A LIGHT-SCATTERING MEASUREMENT OF MEMBRANE VESICLE PERMEABILITY

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ABSTRACT Light-scattering/intensity autocorrelation measurements of vesicle diffusivity were used to follow the time course of the osmotic response of lobster abdominal sarcoplasmic reticulum vesicles to five lipophobic nonelectrolytes. Steady-state portions of the resulting time traces show these vesicles to be permeable to ethylene glycol and glycerol and impermeable to erythritol, glucose, and sucrose. Using measured values of the hydrodynamic radii of these nonelectrolytes, it is concluded that under passive transport conditions, these vesicles may be thought of as having pores whose radii lie between 3.1 and 3.5 Å. In addition, the results presented here indicated that above a certain impermeable nonelectrolyte concentration, vesicles did not respond osmotically even though they had not collapsed. This suggests that at least under the experimental conditions reported here, vesicles behaved as if rigid when their average volume had decreased to about 50% of its original isotonic value.

INTRODUCTION

The osmotic effects of charge-neutral, lipid-insoluble molecules on membrane vesicles can be used to characterize membrane permeability under passive transport conditions.

Electron microscopy and light-scattering/turbidity measurements have been used for some time to study osmotic effects on suspensions of membrane microsomes. In this paper, another light-scattering technique has been used, in conjunction with electron microscopy, to probe osmotic effects on sarcoplasmic reticulum (SR) membrane vesicles. Specifically, the intensity autocorrelation spectra of light scattered quasi-elastically from dilute suspensions of lobster abdominal SR vesicles were used to follow the long term temporal evolution of the osmotic response of these vesicles to 1 M hypertonic solutions of sucrose, glucose, erythritol, glycerol, and ethylene glycol. Analysis by light scattering was also used to examine the steady-state osmotic effects on vesicles of sucrose solutions of different concentrations and to assess the long-term permeability of these vesicles to the charged buffering reagent, TES. Because of its structural simplicity and functional specificity, lobster abdominal muscle SR is ideally suited for this type of investigation (1, 2). These light-scattering spectra were then analyzed using the methods developed in ref. 3. Applying the methods of ref. 3, each spectrum analyzed provided a pair of values; the vesicle Z-averaged diffusion coefficient, $<D>_Z$, and the relative dispersion about that average, δ_Z. Since a vesicle's diffusion coefficient varies inversely as its radius, plots of viscosity-corrected (see Data

Analysis section) $<D>_Z$ values vs. time were used to follow osmotically driven changes in vesicle size. In addition, using equations derived in ref. 4, selected ($<D>_Z$, δ_Z) pairs were used to compute the vesicle number-averaged radius $<R>_N$, and the relative dispersion about this average, δ_N, so that a direct size comparison between isotonic and hypertonic solutions could be made. An alternative way of analyzing vesicle QELS spectra to determine $<R>_N$ has recently been proposed by Aragón and Pecora (5).

Presented here, then, are the light-scattering and electron microscopic results as well as the analyses and arguments used both to characterize passive SR membrane permeability and measure pore size and to infer an apparent vesicle rigidity under certain osmotic conditions.

EXPERIMENT

Time Course Measurements

The experimental approach was to inject, at some reference time, a dilute, aqueous, buffered SR vesicle suspension with a solution of the osmotic agent of interest sufficiently concentrated to bring the sample to a final concentration of 1 M in the injected solute. For a given study, i.e. a given solute, each sample and its controls had the same vesicle concentration. As vesicles formed originally in a 10 mM TES solution designed to hold the suspension pH at 7.0 and as all subsequent dilutions (osmotic agents excepted) are made using 10 mM TES, the isotonic control solutions mentioned throughout this paper refer, at least nominally, to 10 mM TES solutions. Correlation spectra of light scattered from the sample were then taken intermittently for about 6 h. During this 6 h period, spectra of an isotonic control sample and a 1 M sucrose control sample which had reached osmotic steady state were also taken for reference and comparison. The controls were identical to the hypertonic sample with the exception that the injected volume of the osmotic agent was replaced with an equal volume of either 10 mM TES or sucrose solution of the appropriate concentration. Graphs of viscosity-corrected $<D>_Z$ values vs. time for each hypertonic solution and its two controls were then plotted and used both to present and to aid in the interpretation of the experimental results. An electron microscopic study of the effect of a 1 M sucrose environment on vesicle shape was also made and the resulting micrographs used to help interpret the light-scattering results.

Sucrose Concentration and 1 M TES Permeability Studies

To assess the long term osmotic effect of different hypertonic sucrose concentrations on vesicles, vesicles were incubated for several hours in 0.1 M, 0.5 M, and 1 M sucrose solutions and light-scattering spectra then taken of these samples along with an isotonic control from the same preparation. In addition, the osmotic effect on vesicles incubated for several hours in 1 M TES was examined using light scattering.

