

Calcium Accumulation by the Sarcoplasmic Reticulum in Two Populations of Chemically Skinned Human Muscle Fibers

Effects of Calcium and Cyclic AMP

G. SALVIATI, M. M. SORENSON, and A. B. EASTWOOD

From the Laboratories of Muscle Physiology and Muscle Morphology, H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Diseases; and the Departments of Neurology and Anatomy, College of Physicians and Surgeons of Columbia University, New York 10032

ABSTRACT In previous efforts to characterize sarcoplasmic reticulum function in human muscles, it has not been possible to distinguish the relative contributions of fast-twitch and slow-twitch fibers. In this study, we have used light scattering and ^{45}Ca to monitor Ca accumulation by the sarcoplasmic reticulum of isolated, chemically skinned human muscle fibers in the presence and absence of oxalate. Oxalate (5 mM) increased the capacity for Ca accumulation by a factor of 35 and made it possible to assess both rate of Ca uptake and relative sarcoplasmic reticulum volume in individual fibers. At a fixed ionized Ca concentration, the rate and maximal capacity (an index of sarcoplasmic reticulum volume) both varied over a wide range, but fibers fell into two distinct groups (fast and slow). Between the two groups, there was a 2- to 2.5-fold difference in oxalate-supported Ca uptake rates, but no difference in average sarcoplasmic reticulum volumes. Intrinsic differences in sarcoplasmic reticulum function (V_{\max} , $K_{0.5}$, and n) were sought to account for the distinction between fast and slow groups. In both groups, rate of Ca accumulation increased sigmoidally as $[\text{Ca}^{++}]$ was increased from 0.1 to 1 μM . Apparent affinities for Ca^{++} ($K_{0.5}$) were similar in the two groups, but slow fibers had a lower V_{\max} and larger n values. Slow fibers also differed from fast fibers in responding with enhanced Ca uptake rates upon addition of cyclic AMP (10^{-6} M, alone or with protein kinase). Acceleration by cyclic AMP was adequate to account for adrenaline-induced increases in relaxation rates previously observed in human muscles containing mixtures of fast-twitch and slow-twitch fibers.

Address reprint requests to Dr. G. Salviati, Istituto de Patologia Generale, Via Loredan 16, 35100 Padova, Italy.

INTRODUCTION

This study was undertaken to characterize Ca accumulation by the sarcoplasmic reticulum (SR) of normal human muscle. Information from previous studies of human muscle SR has been limited by the small quantities of tissue available, and because most human skeletal muscles contain a mixture of fiber types. In other mammals, muscles can be found that are composed almost entirely of either fast-twitch or slow-twitch fibers. These can be classified histochemically as type II or type I, respectively, by selective staining for myosin ATPase. Substantial differences have been reported for the SR from these two types of muscle. Slow-twitch muscle SR is less abundant (Luff and Atwood, 1971; Eisenberg and Kuda, 1975; Van Winkle and Schwartz, 1978; Schmalbruch, 1979); also, *in vitro*, it has a lower rate of Ca uptake (Harigaya et al., 1968; Sreter, 1969; Fiehn and Peter, 1971; Margreth et al., 1972, 1974; Briggs et al., 1977; Van Winkle et al., 1978; Wang et al., 1979); and the membranes are more heterogeneous in protein and enzymatic composition (Margreth et al., 1977; Schwartz et al., 1976). The lower rates of Ca transport and lower SR volumes are consistent with the physiological observation that slow-twitch muscles relax more slowly than fast-twitch muscles (Barnard et al., 1971; Close, 1972; Brody, 1976; Van Winkle and Schwartz, 1978) and are slower to reduce cytoplasmic Ca to resting levels after stimulation (Eusebi et al., 1980).

In contrast, study of SR isolated from human muscles that have different proportions of fast-twitch and slow-twitch fibers revealed much smaller differences in Ca transport activity (Samaha and Gergely, 1965).¹ The authors pointed out that the differences could have been smaller because of the mixture of fast-twitch and slow-twitch fibers. However, there may be smaller intrinsic differences in the SR of fast-twitch and slow-twitch fibers from human muscle. Evaluation of both of these factors is important because in different pathological states, both SR function and the proportions of fast and slow fibers may be altered (Engel, 1970; Baloh and Cancilla, 1972).

A second part of this study was done because of indirect evidence that an intracellular regulatory mechanism in slow-twitch muscles of other mammals may be of physiological importance in human muscle. In SR vesicles isolated from rabbit slow-twitch muscle, Ca transport can be accelerated by phosphorylation of a low-molecular weight regulatory protein intrinsic to the SR membranes (Katz, 1979). The action of catecholamines in accelerating relaxation of slow-twitch muscles *in vivo* has been interpreted as the physiological consequence of an intracellular requirement for cyclic adenosine 3':5'-monophosphate (cAMP) in this phosphorylation reaction (Bowman and Nott, 1969; Katz, 1979). Adrenaline also accelerates relaxation in human skeletal muscles (Marsden and Meadows, 1970). Until now, however, there has been no direct demonstration that cAMP affects Ca uptake in human muscle SR, although alterations in cyclic AMP metabolism are a prominent feature of some

¹ In human muscles, evidence for a correlation between contraction time and histochemical fiber type is indirect (Buchthal and Schmalbruch, 1980), but we adopt standard practice of referring to type II fibers as fast twitch and type I as slow twitch.

myopathies that also show abnormal SR function in vitro (Canal et al., 1975; Willner et al., 1979; Cerri et al., 1981).

In our approach to the problem of describing SR function in normal human muscle, we have used single, chemically skinned fibers. In chemically skinned fibers of mammalian muscle, the SR remains functional and retains a nearly normal configuration within the sarcomeres, but the sarcolemma no longer provides a barrier to diffusion. Soluble myoplasmic constituents are lost (Eastwood et al., 1979*b*; Wood et al., 1980), and the composition of the medium bathing the SR can be adjusted according to experimental needs. Recently, an optical technique has been developed in this laboratory for measuring both the net rate of Ca uptake and Ca accumulation capacity in single skinned fibers in the presence of a Ca-precipitating anion (Sorenson et al., 1980). Based on differences in rates of Ca uptake and in response to cyclic AMP that we observe with this technique, two populations of fibers can be distinguished in normal human skeletal muscles. Although we have not identified the two populations histochemically, we believe that the faster and slower rates of Ca uptake can be assigned to fast-twitch and slow-twitch fibers, respectively. The differences in Ca transport capabilities of the SR in these two populations are indeed less than for SR from predominantly fast-twitch and slow-twitch muscles of other mammals. The differences are diminished still further by cAMP, which activates Ca transport only in the slow fibers. Preliminary reports of some of these data have been presented elsewhere (Salviati et al., 1980*a* and *b*).

METHODS

Preparation of Chemically Skinned Fibers

Normal tissue was dissected from grossly uninvolved pectoralis muscles obtained from radical mastectomies on five adults. One normal quadriceps specimen was obtained from an adult man undergoing orthopedic surgery. The data of Fig. 6 include four other quadriceps specimens that were histologically normal and were obtained from diagnostic biopsies from patients who were deemed to have psychogenic disorders. The muscles were immediately placed on ice, and when they reached the laboratory (15 min) were completely relaxed. Bundles of 100–200 mg (2–3 mm in diameter, 20 mm long) containing several hundred fibers were excised from the bulk of the muscle after being tied to a wooden stick and stretched to 110–120% of slack length. Each bundle was chemically skinned by exposure to a “skinning” solution containing 5 mM K₂EGTA, 170 mM K propionate, 2.5 mM K₂Na₂ATP, 2.5 mM Mg propionate, and 10 mM imidazole propionate, pH 7.0 (Reuben et al., 1974; Wood et al., 1975; Eastwood et al., 1979*b*). After 24 h at 0°C, the bundles were transferred to skinning solution made up in 50% glycerol (Reuben et al., 1977; Eastwood et al., 1979*b*), and stored at –20°C until used. Most measurements were made within 4 wk.

In previous studies of mammalian muscle, this chemical skinning procedure was shown to have little effect on SR morphology but, within 24 h, it leads to extensive alterations of the plasma membrane (Eastwood and Bock, 1979 and 1981; Eastwood et al., 1979*b*). As previously shown for rabbit psoas fibers (Sorenson et al., 1980), in glycerol-stored human skinned fibers both rate of uptake and maximal Ca capacity of the SR were stable for at least 7 wk (Fig. 1) when the solution was changed several

times during the first 24 h in skinning solution. In one preparation, half of the fibers were skinned and stored in the presence of pepstatin (10 mg/ml). The average rate of uptake was identical for both groups of fibers when they were tested after 3.5 wk of storage.

