Volume Changes in Sarcoplasmic Reticulum of Rat Hearts Perfused with Hypertonic Solutions

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SUMMARY To explore whether morphometry of intracellular membrane-limited subcompartments can be used to follow physiological volume changes in such subcompartments in hearts rapidly fixed by perfusion fixation, we have measured osmotically induced volume changes in electron micrographs of longitudinally oriented sarcoplasmic reticulum (LSR) and terminal cisterns (TC) of rat left ventricular myocardial cells. Vascular perfusion with solutions whose osmolality varied from 0.67 to 1.88 isosmolar showed that in the hypertonic range LSR volume decreased linearly. Approximately 79% of LSR luminal volume participated in the osmotic response, but 21 ± 11% (SD) of LSR luminal volume was osmotically unresponsive. By contrast, we found that the TC responded by dilation when hearts were perfused with hyperosmolar NaCl, NaI, LiCl, or sucrose. Furthermore, with hyperosmolar NaCl the dilation developed within 1 minute; its rate and extent of development were concentration-dependent; it manifested an obligate association with prior or concomitant T-tubular dilation and was not readily reversible. We conclude that (1) the technique sensitively measures in situ changes of LSR volume; (2) most of LSR luminal water is osmotically responsive, but a significant fraction may not be; (3) exposure to hyperosmolar solutions may bring about (perhaps irreversible) structural changes in the diadic membrane complex, leading to changes in its solute permeability.

THE MYOCARDIAL cells of mammalian ventricular heart muscle, like most other muscle cells, contain multiple subcompartments bounded by membranes with different permeabilities. Changes in the volumes of these subcompartments in response to changes in sarcoplasmic osmolality and ionic composition might be expected to provide information about the properties of the subcompartments and their limiting membranes in situ. The availability of morphometric techniques for estimating the volumes of sarcoplasmic reticulum (SR) and other organelles in electron micrographs of heart muscle and skeletal muscle encouraged us to explore the possibility of studying morphometrically changes in SR volume in response to perturbations in extracellular osmolality and ionic composition.

In this paper we present evidence that these methods provide reproducible values for the cross-sectional area of the longitudinally oriented SR (LSR) in rat ventricular myocardial cells. These values change sensitively in response to perturbations in external osmolality and can be used to estimate the fraction of SR luminal volume that responds to an osmotic stimulus. By contrast, the osmotic response of the terminal cisternal portion of the SR (TC) to an increase in extracellular osmolality appears anomalous. This anomalous response, which is associated with prior or concomitant T-tubular dilation, may reflect an abrupt change in the structure and permeability of the diadic membrane complex.

METHODS

DESCRIPTION OF EXPERIMENTS

Rats were injected with heparin and stunned. Their left ventricles were then excised and perfused through the coronary vessels at 37°C using the Langendorff cannula as previously described. The procedure was to perfuse for a predetermined period of 1–20 minutes from a reservoir containing the solution of the osmolality and ionic composition being tested. The perfusion was then switched to a second reservoir containing an isosmolar solution whose chemical composition was identical to that of the test solution except that 62.4 mEq of NaCl were replaced (in 1 liter of solution) with 32.7 mmol of OsO₄ buffered with Na cacodylate. In selected experiments, the perfusing solution used for primary fixation contained 60 mm glutaraldehyde instead of OsO₄. The compositions of the various perfusing solutions and the corresponding solutions containing fixative were as follows:

**Isotonic control solution (mM):**
- NaCl, 153.4; KCl, 5.93; CaCl₂, 1.40; MgCl₂-6H₂O, 0.56; tris(hydroxymethyl)aminomethane (Tris) maleate, 2.0; N-acetylglycine, 2.0; total osmolality, 322 mOsmol/kg of water. The corresponding isotonic control fixative contained NaO₄, 32.7; NaCl, 91; KCl, 5.93; CaCl₂, 1.40; MgCl₂-6H₂O, 0.56; and Na cacodylate, 50.0.

