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Permeability of Barnacle Muscle Fibers to Water and Nonelectrolytes

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Summary. The permeability of isolated muscle fibers of the giant barnacle *Megabalanus psittacus* to water and nonelectrolytes was studied by determining their reflection and permeability coefficients. Reflection coefficients were obtained by comparing the osmotic fluxes produced by a test molecule to that produced by the impermeant sucrose molecule. Permeability coefficients were determined for measurements of tracer uptake.

The results indicate that, in these fibers, nonelectrolyte permeability is closely related to lipid solubility and molecular size.

A value of 2.16×10^{-12} cm^3/sec dyne for the hydraulic conductivity and a value of 10.45×10^{-4} cm/sec for ^{3}HHO permeability coefficient were obtained.

The effect of membrane surface invaginations and clefts on the determination of permeability coefficients is discussed.

Studies of the rates of diffusive permeation of several nonelectrolytes varying in size and physico-chemical properties have provided useful information about the transport mechanisms of cell membranes [6, 27].

On the other hand, information about membrane discrimination between solutes and water, pore sizes, etc., has been obtained from the determination of reflection and filtration coefficients [20].

Since permeability, reflection, and filtration coefficients should specify the transport properties of a cell membrane, we have considered it of interest to measure them in single barnacle muscle cells. The large size of this cell makes the simultaneous measurement of different parameters feasible. The results of this study may be affected by the complex transverse tubular system (*TTS*), which is highly developed in the barnacle muscle cells [12].

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The aims of this paper are:

1. To measure the permeability of the sarcolemma to nonelectrolytes of different chemical and physical characteristics and the magnitude of the unstirred layer.

2. To determine the water permeability with tracers and osmotic methods.

3. To test different methods to obtain reflection coefficients.

4. To measure the extracellular space enclosed in the cell perimeter, as well as its filling, as a function of time.

To estimate the above mentioned parameters, volume, electrical, and optical changes were measured in the same cell. Radioactive tracers were also used for the permeability studies.

Materials and Methods

Single fibers from the barnacle *Megabalanus psittacus* were isolated from the depressor muscles. The fibers were cut free from the shell and firmly tied with a silk thread. The tendon was also tied. The fiber was then placed in barnacle artificial sea water (*ASW*) of the following composition (in mM): NaCl, 461; KCl, 8; $MgCl_2$, 12; $CaCl_2$, 20; Tris, 10. The pH was adjusted to 7.6 and the temperature kept at 16 °C. Hyperosmotic solutions were prepared by including the required amount of the test solute while the rest of the components were kept unchanged. Three types of chambers were used for the different kinds of experiments. They are described in Fig. 1 *a*, *b* and *c*.

Methods to Obtain Reflection Coefficients

The volume flux (J_v), caused by a concentration gradient of a molecule i across a membrane when the hydrostatic pressure in all parts of the system and the concentration differences of all the other solutes are kept constant, is given by [13]:

$$J_v = \sigma_i L_p R T \Delta C_i \tag{1}$$

where σ_i is the reflection coefficient of species i. L_p is the filtration coefficient, R the gas constant, T the absolute temperature and ΔC_i the concentration difference of i across the membrane.

Experimentally, the reflection coefficient can be measured by comparing the volume flux caused by a hypertonic solution of the test molecule i with the one caused by the same concentration of a reference molecule for which $\sigma = 1$. Three different techniques were used to estimate volume fluxes:

1. Measurement of the cell diameter. The rate of volume change of the fibers was followed by measuring their diameter at different times. To this end the fibers were immobilized, a slide cover was placed over the area to be observed, and the chamber was placed under a microscope.

To obtain the filtration and reflection coefficients the cells were assumed to be cylinders of diameter D and length L. The volume V would then be given by:

$$V = 1/4\,\pi D^2 L. \tag{2}$$

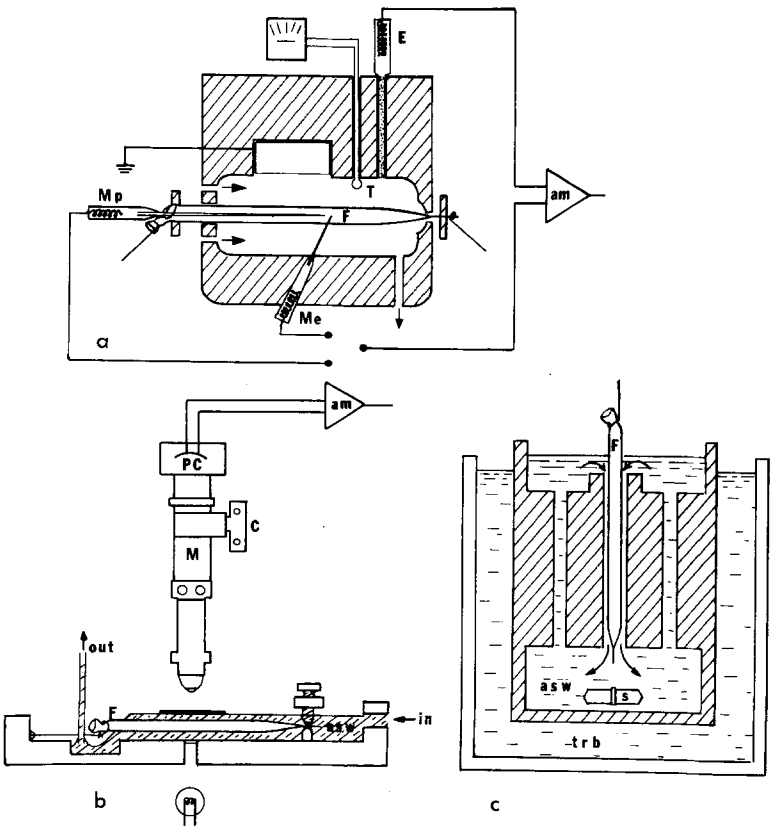

Fig. 1 (*a*). Diagram of the chamber used in the measurements of membrane potentials in barnacle muscle fibers. *F*, fiber; *E*, Ag/AgCl electrode connected to the chamber through agar KCl bridges; *Mp*, micropipette; *Me*, microelectrode; *am*, amplifier system; *T*, telethermometer. Arrows indicate the inflow and outflow of artificial sea water bathing the fibers. (*b*). Diagram of the system used in the measurements of optical density changes of barnacle muscle fibers exposed to hyperosmotic solutions of nonelectrolytes. *F*, fiber; *M*, microscope; *PC*, photoelectric cell; *C*, photographic camera; *am*, amplifier; *asw*, artificial sea water. Arrows indicate the inflow and outflow of perfusing solution. (*c*). Sectional view of the chamber used in the experiments of uptake of tracers by barnacle muscle fibers. The fiber (*F*) was introduced in a central cylindrical channel and maintained hanging during the experiment. The chamber was filled with artificial sea water (*asw*) and introduced in a thermoregulated bath (*trb*) placed on a magnetic stirrer. The spinning of the teflon stirrer (*s*) produced a vortex that forced the solution down through the central hole and produced a flow of solution through the lateral channels, allowing a continuous perfusion of the fiber

Assuming the length was constant

$$dV/dt = 1/2\,\pi LD\,\frac{dD}{dt}. \tag{3}$$

Dividing by the surface area of the cylinder which is πDL we get:

$$J_v = 1/2\,\frac{dD}{dt} \quad \text{where } J_v = \text{volume flux}. \tag{4}$$

Since in our experiments the change in diameter was demonstrated to be an exponential function of time, we can write:

$$(D_t - D_\infty) = (D_0 - D_\infty)\, e^{-\gamma t} \tag{5}$$

where γ is the rate constant for the decrease in diameter. D_t is the diameter at time t, D_0 and D_∞ are the initial and final diameter

$$\frac{dD_t}{dt} = \gamma (D_\infty - D_0)\, e^{-\gamma t}. \tag{6}$$

From Eqs. (4) and (6), J_{v0}, the volume flux at time zero, can be obtained:

$$J_{v0} = \frac{\gamma}{2}(D_\infty - D_0). \tag{7}$$

γ was determined from the plot of Ln $\left(\frac{D_t - D_\infty}{D_0 - D_\infty}\right)$ versus t. $(D_\infty - D_0)$ was measured directly in each experiment.

Since at $t=0$ and at all t's for an impermeant, $\Delta C = C_{\text{out}}$, L_p can be calculated from Eqs. (1) and (7) by using a molecule like sucrose whose σ is 1,

$$L_p = J_{v0}/RTC_{\text{out}}. \tag{8}$$

Once L_p is known, σ for the rest of the solutes can be determined.

J_{v0}/C_{out} was measured at different concentrations and plotted against the concentration.

2. Changes in transmembrane electrical potential. Water transfer across a cell membrane can generate a streaming potential or a concentration potential or both [25]. Since this effect has been shown to be proportional to the water flow produced by a solute, it has been used to determine reflection coefficients [19, 23, 26].

The ratio of ΔE_i produced by a solute i to that produced by an impermeant one, i.e., sucrose, should give us a value for σ_i.

The resting membrane potential was measured by means of glass micropipettes or microelectrodes filled with 3 M KCl and connected through calomel or silver chloride electrodes to a differential amplifier of $10^{14}\,\Omega$ input impedance. The potentials were recorded on a strip chart recorder (Fig. 1 *a*).

The changes in potential produced by urea, glycerol, ethylene glycol, glucose, and raffinose were then determined. Concentrations ranging between 0.2 and 0.4 M were used. The temperature had to be kept under careful control as a $\frac{\Delta E}{\Delta T}$ of 0.75 mV/deg was found.

3. Changes in optical density (O.D.). It has been claimed that volume changes of single muscle cells can be followed by measuring the change in time of the optical density of the cells [21]. We thought it worthwhile to try this method in the barnacle muscle cells.

The measurements were made with a Leitz Ortholux microscope. The chamber with the preparation was mounted on the plate of the microscope (Fig. 1 *b*). The bottom of the chamber consisted of thin glass to assure a high light transmittance. A glass slide cover was placed over the groove allowing solution to completely fill the space in between the bottom of the groove and the slide cover. A system of lamp, mirrors and diaphragms allowed an incident beam of white light to be focused on the middle of the cell. After being colimated this light passed through a narrow band pass filter of 621–623 mμ wavelength. The intensity of the transmitted light was detected by a phototube whose output was fed into a strip chart recorder.