Ca Uptake

Segments of single skinned fibers were dissected from the biopsy and mounted in a chamber containing "relaxing" solution (5 mM K_2EGTA , 170 mM K propionate, 2.5

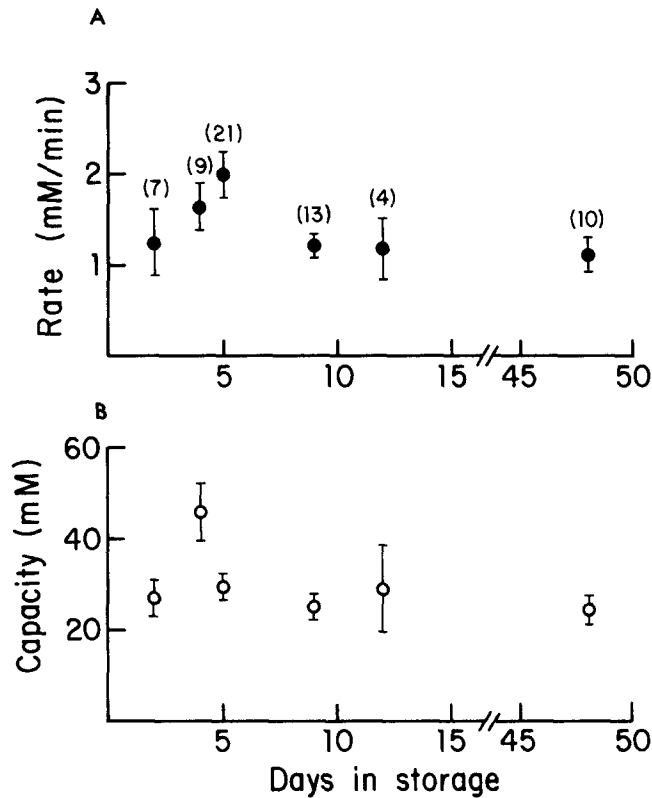


FIGURE 1. Stability of SR function during storage. Small bundles of pectoralis fibers were "skinned" by 24 h exposure to four changes of "skinning" solution (see text) at 0°C. Bundles were transferred on day 0 to a similar solution made up in 50% glycerol and stored at -20°C. At various intervals, oxalate-supported Ca uptake rates (A) and capacities (B) were tested at 25°C in the standard loading medium as shown in Fig. 2, using single fibers isolated from the stored bundle. Values are \pm SEM; number of fibers shown in parentheses in A also applies to B.

mM Mg propionate, 5 mM K_2Na_2ATP , and 10 mM imidazole propionate, pH 7.0). For Ca uptake in the absence of oxalate, fibers were tacked by their ends to the bottom of a plastic petri dish and the slack length (usually 6–8 mm) was measured with the aid of a dissecting microscope and an ocular micrometer. For Ca uptake in the presence of oxalate, the chamber was that described by Sorenson et al., (1980) and the fibers were stretched to 120–130% of slack length between two clamps. In

both cases the solution was vigorously stirred. Diameters were measured in several places along the length of each fiber using a compound microscope at 300 \times magnification.

After skinning, pectoralis muscle fiber diameters ranged from 31 to 82 μm . In three specimens the mean diameters (\pm SEM) were 56 ± 1 ($n = 16$), 65 ± 3 ($n = 12$), and 48 ± 1 ($n = 99$). In one quadriceps specimen, diameters ranged from 56 to 151 μm , with a mean of 97 ± 2 ($n = 102$). No significant differences were found between diameters of fast-uptake and slow-uptake fibers. These figures are probably not equal to the diameters before skinning, because in other skinned-fiber preparations, skinning causes some increase in diameter (Godt and Maughan, 1977). Pectoralis fiber volumes were calculated on the assumption of a right circular cylinder. Many of the quadriceps fibers were elliptical; their volumes were calculated using the average of the major and minor diameters.

Ca Uptake in the Absence of Oxalate

Fibers were transferred from relaxing solution to 5 ml of the standard loading solution containing 170 mM K propionate, 2.5 mM Mg propionate, 5 mM $\text{K}_2\text{Na}_2\text{ATP}$, 2.15 mM ^{45}Ca -EGTA, 2.85 mM K_2EGTA , and 10 mM imidazole propionate, pH 7.0. Specific activity was 50,000 cpm/nmol Ca. The concentration of free Mg ions in this solution is 0.09 mM and free $[\text{Ca}^{++}]$ is 0.39 μM (pCa 6.4), calculated using apparent association constants of 1.919×10^9 , 40, 5×10^3 , and 10^4 M^{-1} for Ca-EGTA, Mg-EGTA, Ca-ATP and Mg-ATP, respectively (for references see Orentlicher et al., 1977). This concentration of free Ca ions was chosen because it is at or below the threshold for contraction (Wood et al., 1978). Incubation was carried out at room temperature (22–24°C) for 1, 5, or 10 min. After incubation, the fiber was washed for 10 s in a relaxing solution modified to contain 0.25 mM EGTA and 10 mM Mg propionate. The fiber was then incubated overnight in 100 μl of 0.3 M acetic acid. The entire acid incubation mixture was spotted on a strip of filter paper and counted in a liquid scintillation counter. For Ca bound to contractile proteins, fibers were preincubated for 10 min in relaxing solution containing a detergent (Brij 58, 0.2% wt/vol). After 10 s in the ^{45}Ca -containing loading medium, fibers were processed as described above.

Ca Uptake in the Presence of Oxalate

In the presence of oxalate, active transport of Ca leads to formation of Ca oxalate crystals in the lumen of the SR (Hasselbach and Makinose, 1961) and to a progressive increase in light scattering by the fiber that is proportional to the increase in Ca content (Sorenson et al., 1980). The experimental setup was slightly modified from that described previously (Katz et al., 1978), with a photodiode replacing the photomultiplier as detector of the scattered light (Katz et al., 1979). The reaction chamber was mounted on the stage of an inverted microscope and the light scattered by the fiber was packed up from below through a long-working distance, 20 \times objective focused on the fiber. Ca uptake was measured by following the increase in light scattering after the addition of oxalate (final concentration, 5 mM) to 0.5 ml of the standard pCa 6.4 loading medium already described. When other ionized Ca concentrations were used, the ratio of Ca-EGTA to K_2EGTA was changed, keeping the total EGTA concentration constant. For Ca^{++} concentrations higher than pCa 6.4, fibers were first stretched to 180% of slack length to avoid interference in the light-scattering measurements caused by actin-myosin interactions (Katz et al., 1978). Control experiments at pCa 6.4 on rabbit psoas fibers have shown that stretching does not affect the rate of uptake (Sorenson et al., 1980; see also Applegate and Homsher,

1981). Temperature was maintained at 25°C, with the exception of some of the earliest data obtained on quadriceps fibers at 22–27°C (Fig. 6).

⁴⁵Ca Calibration of Light Scattering

Scattering by a relaxed fiber (S, Fig. 2) is proportional to its protein content, which in turn is proportional to the volume of the fiber (Katz et al., 1978; Sorenson et al., 1980). During Ca uptake, the increase in scattering as Ca oxalate precipitates inside the SR (ΔS , Fig. 2) is proportional to the amount of Ca accumulated (Sorenson et al., 1980). Therefore, the relative increase in scattering ($\Delta S/S$) is proportional to the Ca concentration inside the fiber. The proportionality factor was determined for each biopsy by measuring fiber volume, $\Delta S/S$ and ⁴⁵Ca content of 10–12 maximally loaded fibers using ⁴⁵Ca at a specific activity of 10,000 cpm/nmol Ca. The procedure for loading, rinsing, and counting the fibers was essentially that described earlier (Sorenson et al., 1980). In the different pectoralis muscle specimens, the proportionality factor ranged from 14 to 21, with a mean of 15.7 mM (mmol/liter fiber) per unit

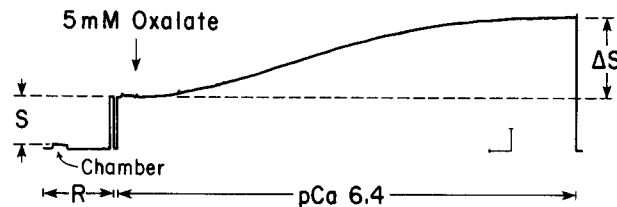


FIGURE 2. Time course of light-scattering increase due to Ca oxalate accumulation. Pectoralis fiber was exposed first to a relaxing solution (R) to obtain a reading for S, then to the standard loading medium (pCa 6.4). Vertical deflections indicate closing of a shutter in the light path. After 2 min, oxalate was added. Maximum rate was attained within 10 min and remained constant for ~9 min before approaching a plateau. At left, scattering signal from chamber containing relaxing solution, before the fiber was mounted in the light path. Horizontal bar, 2 min. Vertical bar, 0.5 V. This and all subsequent light-scattering records shown are tracings.

increase in $\Delta S/S$. Since the proportionality factor was independent of rate of loading, the same factor was used for all fibers from a given specimen.

Protein Determinations

Single fibers were solubilized by incubating overnight in 100 μ l 0.5 N NaOH, after measuring fiber volume at slack length. The total protein concentration in 50 pectoralis fibers was 70 ± 4 mg/ml fiber (\pm SEM), determined according to Lowry et al. (1951) in a final volume of 0.6 ml using bovine serum albumin as a standard. This value is somewhat lower than that reported for rabbit psoas fibers.² In 10 fibers, noncollagen protein was measured after solubilization in 0.1 N NaOH (Lowry et al., 1941; Lillenthal et al., 1950). The value obtained (71 ± 6 mg/ml fiber) was not significantly different from the measurement of total protein concentration in the larger group of fibers.

² The protein content recorded in Sorenson et al. (1980) was erroneously stated to be 136 μ g/ml fiber rather than 136 mg/ml.

