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Osmotic Water Movement across the Sarcolemma of Frog Skeletal Muscle Fibers

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Summary. The permeability coefficient for osmotically induced water flux across the sarcolemma of frog skeletal muscle fibers was determined. A new method for measuring the fiber volume change was applied, based on the fact that the resting tension of a slightly stretched muscle fiber depends on the bathing solution tonicity. Thus, after a quick change in tonicity, the volume change can be derived from the simultaneously occurring tension change. Fitting a theoretical curve to the experimentally obtained values yielded a filtration permeability coefficient for water of 0.54 ± 0.12 cm^4/osmol sec (mean $\pm$ SD, n = 12). Doubling the driving force did not alter the product $P_W \times$ membrane area. The P_W value found in the present work is compared with that for muscle fibers and other cells given previously.

According to Jacobs (1952), water movement across membranes can be classified into a diffusional water flux, comparable to the movement of solutes across membranes, and an osmotically induced water flux. The determination of the permeability coefficient P for the diffusional water flux and P_W for the osmotic water flux requires different experimental procedures, since the osmotic water flux causes a significant change in cell volume, while the diffusional flux does not. Since the driving force for the osmotic water flux is not a linear function of cell volume (Jacobs, 1952), neither the volume change nor the associated water movement is an exponential function of time, as is the case for net diffusional water flux. Thus, to calculate P_W, the relation between volume and time must be known. This relation can be obtained by integrating the differential equation for water flux and driving force given by Jacobs (1952) and Kedem and Katchalsky (1958).

Values of P_W for frog skeletal muscle fibers were reported in two earlier papers. The estimate of P_W given by Hodgkin and Horowicz (1959) is based on the fact that after equilibration in high KCl solution the osmotic water

flux causes a change in the membrane potential. Zadunaisky, Parisi and Montoreano (1963) measured the change in fiber width by photographing fibers at various times after a quick change in the tonicity of the external solution. Since the fiber normally has an irregular circumference and alters its shape during the volume change (Blinks, 1965), P_W determined by this method may be subject to considerable error.

In this paper a new method of measuring the volume change of frog skeletal muscle fibers is used. The method is based on the observation that the osmotically induced volume change is accompanied by a small change in the resting tension (Blinks, 1965; Lännergren & Noth, 1973*a*). It will be shown that the time course of the tension change fits well to the theoretical expression for volume as a function of time, resulting in somewhat higher P_W values for water than have been previously reported for muscle fibers.

Materials and Methods

Preparation and Mounting

The experiments were carried out on frog single muscle fibers dissected free from the dorsal head of the semitendinosus muscle. The frogs (*Rana temporaria*) were kept at 4 to 7 °C until use during the winter months. The isolated fiber was horizontally mounted in a Perspex chamber between a fixed steel hook and the arm of a force transducer. The characteristics of the force transducer and details of the mounting device are described in previous papers (Lännergren & Noth, 1973*a*, *b*). Ringer's solution was continuously running through the chamber (cross-section area 6.25 mm^2) in the direction of the fiber axis. Before introducing the test solution the mean velocity of the Ringer's solution was increased to 12 mm/sec, and with the aid of a stop-cock system the test solution was introduced with the same velocity avoiding artefacts on the sensitive tension recording system. The bulk of the test solution reached the distal end of the fiber 2 sec after turning the arm of the cock which was tested by two different methods (Lännergren & Noth, 1973*a*). The delay of 2 sec is also obtained by calculating the mean residence time T' from the equation $T' = \frac{\text{volume of chamber}}{\text{flow rate}}$, where the fiber volume is neglected and the volume of the chamber was estimated from the stop cock to the distal end of the fiber. The same delay took place after changing back to Ringer's solution.

Microscopy

At each fiber third, the largest (a) and smallest (b) fiber diameter was measured by means of a dissecting microscope with ocular micrometer. The cross-sectional area S was approximated by $\frac{\pi a b}{4}$, the mean diameter D by $(ab)^{\frac{1}{2}}$, and the elliptical circumference C_e by $\frac{3\pi(a+b)}{4} - \frac{\pi(ab)^{\frac{1}{2}}}{2}$. The means $\bar{S}$, $\bar{D}$ and $\bar{C}_e$ were calculated from the corresponding values obtained at the three different fiber points. All experiments were

performed at fiber lengths corresponding to 2.3 μm sarcomere spacing, which was determined with the aid of a high power microscope (1,000 × magnification).

