EFFECTS OF HYPEROSMOTIC SOLUTIONS ON THE FILAMENT LATTICE OF INTACT FROG SKELETAL MUSCLE

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ABSTRACT The effect of increasing the osmotic strength of the extracellular solution on the filament lattice of living frog sartorius and semitendinosus muscle has been studied using low-angle x-ray diffraction to measure the lattice spacing. As the extracellular osmotic strength is increased, the filament lattice shrinks like an osmometer until a minimal spacing between the thick filaments is reached. This minimal spacing varies from 20 to 31 nm, depending on the sarcomere length. Further increase in the osmotic strength produces little further shrinkage. The osmotic shrinkage curve indicates, for both muscles, an osmotically-inactive volume of ~30% of the volume in normal Ringer's solution. Shrinkage appears to be independent of temperature and the type of particle used to increase the osmotic strength (glucose, sucrose, small ions). The rate at which osmotic equilibruim is reached depends on muscle size, being slower for greater muscle diameters. Equilibrium spacings are approached exponentially with time constants ranging from 20 to 60 min. Independent of osmotic equilibrium, the lattice tends to shrink slowly by ~3% over the first few hours after dissection, probably because of a leakage of K^+ ions from inside the muscle cells. This can be partly prevented by using an extracellular solution which contains a higher concentration of K^+ ions or which is hypoosmotic. The volume of the muscle filament lattice ($1.155\ d_{10}^2 \cdot S$) is constant over a very wide range of sarcomere lengths, and is equal to $\sim 3.6 \times 10^6\ nm^3$ for a range of amphibian muscle types.

INTRODUCTION

In their study of the filament lattice of toad skeletal muscle stimulated over long periods of time, Elliott et al. (1967) observed that the filament lattice of resting muscle shrank by ~3%. Similarly, Haselgrove and Huxley (1973) observed that frog sartorius muscle shrank a few hours after dissection. In both cases, the authors concluded that when allowance was made for this slow shrinkage of the filament lattice, there was no change in the lattice spacing when the muscle contracted without changing its length. Neither set of authors could explain the former phenomenon, although Elliott et al. (1967) suggested that it could be caused by a decrease in the internal pH of the muscle fibers by ~0.4 U.

The original purpose of the research reported in this paper was to clarify the above phenomenon. In addition, we wished to check the effects of using glucose in the external solution as had been done by Haselgrove and Huxley, where the amount used (12 g/l) would be expected to cause considerable osmotic shrinkage of the filament lattice. We wished to

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verify this and to see if such shrinking was related to the slow shrinkage that had been observed in the resting filament lattice.

The effects of hyperosmotic solutions on the volume of whole muscle fibers have been studied using ionic solutions (Boyle and Conway, 1941; Sato, 1954) and sucrose (Dydynska and Wilkie, 1963; Blinks, 1965). The effects of such solutions on contraction have also been studied (Howarth, 1958; Edman and Hwang, 1977). To our knowledge, the only study of the direct effects of hyperosmotic solutions on the filament lattice of intact vertebrate skeletal muscle is by Rome (1968), and she used solutions made hypertonic by increasing the ions in the external fluid.

In the present work, we have studied the amount and rate of shrinking in the filament lattice of frog skeletal muscle using hyperosmotic solutions containing glucose or sucrose and also solutions of increased ionic strength. We have compared our observations with earlier experiments on muscle fiber volume and with other estimates of filament lattice volume.

MATERIAL AND METHODS

Sartorius and semitendinosus muscles were dissected from the legs of adult frogs (bullfrog [*Rana catesbeiana*] or leopard frogs [*Rana pipiens*]). *R. pipiens* were used for all experiments except for most of those where the rate of osmotic equilibration was determined. The only difference we observed between the two species was in the size of the muscles and in the rate of osmotic equilibration (Table II). Cotton threads were tied to the tendons or the pelvic bone at the end of the muscle. Each muscle was then placed in a perspex chamber at a fixed length and kept in a Ringer's solution of composition (millimolar): NaCl, 115.5; KCl, 2; $CaCl_2$, 1.8; Na phosphate buffer, 2.0 (pH = 7.0); solid glucose or sucrose had been added as required. The osmolarity of the Ringer's solution was calculated from individual ionic concentrations as 0.229 osm; osmolarities of the other solutions were calculated from their total ionic or molecular compositions. In experiments determining the extent of lattice shrinkage with hypertonic solutions, the solution was left on the muscle for an hour or more before each x-ray diffraction pattern was obtained, and usually the solution was replaced at least once during this period (particularly with high osmotic strength solutions).

The perspex chamber had a pair of thin mylar windows, separated by 1.5 mm, between which the muscle was placed and through which the x-ray beam could pass. The chamber held ~25 ml of solution and this could, if necessary, be circulated through the chamber from an external reservoir. With the external reservoir, the muscle could be cooled (to 8°C) or left at room temperature (20–22°C). Unless otherwise specified, the experiments were done at room temperature.

Equatorial x-ray diffraction patterns were obtained on photographic film (Ilford Industrial G [Ilford Limited, Basildon, Essex, England] or Kodak Medical X-ray [Eastman Kodak Co., Rochester, N.Y.]) using mirror-monochromator, focusing, low-angle cameras of design like that described by Huxley and Brown (1967). The x-ray source was an Elliott GX6 rotating anode x-ray generator (Elliott Automation, Boehrum Wood, England). The camera was normally used in "line focus" mode at a specimen-film distance of either 22 or 36 cm. The former gave good equatorial patterns with an exposure of 5–10 min and was used to determine the time-course of lattice shrinking after a change of solution. The latter required longer exposures (20–60 min) and was used for most of the other experiments. Photographic films were measured on an optical comparator (Scherr Tumico, St. James, Minn.), usually by at least two people, and the measurements averaged. In general, spacings could be determined to an accuracy of better than 0.5%.