100% transmittance was set by focusing an area of the solution close to the cell. The transmittance was again measured after the chamber was moved to a position in which the incident light illuminated the center of the fiber. A camera attached to the microscope allowed the simultaneous recording of volume changes.

Methods to Obtain Permeability Coefficients

To follow the uptake of radioactive tracers, several muscle cells from the same bundle were immersed in *ASW* solution containing a radioactive test solute and radioactive tracer amounts of sucrose. The latter was used to measure the extracellular space (*TTS*) in each experiment. The radioactive isotopes were obtained from New England Nuclear Corporation. The fibers were placed in a special chamber allowing circulation and strong magnetic stirring of the solution (Fig. 1*c*). Two of these chambers were used simultaneously to provide a pair of measurements for each time period. The times were 0.5, 1, 2, 3, 4, and 20 min for tritiated water and 1, 2, 5, 10, 30, 50, and 70 min for the nonelectrolytes. As each time period was completed, the fibers were rapidly removed from the chamber, rinsed with *ASW*, blotted in filter paper and weighed. They were then dissolved in 0.1 N nitric acid for 24 hr. One ml of the acid and 1 ml of diluted samples of the incubation solution were separately diluted in 10 ml of scintillation liquid and then counted in a Packard 526 refrigerated liquid scintillation counter. Two energy bands were used to simultaneously count tritium and carbon. To obtain the intracellular concentration of the tracer, the amount of radioactive material in the *TTS* had to be calculated and discounted from the total number of counts. To do this it must be assumed that the ratio of concentrations of sucrose and the solute in the extracellular space is the same as in the bulk solution. Therefore:

$$M^*_{\text{intracell}} = M^*_{\text{total}} - \frac{S^*_{\text{excell}}}{[S^*]_{\text{sol}}} \cdot [M^*]_{\text{sol}} \tag{9}$$

M^* = radioactivity of the test solute in count/min; S^*_{excell} = count/min of sucrose in the extracellular space; $[S^*]_{\text{sol}}$ = sucrose count/min per milliliter in the solution; $[M^*]_{\text{sol}}$ = test solute count/min per milliliter in the solution.

The intracellular concentration of the tracer was obtained by dividing M^* by the volume of solvent water in the cell. This was estimated by dehydration of the fibers at 110 °C for 24 hr. A value of $79 \pm 1.8\,\%$ for (water volume/wet weight) $\times$ 100 was obtained. From this the volume of the water in the *TTS* as a fraction of the wet tissue volume could be estimated to be 11 %. The remaining 68 %, which is the percentage of water in the cells, has to be multiplied by a factor of 0.8 to obtain the unbound or effective solvent water in the sarcoplasm [5]. Intracellular concentrations at different times (C_t) and at equilibrium, (C_∞), were calculated and the results expressed as concentration ratios, C_t/C_∞.

To estimate the permeability coefficients for the solutes the equation for diffusion into a cylinder surrounded by an external membrane was used. The solution as given by Crank [7] is the following:

$$\frac{C_t}{C_\infty} = 1 - \sum_{i=1}^{i=n} \frac{4L^2 \exp(-\beta_i^2 D\, t/r^2)}{\beta_i^2(\beta_i^2 + L^2)} \tag{10}$$

where $L = Pr/D$ and β_i are roots of the equation $\beta J_1(\beta) - LJ_0(\beta) = 0$, where J_1 and J_0 are the Bessel functions of the variable β. P is the permeability coefficient, r, the fiber radius and D, the diffusion coefficient of the test molecule inside the cell. The latter was assumed to be equal to the free diffusion coefficient of the molecule.

To solve this equation a program for an IBM 360 computer was made. Introducing different values of P several curves of C_t/C_∞ as a function of P could be obtained for different times. The value of P which gave the best fit for C_t/C_∞ for all the times used was considered the closest to the real one.

The above mathematical treatment used to determine permeability coefficients applies to a cylinder surrounded by a membrane. Permeation through the latter and diffusion inside the cylinder determine the rate of uptake of material for a given concentration gradient. Since diffusion inside of the cell has been considered in the treatment, the internal unstirred layer, which is by far the thickest (i.e., about 1 mm), has been already taken into account.

An attempt to quantify the dimensions of the external unstirred layer in order to correct the permeability coefficients was made. To this end the time course of the change is membrane potential caused by solutions made hyperosmotic by the addition of sucrose was used. An equation given by Diamond [9] relates the half-time $t_{1/2}$ of the rise in potential to the thickness d of the unstirred layer and to the free diffusion coefficient D of the test solute:

$$t_{1/2} = \frac{0.38 \cdot d^2}{D}. \tag{11}$$

Results

Reflection Coefficients

A description of the results obtained with the different methods tested is next given.

