A Fixed Charge Model of the Transverse Tubular System of Frog Sartorius

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ABSTRACT Volume changes of the transverse tubular system (T system) of frog sartorius in different solutions can be explained by a model which assumes fixed negative charges in the T system lumen, an open T system mouth, and a Donnan equilibrium between the T system and external solution. The T system volume is regulated by the osmotic pressure difference between the lumen and external solution, as well as by constraining forces whose nature is as yet unclear. The decreased swelling tendency produced by hypotonic solutions and increased tendency produced by some hypertonic solutions are ascribed to changes in the pressure constraint from the sarcoplasm. Fixed charge concentration was estimated tentatively from swelling and resistivity data to be between 0.1 and 0.4 M.

INTRODUCTION

The volume of the transverse tubular system (T system) of frog sartorius appears to depend on ionic strength, chemical composition, and osmotic pressure of the bathing solution (Huxley, Page, and Wilkie, 1963; Freygang, Goldstein, Hellam, and Peachey, 1964; Foulks, Pacey, and Perry, 1965; Rapoport, Peachey, and Goldstein, 1969). In the latter communication, we described the T system swelling which took place when the ionic strength but not the osmotic pressure of the bathing solution was reduced (Fig. 1).

In this paper, we shall elaborate a hypothesis that the T system has fixed charges in order to interpret the observations mentioned above. The equations derived from the fixed charge hypothesis are given in the Appendix, and we state here its six important assumptions:

1. Negative fixed charges are distributed throughout the T system lumen attached to a polyelectrolyte matrix. This matrix may be held within the lumen perhaps by interaction between it and the T system wall or by another constraint about which we can at best speculate.

2. The T system is tubular. The longitudinally oriented dimension of the T system cross-section (*b*, Fig. 2) increases with swelling; the transverse dimension *a* remains at 800 A (Rapoport et al., 1969).

3. The mouth of the T system is open to ions, sucrose, dyes, and ferritin at the muscle surface (Huxley, 1964; Page, 1964; Endo, 1966) and will permit free solute entry into the T system.

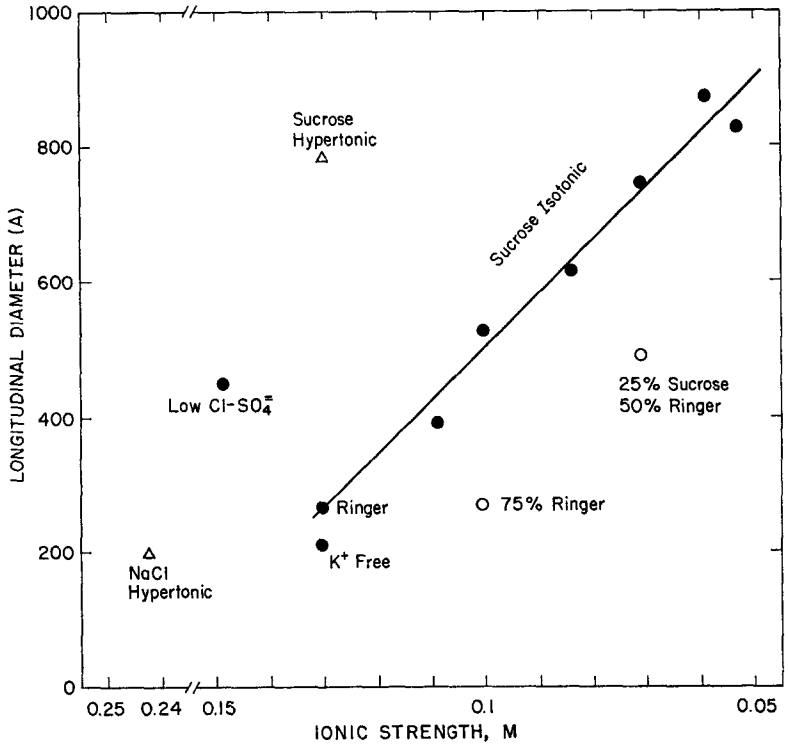

FIGURE 1. Relation between longitudinal diameter of T system cross-section and ionic strength of bathing solution. For the sucrose isotonic solutions (NaCl is partially replaced by sucrose) and low K^+ solution ionic strength is approximately equal to NaCl concentration. Data were taken from Freygang et al. (1964), Freygang et al. (1967), and Rapoport et al. (1969). The regression line is fit visually to the sucrose isotonic values. Filled circles denote solutions isotonic with Ringer, open circles hypotonic, and open triangles hypertonic. Transverse diameter = 800 A.