For experiments involving lattice changes over many hours, x-ray diffraction patterns were obtained automatically over hour or half-hour intervals by an automated film transport mechanism (Racey, 1976). Sarcomere lengths were determined from optical diffraction patterns obtained with a He-Ne laser.

RESULTS

Muscles in Normal Ringer's Solutions

When an intact frog skeletal muscle was placed in Ringer's solution at room temperature, the filament lattice shrank during the first 3–4 h by ~3%. Thereafter, although the scatter of individual results tended to increase with time, the lattice spacing remained approximately constant for at least a day. In the experiments used for Fig. 1 *a*, eight of the muscles were in a closed chamber containing ~25 ml of solution at 22°C. Three experiments were done using a steady flow of solution through the chamber: one experiment at 22°C and two at 8°C. The results from the latter experiments were not significantly different from results without the solution flow. Those from the single experiment at 22°C were included with the other data at 22°C, but those at 8°C were plotted separately (Fig. 1 *a*).

The cause of the initial lattice shrinking was explored further in a series of experiments

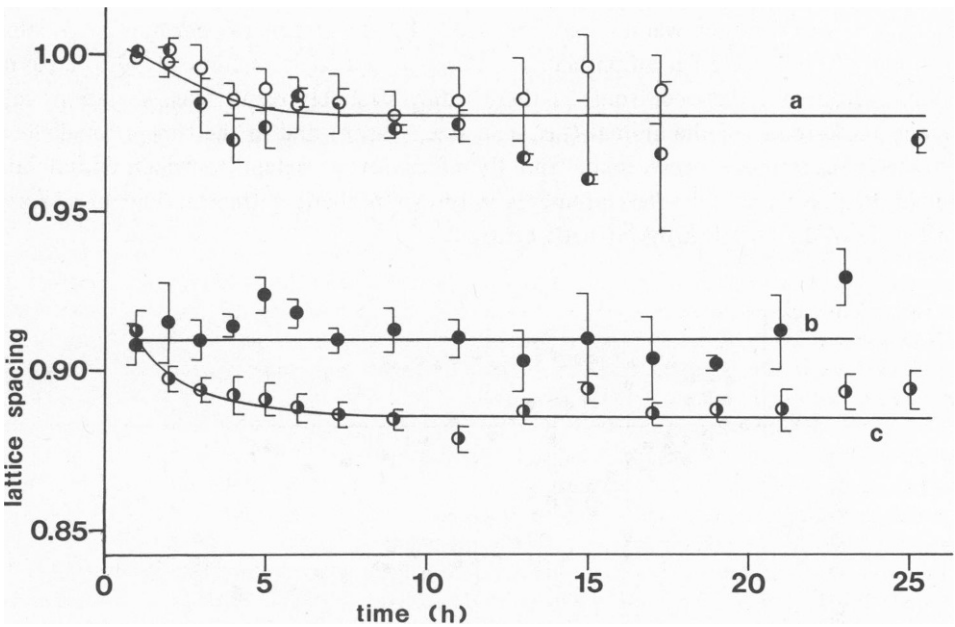

FIGURE 1 Change of filament lattice spacing with time after dissection for frog sartorius muscle (leopard frog). Dissection was complete 15–20 min after the frog was killed. All lattice spacings plotted relative to the spacing at the start of the experiment. For each muscle, the x-ray diffraction patterns were normally obtained every half hour for the first seven hours and every hour thereafter. Points shown are the averages from all x-ray patterns available for the given time interval from all muscles with the calculated standard errors shown as bars. (The actual number of measurements for each point varied from 2 to 19.) The number of muscles used and their sarcomere lengths for each series are as follows:

		Temperature		*Sarcomere lengths*
(*a*)	Normal Ringer's	20–22°C	○ 9 muscles	2.15–2.65 μm
	Normal Ringer's	8°C	◐ 2 muscles	2.45–2.55 μm
(*b*)	Glucose Ringer's	20–22°C	● 2 muscles	2.55–2.60 μm
(*c*)	Glucose Ringer's	8°C	◑ 6 muscles	2.40–2.55 μm

where the ionic composition of the bathing solution was varied (Fig. 2). Boyle and Conway (1941) found that if the external solution contained the same amount of potassium as frog plasma (2.5 mM), there was a slow leakage of potassium from the muscle fiber (and a smaller uptake of sodium), but that higher potassium concentrations (11 mM at 2–3°C or 30 mM at room temperature) could prevent this potassium loss. When we increased the potassium concentration in the bathing solution, the lattice shrinking in the first few hours after dissection was reduced, but after this period the lattice still shrank by ~3% (Fig. 2 *b–d*). On the other hand, when a hypoosmotic solution was used (NaCl concentration reduced to 103 mM), the lattice spacing remained more nearly constant (within 1.5%) over a 24 h period (Fig 2 *e*). In this case, however, the lattice spacing increased initially, before returning to a nearly constant level. It thus appears that a more stable lattice spacing can be obtained by using a bathing solution which is ~10% hypoosmotic.