**1.88 × hypertonic NaCl-Ringer:**
- NaCl, 308.4 mM; composition otherwise as for isotonic control. The corresponding fixative contained NaCl, 246 mM; otherwise as for isotonic control fixative (605 mOsmol).

**1.45 × hypertonic NaCl-Ringer:**
- NaCl, 230 mM; composition otherwise as for isotonic control. The corresponding fixative contained NaCl, 168 mM; composition otherwise as for isotonic control fixative (467 mOsmol).

**Hypotonic NaCl-Ringer:**
- NaCl, 102.21 mM; composition otherwise as for isotonic control. The corresponding fixative contained NaCl, 60 mM; otherwise as for control fixative (212 mOsmol).

**Hypertonic LiCl-Ringer:**
- LiCl, 308 mM (no NaCl); otherwise as for hypertonic NaCl solution; the corresponding fixative contained LiCl, 248 mM (no NaCl); otherwise as for isotonic NaCl fixative (605 mOsmol).

**Hypertonic NaCl-LiCl:**
- NaCl, 308 mM (no NaCl); otherwise as for hypertonic NaCl-Ringer (600 mOsmol). The
corresponding fixative contained NaCl 248 mm (no NaCl), otherwise as for isotonic NaCl fixative.

**Hyperonic Sucrose-NaCl-Ringer**: NaCl 153.4 mm; sucrose. 273 mm; composition otherwise as for isotonic control. The corresponding fixative contained NaCl 91 mm; sucrose. 273 mm; composition otherwise as for isotonic control (605 mOsmol).

The pH of all solutions was 7.3–7.4. The osmolality of the various solutions was determined by measuring their freezing point depressions against NaCl standards of defined osmolality on an osmometer, the desired osmolality being obtained by adjusting the concentration of the dominant salt (NaCl, LiCl, NaI, etc.).

The perfusion system was provided with a special drain through a three-way stopcock directly above the aortic insertion of the cannula. By briefly opening and then closing this stopcock at the time of switching to the reservoir containing the fixative, the time lag due to change of solution in the “dead space” of the connecting tubing could be reduced to 10–15 seconds. After perfusion with fixative for 6–10 minutes, the hearts were removed from the cannula, cut into small blocks, and rinsed free of OsO4 by immersion at 2°C in the original perfusing solution. Tissue that did not turn black during perfusion with fixative was rejected. Postfixation en bloc with uranyl acetate, dehydration, and embedding in Epon were done as previously described. Two or more blocks were processed for analysis from each heart. As in previous studies, the blocks were taken from the subepicardial myocardium of the left ventricular free wall exclusive of the apex.

Very thin cross sections or longitudinal sections, prepared by orienting the blocks under microscopic observation, were cut to give a gray interference pattern, stained with uranyl acetate, dehydration, and embedding in Epon were done as previously described. Two or more blocks were processed for analysis from each heart. As in previous studies, the blocks were taken from the subepicardial myocardium of the left ventricular free wall exclusive of the apex.

**Morphometric Analysis**

**Morphometry of Longitudinal Portion of the Sarcoplasmic Reticulum (LSR)**

The analysis was performed on prints of very thin cross sections of myocardial cells at a final magnification of 135 to 170 times 10^3. The prints were covered with tracing paper, and the rounded profiles of the sarcotubular cross sections were traced with a sharp, hard (no. 3) pencil. The trace was made on the inner edge of the sarcotubular membrane so as to give an outline of the apparent sarcotubular lumen. Only sarcotubular profiles whose traces were closed and not significantly elongated were selected for measurement. The object of the measurement was to determine the cross-sectional diameter of SR tubules oriented parallel to the long axis of the myocardial cells. The terminal cisternal portion of the SR was therefore excluded from the analysis. Elongated or unclosed traces of sarcotubules were excluded because their apparent surface to volume ratio would not accurately reflect that of the tubular cross section. By excluding unclosed traces or roughly elliptical traces whose major axes were more than 2 x longer than the minor axes, we were able to restrict the measurement to an easily defined population of tubular profiles. Since the purpose of these measurements was to compare the volumes of these structures in different solutions, it was assumed that any bias in the selection of structures for measurement would be similar over the range of volume changes studied.

