

CALCIUM UPTAKE BY BOVINE MUSCLE MITOCHONDRIA AND SARCOPLASMIC RETICULUM

R. C. WHITING

ABSTRACT

Mitochondria and sarcoplasmic reticulum were isolated from prerigor bovine muscle, and their anaerobic calcium uptake activities and stabilities were determined at temperatures, pH values, and ATP concentrations selected to simulate conditions in postmortem muscle. Both organelles were relatively active at 1 mM ATP and showed optimum activity at 3 mM. The pH optimum was 7.2 for mitochondria and 6.0–6.5 for sarcoplasmic reticulum; activities of both increased with increasing temperatures. Under simulated postmortem conditions both organelles initially increased in activity, but mitochondria were first to decline below their initial value. Stabilities declined with decreasing pH values and increasing temperatures, especially above 25°C for mitochondria and above 40°C for sarcoplasmic reticulum.

INTRODUCTION

THE RELEASE of calcium into the actomyosin contractile system during the onset of rigor, cold shortening, or thaw rigor will initiate a shortening of the muscle with the subsequent toughening of the meat (Lawrie, 1977; Marsh, 1977). The sarcoplasmic reticulum regulates the intracellular calcium concentration in living fibers, and this organelle has been assumed to play a major role in postmortem muscle, particularly during thaw rigor conditions in the white fibered muscles, which have a highly developed sarcoplasmic reticulum (Greaser, 1974; Lawrie, 1977). Calcium can also be sequestered by mitochondria by use of oxidative phosphorylation when substrates and oxygen are available or by use of ATP when anaerobic conditions exist (Brierley et al., 1963). Mitochondria may be a source of calcium during the cold shortening of red fibers which have more mitochondria and a less developed sarcoplasmic reticulum than white fibers (Carafoli et al., 1969; Patriarca and Carafoli, 1969; Buege and Marsh, 1975).

Several investigators have examined the postmortem conditions in muscle that cause mitochondria and sarcoplasmic reticulum to lose their ability to sequester calcium. Greaser et al. (1967, 1969a, b), Goll et al. (1971) and LaCourt (1971) reported that after 24 hr they were unable to isolate organelles from mammalian muscle with significant calcium uptake capabilities, although ATPase activity by both organelles (Greaser et al., 1969a, b; Goll et al., 1971; LaCourt, 1971) and respiratory control in mitochondria (Cheah and Cheah, 1971, 1974; Cheah, 1973) have been reported to survive much longer. In poultry muscles, the sarcoplasmic reticular calcium uptake activity survives through the rigor period (Hay et al., 1973; Whiting and Richards, 1978). The activity of isolated bovine sarcoplasmic reticulum declines with decreasing temperature and pH (Kanda et al., 1977). The stability of the sarcoplasmic reticular calcium uptake and mitochondrial respiratory control was greatly reduced at pH values below 5.5, particularly at high temperatures (Greaser et al., 1969b; Cheah, 1973).

In this paper the calcium uptake abilities of sarcoplasmic reticulum and mitochondria isolated from prerigor bovine muscle samples at pH values, temperatures, and ATP concentrations similar to conditions found in postmortem muscle are compared. The stabilities of these isolated organelles are also discussed. These data will help determine which organelles and postmortem factors are critical for the postmortem regulation of intracellular calcium.

EXPERIMENTAL

Organelle isolation

Muscle samples were excised at approximately 30 min postmortem from the Biceps femoris of A maturity cattle and placed in ice during transport to the laboratory. An adjacent sample was frozen in dry ice-acetone for evaluation of succinic dehydrogenase activity and fiber typing as described by Chayen et al. (1973).

The organelle isolation was similar to that used by Martonosi and Feretos (1964) and Greaser et al. (1969a, b). Within 2 hr postmortem, 15.0 g B. femoris was diced, added to 225 ml cold modified Chappell-Perry solution (0.10M KCl, 0.05M imidazole, 5 mM MgSO₄, 1 mM ATP, 1 mM EDTA, pH 7.2) as described by Ernster and Nordenbrand (1967) and intermittently homogenized (Servall Omni-Mixer) for 50 sec. All solutions and organelle suspensions were kept on ice. The slurry was centrifuged at 1,000 × G for 10 min (4°C), and the supernatant was filtered through four layers of cheesecloth and re-centrifuged. The supernatant was again filtered through cheesecloth and centrifuged at 8,000 × G for 20 min. The resulting mitochondrial pellet was suspended in 20 ml of a cold mitochondrial suspension buffer (0.25M sucrose, 0.01M imidazole, pH 7.2), the suspension was centrifuged at 8,000 × G for 20 min, and the pellet was resuspended in 5–10 ml of the buffer. The supernatant from the original 8,000 × G centrifugation was centrifuged at 30,000 × G for 45 min and the pellet was suspended in 30 ml of cold 0.6M KCl, 0.01M imidazole (pH 7.2). After 30 min on ice, this was re-centrifuged at 30,000 × G and the sarcoplasmic reticular pellet was suspended in 5–10 ml of cold sarcoplasmic reticular suspension buffer (0.1M KCl, 0.01M imidazole, pH 7.2). The preparations were kept on ice and assayed the day of preparation. Protein was measured by the Biuret method (Gornall et al., 1949).

