Calcium Uptake by Isolated Sarcoplasmic Reticulum Treated with Dithiothreitol

Abstract. *Isolated vesicles of the sarcoplasmic reticulum are known to take up calcium when provided with magnesium adenosine triphosphate as an energy source. Preparations of high activity are obtained by keeping the vesicles in 5 millimolar dithiothreitol (a reagent that reduces disulfide groups), and these preparations retain activity for a week or longer. The highly active preparations lend themselves to a spectrophotometric method for following calcium uptake, and continuous uptake kinetics are readily obtained. Calcium uptake appears to follow Michaelis-Menten kinetics* ($K_m = 8 \times 10^{-6}$; $V_{max} = 7 \times 10^{-7}$ *mole per second per milligram of protein). These preparations are also useful for studying the effects of inhibitors of uptake, such as quinine. When extrapolated to the intact muscle, the results from these isolated vesicles should give a better estimate than has been available of the actual rates of calcium uptake and of the physiological effect of inhibitors of uptake.*

Muscle contraction is triggered by the release of Ca^{++} into the sarcoplasm. It is ended when the Ca^{++} is sequestered by the longitudinal sarcoplasmic reticulum (*1*). Isolated vesicles —presumably derived from the sarcoplasmic reticulum—take up Ca^{++} when supplied with Mg^{++} and adenosine triphosphate (ATP) (*2*). However, the vesicle preparations lose activity quite rapidly; new preparations must be made frequently and it is difficult to study inhibitors of Ca^{++} uptake on a deteriorating system. The vesicles can be stabilized somewhat by suspending them in 45 percent sucrose, which probably acts by inhibiting lysosomal enzymes that destroy the vesicles (*3*).

Table 1. Initial Ca^{++} uptake rates by vesicle preparations. For experimental conditions see the legend to Fig. 1. With the less active preparations protein concentrations were increased to give more accurate readings. The uptake rate is expressed in moles per second per milligram of protein.

Day after isolation	Uptake rate	
	Vesicles stored in DTT	DTT added before assay
1	63×10^{-8}	3.3×10^{-8}
2	63×10^{-8}	1.0×10^{-8}
10	54×10^{-8}	0
16	5×10^{-8}	0

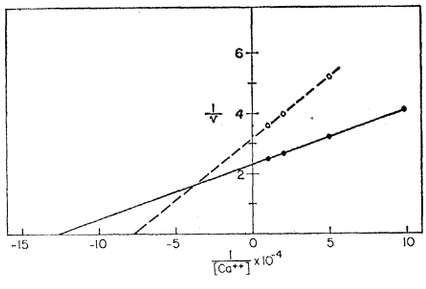

Fig. 1. A plot of the reciprocal of the initial velocity (v) of uptake (arbitrary units) as function of the reciprocal of $[Ca^{++}]$. ●——●, Control; ○— —○, in the presence of $10^{-3}M$ quinine sulfate. The spectrophotometer cell initially contained 2.5 ml of a solution of 20 mM histidine (pH 7.0), 30 mM KCl, 5 mM K oxylate, 5 mM $MgCl_2$, and 5 mM ATP, and 1.6 μg of vesicle protein, all at room temperature. At time 0, 100 μl of $CaCl_2$ solution was added, bringing the $[Ca^{++}]$ to 0.1 mM. The change in optical density at 375 nm was recorded. Initial uptake rate was estimated by measuring the slope.

Sulfhydryl groups are important in the Ca^{++} uptake system (*4*). Acting on a suggestion by Professor H. M. Levy, dithiothreitol (DTT) was tested because it is an extremely effective reagent for preventing and reversing sulfhydryl oxidation (*5*). Vesicles were isolated by the differential centrifugation of homogenates of lobster muscle (*6*). Most experiments were performed in the fraction sedimenting between 8,000 and 16,000g. Once spun down, the vesicles were suspended in a mixture of 0.3M sucrose and 0.005M histidine (pH 7.0) or in the same solution containing 5 mM DTT and stored at 4°C. Uptake of Ca^{++} by the vesicles was measured by the spectrophotometric method of Fairhurst and Jenden (*7*), with occasional checks by the use of solutions containing ^{45}Ca and by removing the vesicles by Millipore filtration. The results of assays on different days following the isolation of the vesicles are shown in Table 1. Just before the assay DTT was added to the DTT-free preparation to a final concentration of 5 mM. One day after preparation, the vesicles stored in DTT took up Ca^{++} 19 times faster than the controls. The activity of the vesicles kept in DTT remained virtually unchanged for 10 days; during this period the control sample lost all activity. Clearly, DTT is a useful and effective reagent for preserving the Ca^{++} uptake in vesicle preparations, although it has no effect on restoring activity to deteriorated preparations.