Traces from several prints were collected on a piece of tracing paper. The traces were then measured with the Zeiss model TGZ3 particle size analyzer using a magnifier to enlarge the trace 2-fold for more precise estimation. This instrument gives the diameter of the circle equivalent in area to the area of the (not necessarily circular) cross section of the closed structure being measured.

**Analysis of Terminal Cisterns (TC)**

Quantitative analysis of volume changes in the TC is particularly difficult for two reasons: First, the TC are normally relatively small as compared to the thickness of section, even when sections are cut as thinly as possible; second, the orientation of the TC of ventricular myocardial cells may be parallel, perpendicular, or oblique to the long axis of the cell. The relatively large section thickness increases the probability of tangential sections and leads to underestimation of the mean value of cisternal luminal volume; and the relatively unpredictable cisternal orientation does not permit one to orient the plane of section systematically so as to cut all the TC in cross section. Moreover, the shape of these structures may be ellipsoidal, so that even a true cross section would vary in area depending on where the section intersected the ellipsoid.

These difficulties appeared to preclude an exact volume measurement of the TC. Nevertheless, it seemed worthwhile to attempt a quantitative approach of the following sort: It was assumed that (for a given thickness of section) an increase in the volume of the TC would be associated with an increased probability of visualizing the cisternal lumen. With the preparative technique used by us, this lumen appears white and can thus be unequivocally identified. Accordingly, all TC were examined on prints at final magnifications of 29 times 10^3 to 33 times 10^3. They were then classified as open (lumen visible), closed (lumen not visible), or ambiguous (decision as to open or closed state of lumen not clear). This classification was carried out for hearts perfused with isotonic Ringer’s solution, for hearts perfused for variable durations with hypertonic NaCl-Ringer, and for hearts perfused with hypertonic sucrose-NaCl-Ringer. The data obtained by this method lend themselves to a comparison of the results of different perfusion conditions by a chi square analysis.

**Results**

**Qualitative Description**

Figures 1 and 2 are low magnification surveys of longitudinal sections of rat left ventricular free wall perfused for 5 minutes with buffered NaCl-Ringer containing, respectively, 153.4 mm and 308.4 mm NaCl (osmolality physiological and 1.88x physiological, respectively) and then fixed with an OsO4-containing solution of the same osmolality. Figure 3 and inset are the corresponding cross sections of myocardial cells from hearts perfused for 5 minutes with 308.4 mm NaCl (osmolality physiological). The orientation of the TC was determined by chi square analysis.
sections magnified to allow a comparison of the lumina of the LSR. The hypertonic NaCl solution arrested the spontaneous contractions of the heart, but perfusion through the coronary vessels remained sufficient to permit subsequent uniform fixation on switching to osmium-containing solution. Figure 2 shows the dilation of the T-system previously described by others for skeletal muscle and heart muscle exposed to hypertonic solutions. The shrinkage of the cells as a whole and of the mitochondria is also evident. In addition, Figure 2 shows a remarkable dilation of those TC which are associated with dilated T-tubules. Representative high magnification micrographs of such dilated TC are illustrated in Figures 4 and 5. In these preparations the lumen of the TC appears white and conspicuously widened, and the diadic membrane complex (the plasma membrane of the T-tubule, the underlying outer membrane of the TC, and the intervening region) bulges into the T-tubular lumen.

QUANTIFICATION OF VOLUME CHANGES IN THE LSR

To examine the effects of changes in extracellular osmolality on the volume of the LSR, it was necessary to validate the method. Figure 6A shows the frequency distribution of equivalent cross-sectional diameters (d') for four hearts each perfused for 5 minutes with isosmolar control solution at 37°C. The 5-minute perfusion was chosen on the basis of experiments summarized in Table 1, which indicated that early transient volume changes during
osmotic perturbations approached completion within 2.5 minutes.

