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$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

where for $\delta^{13}C$, $R = {}^{13}C/{}^{12}C$; for $\delta^{18}O$, $R = {}^{18}O/{}^{16}O$; and for δD, R = D/H. The standard for carbon isotopes is Pee Dee belemnite (PDB); the standard for oxygen and hydrogen isotopes is standard mean ocean water (SMOW).
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$$\frac{({}^{18}O/{}^{16}O)_{calcite}}{({}^{18}O/{}^{16}O)_{water}} = 1.0347 = \frac{1 + \frac{15.7}{1000}}{1 + \frac{x}{1000}}$$

where x is the $\delta^{18}O$ of lee-side water.
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$$\frac{({}^{18}O/{}^{16}O)_{calcite}}{({}^{18}O/{}^{16}O)_{ice}} = \frac{1.0347}{1.003} = 1.0316$$

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25. We thank T. Coplen, W. Dansgaard, D. Ford, I. Kaplan, K. Krauskopf, M. Meier, J. Nye, R. Shreve, T. Wigley, and I. Winograd for stimulating discussions and manuscript criticism. We also thank R. Goldthwait for locating Pleistocene-age subglacial precipitate areas for us in Ohio. We especially thank J. Woodward and T. Coplen for the stable isotope analyses. We are grateful for the help of R. Frauson of the U.S. National Park Service in gaining access to the study areas. Part of this report was presented at the Second International Symposium on Water-Rock Interaction, Strasbourg, France, 17–25 August 1977.

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Excitation-Contraction Coupling in Skeletal Muscle: Blockade by High Extracellular Concentrations of Calcium Buffers

Abstract. *High concentrations (80 to 90 millimolar) of the calcium buffers EGTA and citrate (less than 10^{-7} molar free calcium ion) reversibly block excitation-contraction coupling in intact frog skeletal muscle fibers, but do not block caffeine-induced contractures. Solutions containing the same free calcium concentration but lower concentrations of calcium buffer (1 millimolar) do not block excitation-contraction coupling. These results suggest that excitation-contraction coupling requires the presence of calcium in a "protected" extracellular compartment, probably the transverse tubular network, and that calcium is actively transported into this compartment from the muscle cell cytoplasm.*

There has been considerable debate over whether extracellular calcium is essential for excitation-contraction coupling in skeletal muscle. The following proposed mechanism for excitation-contraction coupling (*1, 2*) assigns a central role to extracellular calcium: the muscle action potential depolarizes the transverse tubules, increasing their calcium permeability, and the resultant flux of calcium from the transverse tubular lumen into the myofilament space either directly or indirectly induces release of calcium stored in the terminal cisternae of the sarcoplasmic reticulum. This hypothesis fell into disfavor with the demonstration that excitation-contraction coupling persists in solutions containing no added calcium plus 1 m*M* of the calcium buffer, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) (free $[Ca^{2+}] < 10^{-8}M$) [see (*3*)].

However, we suggest that 1 m*M* EGTA does not remove all free calcium from the transverse tubular system, since diffusion within this narrow convoluted system is almost certainly restricted (*4*) and since transverse tubular membranes may actively transport calcium (*5*). We demonstrate here that higher bath concentrations (80 to 90 m*M*) of the calcium buffers EGTA and citrate (free $[Ca^{2+}] < 10^{-7}M$) do reversibly block excitation-contraction coupling in frog skeletal muscle. These results strongly suggest that excitation-contraction coupling requires the presence of Ca^{2+} in some "protected" extracellular compartment, and are compatible with the hypothesis that calcium release from the sarcoplasmic reticulum is normally dependent on calcium influx across transverse tubular membranes.

Single muscle fibers were dissected from the cutaneous pectoris muscle of small grass frogs (*Rana pipiens*). An isolated muscle fiber was impaled with two micropipettes filled with 0.2*M* K_2SO_4; one pipette injected current, the other recorded transmembrane potential. Action potentials were evoked by applying pulses of depolarizing current. In solutions where the resting membrane potential was depolarized, the depolarizing current pulses were superimposed on a steady hyperpolarizing current. Contractions of the impaled muscle fiber were monitored visually (at 600×), and were also apparent as movement artifacts in the voltage record. The normal Ringer solution contained 120 m*M* NaCl, 2.5 m*M* KCl, and 1.8 m*M* $CaCl_2$. In modifications of this Ringer solution all or part of the calcium was omitted and sodium EGTA, sodium citrate, Na_2SO_4, tetramethylammonium chloride, or sucrose was isotonically substituted for all or part of the NaCl. All solutions were adjusted to *p*H 7.2 to 7.3, using PIPES (1.4-piperazinediethanesulfonic acid) or citrate as an H^+ buffer and phenol red (5 mg/liter) as a *p*H indicator. Most solutions contained 2.5 m*M* K^+ and 0.5 g of glucose per liter. Experiments were performed at 15° to 20°C.