Sample Preparation

Samples were prepared essentially as described in ref. 3, but the following improvements are worth noting:

(*1*) Sample dust contamination levels were further reduced by filtering the 10 mM TES buffer—used in preparing all final vesicle samples and with which all hypertonic and sucrose control solutions were prepared—five times successively through a 0.22 μm Millipore filter and then twice through two 50 nm Millipore filters. Subsequently, sucrose and glucose solutions were filtered twice through two 0.22 μm Millipore filters while erythritol, glycerol, ethylene glycol and 1 M TES were filtered once through 50 nm Millipore filters. In addition, before being sealed to await filling, scattering cells after detergent, cleaning acid, and distilled water washes were bathed continuously inside and out with ethanol for at least an hour in a reflux washing apparatus designed for that purpose.

With the exception of the preparations used in the glycerol study and in the sucrose concentration and 1 M TES studies, all vesicle suspensions were filtered twice through two 0.45 μm Millipore filters immediately after sonication and were analyzed by light scattering within hours of vesicle isolation. Suspension sonication was carried out at 0°C using a Biosonik probe sonicator (thin probe) (Bronwill Scientific, Rochester, N.Y.) at a power setting of 45. All suspensions were sonicated at a concentration of about 2 mg/ml and each experienced ten 10-s sonication bursts spaced 1 min apart. The glycerol study preparation was sonicated and initially filtered once through a 0.45 μm Millipore filter, but light-scattering spectra taken a few hours later showed vesicle size and polydisperity to be too large for reliable analysis. The following day, an acceptable sample was made by filtering a portion of the sonicated, filtered preparation of the previous day, which had been kept at 0°C overnight, once again through a 0.45 μm Millipore filter. The vesicle preparation used for both the sucrose concentration and 1 M TES permeability studies was kept at 0°C for about 24 h before samples were made up for light-scattering analysis. With this exception, these samples were prepared exactly as those used in the sucrose/glucose and ethylene glycol time course studies.

(*2*) All sonication, filtration, and final vesicle sample loading was carried out in the "dust-free" environment of a laminar flow bench. In the time course studies, filtered osmotic solutions were injected into the sealed scattering samples at the light-scattering spectrometer using sterilized disposable syringes and needles. For the sucrose concentration and the 1 M TES permeability studies, filtered osmotic solutions were injected at the laminar flow bench. The sample cells were then resealed with parafilm.

(*3*) Using the techniques used in ref. 3, suspension ATPase activity and Ca^{++} uptake were checked for all preparations both before and after sonication and filtration to insure that all samples contained a high proportion of functioning, transport-viable vesicles. Vesicle integrity and transport viability in 1 M sucrose was verified by measuring vesicle Ca^{++} uptake using methods described in ref. 1. From this result, it was assumed that vesicles in the other 1 M solutions were transport-viable as well.

(*4*) Based on an earlier study of vesicle diffusivity dependence on vesicle concentration, it was concluded that vesicles in suspension may be considered as diffusing independently for vesicle protein concentrations $\lesssim 0.1$ mg/ml. For this reason, all suspensions used in the scattering experiments reported here had protein concentrations $\lesssim 0.1$ mg/ml. Using a lobster abdominal SR membrane density of 1.1 g/ml, a protein/lipid weight proportion of 50/50 and a membrane thickness of 80 Å as reported by Branton and Deamer (6), the center-to-center spacing of vesicles having a mean radius of 450 Å (representative of isotonic suspensions reported here) at a suspension concentration of 0.1 mg/ml is about 10,000 Å. In addition, the mean time between collisions of such vesicles, t, can be calculated using the equation:

$$t = (8\pi\rho_N DR)^{-1}, \tag{1}$$

where ρ_N, D, and R are the vesicle number density, diffusion coefficient, and radius, respectively. Note that Eq. 1 is different from Eq. 56 derived in Appendix IV of ref. 3. It was pointed out in private conversation by D. P. Y. Chang that Eq. 56 of ref. 3 is in error because its derivation relies on an expression for vesicle mean free path (Λ in that derivation) that does not account for the diffusive nature of vesicle motion. The correct expression for the mean time between collisions of diffusing Stokes spheres has been known for some time (see, for example, ref. 7) and is Eq. 1. Using typical vesicle experimental values, it is found that the mean time between vesicle collisions was of the order of 0.1 s while vesicle relaxation times were of the order of 10 ms. This result supports the assumption that vesicles were moving independently and diffusing freely in suspension in the experiments reported here.

Light-Scattering Measurements

The scattered light correlation measurement apparatus is the same as that described in ref. 3. All measurements reported here were made at a scattering angle of 12° and a thermostatically maintained temperature of 20.0 ± 0.5°C. At 20°C, lobster abdominal SR is reported to be in its more fluid-like state (8). All samples were kept refrigerated at 0°C between measurements and were removed from the refrigerator and allowed several minutes to come to thermal equilibrium at 20°C before a scattering spectrum was taken.

To keep vesicles "fresh," the time spent by samples at 20°C in the spectrometer was minimized by returning them to 0°C after each run. The D_c results indicate that at least as far as the light-scattering measurements of vesicle osmotic response are concerned, this cycling between 0°C and 20°C had no detrimental effects on sample vesicles.