Solutions

Ringer's solution of the following composition was used (mM): NaCl 115; KCl 2.5; $CaCl_2$ 2.0; Na_2HPO_4 2.15; NaH_2PO_4 0.85. For the preparation of hypertonic solutions solid sucrose was added to Ringer's solution as described earlier (Lännergren & Noth, 1973*a*). Hypotonic solutions were obtained by omitting the appropriate amount of NaCl from Ringer's solution. The tonicity (T) of the test solution will be given relative to that of Ringer's solution, which had 234 mosm/kg H_2O. Tetracaine added to the test solution as 2% stock solution was used to block the hypertonicity contracture (Lännergren & Noth, 1973*a*). It was given to hypertonic solutions without compensation in osmolality in the following concentrations (mM): 0.05 at 1.25 T; 0.1 at 1.5 T; 0.2 at 1.75 T; 0.3 at 2 T.

Criteria for Viability of Muscle Fibers

After mounting the fiber in the test chamber the responsiveness to electrical stimulation (100 imp/sec for 500 msec) was tested. Fibers which failed to develop fused tetanic tension were rejected. Between tests with hypertonic or hypotonic solutions the fiber was allowed to rest for at least 15 min. Experiments were terminated when fused tetanic tension no longer could be obtained or when local contractures as a result of membrane damage became visible.

Results

Tension Change Following a Change in the Bathing Solution Tonicity

The isometric tension of a slightly stretched skeletal muscle fiber at rest depends on the tonicity of the bathing solution (Blinks, 1965; Lännergren & Noth, 1973*a*). The records of Fig. 1*A* show that after a change from Ringer's solution to a hypertonic solution, a muscle fiber develops extra tension with a roughly exponential time course. The tension change is fully reversible, as demonstrated by reimmersion of the fiber in Ringer's solution. In solutions with tonicities higher than about 1.5 T, skeletal muscle fibers develop contracture tension. At 2.5 T, the peak of this hypertonicity contracture can reach 30% of the tetanic tension in Ringer's solution, suggesting an activation of the contractile machinery by release of calcium into the myoplasm. This hypertonicity contracture can be completely blocked in the presence of tetracaine (Saito, 1971; Lännergren & Noth, 1973*a*) while the small tension development shown in Fig. 1 is unaffected by tetracaine. Thus, in this work tetracaine was added to Ringer's solution 1 min before the change to the hypertonic test solution and was also present during the test period in concentrations given in Materials and Methods. It was found that tetracaine in concentrations up to 0.5 mM had no effect on height or time course of the small tension increase seen in Fig. 1.