Lattice volumes for both sartorius and semitendinosus muscles in Ringer's solution are shown in Table I and compared with other values from the literature. The lattice volume (which we have defined as the product of the unit cell area and the sarcomere length, i.e., $1.155\ d_{10}^2 \cdot S$) was constant over a range from 1.9 to 4.3 μm. In general, our measurements are very similar to those for other amphibian muscles, giving a lattice volume of ~3.6×10^6 nm^3. The small differences between some of these values probably result from variations in the state and background of the animal (i.e., age, size, season) and in the time after dissection when the measurements were made and therefore in the extent to which initial lattice shrinking had occurred. Our measurements were mostly made within an hour of dissection, before much of the initial shrinking had occurred.

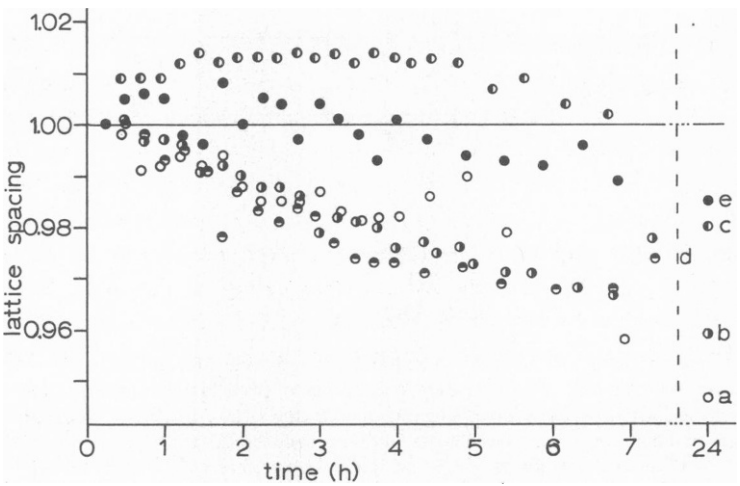

FIGURE 2 Change of filament lattice spacing with time after dissection for frog sartorius muscle in different bathing solutions. All lattice spacings are plotted relative to the spacing at the start of the experiment. Each point is the average from two or three muscles. The total spread in measurements was <1.5% for the early points and <2.5% for the later points. The final points were taken between 23 and 26 h after dissection. ○ (*a*) Muscles in normal "iostonic" Ringer's (i.e., with 2 mM KCl); (*b–d*) muscles in "isotonic" bathing solutions as *a*, but with some NaCl replaced by KCl. ◑ (*b*) total KCl = 7 mM; ◐ (*c*) total KCl = 12 mM; ⊖ (*d*) total KCl = 7 mM, but with temperature = 25°C; ● (*e*) muscles in "hypotonic" Ringer's (NaCl reduced to 103 mM). All experiments except *e* were at a temperature of 8°C.

TABLE I

VOLUME OF THE FILAMENT LATTICE IN AMPHIBIAN SKELETAL MUSCLE

Muscle type	Lattice volume		Sarcomere lengths	Osmolarity of bathing solution‡	Temperature	Reference
	($nm^3 \times 10^6$) (= $1.155\, d_{10}^2 \cdot SL$)		(μm)	(osm)		
Frog sartorius						
Leopard frog	3.52 (29)*	0.20§	2.0–3.0	0.229	8 and 20°C	this paper
Bullfrog	3.60 (11)	0.23	2.1–2.8	0.229	20°C	this paper
	3.16		2.0–3.0	0.304	4°C	Haselgrove and Huxley (1973)
Toad sartorius	3.70 (31)	0.33	2.1–3.7	0.229	5°C	Elliott et al. (1967)
	3.54 (18)	0.28	2.0–3.4	0.239	20°C	Rome (1968)
Frog semitendinosus	3.63 (36)	0.37	2.0–2.9	0.229	8 and 20°C	this paper
	3.89 (12)	0.28	3.0–3.6	0.229	8 and 20°C	this paper
	3.83 (30)	0.39	>3.6	0.229	8°C	this paper
	3.43 (12)	0.25	1.9–3.8	0.230	5°C	Elliott et al. (1963)
Single fibers	3.44 (19)	0.30	2.0–3.2	0.229	20°C	Matsubara and Elliott (1972)

*Number of muscles.

‡Calculated from the osmolarities of the individual constituents.

§Standard deviation.

The one volume measurement significantly different from the others is Haselgrove and Huxley's (1973), which is smaller by ~12%. Haselgrove and Huxley, however, used a bathing solution with 12 g/l glucose added, i.e., with an osmolarity 1.33 times that of normal Ringer's solution. When their volume is corrected for osmotic shrinkage, it becomes 3.8×10^6 nm^3, within the range expected from the other measurements. Furthermore, Haselgrove and Huxley found, unlike previous workers (Elliott et al., 1963; 1967; Rome, 1968; Matsubara and Elliott, 1972), that their muscles did not maintain a constant lattice volume as the sarcomere length was varied (Haselgrove and Huxley, 1973, Fig. 6 *a*). But at the longer sarcomere lengths studied by Haselgrove and Huxley, the lattice spacings were approaching the limit where behavior of the lattice volume does not depend only on the osmotic pressure across the cell membrane. Furthermore, volume changes in Haselgrove and Huxley's preparations may have been modified by data scatter introduced through shrinkage of the lattice in the glucose Ringer's solution. Their experiments were carried out when rapid shrinkage must have been taking place (i.e., soon after putting muscle into the glucose solution), and a slight unevenness in the timing of the x-ray measurements might have caused a significant scatter or even a systematic shift in the lattice spacing at some sarcomere lengths.

We conclude that in normal Ringer's solution, the filament lattice shows a constant volume behavior over a wide range of sarcomere lengths and that the results observed by Haselgrove and Huxley resulted from their use of hypertonic bathing solution.