Time course study hypertonic solutions were initially examined continuously for a period of about 1 h after solute injection by taking back-to-back scattering spectra of these samples. They were then returned to the refrigerator and brought out for subsequent measurements at roughly 1 h intervals. The total time taken for each study was about 6 h. Isotonic buffer control measurements were made between hypertonic solution measurements and at least two sucrose control measurements were fitted between

these two for each study. The light-scattering results of these studies are presented in Figs. 1–4.

The sucrose concentration and 1 M TES permeability study solutions were each measured in back-to-back fashion so that the longest period any sample experienced at 20°C was about 30 min. The total time taken for these studies was about 6 h. The results of the sucrose concentration study (open circles) are presented graphically in Fig. 5. The results of the TES permeability study are discussed in the Discussion of Results section.

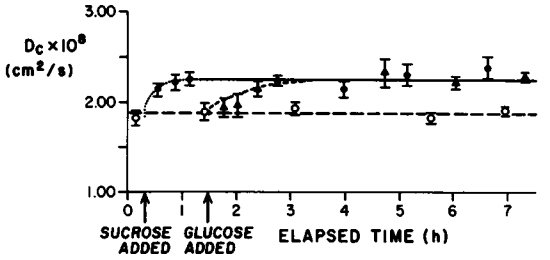

FIGURE 1 Light-scattering D_c results for both the sucrose (solid circles) and the 1 M glucose (solid triangles) studies. In this case, the sucrose is its own hypertonic control. Average D_c for the isotonic control (open circles) is shown as a flat dashed line.

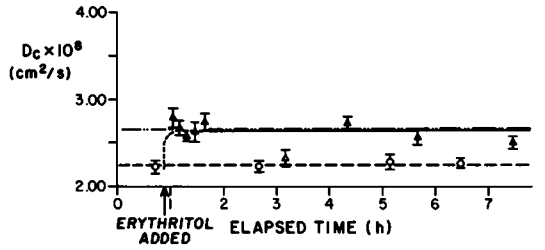

FIGURE 2 Light-scattering D_c results for the 1 M erythritol (solid triangles) study. Average D_c for the hypertonic and isotonic (open circles) controls are shown as a flat dashed-and-dotted line and a flat dashed line, respectively. Since it was considered to be anomalously low, the point represented by the open triangle was deleted from the data set used to compute erythritol steady-state averages.

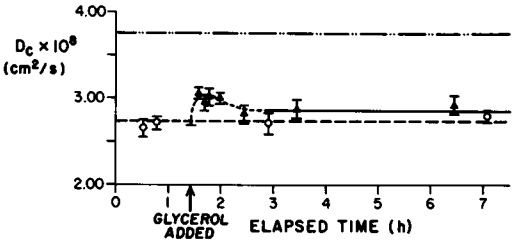

FIGURE 3 Light-scattering D_c results for the 1 M glycerol (solid triangles) study. Average D_c for the hypertonic and isotonic (open circles) controls are shown as a flat dashed-and-dotted line and a flat dashed line, respectively.

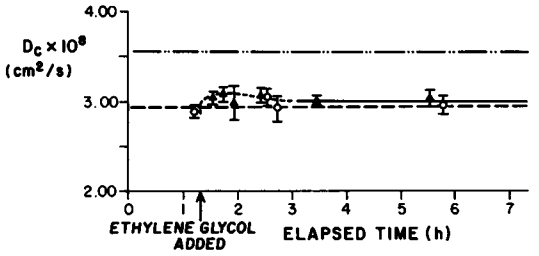

FIGURE 4 Light-scattering D_c results for the 1 M ethylene glycol (solid triangles) study. Average D_c for the hypertonic and isotonic (open circles) controls are shown as a flat dashed-and-dotted line and a flat dashed line, respectively.

Electron Microscopic Measurements

To study the effect of a 1 M sucrose solution on vesicle shape, a sample prepared in a manner identical to those used in the light-scattering studies—except that in order to obtain sufficient sample, all EM preparations had a final vesicle concentration of ≈ 2 mg protein/ml—was incubated in 1 M sucrose at 0°C for approximately 2 h and then left at room temperature for about 1 h to simulate the longest time hypertonic samples remained at 20°C in the light-scattering studies. This sample was then fixed in OsO_4 and sectioned. For EM comparison purposes, an isotonic control of the same sample was prepared in the same way. These EM results are presented as Figs. 6 and 7. To check short-time effects, a portion of the erythritol study preparation was incubated in 1 M sucrose for 2 min, fixed in OsO_4, and sectioned (Fig. 8). Then, to check the effect of fixation on vesicles in 1 M sucrose, another sample prepared as for investigation by light scattering was incubated in 1 M sucrose for several minutes then fixed with a milder fixative, gluteraldehyde, before final fixation with OsO_4. This sample was then sectioned and micrographed.

