IDENTIFICATION OF OXALATE PRECIPITATES IN STRIATED MUSCLE FIBERS

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The location of calcium in muscle fibers is of interest, since the contraction-relaxation cycle is controlled by intracellular movements of this ion (Ebashi and Lipmann, 1962; Hasselbach and Makinose, 1963; Weber et al., 1963; Podolsky and Costantin, 1964). Treatment of the cell with oxalate produces electron-opaque deposits localized in the terminal sacs of the sarcoplasmic reticulum (SR) that have been assumed to be calcium oxalate (Costantin et al., 1965). Although this interpretation is consistent with the observation that the deposits are more abundant in preparations that have been loaded with calcium ions before treatment with oxalate, direct evidence that the precipitate contains calcium is still lacking. In this connection it is worth pointing out that muscle fibers contain, in addition to calcium, appreciable amounts of other metals that form oxalate precipitates in vitro and could possibly give rise to oxalate deposits in the cell, particularly if the ion were concentrated in the SR (Table I). It seemed worthwhile, therefore, to determine the calcium content of the precipitates resulting from oxalate treatment of the normal, unloaded cell. This was done in the present study by spectroscopic analysis of the X-rays generated by bombardment of the deposits with an electron microprobe (Anderson, 1967; Hall, 1968; Hall and Höhling, 1969).

MATERIALS AND METHODS

Small bundles of fibers were dissected out of the semitendinosus muscle of the frog, *Rana pipiens*, and covered with paraffin oil. A segment of a single fiber was separated from the bundle and the sarcolemma was dissected away. The surface of the "skinned" fiber was then perfused with about 1 mμl of oxalate solution (10 mM sodium oxalate, 140 mM KCl) over a length of about 200 μ. The fibers were prepared for electron microscopy as described previously (Costantin et al., 1965) and embedded in Maraglas (Polysciences, Inc., Rydal, Pa.). Sections of about 2000 A thickness were mounted on carbon-coated grids. The deposit content of the sections was checked by electron microscopy, and some characteristic areas were photographed (Fig. 1). The properties and localization of the deposits have been described in detail elsewhere (Costantin et al., 1965).

Sections containing deposits were analyzed in a combination electron microscope-microanalyzer (Duncumb, 1966). In this instrument the section is viewed by means of the conventional transmission electron image, and the electron-illumination is then focused into a microprobe. The operating voltage was 40 kv, and the probe diameter was kept in the range 0.2–0.3 μ. X-ray spectroscopic analysis was performed with a lithium fluoride diffractor.

RESULTS

Data from three sections are given in Table II. When the probe was on a deposit, a strong signal was detected at the Bragg angle for calcium. When the probe was moved off the deposit without change in size or intensity, the count at the calcium Bragg angle generally dropped to close to the background level, so that the calcium concentration away from the deposit was clearly much less than at the deposit. Fields adjacent to one of the

TABLE I

Precipitability of Metals in Frog Muscle Fibers by Oxalate

	Ca	Mg	Zn
Average concentration, C (mM)*	1.5	7	0.2
Solubility product, K (mM^2)‡	1.8×10^{-3}	86	1.3×10^{-3}
Oxalate concentration required for precipitation, K/C (mM)§	0.0012	12	0.007

* Bianci, 1968. p. 16.

‡ For the oxalate salt.

§ Calculated on the assumption that the metal is ionized and uniformly distributed in the cell.