4. Sucrose and NaCl act osmotically across the fiber membrane (sarcolemma) as impermeant solutes (Dydynska and Wilkie, 1963; Blinks, 1965), and between the T system lumen and the sarcoplasm, but water is freely permeable across the T system membrane and sarcolemma.

5. A Donnan equilibrium is maintained between the T system lumen and external solution (Donnan and Harris, 1911; Donnan and Guggenheim, 1932).

6. As a first approximation, solutions can be treated as ideal dilute solutions.

We will not make assumptions concerning the elastic constraints of the system (cf. Lazare, Sundheim, and Gregor, 1956), but will consider the possibilities below.

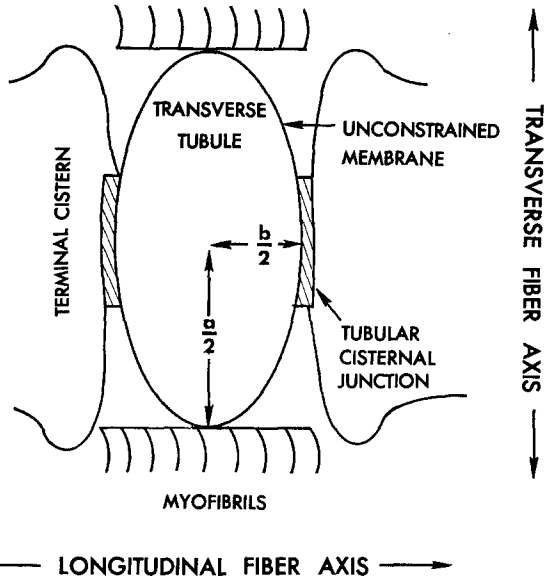

FIGURE 2. Elliptical model of the cross-section of the T system at the level of the triad. In the figure, the diameter b is parallel to the longitudinal axis of the fiber and $b/2$ represents the radius of curvature of the presumably unconstrained portion of the T system membrane. The region at the end of the transverse diameter and in apposition to the myofibrils is considered constrained, the transverse diameter a remaining at about 800 A in all solutions.

ANALYSIS OF DATA

The relation between the longitudinally oriented diameter b and the ionic strength of the bathing solution is roughly linear for isotonic sucrose solutions in which NaCl in Ringer is replaced partially by sucrose (Fig. 1). Swelling is less than expected in the hypotonic solutions and more than expected in the hypertonic sucrose solutions. In Ringer solution, the T system cross-section is rectangular and the tubular volume is

$$V_{\text{Ringer}} = ab \text{ cm}^3/\text{cm length} \quad (1)$$

When swollen, the cross-section is elliptical or circular (Fig. 1, Rapoport et al., 1969), and the volume is

$$V = \pi ab/4 \quad (2)$$

The bathing solutions described by these authors can be approximated by equivalent NaCl solutions whose concentrations are a few millimoles greater than the chloride concentrations given in their Table I, and osmotic pressure and other values can be calculated using the concentrations of the equivalent NaCl solutions (Appendix). The equivalent NaCl concentration of Ringer was 0.122 M, of 50% sucrose isotonic solution 0.063 M.

When sucrose replaces NaCl at constant osmotic pressure of the bathing solution, fiber diameter is unchanged and P^3, π^1, and π^3 can be taken as constant. π^2 should change as a function of external chloride and T system volume (Equation 9). When a new Donnan equilibrium has been established following C^1_{Cl} reduction, then, by Equation 11,

$$\begin{aligned} \pi^2 + \Delta\pi^2 - \pi^1 - (P^2 + \Delta P^2) = 0 \\ \Delta\pi^2 - \Delta P^2 = 0 \end{aligned} \tag{3}$$

where $\Delta\pi^2$ and ΔP^2 are the changes in pressures due to C^1_{Cl} reduction and are balanced at the new equilibrium. Fig. 3 shows how T system volume, as calculated by Equation 10 from the equivalent NaCl concentration at different fixed charge concentrations, would change for different assumptions concerning π^2. If π^2 attained its initial Ringer value at the new equilibrium in each isotonic sucrose solution, less swelling is predicted than was observed for a given initial fixed charge concentration. If π^2 increased with swelling, even less swelling would be expected. However, a close approximation between observed and expected swelling could be had if π^2 decreased with swelling ($\Delta\pi^2 < 0$). The process could be visualized in two steps. After reduction in C^1_{Cl}, π^2 would immediately increase by Equation 9, but as swelling proceeded it would decrease progressively until at the new Donnan equilibrium π^2 would be less than it had been in Ringer. In all the cases of Fig. 3, some swelling would be expected from the fixed charge hypothesis and the equations of a Donnan equilibrium.