Table 1 presents a statistical summary of these four control experiments. It is apparent from Figure 6 that the distribution of $d'$ was similar for all four hearts, and Table 1 shows that the statistical dispersion was small enough to justify proceeding to experiments with anisosmolar solutions.

Table 1 also presents the results of measurements made on hearts perfused for 5 minutes with anisosmolar solutions, then fixed with OsO$_4$-containing solution of comparable osmolality. As the extracellular osmolality was increased stepwise by addition of NaCl to the perfusing solution, $d'$ decreased. Figure 6B shows that the frequency distribution of $d'$ was shifted to the left but did not become bimodal. It was of interest to examine the linearity and other characteristics of the osmotic response. The volume of a unit length of LSR, given by $\pi(d')^2/4$, was plotted against the reciprocal of the relative osmolality of the perfusate (Fig. 7), as described by Blinks for single frog skeletal muscle fibers. Over the range of LSR luminal volumes between the isosmolar controls and the most hypertonic solutions studied, the data can be fitted to a line that extrapolates to a value of the ordinate corresponding to $21 \pm 11\%$ of the LSR luminal volume in the isosmolar control solution. It is thus clear that most of LSR luminal volume responds to an osmotic withdrawal of water from the cell; however, the statistical dispersion does not preclude the presence of a small but significant fraction of osmotically nonresponsive volume.
Figure 3  Cross section of longitudinally oriented sarcoplasmic reticulum (LSR) at magnification used for measurement of equivalent cross-sectional diameter; perfusion for 5 minutes with isosmolar solution as in Figure 1. Inset: Cross section of LSR at identical magnification after perfusion with hyperosmolar (1.88 x isosmolar) NaCl-Ringer. Calibration for both figure and inset = 0.2 μm.
Figure 4  High magnification of terminal cistern (TC) and T-tubule after 5 minutes of perfusion with hypertonic (1.88 x isosmolar) NaCl-Ringer. The arrow is in the lumen of the dilated T-tubule. Proceeding in the direction of the arrowhead the observer encounters, respectively, the traces of the T-tubular plasma membrane, the region intervening between this membrane and the sarcoplasmic reticulum (SR) membrane, the first trace of the SR membrane, the dilated lumen of the SR, the second trace of the SR membrane, the trace of the outer mitochondrial membrane, and the trace of the inner mitochondrial membrane. Calibration = 0.2 μm.
Similar to Figure 4, except perfused for only 1 minute with hypertonic (1.88 × isosmolar) NaCl-Ringer. Calibration = 0.2 μm.
TIME COURSE AND CONCENTRATION DEPENDENCE OF CISTERNAL SWELLING IN HYPERTONIC NaCl-RINGER

To investigate the time course of TC swelling, perfusions with hypertonic NaCl (relative osmolality 1.88 × isosmolal) were carried out for 1, 2.5, 5, 12.5, and 20 minutes, respectively, before the introduction of the fixative solution. These experiments showed that dilation of the TC was already pronounced after only 1 minute of perfusion with hypertonic NaCl, the shortest duration that could be reliably examined. To determine whether the swelling of the TC was already maximal at 1 minute or whether it increased further with time, the TC from the 5-minute isotonic control perfusion and from the 1- and 5-minute hypertonic NaCl perfusions were analyzed according to the “open,” “closed,” and “ambiguous” classifications described under Methods. Table 2 summarizes the results of this procedure. The table shows that the TC from the control experiment were readily distinguishable from those of the hypertonic perfusions by their predominantly “closed” state and the absence of open configurations. In 20 normal rat ventricles perfused with the isotonic control solution, fixed as here described and subjected to morphometry of the SR, we have not seen an “open” configuration of the TC like that in Figures 2, 4, and 5. “Open” configurations were also absent after perfusion with hypotonic (0.67 × isosmolal) NaCl-Ringer. A chi square analysis indicated that, during perfusion with 1.88 × hypertonic NaCl, there was no significant change in the fraction of “open” and “closed” configurations between 1 and 5 minutes of perfusion (P > 0.30). Moreover, the TC were still dilated after 20 minutes of perfusion.