1. Measurements of cell diameter. The change in cell diameter with time after a switch from an isotonic to a hypertonic medium could be

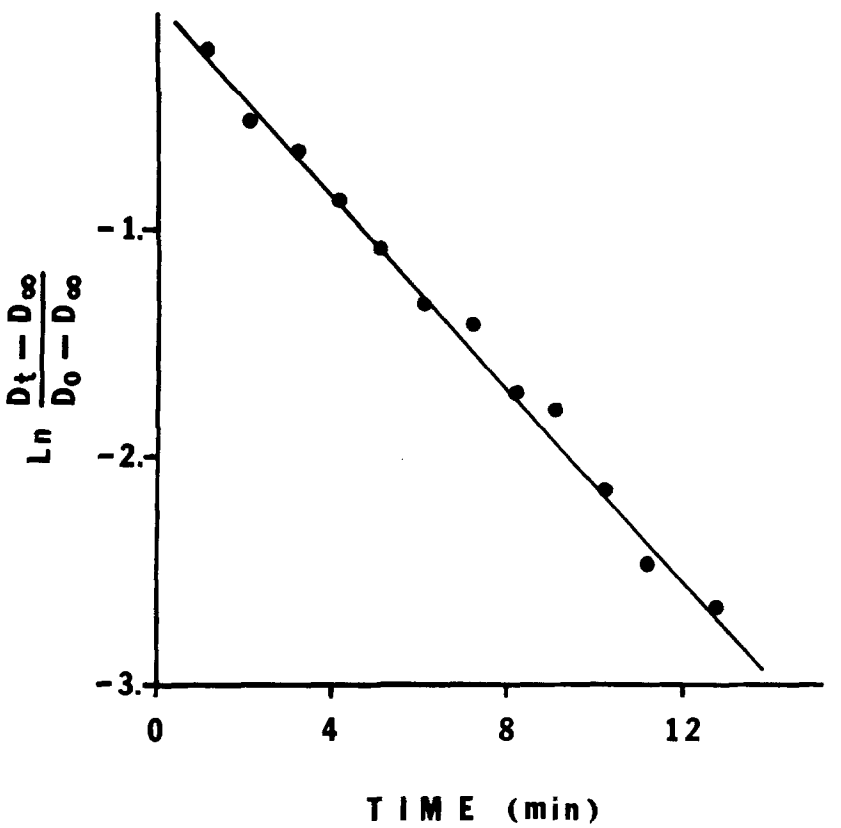

Fig. 2. Logarithmic plot of the diameter change of a barnacle muscle fiber induced by an osmotic gradient of 400 mOsm sucrose. From the slope, the time constant, γ, was calculated. D_0, diameter of the fiber at $t=0$; D_t, diameter at time $=t$ and D_∞, diameter at equilibrium

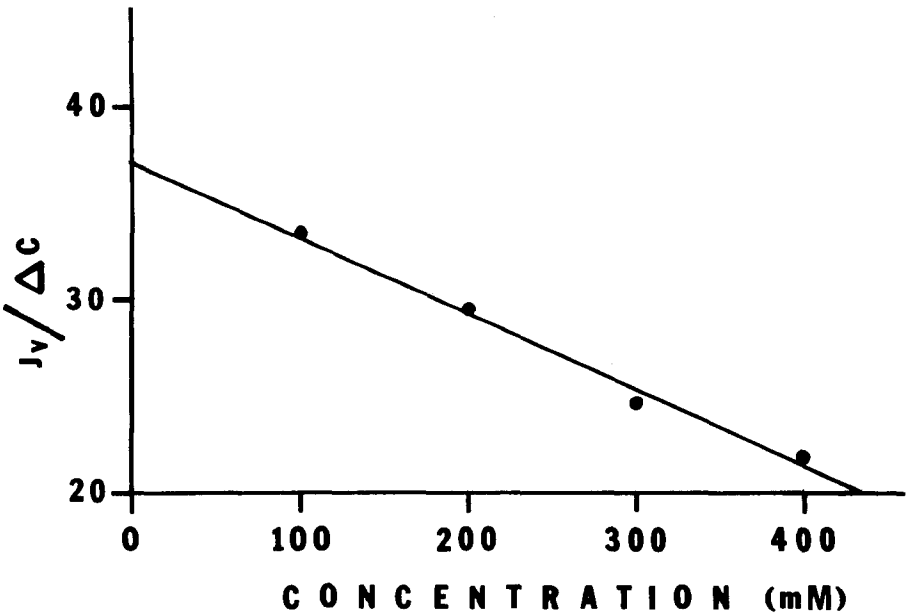

Fig. 3. The effect of the concentration gradient on the ratio between the volume flow (J_v) and the concentration difference (ΔC). $J_v/\Delta C$ is expressed as $\mu \cdot 1/\text{mole} \cdot \text{min}$. By extrapolating to $C_{out}=0$, L_p for the membrane was estimated to be 2.16×10^{-12} cm^3 sec^{-1} dyne^{-1}

fitted by an exponential function as shown in Fig. 2. L_p could be obtained from the experiments done with sucrose as described in the methods. L_p decreased with concentration in a linear fashion as seen in Fig. 3. This change, which was probably caused by unstirred layers, had been previously described [24] and corrected by extrapolating J_{v0}/C_0 to $C_0=0$. After applying the same type of correction, a value of $L_p=2.16 \times 10^{-12}$ cm^3 sec^{-1} dyne^{-1} was obtained. Having a value for L_p, the reflection coefficients could be obtained from the variations of the cells' diameter with time. The values of σ estimated in this manner are listed in Table 1.

2. Transmembrane potentials. The average value of the resting potential (E) in 34 cells was 53 ± 5 mV (mean $\pm$SE). Hyperosmotic solutions hyperpolarized the membrane, i.e., increased E as seen in Fig. 4. The change in membrane potential was a linear function of sucrose concentration between 0 and 400 mM. The ratios of ΔE_i produced by each of five permeant solutes and that produced by sucrose are listed in Table 1. Urea, glycerol, and raffinose show ratios which are not statistically different from 1.