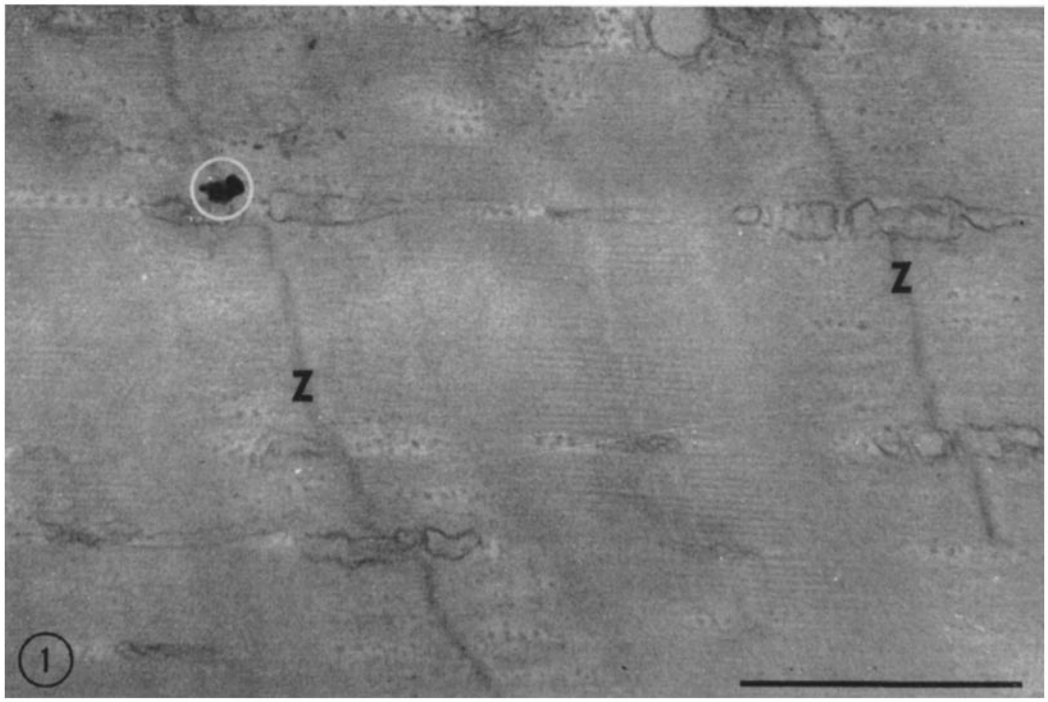

FIGURE 1 Section of oxalate-treated, skinned frog muscle fiber showing typical dense deposit associated with the terminal sacs of the sarcoplasmic reticulum. Sections were not stained since this procedure often dissolved away the deposits; two *Z* lines are marked for orientation. The diameter of the white circle is the minimum probe diameter. Calibration bar, 1 μ. × 37,000.

deposits in Section B gave a small residual calcium signal (measurements 8 and 10), which could have come from diffusely precipitated calcium not visible in the electron microscope.

Zinc was also sought in the precipitates, and no signal was detected (measurements 11 and 12). The sensitivity of the analysis for zinc in absolute terms is similar to that for calcium, but the background is higher at the zinc Bragg angle. The data in Table II show that the deposits do not contain zinc in an amount as large as the amount of calcium present, but that the zinc content of the deposit would not be detectable if the ratio of zinc to calcium were as small as that of the total amounts of these metals in the cell (Table I).

The estimated mass fraction of calcium in fields

TABLE II

Detection of Calcium in Fiber Section

Sections A, B, and C were analyzed both at, and adjacent to, oxalate deposits. *Spectrometer count* is either cps taken from a count-rate meter (measurements 1–6), or recorded count, or average count, in 40 sec (measurements 7–15), with the LiF crystal at the Bragg angle for calcium (*peak, P*), or just offset from this angle (*background, B*); in measurements 11 and 12 the crystal was set at the zinc Bragg angle. Numbers in parentheses give total number of observations where these were repeated, with peak and background alternated to remove the effect of accumulating contamination. *Estimated mass fraction* is taken as the product of the ratio (P–B)/B and 0.07, a proportionality factor derived empirically for the instrumentation used here, based on the use of brehmsstrahlung as a measure of mass per unit area; the estimate is probably good to within a factor of 2 or 3; n.d. means that element was not detected.

Date	Measurement	Field	Spectrometer Count		$\frac{P-B}{B}$	Estimated mass fraction
			Peak, P	Background, B		
						%
31 vii 1968	1	Deposit 1 on A	26	5	4	0.3
	2	Deposit 2 on A	6 (2)	1		
	3	Next to field 2	1	1	0	n.d.
	4	Same as field 2	6	1		
		Average for deposit 2 on A			5	0.3
1 i 1969	5	Deposit 1 on B	4.5	0.5	8	0.6
	6	Next to field 5	0.5	0.5	0	n.d.
	7	Deposit 2 on B	152	16		
	8	Next to field 7	24 (2)	12 (2)	1	0.07
	9	Same as field 7	165	25		
		Average for deposit 2 on B			7	0.5
	10	Next to field 9	23	12 (2)	1	0.07
	11	Deposit 2 on B (Zn)	148	150	0	n.d.
	12	Next to field 11 (Zn)	121	125	0	n.d.
	13	Deposit 1 on C	53 (3)	16 (2)		
	14	Next to field 13	27 (4)	21 (3)	0	0
	15	Same as field 13	45	24		
		Average for deposit 1 on C			1.5	0.1

containing deposits is of the order of 1% (Table II, final column). Since (*a*) the density of the embedding material is 1 g/cm^3, and (*b*) the volume assayed by the probe is 2×10^{-14} cm^3, the mass of calcium in the field is 2×10^{-16} g. If this calcium were contained in a spherical crystal of calcium oxalate, the diameter would be about 0.1 μ, which is close to the average diameter of the deposits (Fig. 1). It is possible, therefore, to account for the observed size of the deposits on the basis of their calcium content, which suggests (but, as the data are only approximate, does not prove) that other elements are not present in major amounts.

CONCLUSION

The deposits found in the terminal sacs of the SR in muscle fibers following treatment with oxalate contain a calcium salt. The amount of calcium estimated to be present in a deposit is in reasonable agreement with that in a calcium oxalate crystal of the same size. These results are consistent with the view that the terminal sacs of the SR are specific regions of calcium ion accumulation within the cell (Costantin et al., 1965; Winegrad, 1965).

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