It seemed worthwhile to establish whether the swelling of the TC upon perfusion with hypertonic NaCl exhibited a dependence on the NaCl concentration. Perfusion for 5 minutes with a significantly less hypertonic NaCl solution (relative osmolality 1.45 ×) strikingly reduced the fraction of TC in the open configuration (Table 2). Prolongation of the perfusion to a total of 12.5 minutes resulted in a slight, though statistically significant, increase in this fraction (P < 0.05); however, the fraction of TC in the open configuration after 12.5 minutes was much smaller than the corresponding fraction achieved after only 1 minute of perfusion with the more hypertonic (1.88 × isosmolal) medium.

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The results described so far were obtained in heart muscle fixed by perfusion with OsO4-containing solutions. Since Birks and Davey have suggested that swelling of

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### Table 1: Mean Equivalent Diameters (d') of Longitudinally Oriented Sarcoplasmic Reticulum (LSR) Cross Sections in NaCl Solutions: Dependence on Osmolality and Perfusion Time

<table>
<thead>
<tr>
<th>Relative osmolality*</th>
<th>Minutes perfused</th>
<th>d' (μm)</th>
<th>No. of profiles measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67</td>
<td>5</td>
<td>0.0430 ± 0.0001</td>
<td>154</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>0.0429 ± 0.0007</td>
<td>216</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>0.0400 ± 0.0006</td>
<td>223</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>0.0379 ± 0.0007</td>
<td>146</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>0.0400 ± 0.0006</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group mean†</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>1.45</td>
<td>5</td>
<td>0.0351 ± 0.0007</td>
<td>161</td>
</tr>
<tr>
<td>1.88</td>
<td>1</td>
<td>0.0290 ± 0.0005</td>
<td>137</td>
</tr>
<tr>
<td>1.88</td>
<td>2.5</td>
<td>0.0318 ± 0.0005</td>
<td>222</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>0.0327 ± 0.0006</td>
<td>168</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>0.0314 ± 0.0004</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group mean†</td>
<td>0.0320 ± 0.0004</td>
</tr>
<tr>
<td>1.00</td>
<td>20</td>
<td>0.0354 ± 0.0006</td>
<td>201</td>
</tr>
<tr>
<td>1.88</td>
<td>20</td>
<td>0.0280 ± 0.0003</td>
<td>258</td>
</tr>
</tbody>
</table>

* Relative to value of 322 mOsmol/kg water for the isotonic control solution.
† Averaged over the number of animals (hearts).

Each line (except the designated group means) represents data from one heart. Dispersions are standard errors of the mean.
tubular systems in skeletal muscle depends on the nature of the fixative, we have also conducted experiments in which the primary fixative was glutaraldehyde (~60 mOsmol/kg as determined by freezing point depression). After isotonic perfusion, TC of hearts fixed in this way were uniformly closed. In glutaraldehyde-fixed hearts perfused with hypertonic NaCl solution, open TC were present as after primary fixation with OsO4.

**Table 2** Quantitative Analysis of Terminal Cisternal (TC) Configuration

<table>
<thead>
<tr>
<th>Relative osmolality*</th>
<th>Minutes perfused</th>
<th>Perfusion solution</th>
<th>Configuration of TC (%) of total cisterns counted</th>
<th>No. analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Open</td>
<td>Closed</td>
</tr>
<tr>
<td>1.00</td>
<td>5</td>
<td>Isotonic control</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>1.88</td>
<td>1</td>
<td>Hypertonic NaCl</td>
<td>38</td>
<td>51</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>Hypertonic NaCl</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>Hypertonic NaCl</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
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<td>5</td>
<td>Hypertonic NaCl</td>
<td>10</td>
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</tr>
<tr>
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<td>12.5</td>
<td>Hypertonic NaCl</td>
<td>15</td>
<td>81</td>
</tr>
<tr>
<td>1.86</td>
<td>5</td>
<td>Hypertonic NaI</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>Hypertonic LiCl</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>1.88</td>
<td>5</td>
<td>Hypertonic sucrose-NaCl</td>
<td>27</td>
<td>55</td>
</tr>
</tbody>
</table>

* Osmolality relative to isotonic control value of 322 mOsmol/kg of water.