3. Optical density changes. Hyperosmotic solutions produced optical density (*O.D.*) changes which differed in sign and magnitude according

Table 1. Reflection coefficients of nonelectrolytes in barnacle muscle fibers[a]

Molecule	σ	$\Delta E_i/\Delta E_s$
Sucrose	1.0	1.0
Glycerol	(8) 0.96±0.11	(13) 1.11±0.18
Urea	(8) 0.83±0.13	(14) 1.08±0.23
Ethylene-glycol	(6) 0.45±0.08	(8) 0.51±0.13
Glucose	– –	(8) 0.84±0.13
Raffinose	– –	(8) 1.01±0.15

[a] The table shows the reflection coefficients σ of several nonelectrolytes as determined by changes in cell diameter. The ratios of the change in the membrane resting potential, $\Delta E_i/\Delta E_s$, produced by a test molecule and that produced by sucrose at the same concentration are also given. Figures in parentheses represent the number of experiments. Mean values ±SE are given.

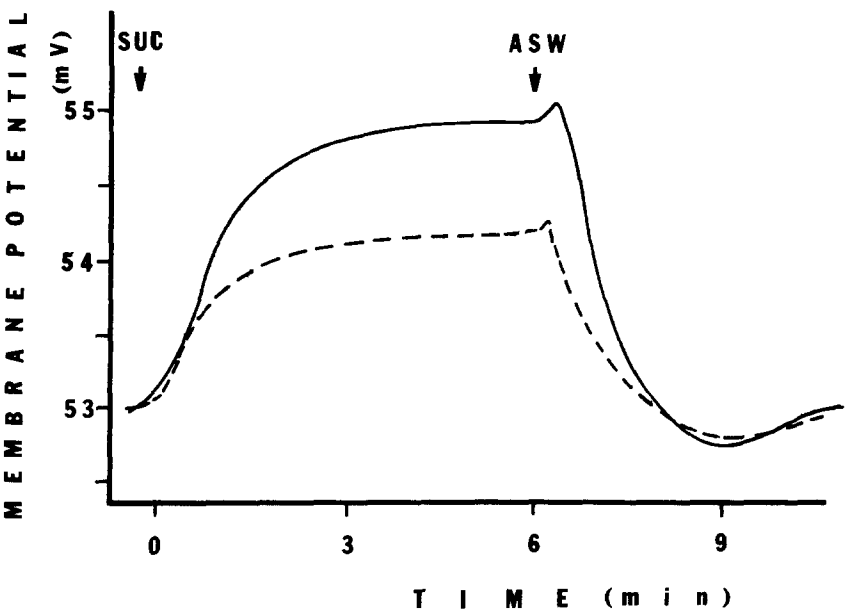

Fig. 4. Time course of the potential change during exposure to hyperosmotic solutions of sucrose. The continuous line shows the effect of 400 mM sucrose and the broken one the effect of 200 mM sucrose. The arrows indicate when the sucrose was added (*SUC*) and when it was replaced by artificial sea water (*ASW*)

to the size of the molecules used. The smaller molecules up to the size of sucrose increased the *O.D.* Molecules larger than sucrose, i.e., raffinose and inulin decreased the *O.D.* Fig. 5 shows experiments done with urea and sucrose.

The effect of sucrose concentration on the *O.D.* was linear up to about 250 mM. At this point saturation was reached.

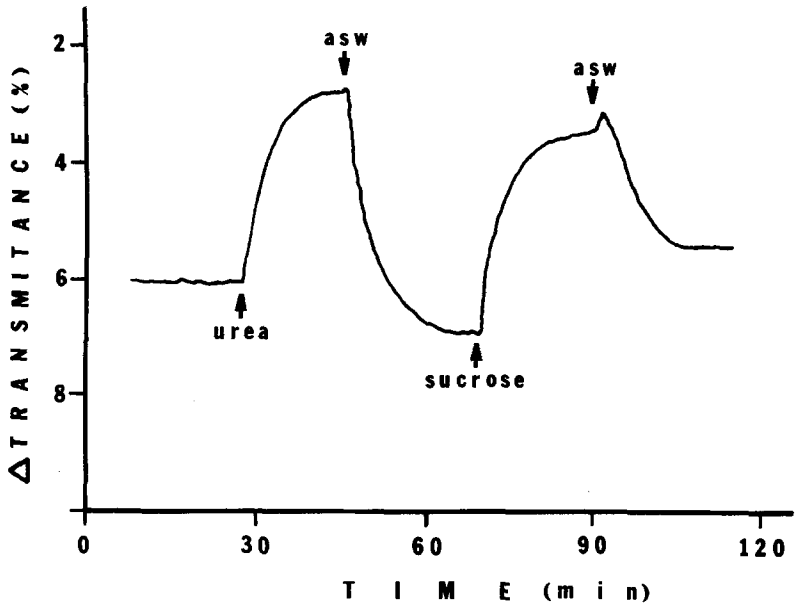

Fig. 5. Time course of the effect of 200 mM sucrose and urea on the optical transmittance of the barnacle muscle fibers. Arrows indicate the addition of the nonelectrolytes or their replacement by artificial sea water (*asw*)

The order of the molecules according to their effect on the magnitude of the *O.D.* change is the following:

Urea > glycerol > glucose > sucrose for the ones which increased *O.D.*

Inulin > raffinose for the ones which decreased *O.D.*

Solute Fluxes

Fig. 6 shows that the concentration of labelled sucrose in the *TTS* reaches an equilibrium level at about 60 min. The volume filled by sucrose at equilibrium as measured in 8 experiments was $10.6 \pm 1.5\%$ of the fiber volume.