Calcium uptake

Calcium uptake was measured by the Millipore filtration procedure (Martonosi and Feretos, 1964). The standard medium used for sarcoplasmic reticulum contained 0.1M KCl, 0.01M imidazole (pH 7.2), 5 mM Na oxalate, 5 mM MgCl₂, and 4 mM ATP. The medium for mitochondria contained 0.25M sucrose, 0.01M imidazole (pH 7.2), 5 mM MgCl₂, 4 mM ATP, and 4 mM Na₂HPO₄. To 10.0 ml of appropriate medium (22–25°C), 0.1 ml of 10 mM CaCl₂ and up to 0.1 mg sarcoplasmic reticulum or 1 mg mitochondria were added. Approximately 5 ml aliquots were withdrawn and filtered (0.45μ Millipore filters) after incubating 20 sec and 15 min. A 3.0-ml sample of filtrate was mixed with 0.5 ml of 10% LaCl₃ · 7H₂O in 3.6% HCl and the unsequestered calcium was determined by atomic absorption spectroscopy. Calcium uptake was determined as the amount of calcium taken from the media between 20 sec and 15 min per mg organelle protein.

RESULTS & DISCUSSION

FIBER TYPING of B. femoris muscle showed an average composition of 33.6% β-red, 60.0% α-white and 6.4% α-red fibers. The yields were 1.2 mg mitochondria and 2.2 mg sarcoplasmic reticulum per g of muscle. The sarcoplasmic reticular isolates accumulated approximately six to ten times as much calcium per mg protein as the mitochondrial

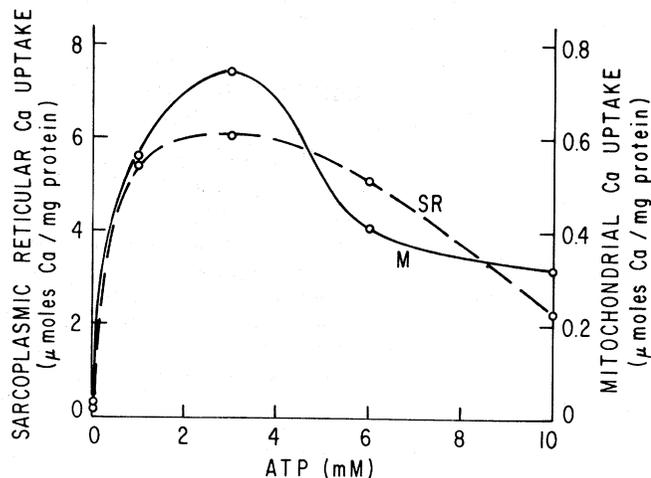


Fig. 1—Calcium uptake activities of sarcoplasmic reticulum and mitochondria from *B. femoris* with varying ATP concentrations. Values are averages of three animals assayed in duplicate. The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 0.17–0.44 and 0.07–0.13 $\mu\text{moles Ca/mg protein}$, respectively.

isolates did throughout this study. This difference may reflect, in part, the degree of purity of the preparations. Preliminary experiments showed that addition of 5 mM sodium azide to the incubation media almost completely inhibited calcium uptake by the mitochondrial preparation and only slightly decreased the uptake by the sarcoplasmic reticular preparation. Since azide is known to inhibit calcium uptake by mitochondria but not by sarcoplasmic reticulum (Fanburg and Gergely, 1965; Batra, 1973), this indicated that a good separation of the two organelles was achieved. The incubation media were chosen to support maximum calcium uptake for each organelle rather than to have identical media which would not necessarily allow demonstration of the organelle's capabilities. Oxalate in the sarcoplasmic reticular medium (Martonosi and Feretos, 1964; Fanburg and Gergely, 1965; Sreter, 1969) and phosphate in the mitochondria medium (Fanburg and Gergely, 1965; Lehninger, 1970) were therefore used to reduce calcium diffusion out of the organelles. The complete cellular microenvironment is not known; nor is it known whether it would be identical for both organelles. Therefore, in this work the organelles, relative responses to comparable changes in environment and treatment were determined.