Electron Microscopy

Bundles of 20–30 fibers were dissected from the biopsy after 24 h in skinning solution and after 1 wk in storage solution. The bundles were fixed overnight in 2% glutaraldehyde at 5°C in skinning or storage solutions, respectively. They were then washed in 0.1 M sodium phosphate buffer (pH 7.2), and postfixed with 1% osmium tetroxide in phosphate buffer for 2 h at room temperature. Finally, the fibers were dehydrated with ethanol and propylene oxide, and embedded in Epon 812. Sections were collected on bare copper grids, stained with lead and uranium, and examined using a JEOL 200CX electron microscope (JEOL Inc., Peabody, Mass.)

Chemicals

All reagents were analytical grade. Brij 58 was from Ruger Chemical Co. (Irvington, N. J.). Ca-EGTA was prepared from CaCO₃ and H₄EGTA and was neutralized with KOH. Sodium azide, Na₂ATP (vanadate-free), cAMP, pepstatin A, and enzymes were obtained from Sigma Chemical Co., St. Louis, Mo. Enzymes used were rabbit muscle protein kinase (3',5'-cAMP-dependent, peak II, of specific activity 0.3 pU/μg protein), and bovine heart phosphodiesterase (3',5'-cyclic nucleotide, activator-insensitive, of specific activity 0.083 U/mg protein). In a few experiments, cAMP-dependent bovine heart protein kinase was used (specific activity 1.4 pU/μg protein).

Calculation of Kinetic Parameters

Estimates of V_{\max} , $K_{0.5}$, and n for the $[Ca^{++}]$ dependence of Ca accumulation rates were obtained as described by Dunne et al. (1973), using a linear transformation of the equation

$$v = \frac{V_{\max}}{1 + (K_{0.5}/Ca)^n}$$

Direct measurements of V_{\max} could not be obtained because saturating concentrations of Ca⁺⁺ (>1 μM) cause Ca oxalate precipitation in the loading medium. Therefore, for each set of points (five or six rates measured on each fiber), V_{\max} and $K_{0.5}$ were calculated after choosing the value for n that gave the highest correlation for a least-squares fit to the following linear transformation:

$$v = V_{\max} - (K_{0.5})^n (v/Ca^n)$$

Only fibers for which the correlation coefficient was >0.90 were used in these calculations. This procedure for estimating kinetic parameters was adopted to avoid the inaccuracies inherent in double-reciprocal plots. It is considered to be superior to other linear transformations for comparisons between two sets of data (Dowd and Riggs, 1965).

RESULTS

Ultrastructure of Human Skinned Fibers

There were distinct gaps in the plasma membranes of chemically skinned human muscle fibers after either 24 h in skinning solution (Fig. 3a) or after 1 wk in storage (Fig. 3b). One to seven gaps around the circumference of most fibers were seen in transverse sections of skinned, stored fibers; the gaps were sometimes several micrometers long. About 10% of the fibers had no visible

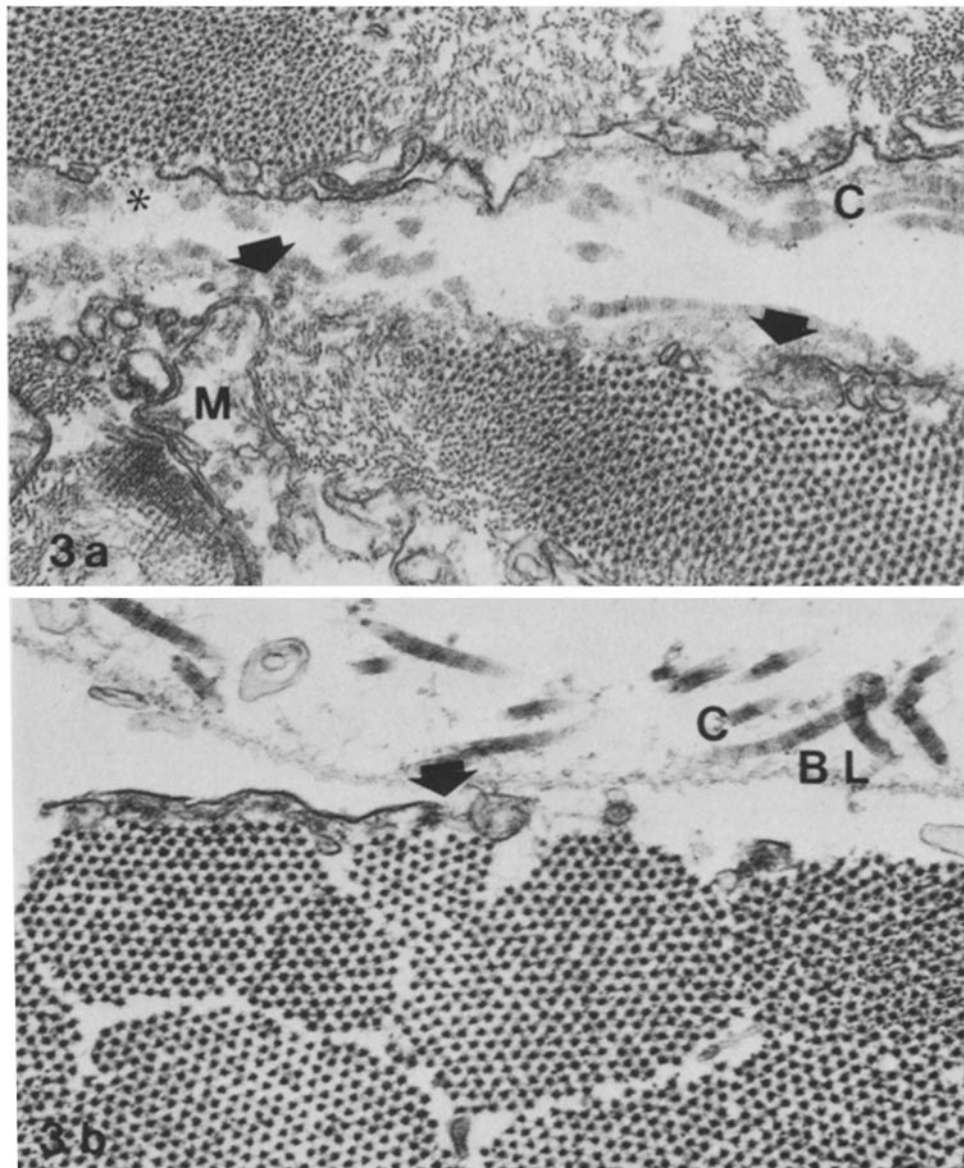


FIGURE 3. (a) Human pectoralis fibers exposed to skinning solution for 24 h at 0°C before fixation. The plasma membrane of the lower fiber is disrupted between the arrows; a smaller gap in the plasma membrane of the upper fiber is marked with an asterisk. A mitochondrion (M) in the lower fiber appears swollen. The extracellular space contains collagen fibrils (C). $\times 60,000$. (b) Human pectoralis fibers skinned for 24 h and then stored for 1 wk at -20°C in skinning solution made up in 50% glycerol. The basal lamina (BL) is lifted from the fiber surface. Between the arrow and the right side of the micrograph, the plasma membrane is disrupted. C, collagen fibril. $\times 60,000$.

gaps in a typical transverse section. In freeze-fracture replicas of chemically skinned rabbit fibers, both fracture faces of the sarcolemma had defects of dimensions similar to the gaps found in thin sections (Eastwood and Bock, 1979).

The morphology of the SR in human muscle was relatively unaffected by chemical skinning and storage; all normal architectural features were recognizable (Fig. 4). The SR tended to vesiculate somewhat in the storage solution, but the vesicles retained the normal relations of SR within each sarcomere, and recognizable triads persisted both in skinned (Fig. 4a) and in stored fibers (Fig. 4b).

Other changes, less significant for physiological studies, included swelling of mitochondria (Figs. 3a and 4a), loss of glycogen from the fibers, and loss of many of the soluble substances that form the myoplasmic background in freshly fixed fibers. Preservation of contractile proteins and other structures in chemically skinned human muscle was generally similar to that found for rabbit muscle, which has been described in more detail elsewhere (Eastwood et al., 1979b).

Ca Uptake

Chemically skinned, human skeletal muscle fibers accumulate Ca in the presence of Mg-ATP. Calcium accumulation by quadriceps fibers was measured using ^{45}Ca at pCa 6.4 in the presence and absence of a precipitating anion (Table I). In the absence of oxalate, uptake was maximal after 5 min of incubation, and no statistically significant changes occurred after 10 min incubation. More than 60% of the maximal uptake was reached in 1 min. Pretreatment with detergent reduced the capacity to 10% of its maximal value, whereas the presence of oxalate in the loading medium increased the maximal capacity for Ca accumulation by about 35-fold. Pectoralis fibers had a lower capacity for Ca oxalate accumulation than quadriceps fibers (21.1 ± 1.1 mmol/l fiber; $n = 124$).

The time course of Ca oxalate accumulation after the addition of oxalate to the loading medium is shown as an increase in light scattering in Fig. 2. In a solution containing ATP and Ca, no increase in scattering occurred until oxalate was added. Then light scattering increased gradually to its maximum rate; subsequently, the increase was linear for several minutes and then approached a plateau. Similar data were obtained using pyrophosphate in place of oxalate, with the rate of increase in light scattering being slower in pyrophosphate.