In some experiments the fibers were exposed for 1 hr to *ASW* whose osmolarity had been raised by the addition of 0.2 or 0.4 M sucrose. Measurement of the extracellular space under this condition showed it had increased to $18 \pm 2.1\%$ and $32 \pm 3.5\%$, respectively.

The permeability coefficients which best fitted the computed solutions for diffusion into a cylinder bound by a membrane are listed in Table 2. These permeability coefficients were then corrected for the effect of an external unstirred layer. The thickness of the latter was obtained from

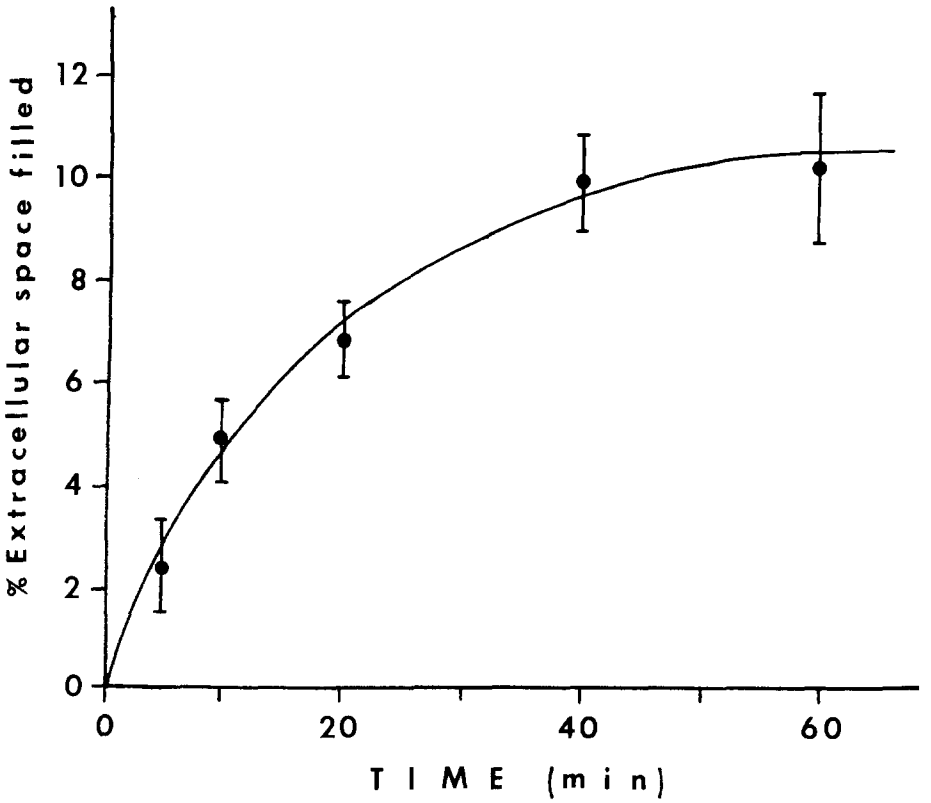

Fig. 6. Plot of the apparent extracellular space occupied by sucrose as a function of time. It reaches an equilibrium level at about 60 min. The volume filled by sucrose at equilibrium was $10.6 \pm 1.5\%$ of the fiber volume. Each point is the average of 4 experiments except for the point at 60 min, which is the average of 8 experiments

Eq. (11). The half time of the rise of the membrane electrical potential induced by *ASW* made hyperosmotic by the addition of sucrose was 44 sec. Sucrose diffusion coefficient was taken as 5.4×10^{-6} cm^2/sec. A thickness of 250 μ was obtained for the external unstirred layer. An equation given by Dainty and House [8] was then used.

$$\frac{1}{P} = \frac{1}{Pm} + \frac{di}{Di} + \frac{de}{De} \tag{12}$$

where P is the experimental and Pm the real permeability coefficients, respectively. D is the diffusion coefficient. The thickness of the unstirred layer, d, suffix i and e account for the internal and external side of the cell.

Since the diffusion inside of the cell is included in the model of diffusion into a cylinder as given by Crank [7], only the de/De term should

Table 2. Permeability coefficients of nonelectrolytes in barnacle muscle fibers[a]

Molecule		Molecular weight (M)	P (cm/sec) $\times 10^4$	$P_{corrected} \times 10^4$	Partition coefficient (κ)
^{3}HHO	(45)	18	5.05 ± 0.12	10.45	0.009
Antipyrine	(12)	188.2	3.85 ± 0.17	4.67	0.032
Methanol	(10)	32.0	2.91 ± 0.08	6.81	0.0078
Acetamide	(15)	59.1	1.52 ± 0.09	1.58	0.00083
Ethylene-glycol	(15)	62.1	0.64 ± 0.01	0.76	0.00049
Glucose	(10)	180.2	0.32 ± 0.01	0.36	$<4.6 \cdot 10^{-5}$
Methylurea	(22)	74.1	0.23 ± 0.02	0.24	0.00044
Urea	(25)	60.1	$0.17 \pm 0,01$	0.18	0.00015
Thiourea	(14)	76.1	0.17 ± 0.01	0.18	0.0012
Glycerol	(30)	92.1	0.12 ± 0.01	0.12	0.00007
Inulin	(8)	5000.0	$0.00 \pm -$	–	–