In agreement with earlier studies (*3*), we found that Ringer solutions containing 1 m*M* EGTA and no added calcium (free $[Ca^{2+}] < 10^{-8}M$) did not block excitation-contraction coupling in isolated muscle fibers. Contractions continued for as long as action potentials could be evoked, usually 20 to 30 minutes. However, when the concentration of EGTA was increased to 85 m*M* (an isotonic solution of sodium EGTA), or when the solution contained isotonic sodium citrate (82 to 90 m*M*), action potentials evoked by applied current were no longer followed by contractions. The solutions with high concentrations of buffer and low $[Ca^{2+}]$ depolarized the muscle fibers, and it was usually necessary to hyperpolarize the fibers before action potentials could be evoked. Figure 1 shows a muscle action potential recorded in 82 m*M* citrate; the action potential has a nearly normal amplitude (80 mV), but it evoked no visible contraction, and no mechanical artifact was evident in the voltage trace. Similar results were obtained in single fiber preparations from more than ten different muscles bathed in isotonic sodium citrate (*6*). The high [buffer]–low $[Ca^{2+}]$ solutions reduced the apparent input resistance of the muscle fibers (*7*), and solutions containing less

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than $10^{-8}M$ free Ca^{2+} eventually rendered the fibers electrically inexcitable. In these blocked fibers even intense, prolonged applied depolarizations (from a holding potential of −100 mV to +60 mV, maintained for 100 msec) evoked no discernible local contraction.

The rapidity and extent of contraction blockade varied with the concentration of the calcium buffer. For example, 90 mM citrate blocked contractions in single muscle fibers more rapidly (1 to 5 minutes) than 40 mM citrate. Contraction blockade was slower in large-diameter than in small-diameter muscle fibers and was slower in small muscle bundles than in single fibers. In whole muscles contraction blockade was incomplete even after 1 hour in isotonic sodium citrate. Contraction blockade was always faster and more extensive at 15°C than at 20°C.

Muscle fibers exposed to 85 mM citrate for less than about 30 minutes still contracted in response to 10 mM caffeine, demonstrating that the calcium buffer did not block excitation-contraction coupling solely by depleting the calcium stores of the sarcoplasmic reticulum. However, some loss of intracellular calcium probably occurred during prolonged exposure to high buffer concentrations. The persistence of caffeine contractures also demonstrates that the sensitivity of the myofilaments to calcium and their ability to contract were not abolished by the high concentration of calcium buffer.

The blockade of excitation-contraction coupling produced by high EGTA and high citrate concentrations was reversible. Excitation-contraction coupling was restored when the bathing solution was switched to normal Ringer, or when extracellular $[Ca^{2+}]$ was raised to $10^{-5}M$ or above by adding calcium [18 mM, as $Ca(OH)_2$] to the citrate solutions. The latter result demonstrates that both the total calcium buffer concentration and the free bath $[Ca^{2+}]$ influence excitation-contraction coupling. In muscles exposed to isotonic citrate for 20 minutes or less, excitation-contraction coupling returned transiently even in solutions containing 0.1 to 1 mM EGTA (free $[Ca^{2+}] < 10^{-8}M$). Coupling was restored in these low $[Ca^{2+}]$ solutions even though muscle fibers still had depolarized resting potentials and low input resistances, which indicates that the original blockade of coupling was not due simply to the altered electrical properties of the membrane. The fact that even low $[Ca^{2+}]$ solutions could restore coupling is a further indication that high citrate concentrations did not block excitation-contraction coupling by exhausting intracellular calcium stores or by irreversibly damaging structures involved in coupling (such as transverse tubules).