Calcium uptake under postmortem conditions

The isolated sarcoplasmic reticulum and mitochondria were studied at ranges of ATP, temperatures, and pH values which have been shown to exist in prerigor beef (Tarrant and Mothersill, 1977). The ATP concentration for maximum calcium uptake by both organelles was 3 mM (Fig. 1). At 1 mM ATP the sarcoplasmic reticulum had 89% of its maximum calcium uptake activity and the mitochondria had 76%. With no ATP both preparations had little activity and at ATP concentrations greater than 3 mM the calcium uptake activity declined. The 5 mM magnesium in the incubation media may have restricted the formation of the Mg-ATP complex at the higher ATP concentrations and limited the calcium uptake by the sarcoplasmic reticulum (Hasselback, 1974) and mitochondria, although inhibition by magnesium has also been observed in heart mitochondria (Carafoli and Crompton, 1978). Cardiac muscle mitochon-

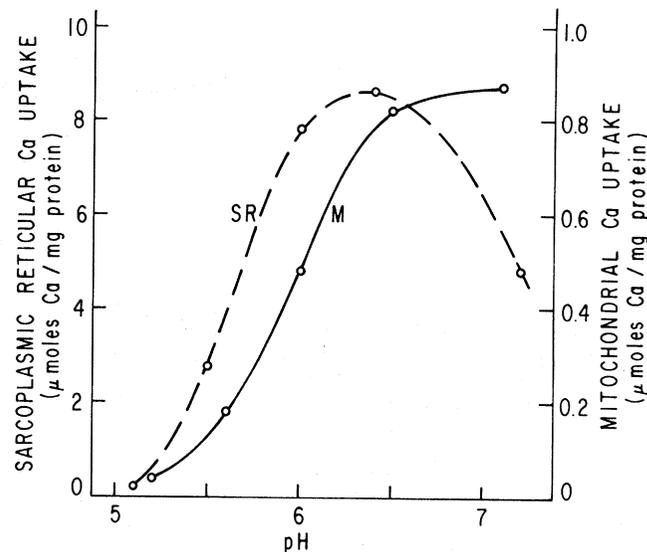


Fig. 2—Calcium uptake activities of sarcoplasmic reticulum and mitochondria with varying pH. Values are averages from *B. femoris* of two animals assayed in triplicate. The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 0.20–0.41 and 0.02–0.13 $\mu\text{moles Ca/mg protein}$, respectively.

dria were reported to have maximum activity at 3 mM ATP and sarcoplasmic reticulum at 6 mM ATP (Fanburg and Gergely, 1965). They also found that cardiac mitochondria had high activity with 1 mM ATP. Martonosi and Feretos (1964) found striated muscle sarcoplasmic reticulum to have high activity with 0.1 mM ATP. All of the data suggest that the postmortem decrease in ATP would not be the cause for calcium release by either organelle until nearly complete exhaustion of the ATP.

The highest mitochondrial calcium uptake was observed at pH 7.2 (Fig. 2). Activity started to decline rapidly at pH 6.5 and was low at pH 5.5. The sarcoplasmic reticular activity increased as the pH declined to 6.5 and then rapidly decreased as the pH decreased below pH 6.0. Both organelles showed essentially no activity near pH 5. This implies that the mitochondria would be the first to lose their postmortem calcium sequestering ability as the pH declines from 6.5 to 6.0. In intact muscle, however, the sarcoplasmic reticulum may be able to compensate for any calcium release by the mitochondria. Reported pH optima for muscle sarcoplasmic reticulum range from pH 6 to 7.3 (Hasselback, 1964; Sreter, 1969; LaCourt, 1971; Kanda et al., 1977). The apparent pH optimum of sarcoplasmic reticular calcium uptake in skinned fibers when determined by tension development decreases with increasing calcium ion concentration (Fabiato and Fabiato, 1978). Sreter (1969) found that the pH optimum decreased with increasing incubation time and that it was higher with oxalate than without. Assay conditions therefore can affect the observed pH optimum of the sarcoplasmic reticulum.

Both organelles increased their calcium uptake activities with increasing temperature (Fig. 3). The sarcoplasmic reticulum initially increased more rapidly than mitochondria but did not increase at 25–37°C. This plateau may result from decreased calcium uptake or increased efflux; however, stability data do not show significant inactivation at 37°C (Martonosi and Feretos, 1964; Sreter, 1969; LaCourt, 1971; Kanda et al., 1977). The mitochondria exhibited an increasing response to increasing temperature. The curves suggest that a decline in muscle temperature into the cold

Whiting and Richards (1978) found that functioning sarcoplasmic reticulum could be isolated from frozen and thawed chicken muscles.

In summary, both organelles were capable of accumulating calcium under conditions simulating those present during the first 4 hr postmortem. The mitochondria lost function more rapidly than the sarcoplasmic reticulum as a result of pH and temperature declines. Mitochondria are generally more labile than the sarcoplasmic reticulum and, therefore, under normal aging and cold shortening conditions could probably be the initial agents of calcium release. Neither organelle survived freezing and thawing well, particularly the sarcoplasmic reticulum. Further work should be done to determine organelle survival in muscles held under various postmortem regimes, to obtain accurate estimates of the amounts of calcium sequestered by each organelle, and to ascertain under what conditions one organelle can compensate for the failure of the other.