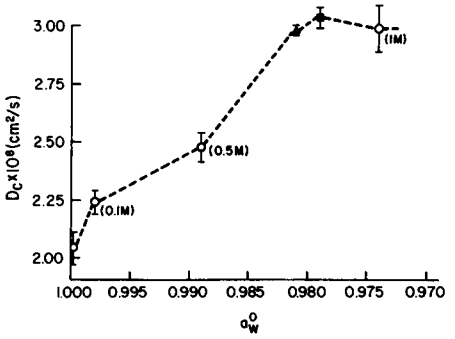

FIGURE 5 Light-scattering D_c results for the sucrose concentration study (open circles). The aqueous activities, a_w^o, of hypertonic sucrose solutions whose molarities are shown on the plot in parentheses are along the abscissa. Also included are the steady-state 1 M erythritol (solid triangle) and 1 M glucose (solid square) time course study results normalized to the 1 M sucrose concentration study value (see Comment section in text).

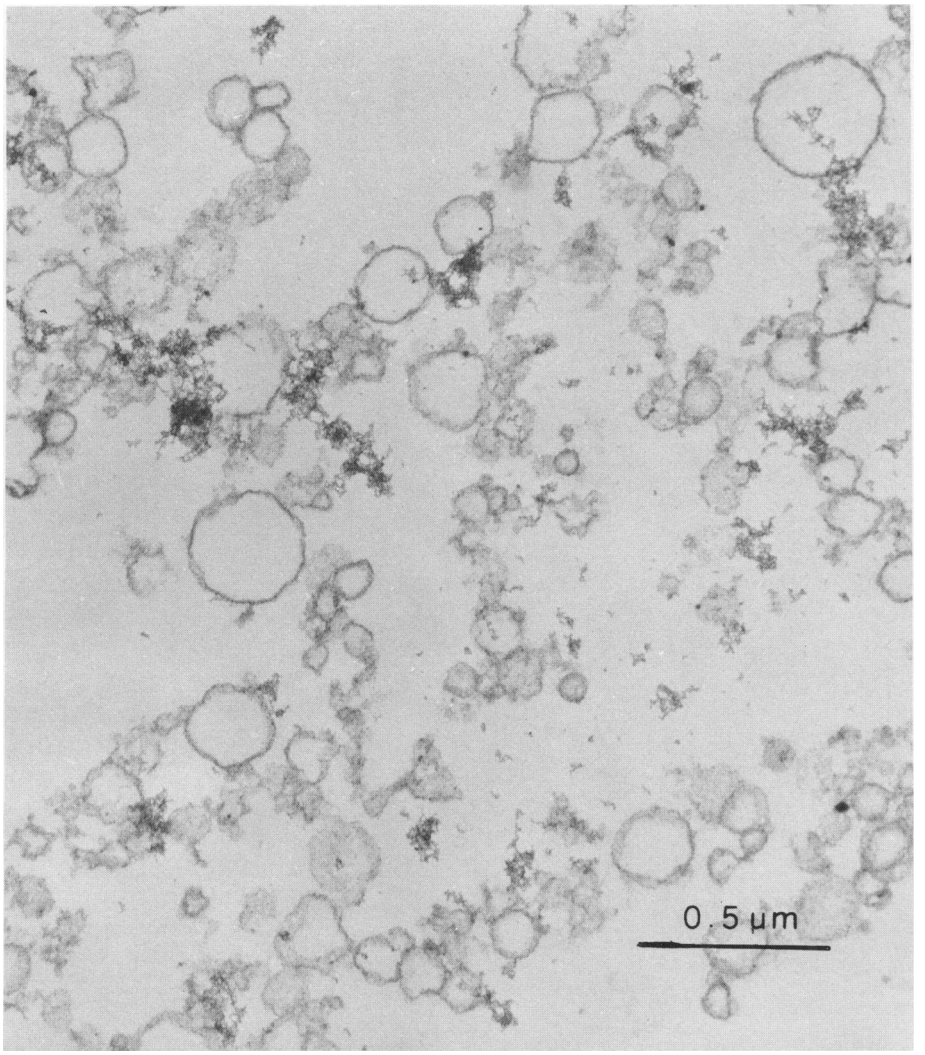

FIGURE 6 Thin-section electron micrograph of vesicles incubated in 1 M sucrose at 0°C for about 2 h, and then left at room temperature for approximately 1 h before fixation and sectioning.

DATA ANALYSIS

The techniques used to analyze the light-scattering data of ref. 3 were extended to analyze the light-scattering results presented in this paper as follows:

(*1*) Values of $< D >_z$ and δ_z were improved by iterating Eq. 24 of ref. 3 until successive values of $< D >_z$ and δ_z agreed to within one part in 10^5. Typically, this took five iterations and decreased $< D >_z$ about 1–2% and increased δ_z about 2–4% from their more approximate values obtained using Eqs. 30 and 36 of ref. 3.