Previously, it was shown that the increase in scattering is proportional to the Ca content of the fiber during net uptake as well as at the plateau. Thus, the slope of the linear part of the curve provides a measure of the rate of Ca uptake, whereas the signal at the plateau level is a measure of the capacity for Ca oxalate accumulation (Sorenson et al., 1980).

At saturating oxalate concentrations, nearly every SR element visible in electron micrographs of skinned fibers is involved in the accumulation of Ca oxalate precipitates (Sorenson et al., 1980). Thus, when loading reaches its

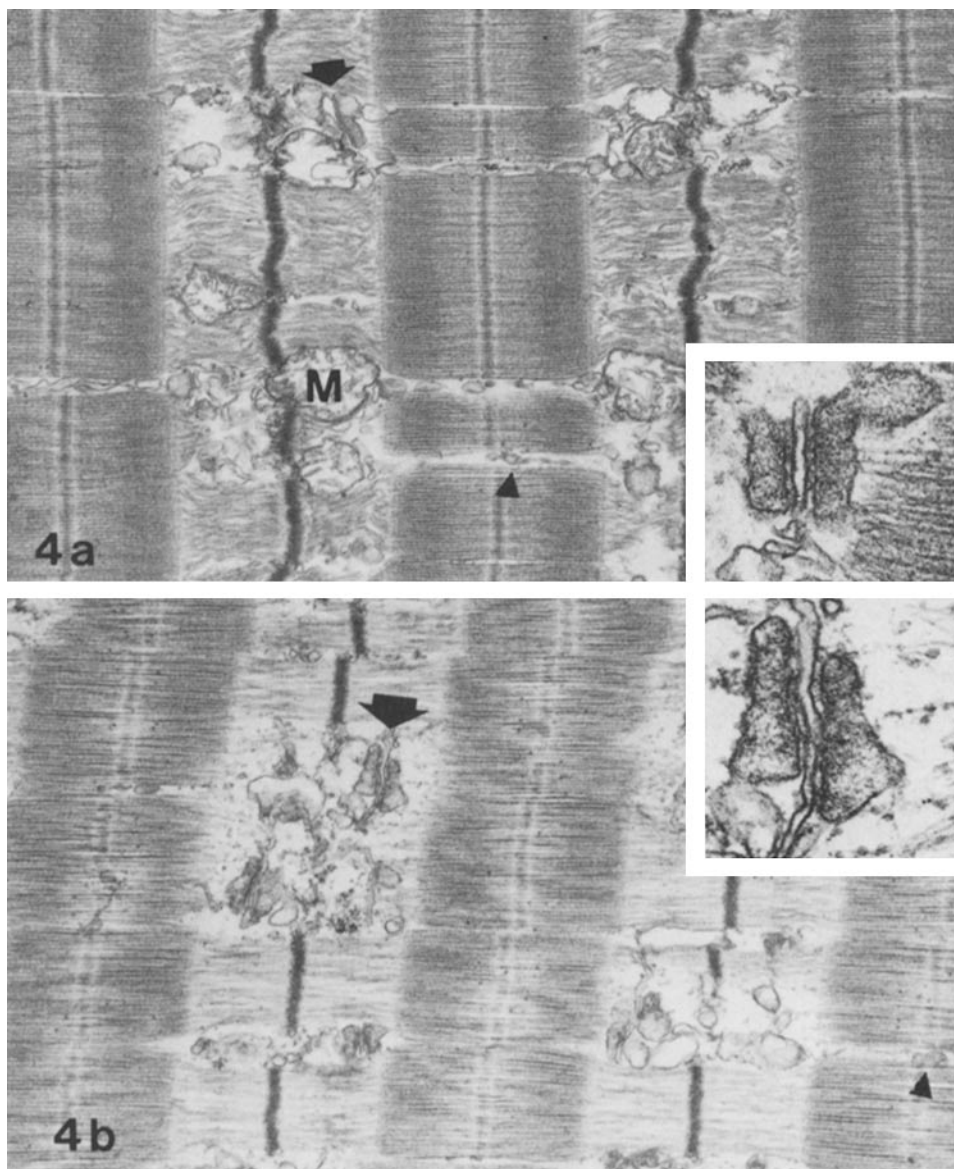


FIGURE 4. SR in human pectoralis fibers after 24 h in skinning solution (*a*) or after 1 wk in glycerol-storage solution (*b*). The normal relationship between myofibrils and longitudinal SR (arrowheads) is retained, and triads (arrows) are clearly recognizable. $\times 20,000$. Insets: higher magnification of triads from a fiber that was skinned for 24 h (*a*), or skinned and then stored for 1 wk (*b*). In *a*, SR "feet" can be seen in the gap between the T tubule and the terminal cisternae of the triad.

final value under these conditions, the SR is filled to its maximum capacity with Ca oxalate, and the Ca content of the fiber is proportional to SR volume (Solaro and Briggs, 1974; Briggs et al., 1977). To establish the maximum capacity for human skinned fibers, in a number of experiments we increased the concentration of oxalate from 5 mM to 10 and 15 mM either during the linear region of the uptake curve or after the light-scattering signal had reached a plateau. No increase was observed in either the rate or the final plateau value. It has already been shown that the plateau level is independent of the Ca concentration in the loading medium (Sorenson et al., 1980). Thus the plateau $\Delta S/S$ reached in 5 mM oxalate represents the maximum SR capacity and provides an index of the total SR volume in human skinned fibers.

TABLE I
Ca UPTAKE IN THE PRESENCE AND ABSENCE OF OXALATE

Condition	Incubation time	Ca taken up <i>mmol/liter fiber</i>	Number of fibers
No oxalate (22–24°C)	10 s	0.08±0.004	37
	1 min	0.54±0.03	35
	5 min	0.87±0.06	33
	10 min	0.73±0.05	15
5 mM oxalate (25°C)	25–45 min	31.6±2.3	55

Values shown are \pm SEM. 10-s and 5-min loads are significantly different from the 1-min load ($P < 0.001$); the 10-min load is not significantly different from the 5-min load ($P > 0.2$).

Chemically skinned quadriceps fibers were incubated in the standard loading medium (pCa 6.4) containing ^{45}Ca and 0 or 5 mM oxalate, vigorously stirred. Fibers incubated for 10 s were first exposed for 10 min to a 0-Ca ("relaxing") medium containing Brij 58 (0.2%, wt/vol). For incubation in the presence of oxalate, fibers were mounted between two clamps and loading was monitored until it reached a maximum, as described for Fig. 2. After incubation, fibers were rinsed and counted as described in Methods.

As controls for precipitation of Ca oxalate in mitochondria or in the myofibrillar space, skinned fibers were treated during net uptake with an inhibitor of mitochondrial Ca transport (Fanburg and Gergely, 1965), or with detergent after loading had reached a plateau. The mitochondrial inhibitor sodium azide (to 10 mM) had no effect on the rate of uptake in three fibers tested (Fig. 5A), and in three other fibers Brij 58 (0.2% wt/vol) added after the plateau had been reached caused immediate loss of the scattering increment (Fig. 5B).

Fiber Heterogeneity

In skinned fibers from rabbit psoas, which are almost all fast-twitch (Weeds et al., 1975) both the rate of increase in fiber Ca and the SR volume, as indicated by maximal Ca oxalate capacity of the fiber, varied over a wide

range. Proportionality between rate and volume was maintained, however, so that the fractional rate of filling the SR (rate of loading normalized to the maximum capacity for each fiber) covered a rather narrow range and showed a normal distribution (Sorenson et al., 1980).

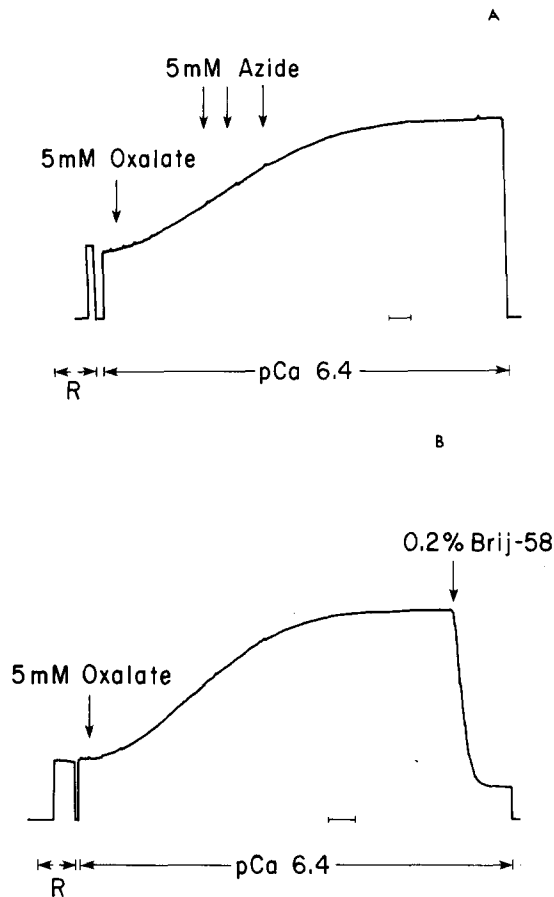


FIGURE 5. (A) Effect of Na azide on Ca uptake. Addition of Na azide to a final concentration of 5 or 10 mM had no effect on rate of uptake. In 15 mM there was some decrease at about the level where loading normally begins to approach a plateau. Similar results were obtained on two other fibers. Horizontal bar, 2 min. (B) Effect of detergent. Addition of Brij 58 to the loading solution caused loss of all the scattering associated with Ca uptake in a fully loaded fiber, and some loss below the level originally recorded in R. Similar results were obtained on two other fibers. Horizontal bar, 2 min.