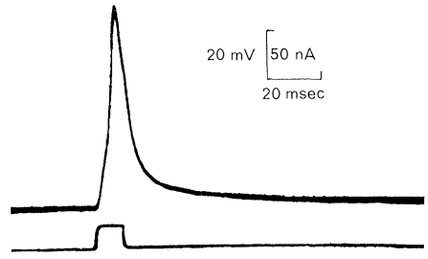

Fig. 1. Action potential (upper trace) evoked by a depolarizing current pulse (lower trace) in an isolated skeletal muscle fiber bathed in 82 mM sodium citrate plus 0.5 mM $CaCl_2$ (free $[Ca^{2+}] < 10^{-6}M$). The depolarizing current pulse was superimposed on a steady current that hyperpolarized the membrane potential to −80 mV compared to the resting value of −40 mV. Calibrations: 20 mV, 50 nA, 20 msec; 15°C. No sarcomere movement (monitored at a magnification of 600 ×) accompanied this action potential. Evoked contractions returned transiently for about 5 minutes when the citrate solution bathing this fiber was replaced by a solution containing 1 mM EGTA and no added calcium. Similar blockade of excitation-contraction coupling also occurred in isotonic sodium EGTA, but resting potentials were more variable and action potentials more prolonged.

The demonstrations that calcium buffers (Fig. 1) and manganese (*8*) reversibly block excitation-contraction coupling in skeletal muscle are consistent with the hypothesis that physiological release of calcium from the sarcoplasmic reticulum requires an influx of calcium. Further evidence that calcium influx can (directly or indirectly) induce internal calcium release in intact skeletal muscle is the slow waves of contraction we frequently observed in solutions containing high concentrations of calcium buffer. Isolated muscle fibers often remained adjacent to other muscle fibers near their insertions, and these end regions often continued to twitch in response to propagated action potentials after excitation-contraction coupling in the isolated central region of the muscle fiber had been blocked. Following these end twitches, a slow wave of contraction, unaccompanied by an action potential, often traveled toward the center of the muscle fiber at a rate of 10 to 100 μm/sec. Similar action potential–independent waves of contraction, attributed to a calcium-dependent internal release of calcium (*2*), have been observed in skinned (*9*) and intact (*10*) cardiac muscle fibers bathed in low $[Ca^{2+}]$ solutions.

The observation that high concentrations of calcium buffer can block excitation-contraction coupling, while low concentrations cannot, suggests that the extracellular calcium essential for coupling is in a protected compartment, probably the transverse tubular network. The narrow external openings of this network (*4*) would slow diffusion between tubules and bath. Diffusion of calcium out of the transverse tubular network would be slowed even further if these openings, or the diffuse "ground substance" that fills them (*4*), contained fixed positive charges. Such fixed charges would also tend to exclude external cations, especially those with a large charge-to-mass ratio, from the tubular lumen, perhaps explaining why the powerful calcium antagonists lanthanum and manganese block excitation-contraction coupling only at relatively high concentrations [0.5 to 1 mM La^{3+} and 10 to 20 mM Mn^{2+} (*8*)]. A loose meshwork of positive charges would have less effect on cations with a smaller charge-to-mass ratio and would not retard neutral or negatively charged molecules; this is compatible with the observations that K^+, horseradish peroxidase, and large anions such as ferritin and the dye lissamine rhodamine B200 move into the transverse tubular network (*11*).

We suggest that calcium is transported from the muscle cytoplasm into the transverse tubular lumen, and that high bath concentrations of calcium buffers (80 to 90 mM) deplete this luminal calcium and, hence, block excitation-contraction coupling by creating within the transverse tubular network a steep gradient of buffer concentration which removes calcium from the tubules faster than it is pumped into the tubules. Reducing the bath buffer concentration would reduce this gradient and thus reduce the rate of calcium removal, accounting for the persistence of excitation-contraction coupling in 1 mM EGTA or citrate. If calcium is actively transported into the transverse tubular network, then tubular $[Ca^{2+}]$ could remain considerably higher than bath $[Ca^{2+}]$, the discrepancy depending on such variables as total buffer concentration, bath-free $[Ca^{2+}]$, and fiber size. The observation that excitation-contraction coupling is more readily blocked at 15°C than at 20°C might be explained by a slower rate of calcium transport into the transverse tubules at the lower temperature.

We do not yet know why transverse tubular calcium is essential for excitation-contraction coupling. One possibility is that transverse tubular membranes are especially sensitive to calcium depletion, such that in low $[Ca^{2+}]$–high [buffer] solutions their permeability

(and thus their electrotonic length) increases so much that deep tubular membranes are, in effect, electrically isolated from the surface membrane. While this effect may occur to some extent, indirect evidence suggests that it is not sufficient to explain the observed blockade of excitation-contraction coupling. For instance, citrate-blocked muscle fibers did not show even local surface contractions in response to action potentials or intense applied depolarization. Also, the action potential evoked in citrate-blocked muscle fibers (Fig. 1) still shows an afterdepolarization, a feature attributed to transverse tubular depolarization which totally disappears in muscle fibers whose transverse tubules have been dissociated from the surface membrane by glycerol treatment (*12*). We think it more likely that transverse tubular calcium contributes to excitation-contraction coupling by crossing depolarized tubular membranes and directly or indirectly enhancing release of calcium from the terminal cisternae. In this regard it is of interest that hypertonic solutions, which also block excitation-contraction coupling, block calcium influx in crab muscle fibers (*13*). Alternatively, tubular calcium may contribute to excitation-contraction coupling by permitting the activation of some other calcium-releasing mechanism.