(*2*) For time course studies, hypertonic solution steady-state and all isotonic buffer

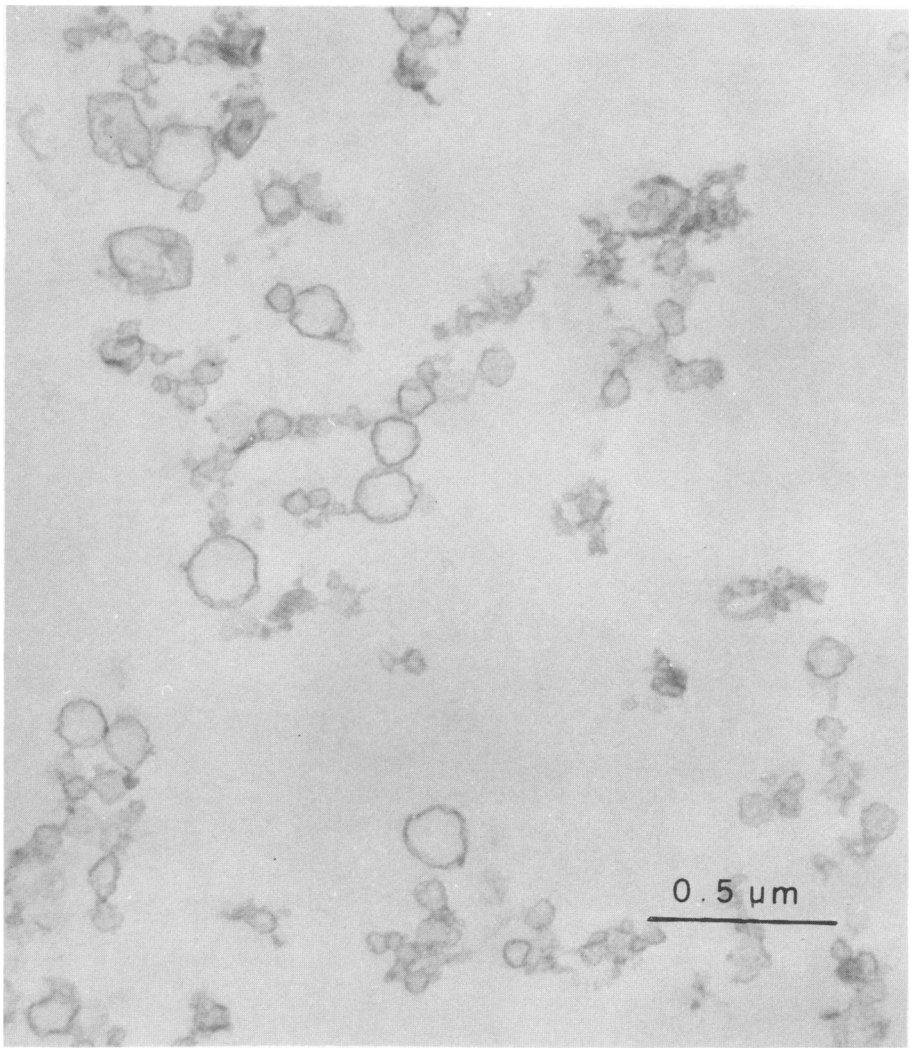

FIGURE 7 Thin-section electron micrograph of vesicles from the isotonic control for the long-term incubation 1 M sucrose sample of Fig. 5.

($<D>_z, \delta_z$) pairs were used to calculate corresponding vesicle number-averaged radii and radius dispersions using Eqs. 7 and 8 of ref. 4. From these equations:

$$<R>_N \simeq \frac{A}{<D>_z(1 + 3\delta_z)}, \tag{2}$$

$$\delta_N \simeq \delta_z. \tag{3}$$

Solution and control $<R>_z$ results were then averaged for each solute using their respective inverse variances as weights. The simple averages of their corresponding δ_N