Lattice Changes in Hyperosmotic Solutions

When glucose is added to the bathing solution, the muscle filament lattice shrinks rapidly because of the osmotic pressure exerted by the extracellular fluid. In muscles at moderate sarcomere lengths (<3.0 μm), this volume shrinkage is proportional to the amount of glucose added, down to the point where the filament lattice has reached a 1,0 spacing of ~27 nm. This occurs at ~40 g/l glucose, corresponding to an osmolarity increase of 0.222 and an osmotic pressure (at 21°C) of 4,084 torr (Fig. 3). Concentrations above 40 g/l glucose (up to 70 g/l) produce little further shrinking of this lattice. If sucrose is used instead of glucose, the same shrinking curve is obtained so long as the lattice spacing is plotted as a function of the increased osmolarity (Fig. 3). Similar results are obtained when the solution is made hyperosmotic by increasing the concentration of all ions in the extracellular solution proportionately (Fig. 3). In fact, Fig. 3 indicates that lattice shrinking is much the same for sartorius and semitendinosus muscles, and independent of the temperature and of the type of particle used to increase the osmotic strength. This shrinkage curve is similar to that obtained for toad sartorius muscle by Rome (1968), where the extracellular osmotic strength was increased by increasing the ion concentration. At sarcomere lengths <3.0 μm, the filament lattice approached a minimal 1,0 spacing of ~27 nm. The average 1,0 spacings observed at net

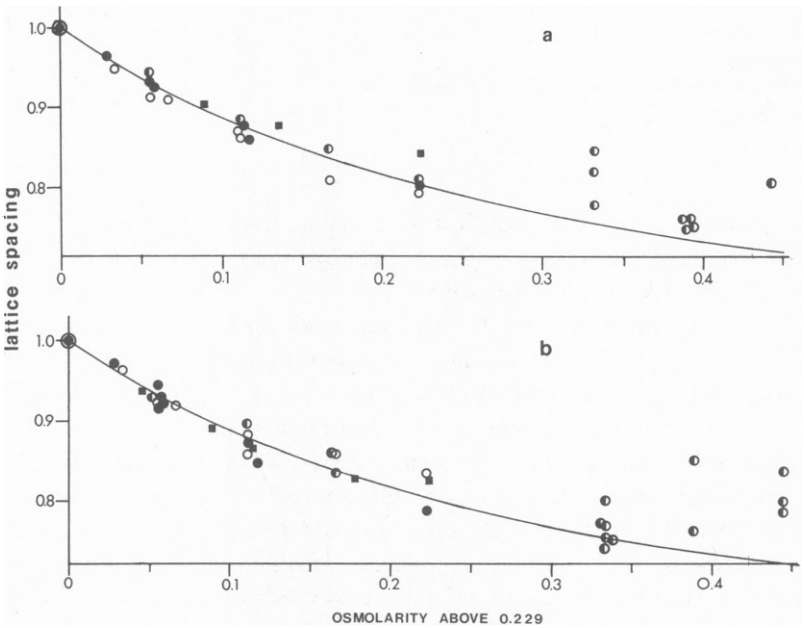

FIGURE 3 Shrinking of the muscle filament lattice in hypertonic solutions (leopard frog). Lattice spacing plotted relative to the spacing in normal Ringer's solution (osmolarity = 0.229). (*a*) Sartorius muscle: sarcomere lengths between 2.5 and 2.8 μm. (*b*) Semitendinosus muscle: sarcomere lengths between 2.3 and 3.5 μm. Each point represents the lattice spacing for an individual muscle averaged from measurements made during increasing and decreasing osmotic strength. ○, Ringer's solution with glucose at room temperature; ◐, Ringer's solution with glucose at 8°C; ●, Ringer's solution with sucrose at room temperature; ■, Ringer's solution at room temparature made hyperosmotic by increasing all ion concentrations proportionately. The lines represent the osmotic shrinking calculated for an osmotically-inactive volume of 25% (Fig. 4).

osmolarities above 0.2 were 27.2 and 27.9 nm from eight sartorius and eight semitendinosus muscles, respectively. At longer sarcomere lengths (3.0–3.6 μm), the minimal spacing was somewhat smaller: the average from four semitendinosus muscles was 25.2 in this case.

When the relative lattice volume was plotted as a function of the inverse of the total osmolarity of the extracellular solution, we obtained a straight line for those experimental points where the total osmotic strength was <0.5 (Fig. 4 *a*, *b*). Points obtained at total osmotic strengths >0.5 osm tended to lie above the straight line, showing that at high osmotic strengths the lattice spacing is no longer determined only by the osmotic pressure. The straight lines indicate that below 0.5 osm the lattice volume is determined osmotically. The intercepts (28.4 ± 1.4 and 30.7 ± 1.9% for the sartorius and semitendinosus, respectively)

