

Specific Uncoupling of Excitation and Contraction in Mammalian Cardiac Tissue by Lanthanum

Kinetic studies

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ABSTRACT Arterially cannulated rabbit interventricular septal tissue was exposed to 5–40 μM La in 2.5 mM Ca perfusate. Immediately following perfusion with La two concurrent events were consistently observed: (a) a rapid decline of active tension to a lesser steady-state value, and (b) an abrupt, release of short duration of tissue-bound Ca. The magnitude of both events was directly related to the $[\text{La}]_o$. If the duration of exposure to La was brief, contractility returned toward normal upon return to the La-free perfusate. Elevation of $[\text{Ca}]_o$ during exposure to La counteracted its effect and induced a concurrent displacement of tissue-bound La. Cellular action potentials recorded during brief perfusion with La demonstrated that essentially normal regenerative depolarization was maintained. Analysis of the quantities of ^{45}Ca released following exposure to 10 μM La indicated that this La-susceptible Ca was being displaced from a homogeneous pool—the one previously shown by Langer to represent contractile dependent Ca. These data led to the following conclusions: During perfusion with 2.5 mM Ca contractile dependent Ca was derived primarily from “superficially” located sites. La effected the release of contractile dependent Ca by modifying the normal permselectivity of this “superficial” membrane for activator Ca. These and other data infer that contractile dependent Ca is derived primarily from superficially located sites.

INTRODUCTION

The role of Ca in the excitation-contraction coupling process is well-established (1–6). This statement amounts to a generalization and does not explain the cyclic movement of Ca from twitch to twitch. For example, the twitch

responses of cardiac and fast skeletal muscle differ markedly when these muscles are subjected to identical interventions (7–10). That a surface-released substance in skeletal muscle fiber could not be expected to serve as an activator of contraction was pointed out by Hill (11) in 1949. The diffusion distances in heart muscle fibers, however, are sufficiently short so that no internal releasing site need be postulated. The relevant question is, where does contractile dependent Ca reside in cardiac muscle?

Winegrad and Shanes (12) in 1962 and Niedegerke (13) in 1963 have extensively studied the kinetics of ^{45}Ca exchange in actively beating guinea pig atria and frog ventricle, respectively. (Note that in mammalian atrial tissue there is a paucity of T tubules and the sarcoplasmic reticulum is located almost exclusively at the cell periphery (14) and that in frog ventricle T tubules are absent and there is a paucity of sarcoplasmic reticulum (15), whereas in the mammalian ventricle there are abundant T tubules and a well-developed intracellular sarcoplasmic reticulum.) Winegrad and Shanes and Niedegerke concluded that contractile dependent Ca was manifested kinetically as a homogeneous fraction. The location of this Ca was identified as being extracellular rather than intracellular. Langer (16) in 1964 demonstrated that each ^{45}Ca washout curve obtained from perfused dog papillary muscle (5.0 mM Ca) could be resolved into a minimum of five phases—in his notation phases 0 to 4. Langer proposed the following phase assignments for the tissue Ca fractions: phase zero, vascular space; phase 1, interstitial space; phase 2, contractile dependent Ca; phase 3, intracellular, perhaps mitochondrial; and phase 4, connective tissue. He consistently observed that when low $[\text{Na}]_o$ perfusion and elevated contraction rate (rate staircase) increased twitch tension there was a net gain in tissue Ca. Analysis of washout curves from muscles responsive to these interventions demonstrated that this extra Ca was always localized to phase 2. Dependent upon the degree of increased twitch tension as much as 1 mmole Ca/liter tissue H_2O could be added to a normal phase 2 content of 1.7 mmoles Ca/liter tissue H_2O (16).