[a] The table shows the permeability coefficients, P, of several nonelectrolytes determined by uptake of tracers. These values were calculated using the equation developed by Crank [7]. The values of oil/water partition coefficients, κ, were taken from Wright and Diamond [27]. Figures in parentheses represent the number of experiments. P_s in the fourth column were corrected for unstirred layers effects. Means $\pm$SE are given.

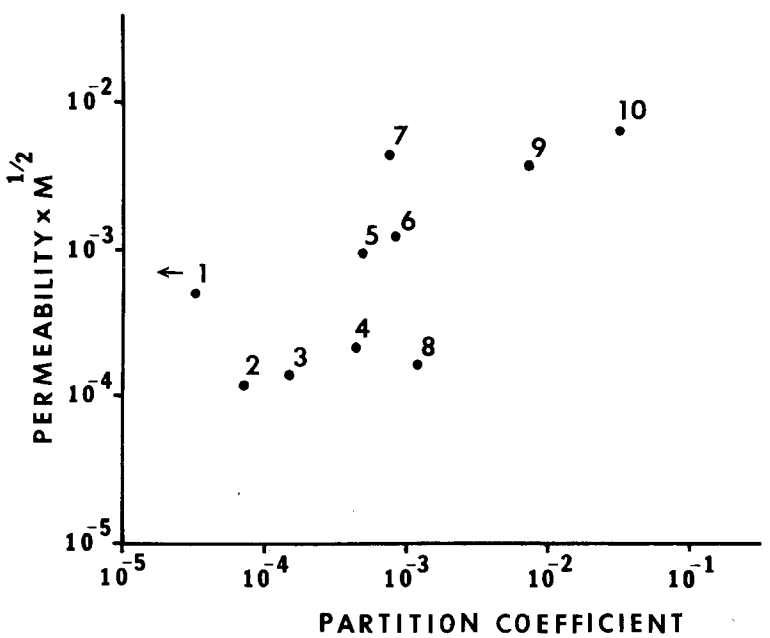

Fig. 7. The permeability of single barnacle muscle fibers to nonelectrolytes of different oil/water partition coefficients. *Ordinate:* Permeability (cm/sec) $\times$ (molecular weight)$^{1/2}$, *Abcissa:* Olive oil/water partition coefficients. *1* glucose; *2* glycerol; *3* urea; *4* methylurea; *5* ethylene glycol; *6* acetamide; *7* water; *8* thiourea; *9* methanol; *10* antypirine. The values of partition coefficients were obtained from Wright and Diamond [27]

be used for the present correction. The results of this correction are given in column 5 of Table 2. As can be seen the external unstirred layer becomes increasingly important at permeability values above 10^{-4} cm/sec. Moreover, it is an important rate contributing factor for water transfer.

In Fig. 7 the product of permeability times the square root of the molecular weight for each solute was plotted versus their oil/water partition coefficient.

Discussion

The permeability coefficients estimated by us should be affected by the structural complexities and large size of the *TTS*. Since the electrical capacitance of the muscle cell membrane of Balanus psittacus is about 20 times higher than that found in other cell membranes [14], the membrane area hidden in the clefts and tubules may be 20 times as large as the geometrical one. This suggests that the permeabilities calculated on the basis of the external surface are higher than the real ones. A simple division of the permeability coefficients by the total area would not be the right correction to apply. This is because the time course for the solutes to reach the whole area should be a function of their diffusion coefficients. Actually, a completely different model instead of the cylindrical one should be used. However, a comparison of the permeability of different molecules can be attempted by assuming that the error introduced by neglecting the *TTS* area is proportional to the permeability coefficients. It is also assumed that the permeabilities of the external and *TTS* membranes are the same. At any rate, the real permeabilities should be smaller than the ones calculated on the basis of the geometrical area. For these reasons we will refer to relative permeability coefficients.

As in other cell membranes [6] the permeability of the nonelectrolytes in barnacle muscle cells show a strong dependance on the partition coefficients of the tested molecules. Glucose relative permeability, however, seems to be well above the line suggesting some special transport mechanism. This is not unexpected as facilitated diffusion for sugars has been shown in different kinds of muscle [17].

On the other hand, urea and thiourea show the same relative permeability. Since the partition coefficient of the latter is about 8 times as large as that of urea while its diameter is only slightly larger, other factors besides lipid solubility or size should be considered. This is assuming that the oil/water partition coefficients are equal to the membrane lipids/water ones. Permeability ratios close to one for urea and thiourea also have

been reported for the choroid plexus [28] and bilipid layers [15]. No special transport mechanism for urea seems to operate in the barnacle muscle cell.

As in skeletal muscle cells [10], the permeability for acetamide is larger than that for urea. Since the former has only one NH_2 group as compared to two in urea, the different permeabilities could be caused by a weaker solute to water interaction of acetamide as compared to urea.