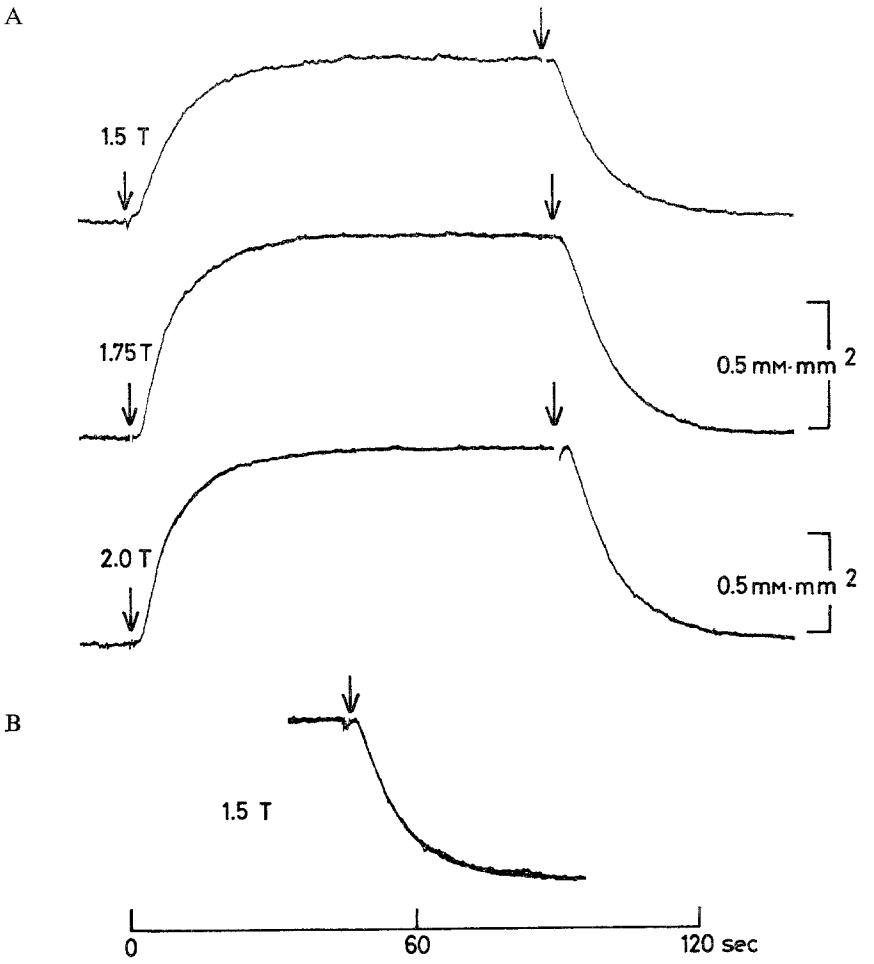

Fig. 1. Time course of isometric tension change caused by changes in bathing solution tonicity. (*A*) First arrow indicates change to hypertonic solution, second arrow return to Ringer's solution. Tonicity (*T*) given relative to that of Ringer's solution. Tetracaine added 1 min before the change to the hypertonic solution. (*B*) Three superimposed records showing the reproducibility of the tension change induced by return from 1.5 *T* to Ringer's solution; 60 min time interval between each record; same tension calibration as for upper two records of *A*. Fiber 24, mean diameter 138 μm

The reproducibility of the osmotically induced tension change is demonstrated in Fig. 1*B*. The fiber responded to three changes from 1.5 *T* to Ringer's solution with the same tension decay. As long as a fiber remained in good condition—as judged by the responsiveness to electrical stimulation—height and time course of the tension development were reproducible.

Conversion of Tension Records into Relative Fiber Volume Values

Two conditions must be satisfied if the tension change shown in Fig. 1 is to serve as a measure for the associated change in fiber volume: Both volume and tension change must occur simultaneously, and they should be linearly related over a sufficient range. The first point was investigated earlier by comparing the half-time of the volume change calculated from change in fiber width with the half-time of the tension change (Lännergren & Noth, 1973*a*). Both values were found to be roughly equal for a given tonicity change. Furthermore, the time from turning the arm of the stop-cock to the first visible tension change was comparable to the time needed for the test solution to reach the fiber, which supports the assumption that no time lag exists between volume change and tension development.

The second assumption, that the relation between fiber volume and tension increase is linear, holds for tonicities between about 1.2 T and 2.0 T for steady-state tension as a function of tonicity, as shown in Fig. 2. Since Blinks (1965) has demonstrated a linear relation between fiber volume and the reciprocal of the relative tonicity of the external solution, the relative tension increase is plotted against $\left(1-\frac{1}{T}\right)$, positive $\left(1-\frac{1}{T}\right)$ corresponding to hypertonic solutions.

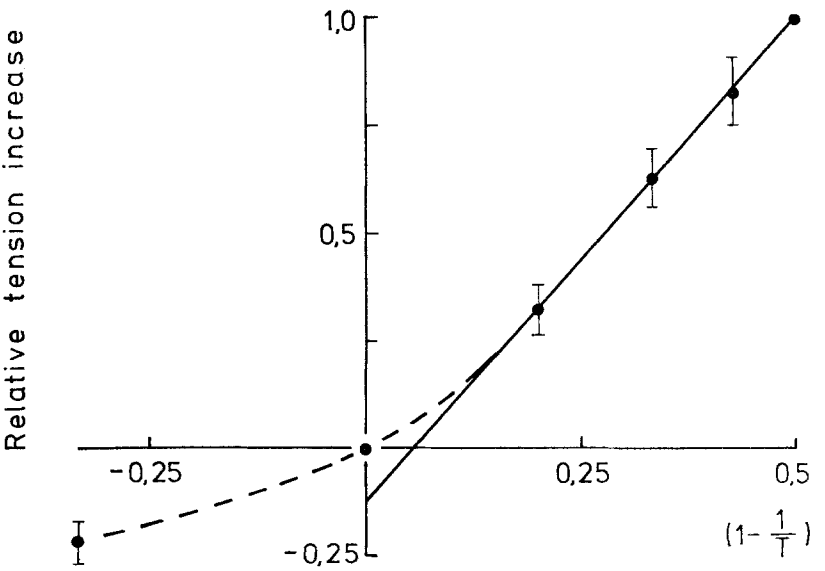

Fig. 2. Relation between relative tension change and reciprocal of tonicity. Tension values obtained 90 sec after solution change. Points represent mean tension values ± SD given relative to tension increase at 2.0 T for 9 different fibers. The negative tension value indicates relative tension loss in 0.75 T. Straight line fitted to the four hypertonic values

The nonlinearity at the beginning of the hypertonic range is the reason for selecting only the swelling phase (i.e., the tension fall) for estimating the permeability coefficient. In this phase, the tension is a linear function of the fiber volume at the start of the volume change, which facilitates the calculation of the fiber volume. The conversion of the original tension records into relative volume curves is described in the Appendix. The aim of the replotting is to correct for the slight nonlinearity between tension and volume.