REFERENCES

- Batra, S. 1973. The effects of zinc and lanthanum on calcium uptake by mitochondria and fragmented sarcoplasmic reticulum of frog skeletal muscle. *J. Cell Physiol.* 82: 245.
- Brierley, G.P., Murer, E., and Bachmann, E. 1964. Studies on ion transport. 3. The accumulation of calcium and inorganic phosphate by heart mitochondria. *Arch. Biochem. Biophys.* 105: 89.
- Brierley, G.P., Murer, E., and Green, D.E. 1963. Participation of an intermediate of oxidative phosphorylation in ion accumulation by mitochondria. *Science* 140: 60.
- Buege, D.R. and Marsh, B.B. 1975. Mitochondrial calcium and postmortem muscle shortening. *Biochem. Biophys. Res. Comm.* 65: 478.
- Campion, D.R., Olson, J.C., Topel, D.G., Christian, L.L., and Kuhlers, D.L. 1975. Mitochondrial traits of muscle from stress-susceptible pigs. *J. Animal Sci.* 41: 1314.
- Carafoli, E. and Crompton, M. 1978. The regulation of intracellular calcium by mitochondria. *Ann. N. Y. Acad. Sci.* 307: 269.
- Carafoli, E. and Gazzotti, P. 1970. Loss and maintenance of energy-linked functions in aged mitochondria. *Biochem. Biophys. Res. Comm.* 39: 842.
- Carafoli, E., Patriarca, P., and Rossi, C.S. 1969. A comparative study of the role of mitochondria and the sarcoplasmic reticulum in the uptake and release of Ca^{++} by the rat diaphragm. *J. Cell Physiol.* 74: 17.
- Chayen, J., Bitenski, L., and Butcher, R. 1973. "Practical Histochemistry," p. 93. John Wiley & Sons, London.
- Cheah, K.S. 1973. Comparative studies of the mitochondrial properties of Longissimus dorsi muscles of Pietrain and Large White pigs. *J. Sci. Fd. Agric.* 24: 51.
- Cheah, K.S. and Cheah, A.M. 1971. Postmortem changes in structure and function of ox muscle mitochondria. 1. Electron microscopic and polarographic investigations. *Bioenerg.* 2: 85.
- Cheah, K.S. and Cheah, A.M. 1974. Properties of mitochondria from ox neck muscle after storage in situ. *Int. J. Biochem.* 5: 753.
- Ebashi, S. and Lipmann, F. 1962. Adenosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.* 14: 389.
- Eletr, S. and Inesi, G. 1972. Phase changes in the lipid moieties of sarcoplasmic reticulum membranes induced by temperature and protein conformational changes. *Biochim. Biophys. Acta* 290: 178.
- Ernster, L. and Nordenbrand, K. 1967. Skeletal muscle mitochondria. *Methods Enzymology* 10: 86.
- Fabiato, A. and Fabiato, F. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J. Physiol.* 276: 233.
- Fanburg, B. and Gergely, J. 1965. Studies on adenosine triphosphate-supported calcium accumulation by cardiac subcellular particles. *J. Biol. Chem.* 240: 2721.
- Fiehn, W. 1978. The effect of phospholipase D on the function of fragmented sarcoplasmic reticulum. *Lipids* 13: 264.
- Goldblatt, M.J. and Romani, R.J. 1977. Maintenance of respiratory control by beef heart mitochondria incubated at 25°C: Response to protective agents and to prior stress. *Arch. Biochem. Biophys.* 183: 149.
- Goll, D.E., Stromer, M.H., Robson, R.M., Temple, J., Eason, B.A., and Busch, W.A. 1971. Tryptic digestion of muscle components simulates many of the changes caused by postmortem storage. *J. Animal Sci.* 33: 963.
- Gornall, A.G., Bardawill, C.J., and David, M.M. 1949. Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.* 177: 751.
- Greaser, M.L. 1974. Sarcoplasmic reticulum and its possible role in postmortem muscle. *Proc. 27th Ann. Recip. Meat Conf., Amer. Meat Sci. Assn.*, p. 337.
- Greaser, M.L., Cassens, R.G., Briskey, E.J., and Hoekstra, W.G. 1969a. Postmortem changes in subcellular fractions from normal and pale, soft, exudative porcine muscle. 1. Calcium accumulation and adenosine triphosphatase activities. *J. Food Sci.* 34: 120.
- Greaser, M.L., Cassens, R.G., and Hoekstra, W.G. 1967. Changes in oxalate-stimulated calcium accumulation in particulate fractions from postmortem muscle. *J. Agr. Food Chem.* 15: 1112.
- Greaser, M.L., Cassens, R.G., Hoekstra, W.G., and Briskey, E.J. 1969b. The effect of pH-temperature treatments on the calcium-accumulating ability of purified sarcoplasmic reticulum. *J. Food Sci.* 34: 633.
- Hamm, R. and El-Badawi, A. 1972. Aktivität und subcelluläre Verteilung einiger Mitochondrien-Enzyme im Skelettmuskel. 3. Einfluss des Gefrierens und Auftauen von Rindermuskel. *Z. Lebensm. Unters.-Forsch.* 150: 12.
- Hasselbach, W. 1964. Relaxing factor and the relaxation of muscle. *Prog. Biophys. Molecular Biol.* 14: 167.
- Hasselbach, W. 1974. Sarcoplasmic membrane ATPases. In "The Enzymes," Vol. 10, 3rd ed., p. 431, Ed. Boyer, P.D. Academic Press, New York, N.Y.
- Hay, J.D., Currie, R.W., and Wolfe, F.H. 1973. Effect of postmortem aging on chicken breast muscle sarcoplasmic reticulum. *J. Food Sci.* 38: 700.
- Kanda, T., Pearson, A.M., and Merkel, R.A. 1977. Influence of pH and temperature upon calcium accumulation and release by bovine sarcoplasmic reticulum. *Food Chem.* 2: 253.
- LaCourt, A. 1971. Action post mortem du pH et de la température sur le captage de calcium et l'activité ATPasique du reticulum sarcoplasmique fragmenté du muscle de bovin. *Ann. Biol. anim. Bioch. Biophys.* 11: 681.
- Lawrie, R.A. 1977. Meat: Current developments and future status. *Meat Sci.* 1: 1.
- Lehninger, A.L. 1970. Mitochondria and calcium ion transport. *Biochem. J.* 119: 129.
- Marsh, B.B. 1977. The nature of tenderness. *Proc. 30th Ann. Recip. Meat Conf., Amer. Meat Sci. Assn.*, p. 69.
- Martonosi, A. and Feretos, R. 1964. Sarcoplasmic reticulum. 1. The uptake of Ca^{++} by sarcoplasmic reticulum fragments. *J. Biol. Chem.* 239: 648.
- Patriarca, P. and Carafoli, E. 1969. A comparative study of the intracellular Ca^{++} movements in white and red muscle. *Experientia* 25: 598.
- Sreter, F.A. 1969. Temperature, pH, and seasonal dependence of Ca-uptake and ATPase activity of white and red muscle microsomes. *Arch. Biochem. Biophys.* 134: 25.
- Tarrant, P.V. and Mothersill, C. 1977. Glycolysis and associated changes in beef carcasses. *J. Sci. Fd. Agric.* 28: 739.
- Whiting, R.C. and Richards, J.F. 1978. Calcium uptake and ATPase activity by sarcoplasmic reticulum of red and white poultry muscles. *J. Food Sci.* 43: 662.