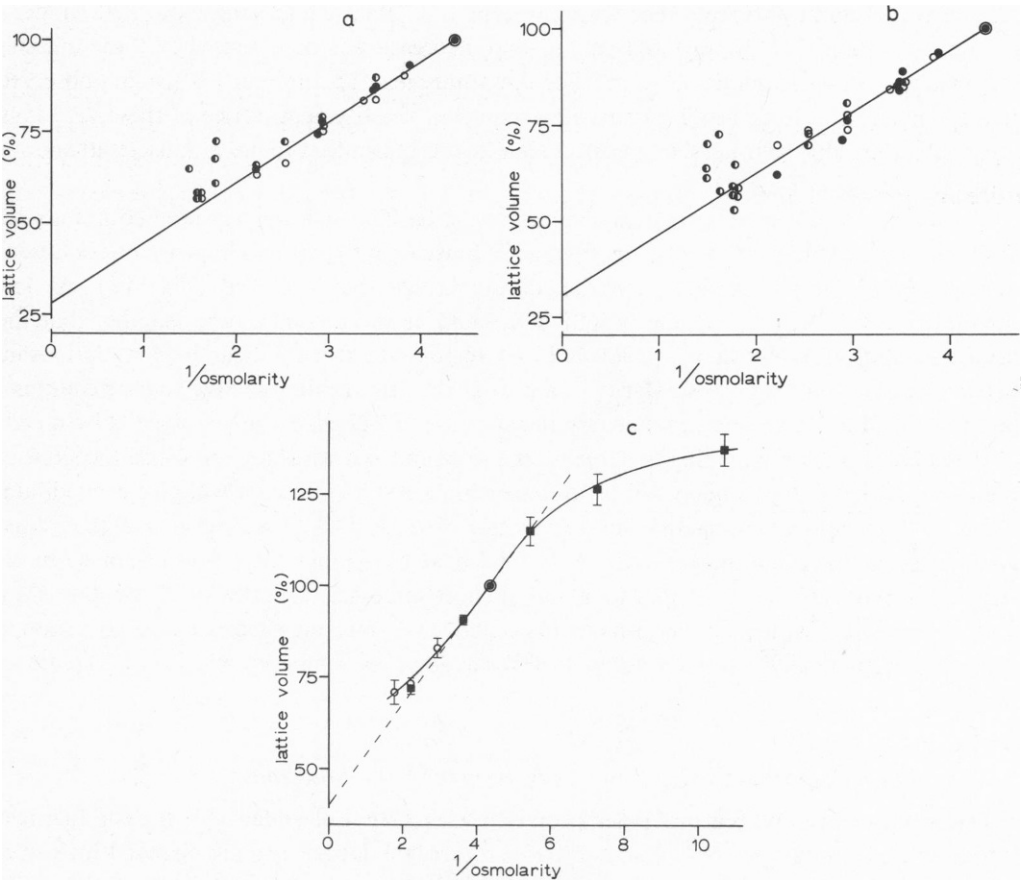

FIGURE 4 Volume of the muscle filament lattice as a function of the reciprocal osmolarity of the bathing solution. Lattice volume is plotted as a percentage of the volume in isosmotic Ringer's solution (osmolarity = 0.229). *a* and *b* are from the same experiments as Fig. 3. (*a*) Sartorius muscle; (*b*) semitendinosus muscle, sarcomere lengths = 2.0–3.2 μm; (*c*) semitendinosus muscle, sarcomere lengths > 3.65 μm. ○, Ringer's solution with glucose at room temparature; ◐, Ringer's solution with glucose at 8°C; ●, Ringer's solution with sucrose; ■, Ringer's solution made hyperosmotic by increasing all ion concentrations proportionately. Lines in *a* and *b* represent the best least-squares fit to the data for 1/osmolarity above 2.0 passing through the point for isosmotic Ringer's solution. Dashed line in *c* is drawn through the points closest to that corresponding to isosmotic Ringer's solution.

give an osmotically-inactive volume of ~30% for both muscles. This value is close to that determined previously for the whole muscle (20%, Boyle and Conway, 1941; 25–30%, Dydynska and Wilkie, 1963) and also for single fibers from frog anterior tibial muscle (26%, Sato, 1954; 33%, Blinks, 1965). A larger value for the osmotically-inactive volume of the filament lattice of toad sartorius muscle (38%) was obtained by Rome (1968), possibly because she included some points at high osmotic strengths which would have tended to shift the intercept to higher values. The smaller values for the osmotically-inactive volume we obtained are closer to the 20% estimated for nonaqueous components (Blinks, 1965) and indicate, as originally suggested by Hill (1930), that most of the water in the muscle fiber is available as solvent.

Semitendinosus muscles stretched beyond filament overlap (3.65–4.3 μm) were also subjected to osmotic shrinking. For these muscles, the 1,0 lattice spacing in normal Ringer's solution was small (27–31 nm) and only a small decrease was observed when these muscles were placed in hyperosmotic solutions. For these muscles, the minimal 1,0 spacing observed from eight muscles averaged 24.0 nm. The volume of the filament lattice of these very long muscles in isosmotic solution did not differ significantly from that found in muscles at shorter sarcomere lengths (Table I).

The change in lattice volume with changes in the bathing solution was studied in muscles stretched beyond filament overlap by placing the muscles in hyper- and hypoosmotic solutions and observing the shrinking or swelling of the lattice that occurred (Fig. 4 *c*). At low osmolarities, we found (as Rome [1968] had found at shorter sarcomere lengths) that the lattice swelling was less than predicted. In no region was there a clear linear relationship between lattice volume and osmolarity^{-1}, and thus the osmotically-inactive volume could not be determined for these muscles. Because the curve, is "S"-shaped, the low slope at both ends of the curve is probably tending to decrease the slope in the central region. Thus, the slope of the curve in the isotonic region will be an upper limit and its intercept will give a maximum value for the osmotically-inactive volume: in this case, of 40%. We believe that the actual volume is close to that in shorter muscles (i.e., 30%), since the total lattice volume in isosmotic solution is almost the same at very long and at short sarcomere lengths, since our data (Fig. 4 *c*) are consistent with a smaller osmotically-inactive volume, and since we have no reason to expect the osmotically-inactive volume to differ at long, as compared with short, sarcomere lengths.