**DILATION OF TC AFTER PERFUSION WITH HYPTERTONIC NaI, LiCl, AND SUCROSE**

It was of interest to determine whether TC swelling could be brought about by raising extracellular osmolality with solutes other than NaCl. Table 2 shows that the TC did indeed dilate after perfusion with hypertonic LiCl, NaI, or sucrose. The table shows a comparison of the effects of hypertonic LiCl and NaI. two alkali metal halides that differ widely in molecular weight, diffusion coefficient, and other characteristics. Although the duration of perfusion and the osmolality of the two solutions were identical, a much larger fraction of TC were in the open configuration after perfusion with LiCl than after NaI. A chi square test indicated that the difference was highly significant ($P < 0.001$). Table 2 also shows that the percentage of dilated (open) TC was significantly smaller after 5 minutes in hypertonic sucrose-NaCl than after only 1 minute in an equiosmolal solution of hypertonic NaCl ($P < 0.001$).

**ATTEMPTS TO REVERSE TC SWELLING**

To test whether swelling of the TC in hearts perfused with hypertonic NaCl was reversible under the conditions of our experiments, two rat ventricles were first perfused for 2.5 minutes with 1.88 $\times$ and 1.45 $\times$ hypertonic NaCl, respectively. The perfusing solution was then changed to isotonic control solution for an additional 2.5 minutes, after which the heart was fixed with isotonic OsO4-containing solution. The spontaneous beating, which had been arrested in either of the hypertonic NaCl solutions, resumed upon reperfusion with isotonic NaCl-Ringer. However, electron micrographs of this material showed extensive membrane disruption both of the TC and LSR.

**ASSOCIATION OF TC AND T-TUBULAR DILATION**

Inspection of the micrographs obtained after perfusion with NaCl solutions of increased osmolality suggested that dilated (open) TC tended to be associated with dilated T-tubes; conversely, TC associated with undilated T-tubules tended to be in the "closed" configuration. To confirm this impression, the micrographs used for the "open" vs. "closed" analysis of the TC at relative osmolalities of 1.00 and 1.88 $\times$ the osmolality of the control solution...
Measurement of SR volume after experimental osmotic perturbations required that the tissue be fixed rapidly enough to avoid or minimize volume changes in the SR during fixation. Perfusion fixation with OsO₄ rather than glutaraldehyde was chosen for three reasons. First, exposure of the plasma membrane to OsO₄ at the concentration used here rapidly destroys the selective permeability of the cardiac plasma membrane and renders the cellular contents accessible to fixative;¹⁴ second, primary perfusion fixation with OsO₄ yields superior and uniform preservation of the fine structure (including SR) in heart muscle;⁷,⁸ and third, because OsO₄ is effective at relatively low chemical concentrations (32 mM or 1%), this fixative made only a small contribution to the total osmolality of the solution used for fixation. The Na concentration and ionic strength of the fixing solution were thus closer to those of the solution used for the initial osmotic perturbation. By contrast, glutaraldehyde must be used at higher chemical concentration and destroys the osmotic selectivity of the sarcolemma more slowly.¹⁴

**PREVIOUS OBSERVATIONS ON OSMOTICALLY INDUCED CHANGES IN SR VOLUME**

Reports in the literature on the response of SR volume to osmotic perturbations are conflicting. Thus Huxley et al.⁴ and Birks and Davey¹⁰ found that hypertonic sucrose and hypertonic NaCl caused dilation of the TC in skeletal muscle, leading the latter authors to conclude that the TC are permeable to extracellular NaCl and sucrose. In heart muscle Sperelakis and Rubbo¹² reported that the SR shrinks in response to an increase in extracellular osmolality; these authors did not differentiate the responses of the TC and the LSR. Our observation of swelling of the TC and concomitant shrinkage of the LSR do not therefore have any exact counterparts in the literature. On the other hand, the osmotic responses of the cells as a whole in both skeletal muscle¹³ and heart muscle¹⁴,¹⁷ suggest that cell volume as a whole diminishes linearly with the reciprocal of osmolality as extracellular osmolality increases above isosmolar values.

