cannot neglect diffusion, since it is the only force opposing the inward flux of convection plus conduction. Eq. 48 can be integrated to obtain

$$\ln\frac{K_e(r)}{K_o} = -\frac{1}{D_e}\left[\frac{1}{\eta_e}p_e(r) + \left(k_e + \frac{1}{Fc_oR_e}\right)\psi_e(r)\right], \quad (49)$$

where Eq. 28 was used to write $u_e$ in terms of hydrostatic pressure and voltage, and the boundary condition $K_e(a) = K_o$ is incorporated in Eq. 49.

Substituting the lens parameters into Eq. 49 yields the curve in Fig. 6 which shows the largest accumulation of potassium with depth. The value of $K_e$ at the lens center is ~21 mM, which is an increase of 18 mM over bath potassium, hence there needs to be an 18 mM depletion of extracellular sodium at the lens center. This depletion corresponds to a 5 mV drop in $E_{Na}$. Thus, even in this extreme situation the radial variation in $e_m$ or $\Delta e_m$ will be small, however we have assumed no active transport of Na-K across the inner cell membranes and this approximation is known to be incorrect (Neville et al., 1978). If we assign some of the active transport of Na/K to the inner membranes, there will be a change in $e_m$ and therefore all other computed parameters will change. However, if we assign just a small percentage of the total active transport to inner membranes, the changes in other parameters will not be large, except for the change in extracellular potassium as demonstrated in Fig. 6.

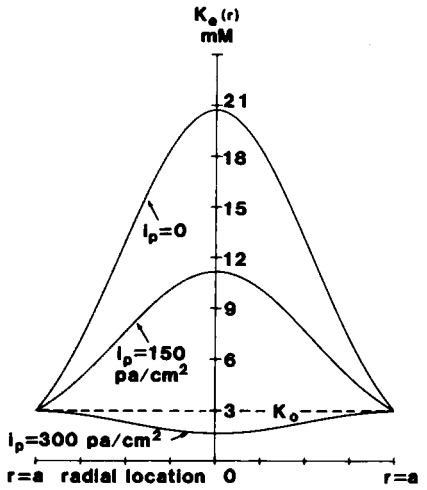


FIGURE 6 Some possible profiles of the radial distribution of potassium concentration within the intercellular clefts. If we assign no potassium permeability and no active transport to the membranes of the inner cells, then the radial voltage distribution, $\psi_e(r)$, will cause the net accumulation illustrated in the uppermost curve. If we assign 2% and 4% of the total active transport to the inner cell membranes we obtain the other two curves representing less accumulation and actual depletion. We see that very little active K transport from small clefts is possible since the clefts concentration will be driven to zero before the transport has much effect on the intracellular concentration.

The two curves in Fig. 6 showing reduced K accumulation and net K depletion with depth were computed by assigning, respectively, 2% and 4% of the total active transport to the inner membranes (this implies a specific pump current density of $i_p$ = 14.4 and 28.8 p$A$/cm$^2$). The values of $u_e$ and $\psi_e$ were not corrected for the small accompanying change in $e_m$, but the point of the calculation is clear. It requires a tiny amount of active transport to completely empty the small intercellular clefts of potassium. Once the clefts are emptied of potassium, the Na-K ATPase must shut off. Chemical studies (Kinoshita, 1963) of the distribution of Na-K ATPase within the lens assigns far more than 4% to the inner membranes, so a more realistic estimate of extracellular ion gradients is that sodium concentration is increased by ~3 mM at the lens center to make up for the 3 mM of potassium present in the bath but absent from the clefts, and the corresponding radial variation in $E_{Na}$ is <0.5 mV. Given that $e_m$ does not depend on $E_K$ (Mathias et al., 1985), the radial variation in $e_m$ and $E_{Na}$ should be similar.

We also have assumed that $g_m$ and $G_S$ are simple constants in the analysis of the lens. This assumption may be incorrect for any of three reasons: (*a*) radial concentration gradients may cause radial variation in conductance; (*b*) radial voltage gradients may cause radial variation in conductance; (*c*) the population of channels may not be spatially uniform, either radially or over the surface.

Patch-clamp studies of the lens (Rae, 1985) or the macroscopic results in the previous paper (Mathias et al., 1985) have shown that channel conductances generally vary with the concentration of permeant ion. The only significant radial gradients are likely to be in $K_e$, as discussed above, but we have found that there are few potassium channels in the inner fiber-cell membranes, hence $g_m$ should not be greatly influenced by radial concentration gradients.

The voltage across the fiber-cell membranes varies from around −70 mV at the lens surface to −40 mV at the center (Mathias and Rae, 1985, or Fig. 2 *B* of this paper). Patch clamp studies or voltage clamp of isolated epithelial cells from the frog lens (Rae, 1985) have generally demonstrated some voltage dependence of the channels, but over this voltage range, the current-voltage curves are probably within ±10% of a linear approximation. However, Rae (1985) reports on channels found in the surface cell membranes and there may be a new population of channels in the fiber cells. The membrane resistance of fiber cells is very high and there is apparantly a low density of channels, consequently patch clamp has not been a feasible technique for studying these membranes. Thus, we have only indirect suggestive evidence that $g_m$ may not have significant radial variation due to voltage gradients.