The high rate of Ca^{++} uptake by vesicles stored in DTT greatly facilitates the use of the spectrophotometric method, because the concentration of vesicles can be kept low. Then there is only a slight absorbance of light at 375 nm before Ca^{++} uptake begins, which enhances the sensitivity of the measurements. The initial rate of uptake can be estimated at different concentrations of calcium. As shown in Fig. 1, Ca^{++} uptake appears to follow Michaelis-Menten kinetics ($K_m = 8 \times 10^{-6}$; $V_{max} = 7 \times 10^{-7}$ mole/sec per milligram of protein). The fastest reported uptake rate of vesicles not kept in DTT is about 1.5×10^{-8} mole/sec per milligram of protein from a solution containing 40 μM Ca^{++} (*8*). By extrapolation, the line in Fig. 1 shows that the initial uptake by DTT-preserved vesicles from a solution containing 40 μM Ca^{++} would be 39×10^{-8} mole/sec per milligram of protein. Sarcoplasmic reticulum is more active in transporting calcium than has hitherto been realized.

Inhibitors can easily be studied by the same methods. Quinine appears to inhibit both competitively, by lowering the binding constant for Ca^{++}, and noncompetitively, by decreasing the maximum uptake rate (Fig. 1). These data can be used to extrapolate to the physiology of intact muscle. In crab muscle at rest, the concentration of Ca^{++} ($[Ca^{++}]$) in the sarcoplasm is about $1 \times 10^{-7}M$. Contraction occurs when $[Ca^{++}]$ rises between 3- and 15-fold (*9*). There are about 2 mg of vesicles per gram of fresh muscle (*6*). In an intact muscle sarcoplasmic Ca^{++} could be reduced from $10 \times 10^{-7}M$ to $1 \times 10^{-7}M$ within 14 msec (*10*). In the presence of $10^{-3}M$ quinine the uptake of Ca^{++} would take about 30 msec, so the active state should be prolonged and there should be an increase in twitch tension; in fact quinine is known to potentiate contraction (*11*).

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10. In these calculations the passive efflux of Ca^{++} back-out from the vesicles is ignored. This outflux would slow the fall in the $[Ca^{++}]$ in the sarcoplasm but would not change the overall conclusion. For a further discussion see (*6*).
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LSD: Autoradiographic Study on the Placental Transfer and Tissue Distribution in Mice

Abstract. *^{14}C-lysergic acid diethylamide administered intravenously passed in a few minutes from the blood into the tissues. In addition to the brain, the adrenals, hypophysis, kidneys, liver, and lungs showed the highest uptake, much higher than the blood concentration. Excretion into the bile started immediately; this was the most important elimination route. In the early stage of pregnancy, 2.5 percent (and in the late stage, 0.5 percent) of the radioactive dose passed the placental barrier into the fetus in 5 minutes. Over 70 percent of this fetal radioactivity was unchanged ^{14}C-lysergic acid diethylamide.*

Lysergic acid diethylamide (LSD) may lead to chromosome damage (*1*) and may have teratogenic properties. Increased rates of abortion and malformations in fetuses have been reported after administration of LSD in early pregnancy in mice (*2*), rats (*3*), hamsters (*4*), and rabbits (*5*). The possible teratogenic effect on two human embryos has been suggested (*6*).

Lysergic acid diethylamide seems to stimulate adrenal activity, to lower metabolism, and to inhibit both thyroid and gonadal function (*7*). Despite the many studies on the behavioral and pharmacological actions of this agent, its kinetics and distribution in the body have had little study (*8*). We used an autoradiographic method to study the transplacental penetration, tissue distribution, and the rate and route of excretion of ^{14}C-LSD in the whole body of mice.

d-Lysergic acid diethylamide tartrate (Sandoz Pharmaceuticals, Hanover, New Jersey), labeled with ^{14}C in the side chain (specific activity, 5.96 μc/mg, 0.5 mg/ml) was concentrated under a stream of nitrogen gas in a water bath (70°C) to 1.7 mg/ml. Its chemical and radiochemical purity was determined with thin-layer chromatography (silica-gel G). Two-way chromatograms, developed first in chloroform and acetone (1:4) and then in methanol, had one spot with an R_F corresponding to that of authentic LSD. Ultraviolet light, fluorescent light, van Urk's reagent (*9*), and autoradiography were used to detect ^{14}C-LSD on the plates.