Under the same conditions, we also found variability both in rate and in capacity, or SR volume, among fibers from pectoralis and quadriceps muscles of humans. Unlike data from rabbit psoas fibers, however, two peaks can be distinguished in a histogram relating number of fibers to fractional rate of

filling (Fig. 6). The frequency distribution of a homogeneous, normally distributed population can be linearized by a probit plot (Colquhoun, 1971). However, a probit plot of the data of Fig. 6 showed two different slopes connected by a nonlinear region between rates of 0.05 and 0.07 min^{-1} . Accordingly, for 179 fibers from both quadriceps and pectoralis muscles, the plot of rate vs. maximum capacity shows points clustered around two lines (Fig. 7).

Fibers with a fractional filling rate $\geq 0.07 \text{ min}^{-1}$ (7% of the maximum capacity per minute), which we will call fast fibers, are grouped around a line of slope $0.086 \pm 0.002 \text{ min}^{-1}$ ($\pm \text{SEM}$; $n = 72$). The other group, which we

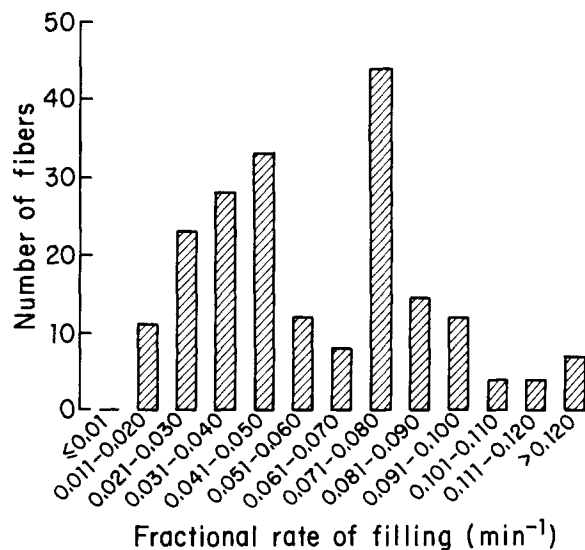


FIGURE 6. Frequency distribution of rate of filling. Rate and capacity were measured under standard loading conditions in 200 fibers from five pectoralis and five quadriceps biopsies, including 21 fibers that were tested at 22–27°C rather than 25°C (see Methods). Fractional rate of filling is the ratio between rate (mmol/liter fiber·min) and maximum capacity (mmol/liter fiber).

will call slow fibers, fall around a line with a slope of $0.037 \pm 0.001 \text{ min}^{-1}$ ($n = 107$). For the five specimens in which nine or more fibers were studied at 25°C, the proportions of fast fibers were 35 (quadriceps), 29, 33, 44, and 69% (pectoralis).

The two populations differed more in rate than in capacity, or SR volume, although there was overlap in both parameters (Fig. 7 and Table II).

Light-scattering records from two fibers that were representative of the two populations are shown in Fig. 8. Both fibers were from the same bundle of pectoralis fibers. The maximal capacity for Ca oxalate in the slow fiber was 67% of the capacity in the fast fiber (16.4 and 24.4 mmol/liter fiber, respectively). However, the rate of loading for fiber I was only 28% of the rate for

fiber II (0.56 and 2.0 mmol/liter·min). Hence the fractional rate of filling fiber I (0.034 min^{-1}) was less than one-half that for fiber II (0.082 min^{-1}).

Since fibers from fast-twitch and slow-twitch muscles of other mammals are known to have different volumes of SR, additional experiments were done to verify the light-scattering data of Fig. 7 and Table II, which indicate little or no difference in SR volume between fast and slow fibers of human muscle. Ca accumulation rates and maximum capacities were measured by the light-scattering method in a smaller sample (26 randomly selected fibers) from a specimen of pectoralis muscle. ^{45}Ca was included in the loading medium, and

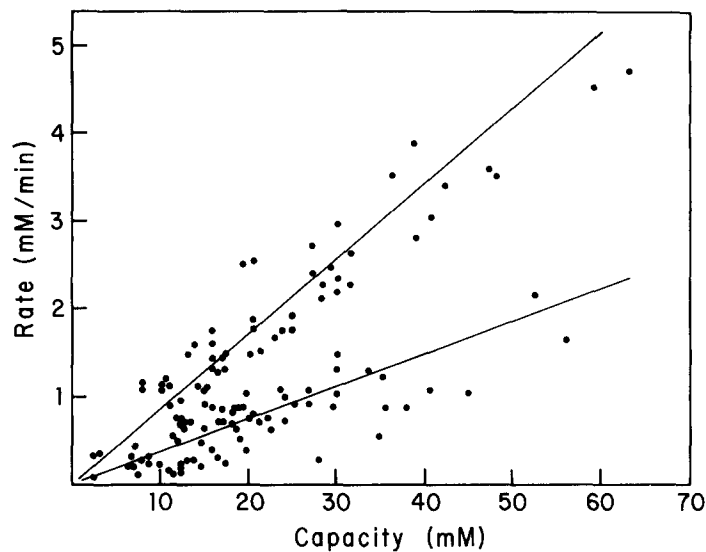


FIGURE 7. Distribution of Ca uptake rates and capacities. Oxalate-supported Ca uptake was measured under standard conditions in 124 fibers from pectoralis and 55 fibers from quadriceps (data from Fig. 6, excluding measurements not made at 25°C). Rates and capacities are calculated from experiments in which the increase in light scattering was related to ^{45}Ca content of the fibers, as described in Methods. The ratio of rate to capacity for each fiber gives fractional rate of filling. The upper line is drawn with a slope of 0.086 min^{-1} (= average of all filling rates $\geq 0.07 \text{ min}^{-1}$); lower line is drawn with a slope of 0.037 min^{-1} (= average of all filling rates $< 0.07 \text{ min}^{-1}$).

at the end of each experiment the fiber was rinsed, digested, and counted as described in Methods. Table III shows that the fibers classified as fast using light scattering (fractional rate of filling $\geq 0.07 \text{ min}^{-1}$) had capacities that were not significantly greater than those for slow fibers, regardless of whether the capacity measurements were based on ^{45}Ca or on $\Delta\text{S/S}$, the final plateau level of light scattering. As expected from morphometric measurements on animal muscles (Eisenberg and Kuda, 1975), similar experiments comparing fibers from slow-twitch and fast-twitch muscles of the rabbit revealed a 2.2-fold higher capacity for fast-twitch fibers by both methods of measurement (M. M. Sorenson, A. B. Eastwood, and J. A. Leavens, unpublished data).

Ca Dependence of the Rate of Uptake

The experiments presented above were all done in the presence of a low free Ca^{++} concentration (pCa 6.4). The different rates of filling in the two populations of fibers might reflect differences in affinity of the Ca pump. This was tested by measuring the Ca uptake rates in single fibers exposed to loading media with free Ca^{++} concentrations increasing from 10^{-7} to 10^{-6} M. Higher concentrations were not used, in order to avoid formation of Ca oxalate crystals in the myofilament space.

A Lineweaver-Burk plot of the fractional rates of uptake at different Ca^{++} concentrations is concave upward for both populations of fibers (Fig. 9), suggesting a stoichiometry greater than one for the reaction of Ca with the pump. In a preliminary report of these data, we concluded on the basis of manually fitted double-reciprocal plots that n was higher for slow fibers than for fast fibers (Salviati et al., 1980a). Using the criteria for fitting the linear transformation described in Methods, a satisfactory correlation was obtained

TABLE II
Ca UPTAKE RATE AND CAPACITY IN FAST AND SLOW FIBERS
OF QUADRICEPS AND PECTORALIS AT pCa 6.4

Muscle	Fast fibers	Slow fibers
Quadriceps		
Rate (mmol/liter fiber · min)	2.59±0.31 (19)	1.20±0.12 (36)
Capacity (mmol/liter fiber)	31.6±3.2 (19)	31.4±3.0 (36)
Pectoralis		
Rate (mmol/liter fiber · min)	1.88±0.13 (53)	0.66±0.05 (71)
Capacity (mmol/liter fiber)	23.2±1.7 (53)	19.3±1.3 (71)

Conditions are as described in Fig. 2 (5 mM oxalate). Quadriceps fibers were from one individual, pectoralis fibers were from five. Values are ± SEM, with the number of fibers in parentheses.

for the data from seven of the fast fibers and four of the slow fibers included in Fig. 9. The range of Ca^{++} concentrations for this analysis was limited to pCa 7–6.2, since only a few of these fibers were tested at pCa 6.0. The calculated values for n , V_{\max} , and $K_{0.5}$ for these fibers are given in Table IV. Rates measured at pCa 6.4 in the same fibers are included for comparison. The results show that the difference in rates persisted in the extrapolation to V_{\max} and that slow fibers had greater n values, whereas Ca affinities were not significantly different in the two groups.