An approximate correspondence is found between observed and calculated volumes (Equation 10) if π^2 were to vary inversely as the longitudinal radius, $b/2$. The empirical relation can be written,

$$\pi^2 - \pi^1 \simeq 2\gamma/b \propto 1/V \tag{4}$$

where γ is a proportionality constant in units of dyne/cm. The error between calculated $\pi^2 - \pi^1$ and $2\gamma/b$ is $< 12\%$ for $\gamma \leq 1$ dyne/cm (Fig. 3).

Fig. 1 shows that the T system also swells in low $Cl^- - SO_4^=$ solution. Calculations with the Donnan assumptions when the divalent ion is taken into account indicated that swelling in this solution could be expected if Equation 4 holds when $N_f/V_{Ringer} > 0.1$ M and $\gamma = 1$ dyne/cm. A volume

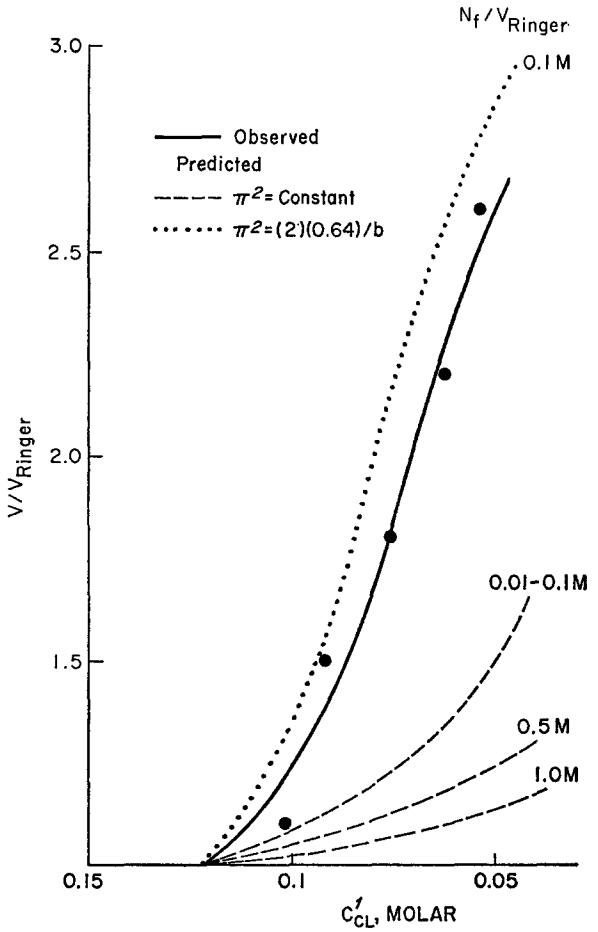

FIGURE 3. Relation of predicted and observed T system volumes to external chloride concentration, when π^1 is unchanged. NaCl equivalent concentrations are taken from Table I of Rapoport et al. (1969). Observed volumes were estimated by Equations 1 and 2 from cross-section dimensions (Fig. 1). Predicted volumes were calculated by Equation 10 for different initial fixed charge concentrations, N_f/V_{Ringer}. The figure shows a series of curves when π^2 was assumed constant and when 0.01 M $< N_f/V_{\text{Ringer}} <$ 1.0 M. If π^2 were to increase with swelling, volume changes would be less. The figure also gives swelling when $\pi^2 - \pi^1 = 2\gamma/b$ (Equation 4), where $\gamma = 0.64$ dyne/cm and $N_f/V_{\text{Ringer}} = 0.1$ M.

change is not expected in Ringer free of KCl, since the ionic strength and osmotic pressure of this solution are the same as in Ringer.

Swelling in Nonisotonic Solutions In these solutions, muscle diameter changes because the fiber acts as a partial osmometer. The effects of tonicity change may be due in part to the nonindependence of P^2 and P^3 in Equation 12 *b*. When fiber diameter decreases in sucrose hypertonic solution, $\Delta\pi^2 =$

$\Delta\pi^s$ if a nonosmotic fiber volume is assumed (Dydynska and Wilkie, 1963; Blinks, 1965). An alternative to some of this nonosmotic volume is to assume that P^s has changed, perhaps in such a way as to promote swelling (cf. "retraction" of Huxley et al., 1963). Conversely, increased fiber diameter in hypotonic solutions would restrict swelling, as is shown in Fig. 1. Lack of swelling in Ringer made hypertonic by NaCl might be due to a balance between the effect on P^s and the increase of C^i_{Cl}, which would tend to decrease T system volume (Equation 10).