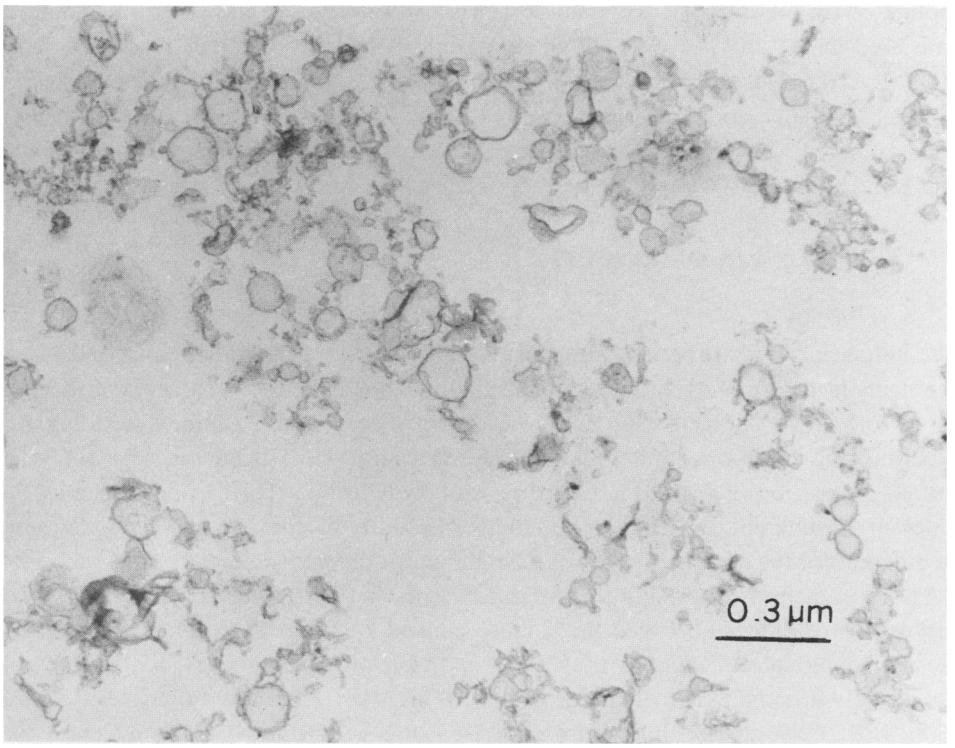

FIGURE 8 Thin-section electron migrograph of the erythritol study vesicles incubated in 1 M sucrose at room temperature for 2 min before fixation and sectioning.

results and δ_N standard deviations were then computed. Simple averages instead of weighted averages and standard deviations of δ_N sets were used because it was felt that due to their greater variability, the dispersion in these data was better represented in this way. Furthermore, since δ_Z enters directly into the calculation of $<R>_N$ and brings its greater uncertainty with it, $<R>_N$ values were compured only for steady-state ($<D>_Z, \delta_Z$) pairs where several measurements were available so that more precise size comparisons between hypertonic solution and isotonic control vesicles could be made.

(*3*) $<D>_Z$ results from measurements of all hypertonic solutions were normalized to isotonic control conditions by multiplying each such value by the ratio η/η_0, where η is the hypertonic solution viscosity and η_0 is the isotonic buffer viscosity. In this way, viscosity-corrected values, $D_c \equiv \eta <D>_Z/\eta_0$, reflect the fact that in the less viscous control environment, osmotically shrunken (swollen) vesicles would have correspondingly greater (smaller) diffusion coefficients. Note that in the isotonic control solution, $D_c \equiv <D>_Z$. By following changes in D_c in time, the evolution of osmotically driven changes in vesicle size could be followed by comparing control and hypertonic solution D_c results. The long-term osmotic effects of different sucrose concentrations and of 1 M TES on vesicles were examined in the same way. Ex-

cepting erythritol and 1 M TES, all 20°C viscosity, refractive index, and osmolality data used in the analysis were obtained by interpolating tabulated, measured data in ref. 9. The 1 M erythritol and 1 M TES data were obtained experimentally using a model HV303 Hoeppler precision viscometer, Fish-Schurman Corp., New Rochelle, N.Y.; a model 10400 refractometer, American Optical Corp., Scientific Div., Buffalo, N.Y.; and a model 5100 vapor pressure osometer, Wescor Inc., Logan, Utah.

DISCUSSION OF RESULTS

General

The initial portions of the time course study D_c curves are dashed to emphasize two problems in determining the true transient time course of D_c in these experiments. First, correlation spectral scan times varied between 5 and 20 min, depending on experimental conditions, and thus averaged out short-time behavior. Secondly, a certain proportion of vesicles in each preparation is leaky. This proportion may be different for different preparations and the permeability of different leaky vesicles may be different for the same solute as well as different for different solutes.

These problems help explain the differing shapes of the transient portions of the D_c curves. The difference between the sucrose control D_c level for glycerol and that of the remaining solutes suggests that the proportion of leaky vesicles in the glycerol sample was less than that of the others (see Figs. 1–4). Because some vesicles are leaky, the 50% vesicle volume shrinkage based on steady-state $<\bar{R}>_N$ averages for the impermeable solutes of Table I necessarily underestimates the extent to which non-leaky vesicles shrank. For this reason, the actual volume decrease of non-leaky vesicles is reported as *approximately* 50% throughout the paper. By comparison, measurements of the radii of 50 vesicles from the electron micrographic study of a 1 M sucrose solution and its control (Figs. 6 and 7) indicated that the average vesicle volume shrank about 60%.

A large variability in the number-averaged sizes of isotonic control vesicles is apparent in Table I. Since samples were always prepared in the same way by the same people, this variability is thought to result from the microscopic size of vesicles which makes it particularly difficult to impose strict size controls on vesicles during the homogenization, sonication, and filtration steps of sample preparation. The experimental results (especially Figs. 1–4 and Table I) do not reveal any obvious difference in vesicle osmotic behavior that might be attributed to isotonic control size differences. As mentioned in the first part of this section, though, to a certain extent sample D_c time course differences and differences in the magnitudes of the final, steady-state osmotic compressions of vesicles are thought to have resulted from differences in sample leakiness. Isotonic control size variability did, however, prompt the use of dual isotonic and hypertonic 1 M sucrose controls in these experiments. In this way, for example, the steady-state erythritol and glucose time study results could be compared with those of the sucrose concentration study (see Fig. 5 and the Comment section).