**VOLUME CHANGES IN THE LSR**

The present observations indicate that most of the LSR (specifically, the lumen of the LSR) participates in the decrease of cell volume as extracellular osmolality increases. The measurement of equivalent diameter used to follow LSR volume was essentially independent of the shape of the lumen. Changes in luminal shape should not, therefore, greatly affect the conclusion that most of LSR luminal volume responds to an osmotic stimulus. Such a conclusion is consistent with the behavior of total cell water in which, as in the LSR, there are likewise significant fractions of osmotically inert water. However, in the present study it was not possible to examine the response at even higher osmolalities because perfusion and especially fixation under these conditions proved nonuniform and unsatisfactory. Conclusions based on the extrapolated intercept must therefore be accepted cautiously. If a portion of the LSR luminal volume is in fact osmotically unresponsive, the interpretation of this observation may be of physiological interest. For example, it is conceivable that what appears in our electron micrographs as "empty"
LSR lumen may have contained proteins or other macromolecular constituents before fixation, and that these constituents may have ion-binding, ion-translocating, or other functions in the intact cell.

SWELLING OF THE TC

The results of this study show that the TC swelled within 1 minute or less when the coronary vessels were perfused with a hypertonic electrolyte solution in which the predominant solute species was NaCl. The swelling of the TC could also be produced by hypertonic NaI, LiCl or sucrose. For NaCl, both the rate and degree of swelling increased with NaCl concentration. For different hypertonic solutions of equivalent osmolality, the rate of swelling of the TC appeared to be faster when the predominant solute was LiCl or NaCl than when it was NaI or sucrose. In all experiments, T-tubular dilation accompanied TC swelling, and TC swelling was not observed in association with undilated T-tubules.

These observations lend themselves to two somewhat different interpretations about the response of the diadic membrane complex (the membrane of the TC subjacent to the sarcolemma, the overlying plasma membrane, and the intervening region). The first interpretation is that exposure to hypertonic solutions produces a permeability change in the diadic membrane complex so as to render this complex permeable to the solutes listed in Table 2. The underlying assumption is that under normal, isotonic conditions this complex is impermeable to NaCl and the other solutes. This assumption is consistent with the failure of freeze-fracture and transmission electron microscopic studies to show channels directly joining the interstices of the TC and the T-tubules under isotonic conditions. According to this interpretation, the swelling of the TC observed in the present study by us and in the skeletal muscle studies of others results from a net movement of solute and water into the TC through channels somehow opened up by the hypertonic exposure. Since there seems to be an obligate association between swelling of the TC and T-tubular swelling, it is at least conceivable that mechanical deformation of the diadic membrane complex might contribute to a permeability change in that complex. A variant of this "mechanical deformation" hypothesis is that the part of the TC membrane which participates in the diadic membrane complex may be pulled by the attached foot processes toward the T-tubular lumen during a transient collapse of the T-tubule. Such a transient collapse might be associated with an initial osmotic T-tubular shrinkage, preceding the subsequent T-tubular dilation.