DISCUSSION

The fixed charge hypothesis to account for T system swelling is based on assumptions which need further testing. Swelling qualitatively is not a fixation artifact since it is reversible and in sucrose hypertonic solution is independent of the sucrose concentration of the fixative (Rapoport et al., 1969). It is correlated with capacitance changes ascribed to the T system membrane (Freygang et al., 1967). Since the volume ratio in Fig. 3 depends critically on the estimate of V_{Ringer} in view of the difficulty in estimating the transverse dimension of the T system cross-section and because of other errors in quantitative electron microscopy (Rapoport et al., 1969), V/V_{Ringer} may be too large. If the transverse dimension is constant, V/V_{Ringer} is independent of it. The relation between observed and calculated curves in Fig. 3 should be taken as tentative in any case.

A polyelectrolyte within the T system lumen must yet be found. (The dimensions of the T system are too large to permit charge, if it is fixed only at the T system membrane, to create a Donnan equilibrium throughout the T system cross-section (Schmid, 1950.)) Perhaps the fact that ferritin volume is about one-third T system volume (Peachey and Schild, 1968) is due to partial ferritin exclusion from the T system lumen. Fawcett and McNutt (1969) have demonstrated recently a protein polysaccharide in the T system lumen of cardiac muscle, a structure analogous to the T system of frog sartorius. The smaller dimensions of the latter might make a polyelectrolyte, if present, more difficult to observe there. A polysaccharide has been postulated to account for swelling between the outside surfaces of the Schwann cell membrane of myelinated fibers and of the mesaxon in solutions of low ionic strength (Robertson, 1959; Blaurock, 1967).

There is not enough information to decide upon the exact nature of the elastic constraints that contribute to P^g. The data suggest that P^g and P^s are not independent. It may be that the elasticity of the polyelectrolyte within the T system decreases with swelling, perhaps because the polyelectrolyte is composed of two dimensional filaments, as suggested for collagen (Kühn, 1962), whose interaction decreases with separation. Such a matrix would differ from the three dimensional network of experimental gels, whose elastic-

ity appears to increase with swelling because of cross-linkages (Helfferich, 1962). Connective tissue composed of collagen and reticulin may swell more than 10-fold with changing pH because of its primary two dimensional network; more swelling obtains when the reticulin fibers which connect the collagen fibers are reduced in number (Jordon-Lloyd and Marriott, 1935).

Another possibility is that γ in Equation 4 is the interfacial tension of the unconstrained T system membrane (Fig. 2), and that Equation 4 represents the Laplace equation which relates pressure in a cylinder to the radius of curvature, $b/2$, and interfacial tension, γ (Joos, 1958). If this were so, fixation of the polyelectrolyte by the T system walls or by other means (assumption 1) would maintain the osmotic pressure difference, $\pi^2 - \pi^1$, but the polyelectrolyte would not necessarily contribute to elasticity. Polyelectrolyte retention by attachment to the walls would be equivalent, thermodynamically, to polyelectrolyte retention by a semipermeable membrane at the mouth of the T system.

Published data on cell surface membranes indicate that γ is between 0.015 and 1 dyne/cm (Cole, 1932; Rand and Burton, 1964; Harvey, 1931). Extrapolating to the intracellular T system membrane, N_f/V_{Ringer} would be between 0.015 and 0.126 M by Equation 4. If V_{Ringer} were larger, we would expect similar values for N_f/V_{Ringer}, but it is apparent that we cannot decide on a satisfactory value from the data without knowing the nature of the elastic constraints and the error in the volume estimates.

Fixed charge concentration may be estimated also from the resistance and volume of the T system lumen by Equation 13. The resistance may be the R_x of the equivalent circuit of muscle as measured by transverse impedance measurements, since R_x is relatively independent of the resistivity of the bathing solution (Fatt, 1964). Its value is about 12,000 ohm cm in Ringer, whose resistivity is 92 ohm cm[2] If the T system volume is 0.5 % of fiber volume (Hodgkin and Horowicz, 1960; Peachey and Schild, 1968), then T system resistivity is about two-thirds that of Ringer fluid. With values of Λ_i taken from the International Critical Tables, N_f/V_{Ringer} was estimated by Equation 13 to be about 0.37 M. This concentration may be an overestimate because of neglect of surface conductivity in Equation 13 (Helfferich, 1962, p. 328). When the data on swelling and the suggested value of N_f/V_{Ringer} from observations in low $Cl^- - SO_4^=$ solution, as well as data on R_x are taken into account, the fixed charge concentration of the T system lumen can be tentatively estimated to be between 0.1 and 0.4 M. By Equation 14, if $N_f/V = 0.37$ M, V^{12} would be -30 mv.