A correlation between reflection and permeability coefficients, though feasible, does not seem rewarding because of the uncertainties caused by the complex geometry of the system.

The hydraulic conductivity L_p here reported, 2.16×10^{-12} cm^3/sec dyne, is smaller than those of crab [21] and crayfish [18] muscles which were 7.2×10^{-12} cm^3/sec dyne and 4.9×10^{-12} cm^3/sec dyne, respectively. This difference is not large, however, considering that the species and methods compared are different. A value of 6 for the ratio of filtration to diffusional permeability coefficient for barnacle muscle was obtained. This ratio was reduced to 3 after correcting for the effect of unstirred layers on the diffusional permeability coefficient. Both values for the ratio are markedly lower than a value of 81 reported for crab muscle [21].

An equivalent pore size can be estimated by using Paganelli and Solomon's treatment [16]. For a water permeability ratio of 3 a value of 5 Å for the equivalent pore radius was obtained. Even though the meaning of the equivalent radius is obscured by the complexity of the cell geometry, a value like the one obtained seems to follow a pattern common to most cells.

The reflection coefficients obtained from the changes in cell diameter seem to have the right sequence according to the size and the lipid solubility of the solutes tested. They do not differ much from those previously reported for glycerol (0.91) and urea (0.87) in barnacle muscle cell [3].

On the other hand, the electrical method did not prove sensitive enough for the measurement of reflection coefficients since the values for glycerol and urea were indistinguishable from that for sucrose. This, we think, is caused by the very special morphology of the cell membrane. The invaginations or clefts and the *TTS* create a barrier for diffusion which lengthens the time for the molecules to reach the whole cell membrane area. The volume of the *TTS* here reported of 10% of the cell volume is slightly higher than the 6 [11] and 8 [12] percent values reported for other species.

A comparison of Figs. 4 and 6 indicates that, while at 6 min the electrical potential rise due to hyperosmolarity is essentially completed, only

about 1/3 of the *TTS* has been filled up by sucrose. This suggests that this extent of penetration into the *TTS* might be enough to produce a withdrawal of water from the cell at a rate faster than the diffusion of the solute into the smaller tubules. If the intracellular diffusion of ions is assumed to be faster than that of sucrose in the *TTS* no osmotic gradient might result across the cell membrane lining the deeper and narrower tubules. Diffusion coefficients in the myoplasm of the barnacle muscle cell of about one half of their free solution values have been reported for K, Na and Cl [4]. A diffusion coefficient about 1/4 of the free diffusion value has been reported for sucrose in the extracellular space of the heart [22]. Although both the *TTS* and the extracellular space contain mucopolysaccharides, the reduction of the free diffusion coefficient could be less marked in the former.

However, since the free diffusion coefficient of Na for instance, is about three times larger than that of sucrose, a difference favorable to the ions could still exist even if the reduction of the diffusion coefficients were the same for ions and sucrose in the myoplasm and *TTS*, respectively.

This difference in diffusion rates would be less for solutes like urea and glycerol, and this might explain why the potential differences and, therefore, the σ's of the smaller solutes were of about of the same value as that for sucrose. Moreover, the previous analysis suggests that the whole external membrane and only a fraction of the *TTS* membrane would participate in the generation of electrical potential differences. Since this would seem to be the case, the present value for the external unstirred layer would be only an order of magnitude correction for the permeabilities of solutes whose rates of diffusion are different than that of sucrose.

The results obtained using the electrical potential method to measure σ in giant squid axons may have been more satisfactory probably because of the simpler geometry of the cell boundary and because the cells were internally as well as externally perfused [23].

Since the same topographic considerations will apply, we may question now the measurement of the diameter as a good method to obtain σ's. We think, however, that in this case the topography does not interfere in the same way. Measuring the diameter will not detect the volume change due to water transferred from the myoplasm to the *TTS*, both being inside of the perimeter of the cell. That this volume transfer does occur is shown by the increase here reported for the *TTS* volume when the cell was immersed in hyperosmotic solutions. This swelling has been observed in electronmicrographs of frog's skeletal muscle [1] but not quantitatively

estimated by the use of extracellular tracers. More recently, it has been reported that the apparent *TTS* swelling might be an artifact of the fixation procedure [2]. It has been claimed, however, that there is an effective swelling of the sarcoplasmic reticulum (*S.R.*). We think that the increase in extracellular volume here reported for the barnacle muscle cell is better explained as a swelling of the *TTS*. The *S.R.* of barnacle cells is apparently too small, only about 0.5% of the cell volume [14] to account for a change from 10 to 30% of the cell volume.

Since, even after 1 hr, the *TTS* volume is still increased in hyperosmotic solution, it seems improbable that a significant rise in the hydrostatic pressure inside the tubules has occurred. This suggests that the *TTS* swelling may be compensated by a simultaneous shrinkage of the adjacent myoplasm. Therefore, water loss through the *TTS* membrane would not be seen as a change in cell diameter. From the above considerations, we conclude that the change in diameter of the cell accounts mainly for the water leaving the cell across its peripheral membrane. If this is true, the measurement of cell diameter would provide a good estimate of σ and L_p.

As the relationship between *O.D.* changes and molecular size was of a very complex nature, no estimation of σ's was made possible by the use of optical methods.

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