An alternative explanation is that (even under normal, isotonic conditions) the diadic membrane complex may be significantly permeable to the ions Na\(^+\), Li\(^+\), Cl\(^-\), and I\(^-\), i.e., very much more permeable than that portion of the high resistance plasma membrane which is not involved in the formation of the diadic membrane complex. Salts made up of these permeant ions could then participate in a rapid net movement across the diadic membrane complex in response to the concentration gradient for the salt. According to this alternative hypothesis the rapid swelling of the TC in hypertonic solutions would be preceded by a transient TC shrinkage as water was osmotically withdrawn from the TC lumen. This early phase could not have been detected by our technique, since the hearts could not be reliably fixed at perfusion durations of less than 1 minute. However, it would follow that the TC, although observed by us only in the swollen state, had already undergone a biphasic osmotic perturbation of relatively large amplitude. Moreover, in the experiment designed to test reversibility, the TC would have been subjected to a second biphasic osmotic transient: on switching back from hypertonic to isotonic perfusion, there would initially have been a net uptake of water by the TC, resulting in further distention of this already dilated structure. This initial event might well explain the rupture of the TC membranes observed in a proportion of these reperfused hearts; it would then be impossible to demonstrate the secondary transient, characterized by an efflux of NaCl and water from the TC into the perfusing solution. The possibility is thus left open that smaller osmotic perturbations of the TC (perhaps associated with physiological events) might in fact be reversible.

Our data do not permit a definitive exclusion of either of these two alternative interpretations. However, the observations that swelling of the TC (1) takes place within 1 minute. (2) appears to require prior or concomitant dilation of T-tubules. (3) can be brought about by such diverse solutes as sucrose and the various alkali halide salts, and (4) is not easily reversible suggest that the process differs from a physiological permeation. Since osmotic perturbations of the magnitude described here have been used for studies on cardiac cellular electrophysiology and contractile performance, it seems worthwhile to pursue these structural questions by other methods; in particular, diadic membrane complexes that have been subjected to osmotic perturbations need to be examined by electron microscopy of freeze-fractured replicas. It should also be useful to study the ion content of the TC in heart muscle by quantitative electron probe analysis. Such a study has recently been made by Somlyo et al. in frog skeletal muscle before and after exposure to hypertonic NaCl. This work has shown that hypertonic NaCl (2,2 isosmolar) produced "electron-lucent vacuoles" containing very high concentrations of Cl (up to 1,500 mmol/kg dry weight); hypertonic sucrose also caused vacuole formation. Somlyo et al. concluded that in hypertonicity treated muscle the excess Cl is compartmentalized in a manner consistent with ion communication of the TC with the extracellular space.

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Length-Dependent Calcium Inotropism in Cat Papillary Muscle

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SUMMARY We studied changes in the relationship of isometric developed force to muscle length when extracellular calcium concentration was altered in isolated cat papillary muscles. In two series of experiments at temperatures of 30°C and 32°C, and rates of 12 and 30 beats/min, 21 muscles were exposed to calcium concentrations of 1.125, 2.25 and 4.5 mM. Muscle lengths were varied between 80% and 100% of the length at which maximum developed force occurred (Lmax). Peak developed force and the time from stimulus to peak force were measured. The data indicate that force is not altered proportionately at all lengths when calcium concentration is changed. Rather, we found that a substantially greater modification of force occurs at short lengths than at long lengths. Similarly, the time to peak force increases with length at a rate which is more than 4 times greater at the lower calcium concentration than it is at the high concentration. Small but consistent shifts of Lmax also are seen. We observed that Lmax is longer when inotropic changes reduce force and shorter when force is increased. These results indicate that the inotropic effect of extracellular calcium concentration changes is dependent on muscle length.

On the other hand, indications that the inotropic effectiveness of some drugs may be altered by changes of muscle length have been available for some time. Furthermore, recent experiments on skeletal muscle have suggested that the process by which the myofilaments are activated may itself be length-dependent. This study was undertaken to elucidate whether or not muscle length and extracellular calcium concentration act independently to determine isometric force development.

Methods
Two series of experiments were conducted to evaluate the relationship between developed force and muscle length in the presence of altered extracellular calcium concentrations. In each series right ventricular papillary muscles were rapidly excised from cats anesthetized with...
Volume changes in sarcoplasmic reticulum of rat hearts perfused with hypertonic solutions.
E Page and J Upshaw-Earley

doi: 10.1161/01.RES.40.4.355

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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