Table 2—Survival after freeze-thawing of calcium uptake abilities of mitochondria and sarcoplasmic reticulum

Protectant (diluted 1:1 with organelle suspension)	Calcium uptake ^a (μ moles Ca/mg protein)	
	Mitochondria	Sarcoplasmic reticulum
Unfrozen control in suspension buffer ^b	0.45 \pm 0.02	5.70 \pm 0.22
Frozen in:		
Suspension buffer	0.14 \pm 0.04	0.05 \pm 0.07
Supernatant in Chappell- Perry solution	0.12 \pm 0.04	-0.20 \pm 0.07 ^d
Incubation buffer ^c	0.36 \pm 0.05	1.26 \pm 0.35
BSA 10 mg/ml in suspension buffer	0.53 \pm 0.05	2.30 \pm 0.15
Glycerol 20% in suspension buffer	0.30 \pm 0.04	4.57 \pm 0.11

^a Values are means and standard errors of three animals assayed in triplicate.

^b Suspension buffers: mitochondria 0.25M sucrose, 0.01M imidazole (pH 7.2). Sarcoplasmic reticulum 0.1M KCl, 0.01M imidazole (pH 7.2).

^c Incubation buffers: mitochondria 0.25M sucrose, 0.01M imidazole (pH 7.2), 5 mM $MgCl_2$, 4 mM ATP, 4 mM Na_2HPO_4 . Sarcoplasmic reticulum 0.1M KCl, 0.01M imidazole (pH 7.2), 5 mM Na oxalate, 5 mM $MgCl_2$, 4 mM ATP.

^d Calcium was released.

Ms received 6/5/79; revised 9/20/79; accepted 9/28/79.