Time-Course of Lattice Changes in Hyperosmotic Solutions

When a muscle was placed in Ringer's solution with .12 g/l of added glucose, the filament lattice shrank rapidly by ~9%. At room temperature, the lattice spacing changed little over the next 24 h (Fig. 1 *b*), but at a tempreature of 8°C, the lattice shrank by a further 2 to 3% over the next few hours and then remained constant for the following 20 h (Fig. 1 *c*). At room temperature, most of the lattice shrinking took place during the first hour of contact with the glucose solution (Fig. 5). The rate of shrinking was exponential (Fig. 6) and depended on the thickness of the muscle (Table II). Bullfrog sartorius muscle equilibrated with a time constant of ~40 min, whereas leopard frog sartorius, with a cross-sectional area about one-fifth that of the bullfrog sartorius, equilibrated almost twice as fast. Some bullfrog sartorius muscles were pared to give a strip ~1 mm thick by 2 mm wide, similar in size to the leopard frog muscles.

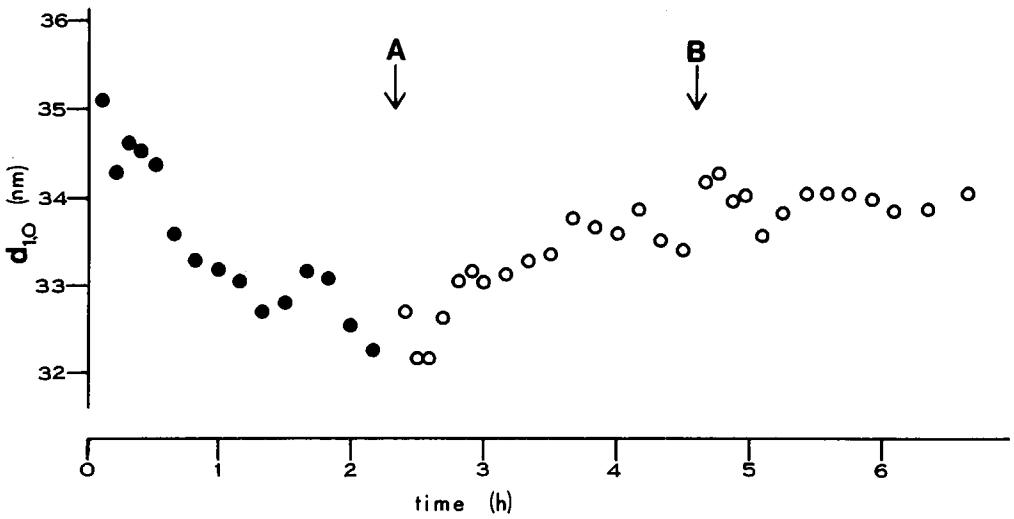

FIGURE 5 Time-course of filament lattice shrinking on shift from normal Ringer's solution to Ringer's containing 12 g/l glucose (●) and on return to normal Ringer's solution (○). The 1,0 lattice spacing was measured from x-ray diffraction photographs obtained every 5 or 10 min. (*A*) Bathing solution changed from glucose to normal Ringer's solution. (*B*) Solution was changed to fresh normal Ringer's solution. Bullfrog sartorius muscle at 22°C; length = 4.5 cm; weight = 0.288 g; sarcomere length = 2.55 μm.

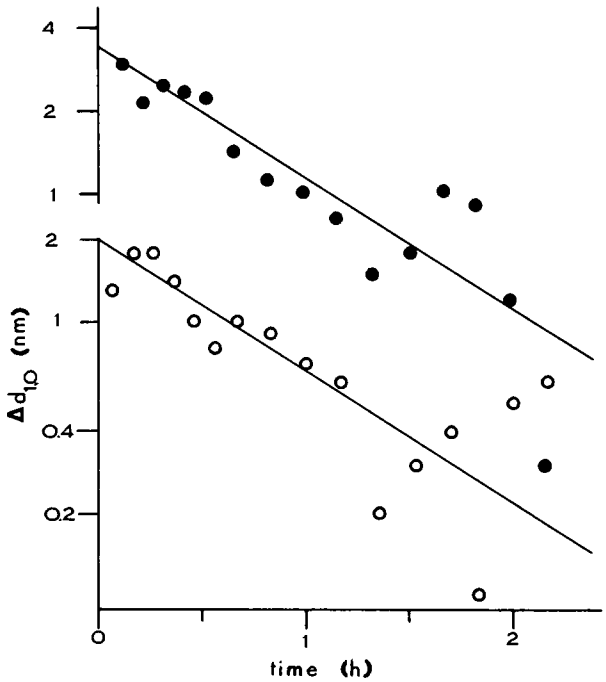

FIGURE 6 Semi-logarithmic plots of the changes in lattice spacing with time after a change of solution. From normal to glucose Ringer's (●) and from glucose to normal Ringer's (○). Δd_{10} is the difference between the observed lattice spacing and the equilibrium spacing. The same muscle and data as used in Fig. 5. The straight lines correspond to a time constant of 53 min.

TABLE II

RATE OF APPROACH TO OSMOTIC EQUILIBRIUM ON CHANGING THE OSMOTIC STRENGTH OF THE BATHING SOLUTION

Muscle	Time constant of exponential approach to equilibrium			Cross-sectional area of muscle		
		(min)			*(mm^2)*	
*Bullfrog sartorius	44	(11)§	14¶		—	
*Leopard frog sartorius	30	(3)	12		—	
‡Bullfrog (intact)	51	(3)	20	7.8	(3)§	1.4¶
‡Bullfrog (pared)	23	(3)	2	1.9	(3)	1.0
‡Leopard frog (intact)	31	(2)	16	2.0	(2)	0.7

*Results from intact bullfrog and leopard frog sartorius muscles.