Comparison between Experimentally and Theoretically Obtained Time Course of Volume Change

Relative volume values are plotted against time in Fig. 3, derived from the 1.5 T record shown in Fig. 1. The curve fitted to the points satisfies the equation

$$Kt=(\alpha-v)-\ln\frac{v-1}{\alpha-1} \tag{1}$$

derived in the Appendix. v is defined as the relative osmotically active fiber volume, which occupies 67% of the total fiber volume (Blinks, 1965). In

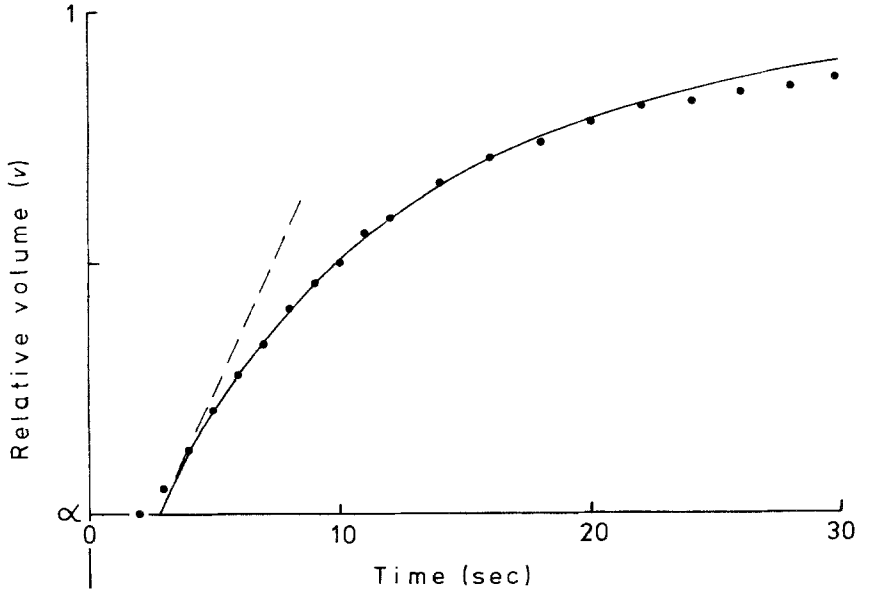

Fig. 3. Adjustment of Eq. (1) to time course of relative volume change obtained experimentally (fiber 24). Ordinate: Relative osmotically active fiber volume ($\alpha=1/T$). At $t=0$, change from 1.5 T to Ringer's solution. Dashed line shows slope of the curve at $v=0$. For details *see text*

Ringer's solution, $v=1$. After equilibration with a hypertonic solution of tonicity T, $v=\alpha\equiv\frac{1}{T}$. t is the time in seconds and K is the constant product

$$K=K_1 D^{-1} C_R P_W \tag{2}$$

(*see* Appendix). P_W is the only constant in Eq. (2) which can be adjusted to give the best fit of Eq. (1) to the experimental points. Another adjustment is the zero time shift t_α of the curve on the abscissa. t_α is the time from turning the arm of the stop-cock to the onset of the hypothetical volume change.

In Fig. 3, the best fit was obtained for $t_\alpha=2.8$ sec and $P_W=0.77$ cm^4/osmol sec. The deviation of the curve from the points at $t>20$ sec is to be expected from the nonlinearity between volume and tension mentioned earlier. The dashed line indicates the slope of the curve at $v=\alpha$ [Eq. (13), Appendix], and it is evident that the determination of P_W from the slope of the points at $v=\alpha$ would lead to a lower P_W value.

The same fiber at 2 T is shown in Fig. 4, where the best fit of Eq. (1) to the experimental points was obtained using $t_\alpha=3.6$ sec and $P_W=0.77$ cm^4/osmol sec, P_W being the same as at 1.5 T. The deviation of the curve from the experimental points at the beginning of the volume change is not typical for all fibers investigated at this tonicity, as the data for another fiber at 2 T show (Fig. 5; $t_\alpha=2.5$ sec, $P_W=0.45$ cm^4/osmol sec).