Presented at the 39th Annual Meeting of the Institute of Food Technologists, St. Louis, Mo., June 10-13, 1979.

The author thanks Devereux Farms, Glenmoore, Pa., for permission to obtain prerigor muscle samples and Arthur J. Miller (Meat Laboratory, ERRC, USDA, Philadelphia, Pa.) for performing the fiber typing.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Table 1—Stability of calcium uptake abilities of mitochondria and sarcoplasmic reticulum from bovine *B. femoris* on incubation

Medium	Calcium uptake ^a (μ Moles Ca/mg protein)	
	Mitochondria	Sarcoplasmic reticulum
Unincubated control in suspension buffer ^b	0.55 \pm 0.05	5.32 \pm 0.37
Incubated in ^c :		
Suspension buffer	0.29 \pm 0.04	1.80 \pm 0.20
Suspension buffer + 4 mM ATP	0.10 \pm 0.02	3.29 \pm 0.61
Suspension buffer + 5 mg/ml BSA	0.50 \pm 0.07	2.96 \pm 0.23
Suspension buffer + 10% glycerol	0.31 \pm 0.05	3.99 \pm 0.27

^a Values are means and standard errors of three animals assayed in triplicate

^b Suspension buffers: mitochondria 0.25M sucrose, 0.01M imidazole (pH 7.2). Sarcoplasmic reticulum 0.10M KCl, 0.01M imidazole (pH 7.2).

^c Incubation: mitochondria ½ hr at 25°C, sarcoplasmic reticulum ½ hr at 43°C.

shown to be relatively stable, surviving several hours at 37°C (Greaser et al., 1969b) or several days at 0–4°C (Ebashi and Lipmann, 1962; Eletr and Inesi, 1972; Kanda et al., 1977).

The ability of the organelles to survive was tested in the presence of ATP, bovine serum albumin (BSA) and glycerol (Table 1). Since in their suspension buffers mitochondria were less stable than sarcoplasmic reticulum to higher temperatures, incubations were for ½ hr at 25°C and 43°C, respectively. Addition of 4 mM ATP increased the sarcoplasmic reticular survival relative to the sarcoplasmic reticulum incubated in suspension buffer but ATP was detrimental to the mitochondria. BSA was effective in stabilizing both organelles and glycerol stabilized the sarcoplasmic reticulum. This indicated that the organelles were very sensitive to their environment. Goldblatt and Romani (1977) found that isolated beef heart mitochondria would maintain respiratory control for 3 days at 25°C if provided a respiratory substrate (α -ketoglutarate) and EDTA and BSA were present and that addition of ATP had little influence on stability. Mitochondrial respiratory control and

aerobic calcium uptake are more stable than ATP driven calcium uptake (Brierley et al., 1963; Carafoli and Gazzotti, 1970). The above observations suggest the use of caution when quantitative inferences regarding in vivo stability are made from data determined in in vitro environments, since the cellular constituents undoubtedly aid in maintaining stability.

Both organelles became increasingly unable to sequester calcium at decreasing pH values or increasing temperatures (Fig. 6). As shown before, mitochondria were more sensitive than sarcoplasmic reticulum to temperature. At pH 7.2 temperature was important for mitochondrial stability, but at pH 5.6 there was relatively low activity after incubation at any temperature. Temperature, however, was not an important factor in sarcoplasmic reticular stability unless it was above 37°C. Greaser et al. (1969b) had also found an interaction between pH and temperatures in porcine sarcoplasmic reticulum. Stability was high at all temperatures when the pH was greater than 6.0 but decreased at higher temperatures when the pH was below 6.0. Fiehn (1978) found muscle sarcoplasmic reticulum to be stable for 2 hr at ambient temperatures when the pH was higher than 5.5. Cheah (1973) and Campion et al. (1975) observed that mitochondria with respiratory control could not be isolated from porcine muscle having a pH value less than 5.5 or 5.9, respectively.

Aliquots of the organelles with various protectants were frozen in air at –13°C, held frozen 1–3 days, and slowly thawed (Table 2). Survival of their calcium uptake abilities was low when suspended in their suspension buffers. Diluting the buffer 1:1 with the supernatant from the 30,000 \times G centrifugation in Chappell-Perry solution had no effect, indicating an absence of soluble protective agents from the muscle fiber. Diluting 1:1 with their respective incubation buffers increased stability of both organelles. The sarcoplasmic reticulum may be protected by the ATP (Table 1). BSA gave excellent protection, particularly for the mitochondria, and glycerol protected both organelles. These data also show that the environment greatly affects the stability observed in vitro. With intact bovine muscle, Hamm and El-Badawi (1972) found an increase in some of the mitochondrial enzymes in the press-juice after freezing and thawing which indicated damage to the mitochondrial membranes.