The solid, flat portions of the D_c curves represent the final, steady-state D_c value

TABLE I

AVERAGE STEADY-STATE VESICLE SIZE AND SIZE DISPERSION AND AVERAGE VESICLE DIFFUSION COEFFICIENT DISPERSION RESULTS

	1M Ethylene Glycol	10mM TES Control	1M Glycerol	10mM TES Control	1M Erythritol	10mM TES Control	1M Glucose	10mM TES Control	1M Sucrose	10mM TES Control
Average steady-state vesicle radius, $\langle \bar{R} \rangle_N$ (Å) (No. of data points used to calculate $\langle \bar{R} \rangle_N$)	415±40(2)	361±32(4)	336±40(3)	333±25(4)	321±16(8)	396±26(4)	423±27(3)	487±28(5)	400±26(5)	487±28(5)
Average relative dispersion about $\langle D \rangle_z$, $\bar{\delta}_z$. (No. of data points used to calculate δ_z - includes all data)	0.34±.11(6)	0.33±.08(4)	0.34±.14(8)	0.39±.13(4)	0.46±.11(8)	0.41±.12(4)	0.40±.18(6)	0.39±.06(5)	0.34±.18(7)	0.39±.06(5)

for each solute studied and the position of this line on each group was calculated by averaging those values considered to represent the steady-state condition. The number of such values used in each study are presented in Table I.

In spite of the problems discussed above, differences in the overall shapes of the D_c curves, the steady-state D_c values and the δ_Z results can be used to draw certain conclusions about the vesicles examined. Based on many experimental observations and measurements for both cells and phospholipid vesicles (see, for example, refs. 10–14), it was assumed that water would have been free to move rapidly in and out of SR vesicles. It was expected that the D_c time curves for impermeable solutes would resemble step functions while those of the permeable solutes would remain flat and more or less coincide with their respective isotonic controls. Keeping the experimental difficulties discussed above in mind, it still appears that vesicles shrank much more slowly than anticipated. Inspection of the D_c curves and the Table I results suggest that under the conditions of the experiments reported here, the net flow of osmotically driven water out of vesicles was sufficiently slow as to require times of the order of minutes for vesicles to shrink in average volume by about 50%. Secondly, the proportion of leaky vesicles in these samples was never large enough to mask the light-scattering determination of their long-term impermeability to sucrose, glucose, and erythritol and their long-term permeability to glycerol and ethylene glycol. In addition, a comparison of isotonic solution and hypertonic solution electron micrographs shows an overall decrease in vesicle size further demonstrating that the degree of leakiness in those samples was not large enough to obscure the osmotic effects of interest. Finally, δ_Z time plots revealed that these values fluctuated about some mean, and averages of δ_Z for *all* measurements of sample and control within a given study, for each solute studied, were found to be essentially the same (See Table I). Since a high proportion of leaky vesicles would presumably have been observed as an increase in the average δ_Z of a hypertonic solution compared with its isotonic control, this uniformity of average δ_Z provided additional confirmation that vesicle leakiness would not prevent meaningful analysis of the experimental results presented here.

Assuming that fixation with OsO_4 did not significantly alter vesicle shape or size, the 1 h, the 2 min, and the gluteraldehyde/OsO_4 electron microscopic studies of vesicles incubated in 1 M sucrose solution show that under the experimental conditions reported here, lobster abdominal muscles SR vesicles remained spherical shells in hypertonic 1 M sucrose solutions. This result is consistent with the fact that the measured rate of Ca^{++} uptake of vesicles in 1 M sucrose from the sample used in the EM study of Figs. 5 and 6 was not less than that of its isotonic control—which it presumably would have been had vesicles collapsed, e.g., to biconcave discs, in the 1 M sucrose solution. This presumption is based on the notion that the dramatically reduced interior volume of collapsed vesicles—whose surface areas presumably had not decreased commensurately—would have resulted in more rapid concentration of Ca^{++} inside vesicles thereby reducing the rate of vesicle Ca^{++} uptake. Moreover, the electron micrograph of vesicles in 1 M sucrose fixed first with gluteraldehyde and

then with OsO_4 (not shown) showed them to be spherical shells as well. Since gluteraldehyde was chosen for the initial fixation because of its relatively gentle fixative character and thus presumably did not alter the shrunken vesicle size population so that final fixation with OsO_4 also preserved this population, this last result indicates that fixation with OsO_4 alone did not significantly affect vesicle shape or size. The effects of the other solutes were not examined using the electron microscope since, presumably, 1 M hypertonic solutions of glucose, erythritol, glycerol, ethylene glycol, and TES do not alter vesicle spherical shapes either. This maintenance of sphericity allows $<D>_z$ and δ_z to be computed in the same way as they are for isotonic suspensions and means that the hypertonic solution D_c data do in fact reflect changes in the average vesicle radius.