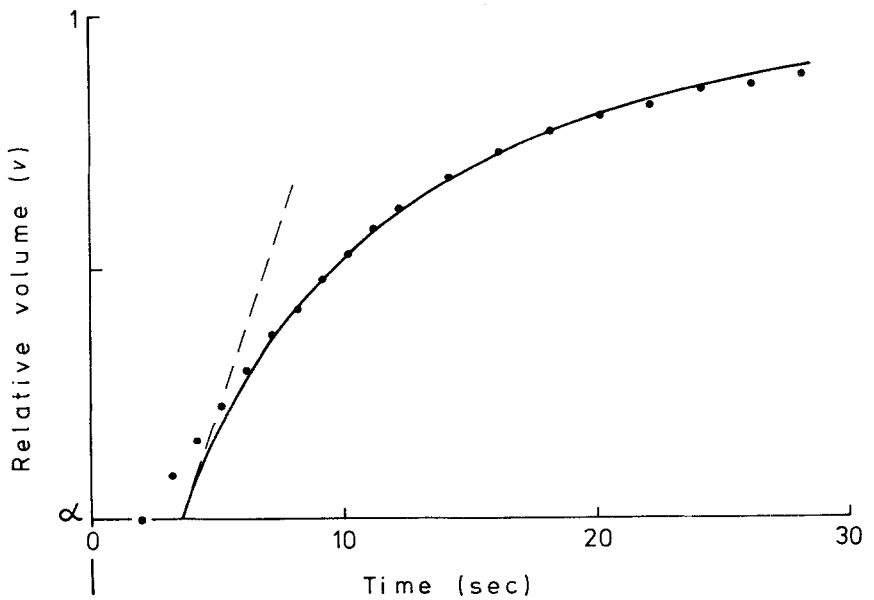

Fig. 4. Same as Fig. 3, except tonicity change from 2.0 T to Ringer's solution. Note deviation of the curve from the points at the beginning of the volume change. Fiber 24

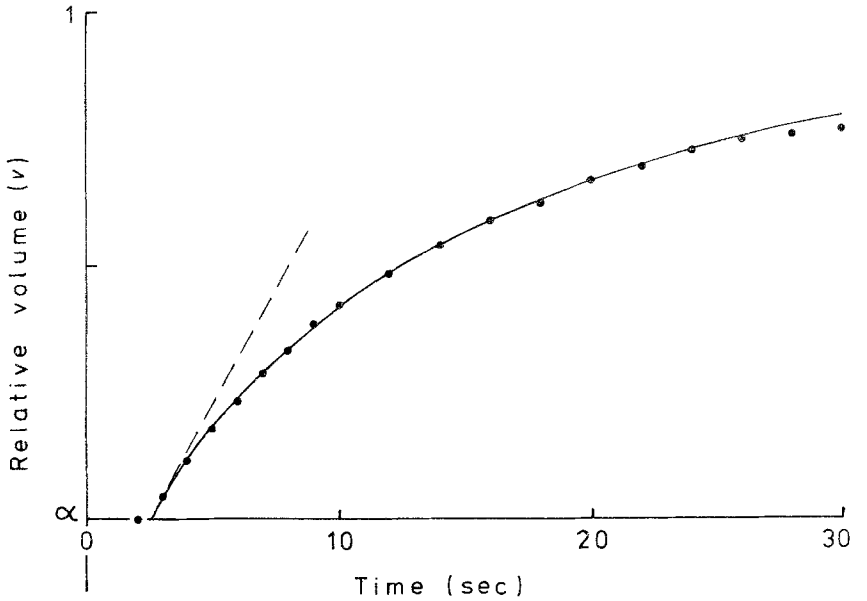

Fig. 5. Change from 2.0 T to Ringer's solution at $t=0$. Fiber 30, mean diameter 133 μm. Otherwise as Figs. 3 and 4

Correction of P_W by Approximating the Fiber Circumference as an Ellipse

Since the circumference of the muscle fibers is actually elliptical and not circular (Blinks, 1965), the ratio A/V_{W_0} in Eq. (8) (Appendix) is too low, thus overestimating P_W. The values of P_W obtained above may be corrected by multiplying the ratio of mean circular circumference $\bar{C}_c$ to mean elliptical circumference $\bar{C}_e$ (*see* Materials and Methods). The ratio $\bar{C}_c/\bar{C}_e$ is less than unity, since for equivalent areas $\bar{C}_e > \bar{C}_c$. The corrected values of the permeability coefficient P'_W are listed in Table 1, which summarizes

Table 1. P'_W values for various fibers and tonicities

Fiber	$\bar{D}$ (μm)	$\bar{C}_c/\bar{C}_e$	1.5 T		1.75 T		2.0 T	
			P'_W	t_s	P'_W	t_s	P'_W	t_s
7	130	0.94	—	—	0.52	0.7	—	—
20	142	0.96	0.47	0.2	0.41	0.8	0.43	0.5
24	145	0.93	0.72	0.8	0.72	1.4	0.72	1.6
25	138	0.95	0.61	3.5	0.49	3.2	—	—
30	133	0.90	0.43	0	0.47	0.3	0.54	1.0