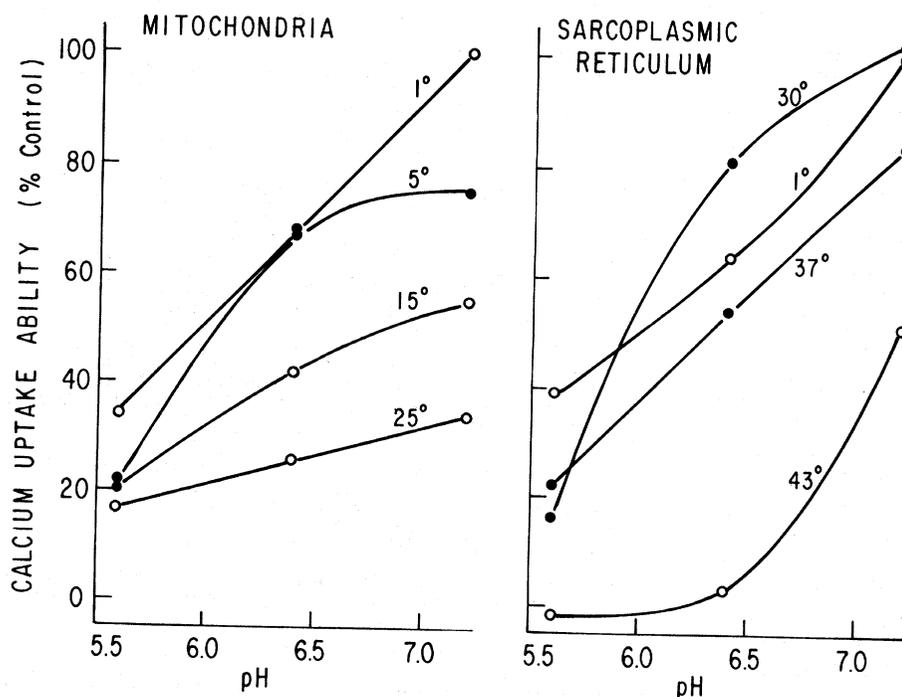


Fig. 6—Stabilities as measured by calcium uptake of sarcoplasmic reticulum and mitochondria after incubation for ½ hr at the indicated temperatures and pH values. Values are averages of *B. femoris* of two animals assayed in triplicate (○) or one animal assayed in triplicate (●). The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 2–9% and 8–30%, respectively.

shortening range may have a more marked effect on the Ca-accumulating ability of mitochondria compared to sarcoplasmic reticulum.

To study the combined effects of ATP concentration, pH, and temperature, expected postmortem values were

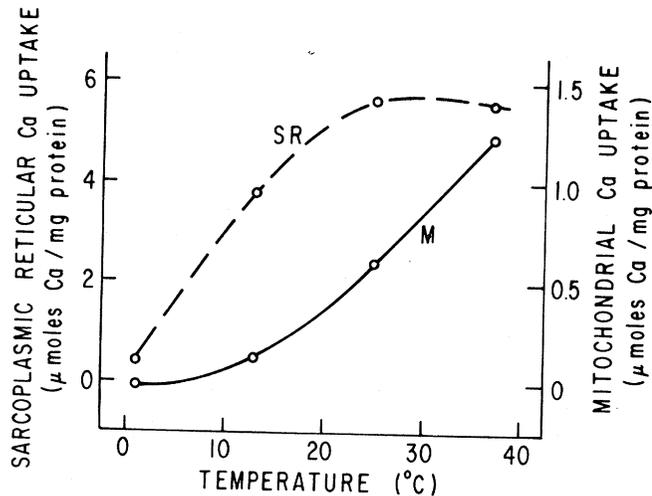


Fig. 3—Calcium uptake activities of sarcoplasmic reticulum and mitochondria with varying temperature. Values are averages of *B. femoris* muscles of three animals assayed in triplicate. The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 0.12–0.49 and 0.02–0.16 μ moles Ca/mg protein, respectively.