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7. The reduction in the measured input resistance in low $[Ca^{2+}]$ solutions was due primarily to current leakage around the sites where the electrodes penetrated the muscle membrane; local microperfusion of this small region with a solution containing normal $[Ca^{2+}]$ restored the input resistance (measured at −80 mV) to normal values and frequently restored the resting potential as well.
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Flake Tools Stratified Below Paleo-Indian Artifacts

Abstract. *In northwest Missouri, Lithic stage flake tools struck from prepared cores have been excavated underlying a Paleo-Indian fluted point assemblage. These assemblages were in two different loesses of the last glaciation. Thermoluminescent analysis of stone tools dates the Paleo-Indian occupations at 8690 ± 1000 B.C. and 12,855 ± 1500 B.C.; the Lithic stage occupations must be older than 13,000 B.C. on the basis of geologic correlation, lithic analysis, and cultural stratigraphy.*

Archeological excavations (*1*) conducted in 1975 at the Shriver site (23DV12) in Daviess County, Missouri (39°58′30″N, 94°5′45″W) (Fig. 1), have recovered stratified remains of a Woodland assemblage and a Paleo-Indian fluted point assemblage overlying a lower stratum of earlier cultural remains. These lowermost artifacts were manufactured predominantly from flakes struck from discoidal-shaped prepared cores, by a technique reminiscent of the Old World Levalloisoid technique. The recovery of such a flake-tool assemblage, deemed an important research goal (*2*), provides important information needed to delimit the early prehistory of North America (*3*) prior to the Paleo-Indian Period (*4*).

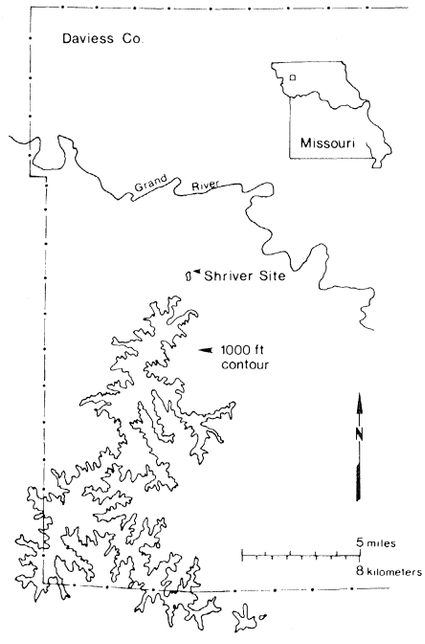

Fig. 1. Location of the Shriver site (23DV12) on a remnant of a Kansan-age till plain.

The Shriver site is located in an open-ground upland setting on a remnant of the eroded Kansan-age till plain (Fig. 1). The site sporadically but recurrently attracted early American inhabitants, probably in part because of a spring just 40 m away and the location of the site at the apex of a spur overlooking the Grand River Valley. Artifacts lie in the uppermost sedimentary units composed of deeply weathered loess.

A research approach including comparative geology, soil analysis, and palynology attempted to facilitate understanding of the geologic and cultural stratigraphy. Comparisons with known regional geologic profiles indicated that several loess depositions had occurred. Soil analysis, incorporating comparison of sand and clay size particle distributions and studies of phosphorus (P_2O_5) and potassium (K) patterns, indicates that the loess at the site could be separated into two deposits. The boundary of these deposits was an erosional surface ±40 cm deep. On this basis, site stratigraphy includes several sedimentary units tentatively identified by stratigraphic correlation as (i) unit 1, Bignell loess 8,000 to 13,000 years before the present (B.P.); (ii) unit 2, Peorian loess, 13,000 to 18,000 B.P.; (iii) unit 3, other glacial and erosional sediments; and (iv) unit 4, Upper Pennsylvanian bedrock. Amounts of fossil pollen preserved were inadequate to permit interpretation of a pollen column.

Cultural stratigraphy includes three

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