P'_W was obtained by multiplying each P_W by the ratio $\bar{C}_c/\bar{C}_e$. The mean corrected permeability coefficient $\bar{P}'_W = 0.54 \pm 0.12$ cm^4/osmol sec (mean $\pm$ SD, n = 12). t_s (sec) is the time shift between the onset of the experimental and hypothetical volume change.

the results on five different fibers. Only those tension records with a resolution high enough for the exact evaluation of the volume change were used. Thus, smaller fibers were disregarded, because their absolute tension changes were too small, due to the linear relation between fiber cross-sectional area and tensile force (Lännergren & Noth, 1973*b*). The average values of P'_W at 1.5, 1.75 and 2 T are of the same magnitude, the mean of all the corrected coefficients in Table 1 being 0.54 ± 0.12 cm^4/osmol sec (mean $\pm$ SD, n = 12).

Discussion

The aim of the present work was to determine the permeability coefficient P_W for osmotic water flux across the sarcolemma of frog skeletal muscle fibers. This has been done by fitting to the experimental values a theoretical expression describing the time course of the volume change caused by a change in tonicity of the bathing solution.

Sources of Error

First, possible methodical errors in the determination of P_W will be discussed. The only fiber parameter, which is to be introduced in Eq. (2) is the mean fiber diameter. This parameter was obtained by approximating the fiber cross-section as an ellipse and calculating the diameter of a circle of equivalent area. While this procedure is certainly the most accurate one when the fiber cross-section cannot be viewed directly, it is still subject to error (Blinks, 1965). This error, together with the fact that only the cross-sectional area but not its shape remains constant along the fiber length (Blinks, 1965), will contribute to the scatter of the P_W values in Table 1.

In comparison, the measurement of tension and thus of relative volume is considerably more accurate. Furthermore, the fit of the theoretical curve to the measured points is very good and depends only on the tension measurements and not on the mean fiber diameter. The latter quantity influences only the estimate of P_W. A large initial deviation of the relative volume values from the hypothetical curve was seen only in one fiber (fiber 25, Table 1). This result can be interpreted by a permeability change during the beginning of the volume change; but it is also conceivable that despite the linear steady-state relation between tension and volume, in this case the tension change lagged behind the initial volume change.

The assumption that only the sarcolemma and not the myoplasmic water diffusion is the rate-limiting factor for water exchange can be tested

by means of an equation presented by Hill (1948, Eq. 2), which describes the diffusion of a substance into a cylinder when no barrier at the surface hinders free diffusion. Taking 100 μm as the mean fiber diameter and $k = 2.4 \times 10^{-5}$ cm^2/sec as the myoplasmic diffusion coefficient for water (Bunch & Kallsen, 1969), the water concentration at the fiber axis would reach half its final value about 0.1 sec after the solution change. This is only 1 % of the half-time found here for the volume change, indicating that the rate-limiting factor is the membrane and not the intracellular diffusion of water. On the other hand, Dick (1964) has postulated a much lower intracellular diffusion coefficient for water. Taking his highest estimate of $k = 2 \times 10^{-8}$ cm^2/sec, the half-time for the concentration change at the fiber axis would be on the order of 100 sec. This half-time is inconsistent with the half-time for the volume change of about 10 sec found in the present work. If the true value for k lies between the values considered above, it is possible that in muscle fibers with large diameters the intracellular diffusion becomes more dominant, since diffusion time varies as

Table 2. P_W values for various membranes

Membrane	P_W (cm^4/osmol sec)	Fiber diameter (μm)	Source
Bimolecular lipid membranes	0.01 – 0.20		Haydon (1970)
Mammalian cells			
fibroblast	0.02 – 0.04		Brues and Masters (1936)
leucocyte (rabbit, man)	0.01 – 0.04		Shapiro and Parpart (1937)
lymphocyte (rat)	0.01 – 0.04		Dick (1964)
erythrocyte (dog, beef, cat, man)	0.22 – 0.72		Rich *et al.* (1967); Farmer and Macey (1970)
Muscle fibers			
crayfish	0.12	100 – 400	Reuben *et al.* (1964)
crab	0.18	200 – 1,000	Sorenson (1971)
frog	0.23	?	Zadunaisky *et al.* (1963)
frog	0.41	70 – 170	Hodgkin and Horowicz (1959)
frog	0.54	130 – 145	This work
Capillary membranes			
muscle (cat, dog)	0.6		
mesenterium (frog)	14		Renkin and Pappenheimer (1957)
glomerulus (mammalian)	57 – 150		

the square of diameter (Hill, 1948), which would lead to lower P_W values (Table 2). Thus, the P_W value obtained in this work could be too low due to this factor.