It seems likely that the worst assumption is *c*, namely that the channel and other transport properties are spatially uniform. Anterior and posterior surface cells differ morphologically and in their transport properties. However, these cells are electrically nearly in parallel so one would not predict a large angular variation in the driving forces and, indeed, Mathias and Rae (1985) show that the resting voltages in the frog lens are nearly radially symmetrical, which implies little angular variation. The anterior surface epithelial cells of the lens differentiate into the fiber cells and there is a zone of differentiating cells that extends radially inward for several hundred microns. We suspect that the transport properties of these differentiating cells changes with depth as does their structure. However, the measured radial variation in steady-state voltage (Mathias and Rae, 1985) agrees quite well with the predictions in Fig. 2 of this paper, at least insofar as its magnitude is concerned. The details of the shape (as a function of radial location) of the resting voltages is not as simple as the theory predicts and this may be due to radial variation of $g_m$. Clearly, the impedance studies in Mathias et al. (1979, 1981, and 1985) measure some sort of a radial average value of $g_m$ and we use this value in the calculations here, thus is seems reasonable that we should predict roughly the correct value of voltage but not the precise radial variation.

## DISCUSSION

The results in Figs. 2–6 illustrate many fluxes that are not usually associated with steady-state conditions. Nevertheless, a true steady state does exist since each flux balances such that cellular volume and solute concentrations are not changing. Given the measurements reported in Mathias et al. (1985) and Mathias and Rae (1985), it seems inescapable that such circulating fluxes exist in the lens, and one suspects that these complexities will be found in other tissues. Our studies of the lens have been mostly electrical, moreover they are the first attempts at sorting out the localization of properties in the lens. We anticipate that more sophisticated electrical experiments and experiments on fluid movement in the lens will quantitatively alter the results presented here, but we feel the general conclusion that steady state can be a dynamic state will survive.

### The Physiological Role of Localized Transport

One presumes there are some physiological reasons for localizing transport properties such that the lens attains the complex steady state we have just described. The calculations for Fig. 6 show that active transport into narrow restricted intercellular clefts will tend to control the composition of the cleft rather than the cytoplasm, so this is one reason to localize active transport at the surface, but in the lens there may be other reasons.

The lens is avascular and it does not have intracellular organelles in the fiber cells comprising its mass. We believe these specializations are essential to transparency, since

the above mentioned structures will scatter light. Accordingly, the fiber cells are not in close proximity with the blood oxygen or glucose like a normal cell and the fiber cells have no mitochondria, hence it is not surprising that they possess little ability to actively transport Na and K. The localization of active transport to the surface cells of the lens therefore seems to be in part a consequence of the need for transparency.

There is an enormous surface area of lens inner-fiber cell membrane, so the specific conductance of these membranes must be very low, or active transport at the surface would not be able to keep up with the passive volume leak. Indeed, the inner-fiber cell membrane conductance is extremely small (Mathias et al., 1979, 1981, or 1985). The surprising observation is that the sodium conductance of the fiber membranes appears to be considerably larger than the potassium conductance (Mathias et al., 1985). The result of this localization of selectivity is to move fluid into the intercellular spaces and along these spaces to the innermost clefts. If the inner membranes had a more typical selectivity such that sodium conductance was less than potassium, then extracellular voltage gradients would be much smaller than those measured (Mathias and Rae, 1985) and the flow of water along intercellular clefts would cease or reverse direction. Presumably, an inward directed extracellular water flow is of some advantage to the lens.

It is not difficult to speculate on the advantages of fluid circulation. For example, oxygen, glucose, and other nutrients are convected from the aqueous and vitreous humors, along the extracellular clefts, and to the innermost cell membranes. Moreover, the intracellular water flow is opposite in direction, so it convects waste products from inner cells to the surface cells where they can be metabolized and extruded. Furthermore, the negative hydrostatic pressure between the lens fiber cells keeps the intercellular spacing to a minimum. This space must be kept smaller than the wavelength of light in order to maintain transparency. There may also be mechanical advantages to having a negative pressure in the intercellular spaces, particularly since in vivo the shape of the lens is continuously adjusted. There are likely to be many other advantages in a circulating fluid flux and one should not a priori extrapolate the rather static steady state of a single cell to any syncytial tissue.

Venosa and Horowicz (1983) reported that the Na-K ATPase in skeletal muscle fibers is localized to the surface membranes and there is little active transport going on in the T-system. Even a single cell can therefore have circulating steady-state fluxes, but because the dimensions of a single cell are comparatively small, the standing gradients needed to drive the fluxes will be correspondingly small. Similarly, standing gradients in epithelia (Diamond and Bossert, 1967) have been difficult to measure, presumably because such gradients are small and not because the resulting fluxes are unimportant; in fact they are the essence of epithelial function. Thus, we anticipate that the analysis presented here has more widespread applicability than just the lens, and the classical descriptions of electrochemical steady-state and volume regulation (Boyle and Conway, 1941) may not adequately describe many important tissues.

I would like to thank Dr. James Rae for providing the inspiration to examine the interesting problem of syncytial steady state. I am also grateful to Drs. R. S. Eisenberg and J. Rae for a careful reading of the original version of this work.

This work was supported in part by National Institutes of Health grants EY03095 and HL29205.

*Received for publication 2 January 1985 and in final form 24 April 1985.*

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