We wish to emphasize that these data are presented for the purpose of comparing the two populations rather than for estimating absolute values. Based on other studies of Ca transport in the SR, it is unlikely, for example, that n is actually >2. Moreover, Dowd and Riggs (1965) have shown that this transformation of the Michaelis-Menten equation tends to underestimate K_m and V_{\max} . Finally, we point out that data from a number of fibers in Fig. 9 were rejected because of failure to converge in the least-squares analysis for Table IV. Most of these rejects could not be fitted by a nonlinear iteration

procedure (Brandt et al., 1980) for the same reason, which indicates that the Ca^{++} concentrations used may not have been high enough to provide clear evidence of saturation.

Effects of cAMP on Ca Transport

In SR vesicles isolated from cardiac muscle and from slow-twitch muscles of various mammals, ATPase activity and oxalate-supported Ca uptake are accelerated by cAMP. Sometimes the addition of cAMP-dependent protein kinase is required for these effects to be seen (Katz, 1979).

An effect of adding cAMP and protein kinase to the loading medium is

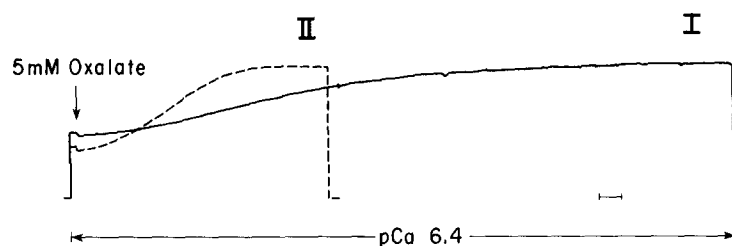


FIGURE 8. Time course of Ca oxalate accumulation in a slow fiber (I) and a fast fiber (II). Horizontal bar, 2 min.

TABLE III
COMPARISON OF LIGHT-SCATTERING AND ^{45}Ca
MEASUREMENTS OF Ca UPTAKE CAPACITY IN FAST AND
SLOW FIBERS OF PECTORALIS AT pCa 6.4

Fiber type	^{45}Ca <i>mM</i>	Light scattering $\Delta S/S$
Fast	22.7 ± 3.2	1.74 ± 0.27 (8)
Slow	17.0 ± 2.0	1.49 ± 0.15 (18)
<u>Fast</u> <u>Slow</u>	1.34	1.17

Conditions are as described in Table I (^{45}Ca , 5 mM oxalate). Values are \pm SEM, with the number of fibers in parentheses.

shown in Fig. 10 for two slow fibers from human muscle.³ Because an endogenous adenylyl cyclase activity capable of producing low concentrations of cAMP has been detected in rabbit skinned fibers (Wood et al., 1980), the fibers were first preincubated with phosphodiesterase (PDE) in relaxing solution. Subsequently, the rate of loading at pCa 6.4 was measured first in the presence of PDE, and then in its absence with cAMP and protein kinase

³ We are indebted to Drs. D. S. Wood and J. H. Willner for communicating the results of experiments on endogenous enzyme activities and caffeine-releasable calcium, which first showed that cAMP affects the SR in chemically skinned mammalian fibers. This information was essential in our decision to test cAMP in human fibers.

added. The rate of loading increased by 65% in the pectoralis fiber (Fig. 10A) and by 50% in the quadriceps fiber (Fig. 10B). In control experiments using fast-twitch fibers from rabbit, neither boiled nor enzymatically active PDE had any effect on Ca uptake (data not shown).

Stimulation by cAMP was partially reversible in a number of fibers tested, but by no means in all of them (Fig. 10B).

Self-controlled experiments like that of Fig. 10A were performed on 43

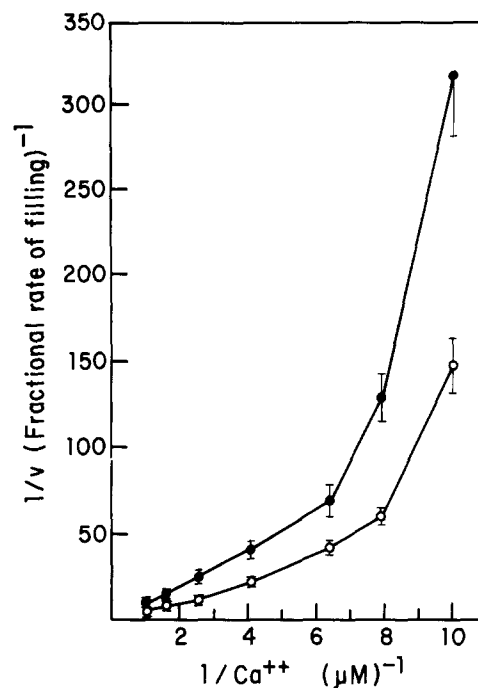


FIGURE 9. Ca concentration dependence of rate of filling. Fifteen fast fibers (○) and 7 slow fibers (●) were each exposed sequentially to several different Ca concentrations in the presence of 5 mM oxalate, and the maximum linear rate was recorded at each concentration. The sequence was terminated and loading was allowed to proceed to its maximal capacity when the rate of increase in light scattering began to fall off. Values plotted are reciprocals of fractional rate of filling (\pm SEM; $n = 4-7$ for slow fibers, 5-15 for fast fibers).

fibers from both fast and slow populations of quadriceps and pectoralis muscles (Table V). Fast fibers were insensitive to added cyclic nucleotide; in slow fibers, the rate of uptake increased, on the average, by 72%. Included in these data are 37 fibers in which the increase in rate ranged from 20 to 160%, and one slow fiber which showed no effect.

The addition of cAMP alone was also effective in stimulating Ca uptake, although the activation was less than when protein kinase was added. In eight quadriceps fibers from the slow population, cAMP alone increased the rate by

77%; in the same fibers, when protein kinase was also added to the solution (2 min later), the activation over the control rate increased to 88%.

Slow fibers that were allowed to load to full capacity in the presence of cAMP and protein kinase showed no increase in capacity over controls (data not shown). As another test for a cAMP effect on capacity, four slow fibers and two fast fibers were exposed to cAMP plus protein kinase after loading had reached a plateau. Capacity was unaffected.

In SR vesicles isolated from slow-twitch and cardiac muscles of other mammals, micromolar concentrations of cAMP had no effect on steady-state Ca loads in the absence of a precipitating anion (Kirchberger et al., 1974; LaRaia and Morkin, 1974; Will et al., 1976). In a similar experiment using ^{45}Ca and a pCa 6.4 loading medium without oxalate, we measured Ca contents of skinned fibers selected randomly from a human quadriceps biopsy. In these experiments, each fiber segment was divided in half transversely, and the two halves were incubated separately in the presence or absence of cAMP

TABLE IV
KINETIC PARAMETERS OF OXALATE-SUPPORTED Ca UPTAKE
IN FAST AND SLOW FIBERS

Fiber type	Fractional rate of filling (min^{-1})		$K_{0.5}$	n
	pCa 6.4	V_{\max}		
			μM	
Fast (7)	0.081 ± 0.006	0.118 ± 0.009	0.26 ± 0.01	2.5 ± 0.1
Slow (4)	0.048 ± 0.003	0.062 ± 0.009	0.24 ± 0.02	3.4 ± 0.2
Fast	1.7	1.9	1.1	0.74
Slow				

Values are \pm SEM. See text for details. Differences in $K_{0.5}$ are not significant; the rest of the parameters are significantly different in the two groups of fibers ($P < 0.005$ by Student's t test).

plus protein kinase. Calcium accumulated after 1–5 min in the segments exposed to cAMP was not significantly different from the control segments (Table VI).

DISCUSSION

This investigation has established that normal human muscle fibers can be described as fast or slow on the basis of ability to accumulate Ca after chemical skinning. Cyclic AMP accelerates Ca uptake only in the slow fibers.