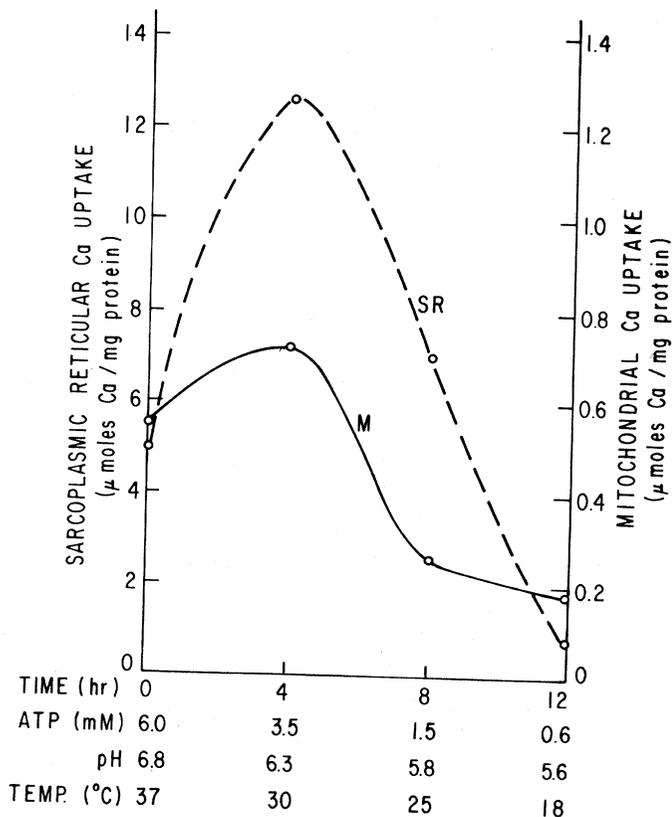


Fig. 4—Calcium uptake activities of sarcoplasmic reticulum and mitochondria at various simulated postmortem times. Values are averages of *B. femoris* of three animals assayed in triplicate. The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 0.26–0.55 and 0.05–0.10 μ moles Ca/mg protein, respectively.

selected from data reported by Tarrant and Mothersill (1977) (Fig. 4). Both organelles showed a marked increase in calcium uptake activity in an incubation media representing 4-hr postmortem from activity in the zero-hr media. The activity of the sarcoplasmic reticulum declined in 8-hr media but still remained 41% greater than at zero hr. However the mitochondria had declined to 47% of their zero-hr activity. With 12-hr media, the activity of the mitochondria was 33% of its zero hr activity while the sarcoplasmic reticular activity was 16%. The increase in activity by the sarcoplasmic reticulum at 4 hr can be attributed to a combination of pH and ATP declines (Fig. 1 and 2), apparently acting synergistically, while the mitochondrial increase is due to ATP decline (Fig. 1). The final decreases would be affected primarily by pH and temperature (Fig. 2 and 3); the ATP would still be adequate for high uptake activity (Fig. 1).

Stability of calcium uptake activities

In the previous experiments we compared the organelles' capability of functioning under postmortem conditions but not the stability or survival of calcium uptake abilities. To measure these stabilities, the organelles were subjected for ½ hr to the various treatments, immediately cooled and, after readjustment of the pH to 7.2 if altered, assayed at the standard conditions. The control organelles were held at pH 7.2 and 1°C in their suspension buffers.

The stabilities of the calcium uptake of the organelles after incubation for ½ hr at different temperatures are shown in Figure 5. The calcium uptake of the mitochondria was relatively stable at temperatures below 20°C, rapidly decreased at higher temperatures, and was virtually non-existent after ½ hr at 37°C. The calcium uptake ability of the sarcoplasmic reticulum survived until incubation temperatures were greater than 37°C, and little activity remained after ½ hr at 49°C. These data agree with results on cardiac and liver mitochondria reported by Brierley et al., (1963, 1964), Fanburg and Gergely (1965), and Carafoli and Gazzotti (1970). Sarcoplasmic reticulum has been

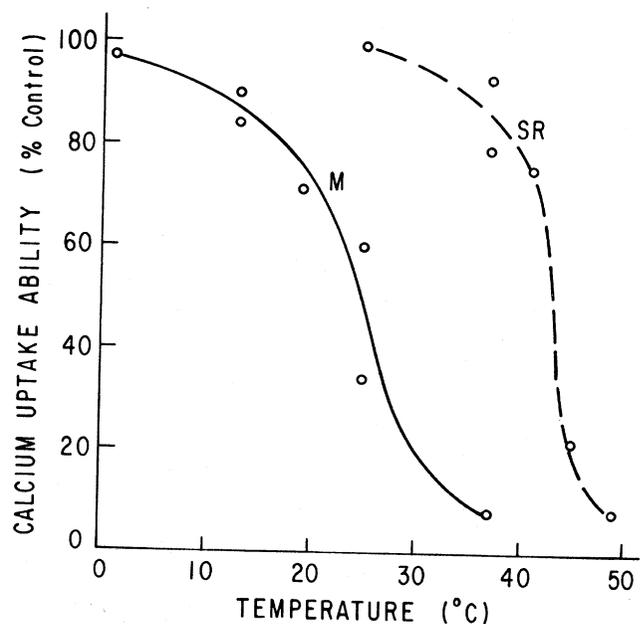


Fig. 5—Stabilities as measured by calcium uptake of sarcoplasmic reticulum and mitochondria after incubation for ½ hr at the indicated temperatures in their respective suspension buffers. Values are averages of triplicate assays. The ranges of standard errors for sarcoplasmic reticular and mitochondrial values were 1–5% and 1–6%, respectively.