The Donnan equilibrium is a true equilibrium. Transient volume changes and bursting may occur when muscle fibers in Ringer to which 400 mM glycerol has been added are returned to isotonic Ringer fluid (Howell and Jenden, 1967). These changes may be caused by the faster entry of water into

than the exit of glycerol from the T system, and would follow the mathematical model of Manegold and Solf (1932).

APPENDIX

The bathing solution is denoted as compartment *1*, the T system lumen compartment *2*, and the sarcoplasm compartment *3*, following the model of Adrian and Freygang (1962). Compartments will be denoted by superscripts. Electrochemical equilibrium of NaCl across the mouth of the T system leads to the approximate equation, neglecting the pressure term and assuming ideality (Lazare et al., 1956).

$$C^2_{Na}C^2_{Cl} = C^1_{Na}C^1_{Cl} \tag{5}$$

where C is concentration and the subscript denotes the ionic species. The electroneutrality condition in compartments *1* and *2* leads to the following, assuming that NaCl is the main ionic component in either compartment,

$$\begin{aligned} C^2_{Na} &= N_f/V + C^2_{Cl} \\ C^1_{Na} &= C^1_{Cl} \end{aligned} \tag{6}$$

where N_f is the negative fixed charge/centimeter length of T system (moles), and V the T system volume/centimeter length when the tubule is considered a cylinder with cross-section as shown in Fig. 2.

The chloride in the T system lumen is obtained from Equations 5 and 6,

$$C^2_{Cl} = \frac{-N_f/V + \sqrt{(N_f/V)^2 + 4(C^1_{Cl})^2}}{2} \tag{7}$$

The osmotic pressure difference between the T system and external solution is approximated by the van't Hoff equation,

$$\pi^2 - \pi^1 = RT(\sum_i C^2_i - \sum_i C^1_i) \tag{8}$$

where i denotes any freely diffusible solute including sucrose. Substituting Equations 6 and 7 into 8, the osmotic pressure difference as a function of C^1_{Cl} and the volume of the T system can be calculated,

$$\pi^2 - \pi^1 = RT(\sqrt{(N_f/V)^2 + 4(C^1_{Cl})^2} - 2C^1_{Cl}) \tag{9}$$

$$V = \frac{RTN_f}{\sqrt{(\pi^2 - \pi^1)^2 + 4RT(\pi^2 - \pi^1)C^1_{Cl}}} \tag{10}$$

Water equilibrium across the mouth of the T system leads to (cf. Equation 9 of

Lazare et al., 1956)

$$\mu_w^1 = \mu_w^2$$
$$\pi^2 - \pi^1 - P^2 = 0 \qquad (11)$$

where μ_w is the chemical potential of water and P^2 is the hydrostatic pressure in the T system lumen with reference to atmospheric pressure. The osmotic pressure difference in Equation 11 must be balanced by a hydrostatic pressure difference produced by elastic constraints of the system.

Water equilibrium across the T system membrane and surface membrane also leads to the following,

$$\pi^3 - \pi^1 - P^3 = 0 \qquad (12\,a)$$

$$\pi^3 - \pi^2 - (P^3 - P^2) = 0 \qquad (12\,b)$$

Although Boyle and Conway (1941) took P^3 as zero, the fixed charges in compartment *3* (Ling, 1962) as well as the observation that the muscle fiber is only a partial osmometer (Blinks, 1965) suggest that P^3 may be considered nonzero in analyzing T system swelling.

T system conductivity can be calculated from the concentration of free ions in the T system lumen, with the use of Equations 6 and 7,

$$\kappa = \frac{(N_f/V)(\Lambda_{Na} - \Lambda_{Cl}) + \sqrt{(N_f/V)^2 + 4(C_{Cl}^I)^2}(\Lambda_{Na} + \Lambda_{Cl})}{2000} \qquad (13)$$

where κ ohm^{-1}cm^{-1} is the specific conductivity and Λ_i ohm^{-1}cm^2 is the equivalent conductance of solute i in the T system (Glasstone, 1946).

The electrical potential, V^{12}, of the T system with respect to the external solution is also calculated from equilibrium conditions,

$$V^{12} = RT/F \ln \frac{-N_f/V + \sqrt{(N_f/V)^2 + 4(C_{Cl}^I)^2}}{2C_{Cl}^I} \qquad (14)$$

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