‡A series of experiments where the muscle's cross-sectional area was calculated from measurements of muscle length and weight. The bullfrog muscles were then pared with a scalpel to give a cross-sectional area similar to that of the leopard frog muscles.

§Number of muscles.

¶Standard deviation.

Bundles of fibers were gently stripped from the surface of the muscle until the desired size was reached. In the authors' experience, this procedure causes very little damage to the remaining tissue. Such paring reduced the time constant for equilibration by a factor of 2 (Figs. 7 and 8; Table II). Replacing a muscle (without a chamber) in a constant flow of solution sometimes reduced the equilibration time constant, but in no muscle was a time constant <20 min observed.

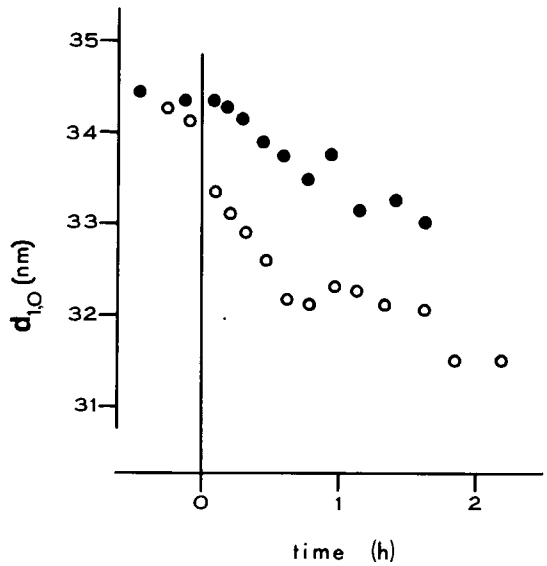

FIGURE 7 Time-course of filament lattice shrinking on shift from normal Ringer's Solution to Ringer's containing 12 g/l glucose. Solutions were changed at time = 0. ●, intact bullfrog sartorius muscle at 22°C; length = 4.5 cm; weight = 0.319 g; sarcomere length = 2.45 μm. ○, same muscle, at the same length, but pared longitudinally to a weight = 0.074 g.

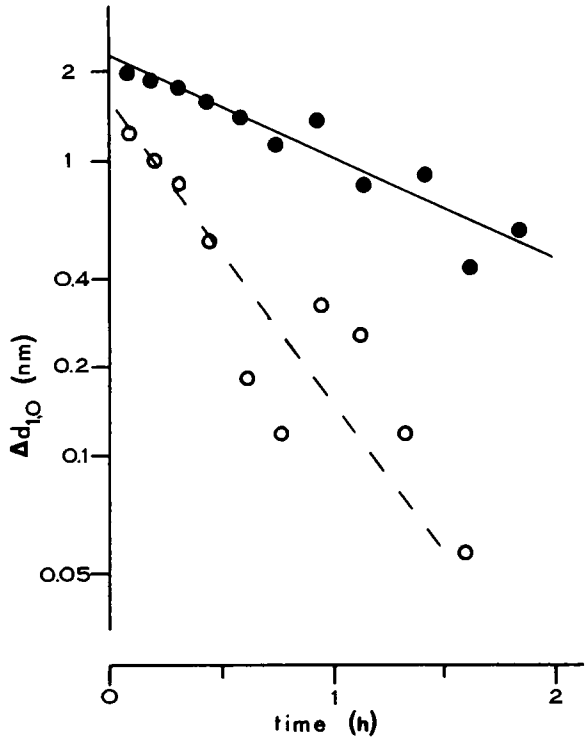

FIGURE 8 Semi-logarithmic plot of the change in lattice spacing with time after a change of solution from normal to glucose Ringer's solution, using the same muscle and data as in Fig. 7. ●, intact muscle; ○, pared muscle. The lines correspond to time constants of 74 min (solid line) and 25 min (dashed line).

Placing a muscle equilibrated in glucose Ringer's solution back into isotonic solution caused the filament lattice to swell back to within ~0.6 nm of the original spacing with a time constant very similar to that found in the shrinking case (Figs. 5 and 6). In general, the spacing recovered after glucose treatment was smaller than that at the start of the experiment, indicating again that there was a small shrinkage of the filament lattice (~2%) during the course of the experiment.

We conclude that osmotic equilibrium (to 5%) is reached within 2 h in large muscles and in shorter times in smaller muscles. These times are very much longer than those found by Blinks (1965) where he observed, using single fibers, that osmotic shrinking was complete in <1 min. From these observations, then, it seems clear that the major osmotic equilibration time for whole muscle involves equilibration of the bathing medium with the fluid between the muscle fibers, with equilibration across the cell membrane being relatively rapid.

DISCUSSION

The Amount of Lattice Shrinking in Hyperosmotic Solutions

We have shown (Fig. 4) that the filament lattice volume of intact muscle shrinks in moderately hyperosmotic solutions as if it were an osmometer, thus showing the same

behavior, as does the whole fiber volume (Boyle and Conway, 1941; Dydynska and Wilkie, 1963). But when the lattice reaches an interfilament spacing of ~31 nm (d_{10} = 27 nm), the filament lattice no longer shrinks with increasing tonicity of the external solution. Even when sarcomeres are stretched to lengths where there is no filament overlap, a somewhat smaller minimum interfilament spacing of 28 nm (d_{10} = 24 nm) was found.