Other Factors which may Influence P_W

In contrast to diffusional flux of water or solutes across membranes of living cells the osmotic water exchange causes significant changes in the cell volume and therefore also in the cell surface. In red blood cells, large variations of the cell volume do not influence the product $P_W \times A$, where A is the actual membrane area (Farmer & Macey, 1970).

The great similarity between experimental and hypothetical volume change found in the present work may indicate that the same is true for muscle fiber membranes, but it must be noted that at the highest osmotic strength ($2\,T$) used here the membrane area is reduced only to about 81% of its value in isotonic solution.

The experiments of Sorenson (1971) on crab muscle fibers, in which P_W is derived from measurements of $\frac{dV}{dt}$ at the beginning of the volume change, neglecting the later time course, indicate a decrease of P_W with increasing osmotic pressure gradient. The discrepancy between Sorenson's results and the present findings on frog muscle fibers showing P_W independent of the driving force may be explained by the much smaller osmotic pressure difference applied in the former work. On the other hand, it seems possible that the change in light absorbance used by Sorenson for monitoring the volume change may lag behind this change, a possibility which is not ruled out by the linear steady-state relation between light absorbance and fiber volume.

Comparison with P_W Values of Other Membranes

P_W values for frog skeletal muscle fibers of different species are listed in Table 2, in which the direction of the water flux was not taken into consideration, which seems to be greater inwards than outwards (Farmer & Macey, 1970; 1972). Using frogs, Hodgkin and Horowicz (1959) found a P_W of about 0.41 cm^4/osmol sec, comparable to the value given here. The P_W value of 0.23 ± 0.02 cm^4/osmol sec obtained by Zadunaisky *et al.* (1963) for frog muscle fibers is significantly lower and has the same magnitude as the values reported for crayfish (Reuben, Girardier & Grundfest, 1964) and crab muscle fibers (Sorenson, 1971). It is noteworthy that muscle fibers showing a low P_W are much larger than those with a high permeability coefficient. This coincidence may indicate that in large fibers not only the

membrane but also the intracellular water diffusion determine the time course of the osmotic volume change as discussed earlier. But it is also likely that the discrepancy reflects distinct membrane properties. In this context, it should be noted that the sarcolemma of skeletal muscle fibers has openings allowing the entrance of solutes and water into the transverse tubular system (Hill, 1964; Huxley, 1964). Therefore, a different extent of tubulization could perhaps account for the differences in P_W.

The inhomogeneity of the muscle fiber membrane due to the tubular system should also be considered when comparing with P_W of other membranes (Table 2). It might at least partially account for the relative high permeability to water in comparison to artificial lipid membranes and to mammalian cells. Red blood cells are an exception, having a P_W similar to that of muscle fibers. Another difference between the membrane of the mammalian cells listed in Table 2 and the muscle fiber membrane is the excitability of the latter and it is conceivable that the special structure of this kind of membrane favors osmotic water flux. Capillary membranes are more permeable to water than muscle fiber membranes, which agrees with the assumption of large intercellular pores in capillary membranes (Renkin & Pappenheimer, 1957).

Appendix

Conversion of Tension Record into Relative Fiber Volume as a Function of Time

Due to the small nonlinearity between steady-state tension and fiber volume (Fig. 2), the tension records cannot be used directly for comparing with the time course of the hypothetical volume change. Thus, for a given tension record (right-hand diagram, Fig. 6) with the initial tension p_0, the tension p at various time intervals was determined and converted into relative volume values as illustrated in the left-hand diagram. This diagram corresponds to that of Fig. 2, but was redrawn for each individual fiber. The straight line fitted to the four stationary tension values can be described by

$$a = cx - b \tag{3}$$

where $x = (1 - 1/T) = (1 - v)$ and the slope c is defined by the condition that $a = 1$ when $x = 0.5$; thus