Four male (20 to 22 g) and six pregnant female mice (Yale Swiss) were injected intravenously with ^{14}C-LSD (19.8 and 9.9 μg/g, respectively). Three females (28 to 32 g) were in the first trimester of pregnancy, and the others (44 to 50 g) were in the last week of pregnancy. The pregnant mice were killed 5, 30, 60, and 120 minutes after injection, by being dropped into hexane cooled to about −70°C with solid carbon dioxide. The male mice were killed at 5 and 30 minutes and 6 and 24 hours after injection. Sagittal sections (30 to 60 μ), through the whole frozen animal, were cut with a model "K" microtome (R. Jung AG, Heidelberg) in a cold room (−10°C). The specimens were picked up with Scotch tape and dried for 24 hours at −10°C (*10*). The sections were then pressed onto Kodak RP/S X-omat medical x-ray film and exposed for 22 to 24 days. Radioactivity in the various animals and tissues was measured (*11*). Fetuses were first homogenized in five volumes of methanol. Unchanged ^{14}C-LSD was then

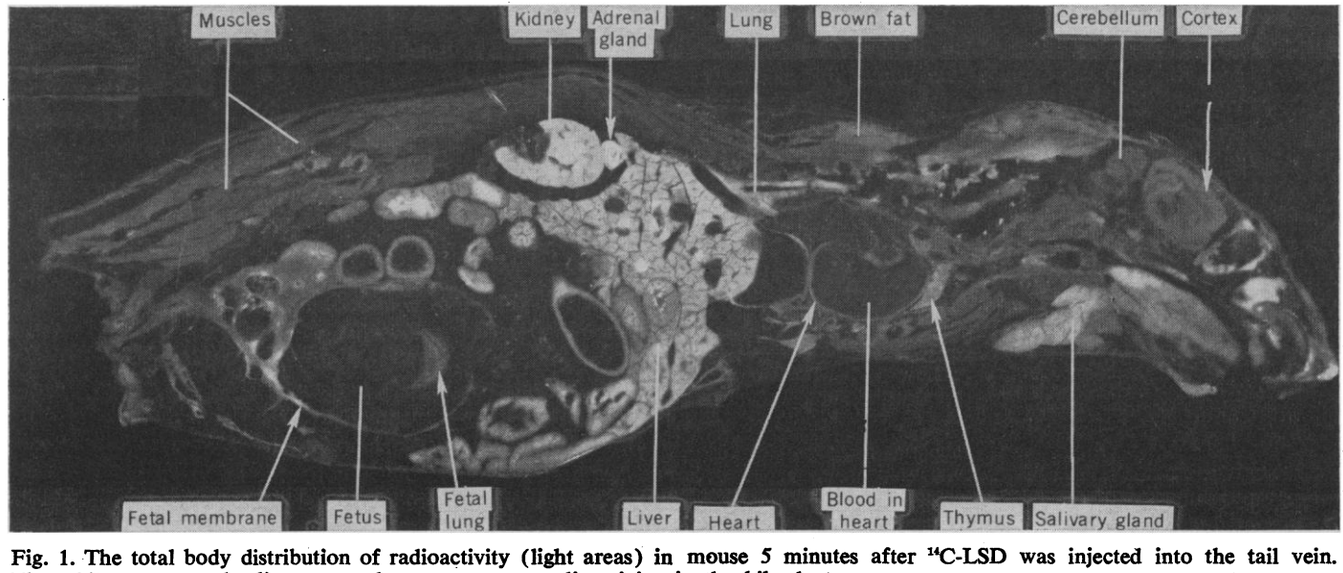

Fig. 1. The total body distribution of radioactivity (light areas) in mouse 5 minutes after ^{14}C-LSD was injected into the tail vein. The white spots on the liver parenchyma represent radioactivity in the bile ducts.