This lower limit to the interfilament distance suggests that physical (e.g., stereochemical) constraints are impeding further shrinkage. We have found very similar limiting interfilament spacings in chemically-skinned frog muscle preparations where shrinking was induced by large polymeric molecules that could not penetrate the filament lattice (Millman and Racey, 1977; Millman and Nickel, 1980; Millman and Irving, 1980). If one assumes normally-accepted values for thick and thin filament diameters of ~14 and 8 nm, respectively, the thick filament backbones at the limiting spacings are far from being in contact. The observed minimal thick to thin filament distance of 17 nm would still leave a gap of ~6 nm between the surfaces of these filaments. It is possible either that the thick filaments have a larger backbone diameter than previously thought (Millman and Nickel, 1980; Millman and Elliott, in preparation), or that the thick filament projections (cross-bridges) may occupy this space and at the minimal lattice spacing the thick (and thin) filaments are pressed together with the projections in between, occupying a 6-nm ring around the thick filament backbone. In either case, the results at very long sarcomere lengths (~3.6 μm) suggest that the effective thick filament diameter is ~28 nm. The observed larger interfilament spacing at shorter sarcomere lengths (31 nm) is just what would be expected by the addition of thin filaments to the lattice. An hexagonal lattice of rods 28 nm in diameter with axes spaced 31 nm apart would leave room at the trigonal points (where the thin filaments are found in vertebrate muscle) for rods of diameter 7.8 nm, very close to the accepted size of the thin filaments. Further experiments using skinned muscle preparations are in progress on this aspect of the lattice structure.

Long-Term Shrinking of the Filament Lattice

The shrinking of the filament lattice that takes place during the first several hours after dissection does not appear to be just a simple osmotic effect. It could be argued that the Ringer's solution used was slightly hypertonic, but the significant increase in spacing observed with "hypotonic" Ringer's during the first few hours (Fig. 2 *e*) suggests that initially water is taken up, followed by a later water loss. The slow shrinking, which was seen under all conditions studied, is greatest at low temperatures in the presence of glucose. We are of the opinion that this effect is not related to the internal pH (as was suggested by Elliott et al., 1967), but rather reflects a decrease in the osmotic strength of the intracellular fluid. If the effect were related to the internal pH, shrinkage should be greater after a long series of contractions when the muscle was fatigued than in the resting muscle. Although both Elliott et al. (1967) and Haselgrove and Huxley (1973) found a small decrease in the lattice spacing of resting muscle with time, they found no significant change in the spacing because of contraction. Furthermore, no change in lattice spacing has been observed in skinned, single, frog muscle fibers when the pH is decreased from 7 to 6 (Matsubara and Elliott, 1972; Fig. 3 *a*).

If the internal osmotic strength of muscle fibers is decreasing, the ion most likely to be involved would be the potassium ion, to which the membrane is relatively permeable (Boyle

and Conway, 1941; Adrian, 1960; Rome, 1968). Potassium is lost during the first few hours after dissection (Adrian, 1960), a period similar to that over which the filament lattice change is observed. It thus seems likely that the slow decrease in lattice spacing is caused by a leakage of potassium ions from the interior of the muscle fiber. The volume changes associated with this leakage can be reversed temporarily by increasing the potassium ions in the bathing solution, but the most stable lattice over a longer period of time is obtained by using a hypoosmotic Ringer's solution. On the basis of our experiments, in cases where a steady lattice spacing of lattice volume is required, we recommend using a bathing solution with the NaCl concentration reduced by ~10%.

If the above explanation for the slow lattice shrinking is correct, it is surprising that the shrinkage is greater at 8°C than at room temperature. It is possible, however, that at room temperature, the shrinkage due to potassium loss is partly reversed by an uptake of other molecules or ions: either glucose, which is generally transported across cell membranes by a carrier-mediated mechanism requiring insulin, or sodium, through a partial failure of the sodium pump at room temperature.

One distinct difference appears here between intact muscle and single fibers. The slow shrinking found in intact muscle has not been found in single fibers. Both Sato (1954) and Matsubara and Elliott (1972) observed that after a few hours in Ringer's solutions, single muscle fibers swell by as much as 24%. Matsubara and Elliott attributed this result to a breakdown in the sodium pump in the dissected fiber, which allowed sodium accumulation within the fiber. This may be caused by mechanical damage to the fiber during separation of the single fiber from the whole tissue. We have found no direct evidence for this such fiber damage in intact muscle.

Conclusions

We have verified the constant volume relationship for frog skeletal muscle over a very wide range of sarcomere lengths and have determined improved values for the lattice volume and the osmotically-inactive volume of the muscle fiber. Above a minimal value (28–31 nm), the thick filament lattice spacing depends on the relative osmotic strengths of the intracellular and extracellular fluids. The lattice spacing can vary with time (e.g., by loss of potassium ions from the intracellular fluid, or by uptake of glucose or sodium ions), and this effect must be considered when measuring lattice volume over long periods of time. The slow initial shrinking of the lattice can be reduced by using a hypoosmotic bathing solution. It should be noted in this respect, that the long term behavior of single fibers and of intact muscles is not the same: single fibers tend to swell with time, whereas the fibers of intact muscle tend to shrink.

We thank Wendy Warden, Tom Irving, and Jackie Marsh for technical assistance.

We are grateful to the National Research Council of Canada for operating grant support.

Received for publication 11 May 1979 and in revised form 30 September 1980.

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