Time Course Measurements

The D_c time plots reveal two distinct behavioral categories. In the first category, as vesicles shrink in 1 M sucrose, glucose, and erythritol solutions, D_c values climb to some final steady-state value and remain there for the duration of the experiment. The final D_c level is about the same for both sucrose control and solute studied in spite of the fact that the osmolalities of these 1 M solutions vary as: sucrose (1.45) > glucose (1.17) > erythritol (1.04). This interesting behavior is discussed further in the Comment section. In the second category, 1 M glycerol and ethylene glycol D_c values rise to a maximum, do not achieve the sucrose control level, and then relax back to within a few percent of the isotonic control $<D>_z$ value. It is concluded that under passive transport conditions, the vesicles studied are permeable to ethylene glycol and glycerol and impermeable to sucrose, glucose, and erythritol. Using the viscometrically measured hydrodynamic radii of glycerol and erythritol reported by Schultz and Solomon (15), it is further concluded that these vesicles may be thought of as having pores whose radii lie between 3.1 and 3.5 Å.

This measured impermeability of lobster abdominal muscle SR vesicles to sucrose contrasts with the results of other researchers, using radioassay techniques, who found rabbit skeletal muscle SR vesicles to be outside-inside permeable to sucrose—although it was explicitly noted that this permeability might be a "consequence of membrane damage suffered during homogenization" (16). A more recent radioassay measurement of the sucrose permeability of rabbit muscle microsomes suggests, however, that these vesicles were essentially inside-outside impermeable to sucrose since after an initial drop (about 30 min), perhaps due to leaky vesicles, the activity level of C^{14} labeled sucrose in vesicles remained essentially constant for about 24 h (17).

It is interesting to note that neither the glycerol nor the ethylene glycol D_c curve relaxed completely back to the isotonic level. This could be because the tabulated 1 M glycerol and ethylene glycerol solution viscosities were a few percent higher than those of the experimental solutions, thus slightly exaggerating the η/η_o correction factors, or it may have been that the vesicles suffered some slight irreversible structural change during shrinkage and were left incapable of expanding completely back to their respective average isotonic sizes.

Sucrose Concentration and 1 M TES Permeability Studies

The results of the sucrose concentration study are presented as open circles in Fig. 5. Each point presented represents the average of two measurements. (The aqueous activity due to 10 mM TES was considered negligible and ignored.) From these data, it appears that at least in the aqueous solvent activity range corresponding to 0–0.5 M sucrose, vesicles behaved as good osmometers (see Comment section) since D_c increased, i.e. vesicles shrank, monotonically as the sucrose concentration was increased.

The steady-state TES D_c value, $(2.59 \pm 0.06) \times 10^{-8}$ cm^2/s, was about 25% above that of its isotonic control, $(2.04 \pm 0.07) \times 10^{-8}$ cm^2/s showing that vesicles were impermeable to TES as well as to sucrose, glucose, and erythritol. Considering the molecular weight (229) and lipophobic nature of TES, this result is not surprising. In contrast to glucose and erythritol, however, the 1 M TES steady-state D_c value was distinctly below that of its sucrose control for which $D_c = (2.99 \pm 0.10) \times 10^{-8}$ cm^2/s. The reason for this difference is not clear, though it is not surprising that TES, a charged lipophobic solute, might result in vesicle osmotic behavior that would be somewhat dissimilar to that of lipophobic nonelectrolytes such as sucrose, glucose, and erythritol.

COMMENT

Assuming that differences in vesicle osmotic behavior in 1 M concentrations of glucose, sucrose, and erythritol can be accounted for by differences in their aqueous activities, the osmotic effects on vesicles of 1 M erythritol (solid triangle) and 1 M glucose (solid square) measured in the time course studies are presented along with the sucrose concentration study results in Fig. 5. The glucose and erythritol D_c values of Fig. 5 were calculated by ratioing the steady-state D_c value for each solute to that of its 1 M sucrose control and then multiplying these ratios by the sucrose concentration study 1 M D_c value.

Considering the number of data points that characterize the steady-state D_c values for erythritol and its sucrose control (eight and three, respectively) and for glucose and its sucrose control (three and five, respectively), it seems reasonable to say that in Fig. 5, D_c increased monotonically for aqueous activities in the range 1 to ≈ 0.990 but had leveled off in the range ≈ 0.980 to ≈ 0.970. This indicates that vesicles behaved as good osmometers for $a_w^o = 1$ to ≈ 0.998 but had become osmotically unresponsive for $a_w^o \approx 0.980$–0.970. Apparently vesicles had stopped shrinking after a_w^o had attained a value of approximately 0.980 in spite of the fact that they had not collapsed and had retained their ability to actively transport calcium. This suggests that vesicles had become effectively "rigid" under these osmotic conditions.

CONCLUSIONS

In accordance with the discussion of the light-scattering and electron microscopic experimental results, it is concluded that under passive transport conditions lobster abdominal SR vesicles are impermeable to sucrose, glucose, and erythritol and permeable to glycerol and ethylene glycol. Based on measured values of the hydro-

dynamic radii of glycerol and erythritol, these vesicles can then be thought of as having pores whose radii lie between 3.1 and 3.5 Å.

In addition, the results presented here indicated that above a certain impermeable nonelectrolyte concentration, vesicles became osmotically inactive although they continued to transport calcium actively and had not collapsed. This suggests that at least under the experimental conditions reported here, vesicles behaved as if rigid when their average volume had decreased to about 50% of its original isotonic value.

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