Although Langer and coworkers (17)¹ have demonstrated the kinetic homogeneity of contractile dependent Ca (i.e., the rate constant of a single kinetic phase, phase 2, is sufficient to describe its exchange with the perfusate) in dog papillary muscle, rabbit ventricle, and frog ventricle, the morphological location of this Ca fraction remains obscure. Langer (3) has stated that the evidence for release of Ca from the sarcoplasmic reticulum in cardiac tissue is circumstantial. It is possible that the sarcoplasmic reticulum acts solely as the site of Ca sequestration (3) (Langer's alternate hypothesis) or that it possesses the capability to perform both functions. To date there has been no definitive research which would clarify the issue. (However, see references 29 and 30 for a definitive examination of the situation in skeletal muscle.) If a substance

¹ Sopsis, J. A. Calcium kinetics in frog ventricular muscle. Manuscript submitted for publication.

could be found that would displace contractile dependent Ca, is biologically compatible (i.e., nontoxic to the tissue in general), and is electron-dense as well, it could be used to displace all or part of this contractile dependent Ca and possibly serve as its marker.

Nayler and Anderson (10) and Ciofalo and Thomas (7) demonstrated that Zn below 0.5 mM reversibly diminished tension in actively contracting cardiac tissue. Since cellular electrical activity was essentially normal, interference of excitation-contraction coupling was suspected. Both groups showed that Ca loss followed tissue exposure to Zn. Takata, Pickard, Lettvin, and Moore (18) investigated Na and K conductance in lobster axon bathed in Ca-free lanthanum (La) seawater. They selected a $[La]_o$ of 11 mM and observed that both g_{Na} and g_K were markedly reduced. They concluded that La acted like "super-calcium" because a $[Ca]_o$ of 210 mM would have been required to duplicate these La-induced effects. Takenaka and Yumoto (19) published a brief paper 2 yr later (1968) in which they discussed data recorded from crayfish giant axons perfused with Ca-free La seawater. They were aware of the Takata et al. paper and hypothesized that if La in lower concentrations were substituted for Ca, the conductance properties of the membrane would remain normal. Takenaka and Yumoto reported that so long as the $[La]_o$ remained between 0.5 and 3 mM essentially normal action potentials were recorded. Mines (20) in a paper published in 1910 discussed the effect of La on perfused frog heart. His twitch tension records clearly demonstrated the inhibitory effect of Y, La, and Ce in concentrations ranging from 0.5 to 10 μ M. In discussing the reversibility of the effect on tension he stated that contractility returned rapidly if the exposure were brief. Thus the expectations were that La in micromolar concentrations would affect the contractile response of the mammalian ventricle as it did the frog ventricle and that, at these low concentrations, La would not impair the electrical excitability of the cell more than did Zn. Preliminary experiments on rabbit interventricular septa and papillary muscle confirmed these expectations and we were, therefore, encouraged to investigate the matter further to determine whether the interruption of excitation-contraction could be due to an unfavorable modification of the Ca permselectivity of the regulating membrane.

METHODS

Adult New Zealand white rabbits of either sex, weighing 3–4 kg, were killed by an overdose injection of heparinized (400 units/ml) sodium pentobarbital. Excision of the heart, cannulation of the septal artery, and perfusion of the interventricular septal wall followed immediately and required from 3.5 to 4.5 min for completion.

Tissue mounting, recording of isometric tension, and perfusate temperature control varied slightly to suit individual preference but were technically equivalent to those methods described by Langer and Brady (21).

Standard perfusate for all but the initial exploratory experiments consisted of the following (mM): NaCl, 140; KCl, 4.0; $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 2.5; $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 1.0; glucose, 5.56; and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (22) (HEPES, Calbiochem, Los Angeles, Calif.), 3.0. After all the compounds had been dissolved in distilled, deionized water the solution was filtered through a sintered glass filter (10 μ pore diameter) and titrated with 3 M NaOH to pH 7.2–7.4. Perfusate prepared in this way was stored in a refrigerator and aliquot portions drawn as needed for weekly experiments. Although the solution maintained excellent pH stability, unused batches were discarded after 4–6 wk of cold storage. HEPES was substituted for HCO_3^- to guard against formation of the insoluble compound $\text{La}_2(\text{CO}_3)_3$. Not only is the pK_a adequate for physiological buffering ($\text{pK}_a = 7.55$ at 20°C) but also Good et al. (22) were able to demonstrate that HEPES is essentially inert, measured by the Hill reaction (ferricyanide reduction by illuminated spinach chloroplasts) and by succinate oxidation by bean hypocotyl mitochondria.