Previous studies of chemically skinned, stored fibers from human muscle have demonstrated that contractile protein function and the SR's response to caffeine remain unchanged over long periods (Reuben et al., 1977; Wood, 1978). Retention of functional and ultrastructural characteristics of the SR (Figs. 1, 3, and 4) provides further evidence of the stability of the human skinned-fiber preparation. Similar evidence has been presented for rabbit muscle (Eastwood et al., 1979b; Sorenson et al., 1980). In the latter study, the

correlation between SR function and the increase in light scattering (Fig. 2) due to formation of Ca oxalate precipitates in the lumen of the SR was extensively documented. The experiments of Figs. 5, 9, and 10 and Table III indicate that the increase in light scattering also provides a measure of SR function in human skinned fibers. In this study, an oxalate concentration that

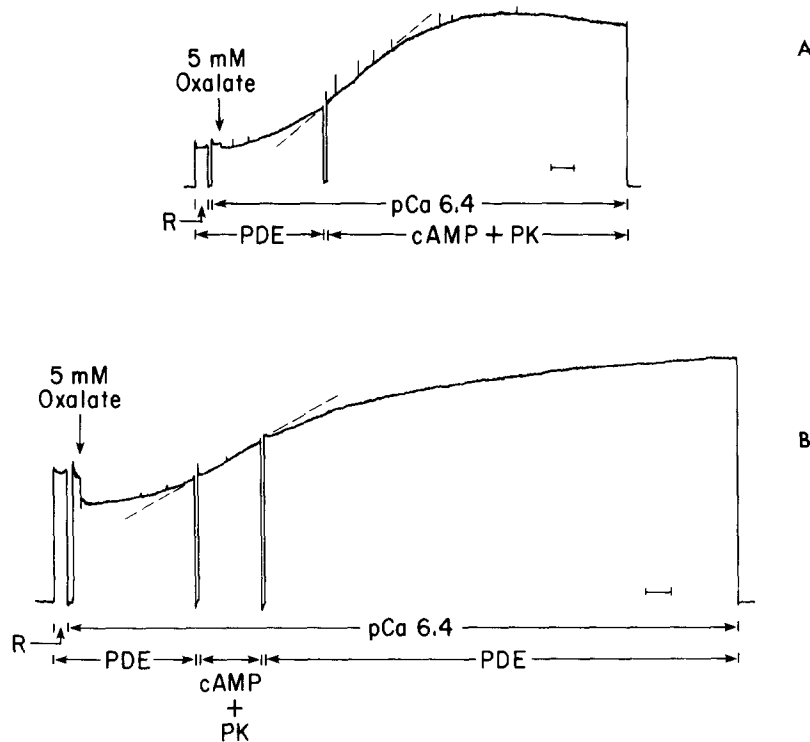


FIGURE 10. Reversible acceleration of Ca uptake by cAMP plus protein kinase. In A, a slow fiber from pectoralis was preincubated for 2 min in R containing 20 μ U/ml PDE, then allowed to accumulate Ca oxalate in a standard loading medium with PDE added. When the solution containing PDE was replaced with one containing 1 μ M cAMP plus 10 μ g/ml protein kinase (PK), the rate increased to 165% of the rate in PDE. Horizontal bars in A and B, 2 min. In B, the experiment was repeated on a slow fiber from quadriceps muscle. The rate of loading increased to 150% of the rate in PDE when cAMP and PK replaced PDE, and returned to 105% of the original rate when cAMP and PK were washed out. Less complete reversals of cAMP acceleration were obtained in 6 of 13 slow fibers tested.

was maximally effective for both types of fibers was used to engage all of the SR, and thus to avoid differences in rate or capacity which might reflect different oxalate concentration requirements for formation of precipitates.

Since we use oxalate as a tool to provide a measurable optical change, it is important to consider whether oxalate itself has any effect on the operation of

the Ca pump or on the Ca permeability of SR membranes. Data have been described that indicate that concentrations of oxalate up to 15 mM did not alter the maximum rate of uptake that was established with 5 mM oxalate. Studies cited elsewhere (Sorenson et al., 1980) have shown that it has no effect on rate of uptake by isolated SR vesicles, the initial rates of transport measured

TABLE V
EFFECTS OF PDE, cAMP, AND PROTEIN KINASE ON RATE OF SR FILLING IN FAST AND SLOW FIBERS

Additions	Fractional rate of filling (min^{-1})	
	Fast fibers	Slow fibers
None	0.086 \pm 0.002 (72)	0.037 \pm 0.001 (102)
Phosphodiesterase (20 $\mu\text{U/ml}$)	0.098 \pm 0.011 (5)	0.036 \pm 0.003 (38)
cAMP + protein kinase (1 μM + 20 $\mu\text{g/ml}$)	0.098 \pm 0.011 (5)	0.062 \pm 0.005 (38)

Rate and maximum capacity of oxalate-supported Ca uptake were measured by light scattering at pCa 6.4; the ratio of rate to capacity provides a measure of the fraction of total SR volume filled per minute. Control data (no additions) are from Fig. 6. The measurements with phosphodiesterase and with cAMP plus protein kinase were made sequentially on the same fiber, as shown in Fig. 10A. Values shown are \pm SEM, with the number of fibers in parentheses. For slow fibers, the increase with cAMP is highly significantly ($P < 0.001$ by a paired t test).

TABLE VI
Ca UPTAKE IN THE ABSENCE OF OXALATE, WITH AND WITHOUT cAMP PLUS PROTEIN KINASE

Incubation time	Ca taken up (mmol/liter fiber)		Number of fibers
	Control	cAMP + PK	
10 s	0.08 \pm 0.004	—	37
1 min	0.41 \pm 0.05	0.44 \pm 0.04	9
5 min	0.82 \pm 0.10	0.86 \pm 0.09	10

For each incubation period, two halves of the same chemically skinned quadriceps fiber were first preincubated for 5 min in R at 22–24°C, then transferred to the standard loading medium (pCa 6.4, no oxalate) containing ^{45}Ca and either no additions (control) or 1 μM cAMP + 10 $\mu\text{g/ml}$ protein kinase. Segments exposed to cAMP during loading also had cAMP plus protein kinase present during the preincubation in R. Fibers incubated for 10 s were first exposed to Brij 58 (data from Table I). After incubation, fibers were rinsed and counted as described in Methods. Values shown are \pm SEM.

by rapid-mixing techniques in the absence of oxalate being consistent with maximal rates of oxalate-supported uptake measured by slower methods. In that paper it was also shown that for chemically skinned fibers of the rabbit, maximal rates of Ca oxalate accumulation compared favorably with rates estimated by using ^{45}Ca in the absence of oxalate in mechanically skinned

fibers of the frog (Ford and Podolsky, 1972). Oxalate appears to act primarily as a sink for the entering Ca, thus preventing much of the increase in ionized $[Ca^{++}]$ in the SR lumen which would occur in its absence.

Ca Uptake in the Absence and Presence of Oxalate

The isotopic measurements (Table I) are most suited for comparison with data on SR vesicles isolated from mixed muscle, where no distinction is made between different fiber types. Subtracting Ca bound to detergent-treated fibers (10-s load) from Ca uptake with SR intact (5-min load) leaves a fiber Ca concentration of 0.79 mM (mmol/liter fiber) that can be attributed to the SR. The capacity was increased to 32 mM by addition of oxalate (Table I). For a rough comparison with data from isolated vesicles, we assume that 1 liter of muscle contains 5 g SR protein (Weber and Herz, 1968). On this basis the data of Table I yield 0.18 $\mu\text{mol}/\text{mg}$ protein for quadriceps SR in the absence of oxalate and 6 $\mu\text{mol}/\text{mg}$ protein with oxalate present (4 $\mu\text{mol}/\text{mg}$ for pectoralis; see Table II). These values for SR capacity in skinned fibers compare favorably with isolated-vesicle preparations from human muscles, where steady-state capacities were 0.08 $\mu\text{mol}/\text{mg}$ protein for loading without oxalate (Takagi, 1971; Scarpa et al., 1978). With oxalate, the capacity of isolated vesicles increased to 2.0 $\mu\text{mol}/\text{mg}$ protein for gastrocnemius plus vastus lateralis (Samaha and Gergely, 1965) and 3.9 $\mu\text{mol}/\text{mg}$ protein for pectoralis (Takagi, 1971).

Oxalate-supported Ca uptake is stimulated by a wide range of Ca concentrations, and eventually reaches the same level of loading in all of them (Hasselbach and Makinose, 1961; Sorenson et al., 1980). However, the steady-state capacity measured without oxalate increases with increasing Ca concentrations (Weber et al., 1966), and so would probably be higher than 0.79 mM if it were measured under the conditions used for the isolated-vesicle studies (0.1 mM Ca) rather than in our standard loading medium (0.39 μM Ca).

Ca binding to detergent-treated fibers (0.08 mM, Table I) is consistent with reports for Ca binding to glycerinated, detergent-treated fibers of rabbit psoas. Maximal Ca binding was about 0.2 or 0.35 mM (mmol/liter fiber) in the presence of 0 or 5 mM free Mg^{++} , respectively (Fuchs and Bayuk, 1976). From these data, we calculate that Ca binding at an intermediate Mg^{++} concentration such as the one we use would be >0.04 mM and <0.17 mM at a Ca concentration near tension threshold.

Rate of Ca Accumulation in Fast and Slow Fibers

On the basis of histochemical stains for myosin ATPase, human pectoralis and quadriceps muscles are mixtures of type I (slow-twitch) and type II (fast-twitch) fibers. One of our goals was to compare Ca uptake in these different fiber types. Samaha and Gergely (1965) had reported that SR isolated from human gastrocnemius and vastus lateralis took up Ca twice as fast as SR from soleus, which has more slow-twitch fibers. We expected that we might find even greater differences if we could compare pure samples of fast-twitch and

slow-twitch fibers. In other mammals, SR isolated from predominantly fast-twitch muscles takes up Ca at rates ranging from 4 to 25 times faster than SR from slow-twitch muscles (see references in Introduction).

Every biopsy we examined showed evidence of two fiber populations (Figs. 6–8). The proportions of slow and fast fibers were in the range reported for type I and type II fibers in these muscles (Edstrom and Nystrom, 1969; Johnson et al., 1973). Average rates for Ca uptake at pCa 6.4 in the two populations differ by a factor of 2.5 (Fig. 6 and Table II). This difference is consistent with the estimate that the half-time for relaxation of type I fibers in human quadriceps is about twice that for type II fibers (Wiles et al., 1979). We conclude, then, that the SR in these muscles can account for differences in their relaxation rates, but that fast-twitch and slow-twitch fibers of human muscle are more similar than are fibers of animal muscles.