$$c = 2(1 - b). \tag{4}$$

After rearrangement we have

$$v = \frac{2 + b - a}{2(1 + b)}. \tag{5}$$

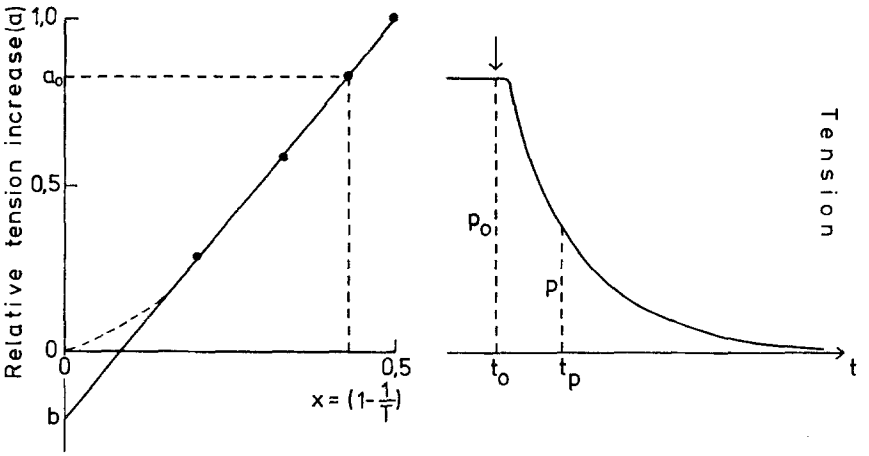

Fig. 6. Schematic diagrams illustrating the conversion of original tension records into relative volume values for an arbitrarily chosen solution change from 1.75 T to Ringer's. Left-hand diagram corresponds to that of Fig. 2. The values a_0 and b were read off for each individual fiber at 1.5, 1.75, and 2.0 T. Right-hand record shows determination of p_0 and p in arbitrary units. p were read off for at least 18 different time intervals. For details *see text*

The relative tension at $t=0$ and a given tonicity T is denoted a_0 and can be read off from the left-hand diagram. Comparing the left- and right-hand diagrams, we see that $a/a_0 = p/p_0$. Substituting into Eq. (5), we finally obtain for $v(p)$:

$$v = \frac{2+b-a_0(p/p_0)}{2(1+b)}. \tag{6}$$

The Derivation of the Function between Relative Fiber Volume and Time

The derivation of Eq. (1) describing the time course of the relative fiber volume change following a quick change in tonicity of the external solution is based on the following assumptions:

1. The muscle fiber is cylindrical in the isotonic solution.

2. The osmotically inactive fiber volume occupies 33% of the total fiber volume and remains constant in the hypertonic solutions used in this work.

3. The shrinkage of the fiber in the hypertonic solution does not alter the product $P_W \times A$.

4. The inhomogeneity of the sarcolemma is neglected.

5. The main barrier for the water flux is the sarcolemma.

6. There is no net flux of ions or sucrose across the sarcolemma during the immersion in the hypertonic solution.

The calculation is based on the differential equation given by Jacobs (1952), describing the kinetics of the volume change of an ideal osmometer under osmotic pressure gradient:

$$\frac{dV_W}{dt} = P_W A \left(\frac{C_R V_{W_0}}{V_W} - C_M\right), \tag{7}$$

where for our purposes: V_W (cm^3) = osmotically active fiber volume, A (cm^2) = surface area of fiber, C_R and C_M (osmol/cm^3) = the solute concentrations in Ringer's solution and in the external medium after the solution change, respectively, and t = time in seconds. Replacing V_W by $v = \frac{V_W}{V_{W_0}}$, one obtains

$$\frac{dv}{dt} = P_W \frac{A}{V_{W_0}} \left(\frac{C_R}{v} - C_M\right) \tag{8}$$

$$= K_1 P_W D^{-1} \left(\frac{C_R}{v} - C_M\right), \tag{9}$$

since for a cylinder

$$\frac{A}{V_{W_0}} = \frac{\pi D l}{0.67 \frac{\pi}{4} D^2 l} = K_1 D^{-1} \tag{10}$$

(D = fiber diameter in cm; l = fiber length; $K_1 = 5.97$; 0.67 = osmotically active fraction of fiber volume).

Using the initial condition $v(t=0) = 1$, integration of Eq. (9) yields

$$Kt = (1-v) - \alpha \ln \frac{v-\alpha}{1-\alpha} \tag{11}$$

with

$$K = K_1 P_W D^{-1} C_M \tag{12}$$

and $\alpha = \frac{1}{T}$. In Eq. (11), α was introduced for $\frac{C_R}{C_M} \cdot \frac{1}{T} \approx \frac{C_R}{C_M}$, with a maximal error of 4% at 2 T due to the difference between osmolality and osmolarity of the hypertonic solution. This error was neglected.

Eq. (11) describes the time course of the change in the relative osmotically active fiber volume following a change from Ringer's to hypertonic solution, and it is seen that a linear term is added to a logarithmic expression.

Eq. (1), describing the time course of v following a return to normal Ringer's solution, is found by integrating Eq. (9), in which $C_M = C_R$, with the initial condition $v(t=0) = \alpha$.

The slope of the curve [Eq. (1)] at $t=0$ is given by

$$\dot{v}(0)=K_1 P_W D^{-1}(C_0-C_R), \tag{13}$$

where C_0 is the hypertonic solute concentration before the change to Ringer's solution. This equation is similar to that used by Sorenson (1971, p. 291), differing only in the units and in the direction of the water flux.

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