For these experiments the perfusion system consisted of a high pressure supply of 100% O_2 , a constant pressure regulator (0–10 psi differential pressure), a gas humidifier, a manifold feeding up to four reservoir bottles, and various valves to direct and control the flow of gas and perfusate. Previously oxygenated solutions of specified composition were stored in the reservoir bottles which were constantly bubbled with 100% O_2 throughout an entire experimental period. Perfusion pressures normally ranged between a calculated 90 and 150 mm Hg at the cannula tip. Dependent upon the degree of vascular resistance of a septum, flow rates varied between approximately 1 and 2 ml per min per g wet tissue. Pressure as high as 190 mm Hg could be tolerated for brief periods, but pressures of 200 mm Hg or greater caused immediate and permanent loss of contractile tension. The mean (\pm SE) per cent tissue water for 28 septa was 83.3 ± 0.3 .

The method of collecting and counting the ^{45}Ca effluent has been described by Langer and Brady (21). Because of the higher rates of perfusate flow in this study, compared to those selected by Langer for his red blood cell perfusion technique, two effluent drops per planchet were caught. The radioactivity of the ^{140}La in each sample of effluent was measured by the following technique. Three consecutive drops were collected in Beckman 20 ml glass scintillation vials, and 15 ml of Bray's solution (23) were added and the isotopic activity (cpm) measured by a Beckman model LS-200B scintillation spectrometer.

Each effluent ^{45}Ca washout curve obtained during this study was resolved into a function of the type $F(t) = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} + A_3e^{-\lambda_3 t} + A_4e^{-\lambda_4 t}$. This was accomplished with the aid of a computer program developed by one of the authors (W.G.S.). $F(t)$ is the function (or curve reconstruction) derived by the computer from selected input data points picked off the "best fit" curve. The identification of each exponential term conforms to the terminology originally designated by Langer (16). Each best fit curve was drawn by eye (with the aid of smooth curves) through the washout data point loci (cf. the continuous dark curve of Fig. 4, panel A). A_1 , A_2 , A_3 , and A_4 and λ_1 , λ_2 , λ_3 , and λ_4 are the zero time intercept and rate constant values, respectively, of each term (also called phase). The term $A_0e^{-\lambda_0 t}$, which Langer identifies with the vascular space, was not calculated by the computer program, but was

obtained by hand subtraction when desired. The hand subtraction method employed by Langer to obtain $F(t)$ has been discussed previously (3, 16). An extensive discussion of the computer program's development, logic steps, and validity will be found in reference 24; only a brief presentation will be undertaken here.

During the initial development of the program multiple solutions for each curve were obtained by assigning values to λ_4 . An extensive study showed that only values ranging from 0.003 min^{-1} to 0.009 min^{-1} yielded solutions with biological relevance and further that the terms $A_2e^{-\lambda_2 t}$ and $A_1e^{-\lambda_1 t}$ varied little as λ_4 took on values between these limits. Therefore, all washout curves obtained during this study were resolved by setting λ_4 equal to 0.004 min^{-1} and then to 0.006 min^{-1} . For comparison, Table I presents the rate constants previously published for dog papillary muscle and rabbit septa and the computer-derived values obtained during this study. Note that

TABLE I
RATE CONSTANTS (MEAN \pm SE) AND TIME
CONSTANTS FOR HAND RESOLUTION (COLUMNS 1 AND 2)
AND FOR COMPUTER RESOLUTION (COLUMN 3)

	I Dog papillary muscle (16)		II Rabbit septa (17)		III Rabbit septa (present study)	
	Rate constant	Time constant	Rate constant	Time constant	Rate constant	Time constant
	min^{-1}	min	min^{-1}	min	min^{-1}	min
Phase 1	0.59 ± 0.14	1.72	0.52 ± 0.06	1.92	0.52 ± 0.04	1.92
Phase 2	0.116 ± 0.004	8.62	0.106 ± 0.004	9.45	0.165 ± 0.009	6.06
Phase 3	0.021 ± 0.001	47.6	0.018 ± 0.001	55.5	0.047 ± 0.002	21.3
Phase 4	0.004^*	250	0.004^*	250	0.004^\ddagger	250

* Approximate value determined from tissue activity curve.