In other mammals, some of the characteristics which clearly distinguish between type I and type II fibers from different muscles of the same animal differ much less in type I and type II fibers taken from the same muscle (Burke et al., 1974; Eisenberg and Kuda, 1977). On this basis, the greater similarity between fiber populations in human muscle may be simply a consequence of sampling from mixed muscles rather than a species characteristic.

Elsewhere it has been suggested that rabbit muscle fibers which can be identified histochemically as IIA (“fast-twitch red”) or IIB (“fast-twitch white”) can also be distinguished from each other on the basis of oxalate-supported Ca uptake (Mabuchi and Sreter, 1978). Our oxalate data show no evidence of subcategories among fast fibers in either human (Fig. 6) or rabbit muscles (Sorenson et al., 1980), although we cannot rule out the possibility of some correlation between rate or capacity and fast fiber sub-type without testing each fiber histochemically. In other studies of mammalian muscle, SR vesicles from fast-twitch red and fast-twitch white muscles were essentially identical (Fiehn and Peter, 1971; Margreth et al., 1974).

SR Volumes

In previous studies of human muscle SR, isolated vesicles were used. Estimates of volume based on yield are unreliable. Several authors have noted that SR content of human fibers appears to be similar in different fiber types (Shafiq et al., 1966; Payne et al., 1975). Limited quantitative data from ultrastructural studies on human fibers gave a figure for SR volume in fast-twitch fibers which was 1.6-fold greater than in slow-twitch fibers (Eisenberg, in press). The use of single fibers makes it possible to evaluate differences in Ca transport capabilities which could exist *in situ* due to different SR volumes. For this purpose we have compared relative SR volumes in different fiber types of human muscle, using maximum capacity for Ca oxalate accumulation as an index of volume (Briggs et al., 1977; Sorenson et al., 1980). Measured in this way, SR volume was not significantly greater in the fast-uptake fibers (Table II), and the results obtained by light scattering are confirmed by use of ^{45}Ca (Table III). We conclude that most of the 2.5-fold difference in Ca uptake rates of fast and slow fibers must be attributed to intrinsic differences in SR function rather than SR volume.

Our results show that the rates of Ca accumulation in both types of fibers can be increased by increasing external Ca^{++} concentrations but not by increasing the concentration of oxalate above that which engages the maximum SR volume (5 mM). These observations suggest that the differences in fast and slow fibers are related to the active transport of Ca rather than to oxalate permeability. From the data of Fig. 9 and Table IV, we infer that important differences in SR function may be reflected in V_{max} and in the stoichiometry of Ca transport. In previous studies comparing SR from fast-twitch and slow-twitch muscles of animals, differences in V_{max} were reported (Briggs et al., 1977; Wang et al., 1979). However, these authors also found differences in Ca affinity. The possibility of different n values appears to have been considered only in a comparison of fast-twitch skeletal and cardiac muscle SR (Hicks et al., 1979); in that study, as in ours, n was higher for the slower type of muscle.

In other mammalian muscles, differences in volume are more important. The study by Briggs et al. (1977) compares most closely with ours in providing a measure of SR function in which the contribution of volume can be distinguished. Per gram of muscle, maximum oxalate-supported Ca uptake rates by the SR were five to six times faster for homogenates of fast-twitch muscles than for slow-twitch muscles of rats and cats. Differences in SR volume, as estimated from maximum capacity for Ca oxalate accumulation, accounted for 30–60% of the differences in rate of Ca uptake. We have obtained similar data using the light-scattering technique to compare skinned fibers from fast-twitch and slow-twitch muscles of the rabbit (Eastwood et al., 1979a). Comparison with Ca uptake rates in the present study indicates that human fast and slow fibers are much slower than their counterparts in rabbit muscle, and that this is largely a consequence of a smaller volume of SR. Volume differences aside, the SR in fast and slow fibers differs by about the same factor in humans as it does in animals (Table II; Briggs et al., 1977; Eastwood et al., 1979a).

Effect of cAMP

Perfusion with adrenaline causes a 20% increase in rate of relaxation of human calf muscles (Marsden and Meadows, 1970). It was postulated that a β -adrenotropic response leading to stimulation of cAMP production might account for the results, but at the same time it was not known that cAMP could affect Ca transport by the SR. Stimulation of SR Ca transport by a cAMP-dependent process is now well established for cardiac and slow skeletal muscles of animals, but an effect of cAMP on human muscle SR has not previously been reported. The stimulation we find for slow fibers (72%, Table V) lies within the range reported for stimulation of oxalate-supported Ca uptake in cardiac SR vesicles (LaRaia and Morkin, 1974; Kirchberger et al., 1974; Schwartz et al., 1976; Kirchberger and Chu, 1976).

We interpret our results in terms of the hypothesis developed by these authors, which has been extended to include the effects on slow-twitch muscle SR (see Katz [1979] for references). It attributes the acceleration of Ca translocation by the transport ATPase to phosphorylation of a small mem-

brane-bound regulatory protein. This reaction is catalyzed by a cAMP-dependent protein kinase which is present to a variable extent in SR preparations from different laboratories (Kirchberger et al., 1974; Katz, 1979). Elsewhere, it has been proposed that intrinsic differences in SR of fast-twitch and slow-twitch fibers might reside primarily in inhibition of the pump in slow fibers by the dephosphorylated form of the regulatory protein, rather than in the pump proteins themselves (Margreth et al., 1975). This hypothesis becomes more attractive because a large fraction (50%) of the difference between fast and slow fibers is eliminated by cAMP plus protein kinase (Table V). However, the mechanism of the postulated interaction between the regulatory protein and the pump protein is unknown, and we cannot rule out involvement of other proteins (Katz and Remtulla, 1978; Le Peuch et al., 1979). Regardless of mechanism, the effect of cAMP on rate of Ca uptake by the SR in chemically skinned human fibers seems more than adequate to account for the increased rate of relaxation observed by Marsden and Meadows (1970).

In SR vesicles isolated from predominantly slow-twitch muscles of other mammals, oxalate-supported Ca uptake rates were increased ~30% by the addition of cAMP plus protein kinase (Kirchberger and Tada, 1976; Schwartz et al., 1976). Lack of an effect in fast fibers (Table V) is consistent with the results of Kirchberger and Tada (1976) rather than those of Schwartz et al. (Schwartz et al., 1976; Bornet et al., 1977). However, it may be that our conditions were not optimal for the effect in fast-twitch muscle SR (Galani-Kranias et al., 1980). Other conditions in which cAMP effects can be demonstrated in skinned fast-twitch fibers (Fabiato and Fabiato, 1978) were not tested.

Our finding of a 72% stimulation for slow fibers (Table V) may indicate better preservation of the cAMP-dependent regulatory system in chemical skinning than in vesicle isolation procedures. Other evidence supports this idea. The immediate partial reversal of the cAMP effect found in about one-half of the slow fibers tested (Fig. 10B) suggests that variable amounts of phosphoprotein phosphatase activity may be preserved. Data have been presented that show that a nearly maximum stimulation can be obtained by addition of cAMP without protein kinase, and we infer that endogenous protein kinase activity accounts for most of the activation by added cAMP. Schwartz et al. (1976) found that the effects of cAMP were doubled when protein kinase was added at the same time.

Calcium concentration is another factor which must be taken into account in comparing relative magnitudes of cAMP effects. Cyclic AMP activation of anion-supported Ca accumulation by cardiac SR is greater at Ca concentrations below $K_{0.5}$ than above it (Tada et al., 1974; Hicks et al., 1979). In a number of fibers, we attempted to compare activation by cAMP at pCa 6.4 with effects of cAMP at other Ca concentrations on the same fiber. Incomplete reversal of cAMP effects prevented us from measuring rates alternately without and with cAMP at more than one Ca concentration. In another protocol, rates were measured at pCa 7.0–6.4 in the absence of cAMP, and

then the measurements were repeated in the presence of cAMP. Cyclic AMP seemed to be more effective at pCa 7 and pCa 6.9 than at pCa 6.4 (Salviati et al., 1980a), but more experiments of this type are needed.

Lack of an effect of cAMP on the amount of Ca taken up in the absence of oxalate (Table VI) has previously been reported for cardiac SR (LaRaia and Morkin, 1974; Kirchberger et al., 1974; Will et al., 1976a and b). This observation might be taken as evidence against the feasibility of extrapolating from experiments with oxalate to the in vivo situation. However, it has been suggested that lack of an effect near steady state would be consistent with stimulation of oxalate-supported uptake rates if cAMP led to parallel increases in both efflux and influx (Will et al., 1976a; Katz, 1979). In this case, cAMP would increase net uptake only when efflux was relatively small compared with influx. Use of oxalate as a sink for Ca entering the SR meets this criterion (Hasselbach et al., 1969), but in the absence of a precipitating anion only the very early stage of Ca uptake represents a condition with minimal back flux. Accordingly, recent studies of Ca binding on a millisecond time scale have shown that initial rates of uptake in the absence of oxalate are also significantly accelerated by cAMP (Will et al., 1976a; Tada et al., 1980).

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