† Lower value assigned for λ_4 . For $\lambda_4 = 0.006$, $\lambda_3 = 0.049 \pm 0.003$, $\lambda_2 = 0.167 \pm 0.010$, and $\lambda_1 = 0.53 \pm 0.05$.

the computer- and the hand-derived λ_3 values are markedly different. This is due to the technique employed for each method; hand-fitting determines one exponential, $A_3e^{-\lambda_3 t}$, to describe the washout curve between 70 and approximately 50 min, whereas computer-fitting determines two exponentials, $A_4e^{-\lambda_4 t}$ and $A_3e^{-\lambda_3 t}$. Thus over this interval, 50–70 min, the hand-determined function, $A_3e^{-\lambda_3 t}$, is approximately equivalent to the sum of the computer-determined function, $A_4e^{-\lambda_4 t} + A_3e^{-\lambda_3 t}$.

RESULTS

Preliminary Investigations

The contractile responses of two arterially perfused septal preparations following a change from standard perfusate to standard plus $5 \mu\text{M}$ La and to standard plus $40 \mu\text{M}$ La are presented in Fig. 1. Within a few seconds after perfusion with La, allowing for dead space clearance of the cannula, both contractile and resting tensions (alternately referred to as active or systolic and diastolic tensions respectively) changed, the former decreasing and the latter increasing.

Papillary muscles, isometrically mounted in a flow chamber (12 cc volume) and perfused with 2.5 mM Ca solution, exhibited qualitatively identical responses; the major difference was a much smaller rise in resting tension (see Fig. 2). After the restoration of perfusion with 2.5 mM Ca, active and resting tensions returned toward their prelanthanum values. Not every septum, briefly exposed to La, totally regained its prelanthanum contractility. The change with time of both these parameters was definitely not linear; a skewed sigmoid curve more appropriately described the responses.

More than 40 septa were perfused with standard plus 5–40 μM La, and in each case the muscle displayed qualitatively similar changes in systolic and diastolic tension. Decline of systolic tension was always the dominant factor. The increase of diastolic tension was relatively minor. In general, the magni-

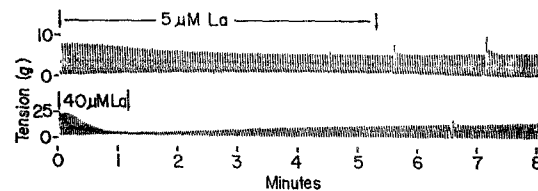


FIGURE 1. The upper trace is a recording from an isometrically mounted rabbit septum during and after exposure to 5 μM La. The lower tracing shows the contractile response during and after exposure to 40 μM La. Dead space clearance times for the two cannulae were 14 and 11 sec, respectively. Although neither tension record indicated a normal recovery by the 8 min mark, both septa eventually recovered to within 90% of their prelanthanum levels. The $[\text{Ca}]_o$ of the perfusate was maintained at 2.5 mM throughout each experiment.

tude of the decline in active tension depended upon three factors: (a) the concentration of La in the perfusate—the higher the $[\text{La}]_o$, the more marked the effect; (b) the number of exposures to La preceding the one in question, i.e. each successive La event was relatively less effective; and (c) the time of year during which the experiment was performed—fall and winter septa were less sensitive to La than were spring and summer septa.

The factors responsible for statement (b) remain obscure at this time. With repeated exposure to La the rate of decline in tension decreased as did the quantity of ^{45}Ca displaced by La. The mechanism of the interaction between La and the myocardial tissue was not systematically investigated during this study. The contractile responses and concurrent ^{45}Ca losses were quantified for three septa, each labeled, washed out, and exposed to 10 μM La three successive times. The data obtained suggest that even though contractile tension may return to control after the initial exposure to La, the La-Ca relationship is altered with respect to subsequent exposures to La.

Three septa were continuously exposed to perfusate which contained 1.25, 2.5, or 5.0 mM Ca with or without 10 μ M La. Although the long-term deleterious effects of perfusion with La hindered direct quantitative comparisons of the contractile responses from the septa, the presence of the La did not abolish the positive inotropic effect due to increases in $[Ca]_o$. To determine whether the observed decline in active tension could have been caused by a change in the electrical activity of the cell membrane, action potentials were continuously recorded from two papillary muscle preparations, perfused in a bath, using KCl-filled microelectrodes. In addition contractile tension and rate of tension development were also recorded. The experimental sequence

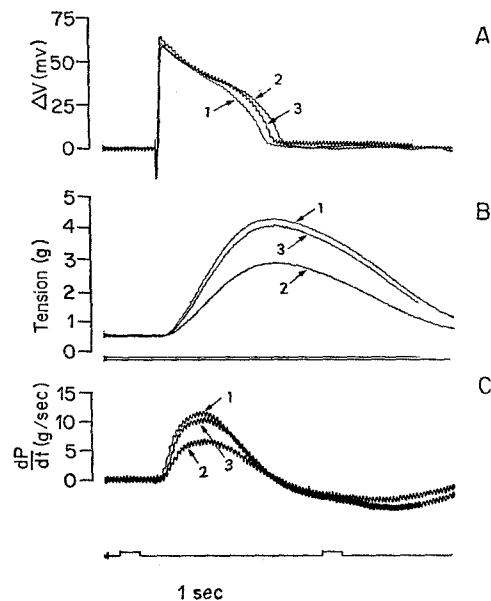


FIGURE 2. The three superimposed sets of tracings were obtained from a rabbit papillary muscle bath perfused with 2.5 mM Ca solution. The numbered traces refer to prelanthanum stabilized parameters (1), maximum effect of exposure to 10 μ M lanthanum (2), and 47 min after returning to La-free 2.5 mM Ca perfusate (3). The letters refer to action potential (A), isometric tension (B), and dP/dt (C). (Authors' correction: The ordinate values of panel A are in error; they should be 50, 100, and 150 mv.)

was as follows: the muscle was stabilized in standard perfusate prior to perfusion with standard plus 10 μ M La (for the second muscle, 40 μ M La) and perfusion with standard perfusate was resumed after systolic tension approached its reduced steady-state value. Fig. 2 is a composite reproduction of the significant results from the first experiment in which the $[La]_o$ was 10 μ M. The numbered curves in each panel refer to stabilized prelanthanum parameters (1), maximum response to La (2), and 47 min postlanthanum (3). A comparison of parameter values at maximum La effect to these prelanthanum values yields the following results: The duration of the action potential lengthened 15.9%, but the amplitude of the membrane depolarization remained constant. Systolic tension fell 38.0%; diastolic tension registered no change; and time to peak tension remained constant. 47 min after the return

to standard perfusate the duration of the action potential was only 4.7% greater than its control value, and systolic tension registered a value 7.3% less than its control value. As before, time to peak tension remained constant. Although the amplitude of the action potential (3) was greater than that for (1) and (2), this was probably not significant. A random sampling of cellular action potentials throughout the experiment indicated no systematic change with time. A second experiment, similar to the first except that $[La]_o$ was 40 μM instead of 10 μM , was terminated at the time of maximum La effect. For this case systolic tension fell 52.0% and the duration of the action potential lengthened only 9.0%. Time to peak tension and action potential amplitude displayed no measurable changes.

In summation, the results of the preliminary experiments indicated the following: (a) active tension (or systolic tension) varied as the ratio, $[Ca]_o/[La]_o$; (b) aside from a 9–16% lengthening of the action potentials, no wave form anomalies were evident; and (c) the decline of contractile tension was associated with decline in the rate of tension development since time to peak tension remained constant.

If contractile tension were, in any way, dependent upon the ratio $[Ca]_o/[La]_o$, this relationship should have been evidenced by changing the $[Ca]_o$ in septa labeled with the La isotope, ^{140}La , and by exposing ^{45}Ca -labeled tissue to La. Both sets of experiments were undertaken.

Effect of Ca upon ^{140}La Washout

The effect of Ca on ^{140}La -labeled tissue is shown in Fig. 3. Seven experiments comprised this set. From the first few experiments the following was determined: (a) tissue activity, monitored by an externally positioned GM probe, registered a linear ^{140}La uptake—the longest labeling period was 261 min and the shortest was 2 min; (b) the mean (\pm SE) uptake rate for 10 labeling periods was 4.12 ± 0.35 $\mu moles$ La/kg wet tissue/min; (c) even though ^{140}La uptake was linear for periods well in excess of 3 hr, the active tension response which followed perfusion with La was manifest only during the first 1–3 min. Panel A of the figure displays the contractile tension response during the brief 2.25 min label with 10 μM ^{140}La in standard perfusate (the solid lines indicate the best fit, by eye, to the data points). Zero time indicates the return to standard, La-free perfusate as well as the beginning of ^{140}La washout. During the 25 min washout the $[Ca]_o$ in the perfusate remained constant. For the next two washouts this was not true. The label and washout sequence remained the same up to approximately 4 min of washout when the $[Ca]_o$ was increased. The $[Ca]_o$ was switched from 2.5 to 5.0 mM at 4.25 min during the second washout and was switched from 2.5 to 7.5 mM at 4.67 min during the third washout.

Immediately following the change to higher $[Ca]_o$ (washout curve 2, panel

B), the data indicated a noticeable alteration of the washout rate. The maximum vertical separation, during the 4.25–7.0 min interval, of the best fit curve and the predicted curve is 20 standard deviations. (Note that the predicted curve, marked by the dashed line (again panel B and also panel C), has been drawn to indicate the situation had not the $[Ca]_o$ been elevated.) Concurrently

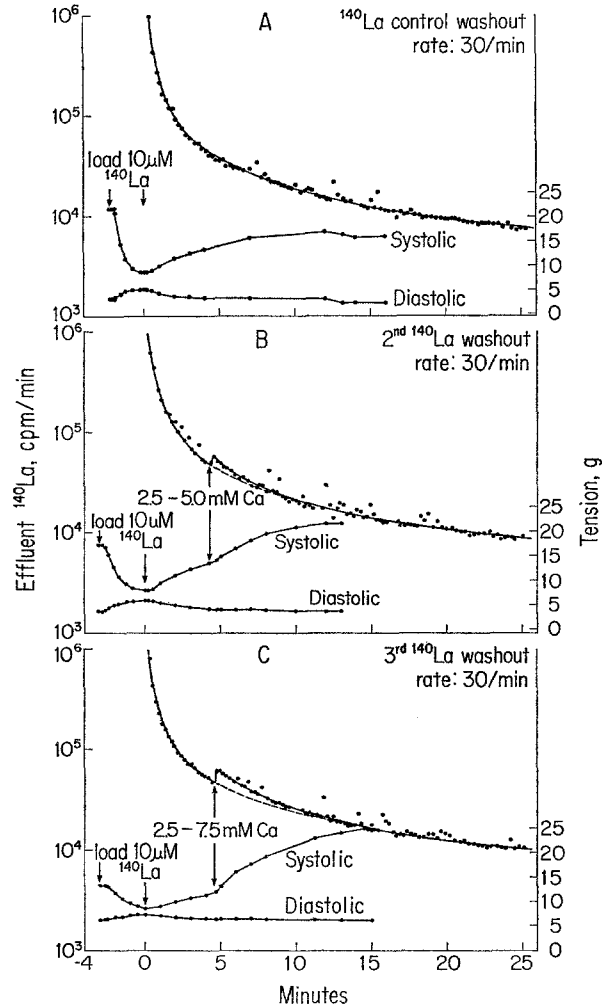


FIGURE 3. The septum (perfused with 2.5 mM Ca) from which these data were obtained was exposed to ^{140}La and washed out with La-free solution three consecutive times (A, B, and C). Panel A displays a normal control, 10 μM La washout curve, and tension record. In panel B the label and washout were identical to those in A except that at 4.25 min the $[Ca]_o$ was increased from 2.5 to 5.0 mM. The third label and washout (panel C) also were identical to those of panel A except the $[Ca]_o$ was increased from 2.5 to 7.5 mM at 4.67 min. Note the location of the ordinate scales; i.e., washout counts per min per min on the left scale and tension in grams on the right scale.

with the transient disturbance in the washout curve there was an abrupt rise in systolic tension. Compared to the control rate of tension recovery, 0.755 g/min, over the same interval, the new rate was 1.97 g/min, an increase of 160%. After the switch to 7.5 mM Ca (washout curve 3, panel C), the maximum vertical separation of the best fit and extrapolated curves is 37 standard deviations. Similarly the rate of tension recovery was 3.03 g/min which was 300% greater than the control value. Although the absolute magnitudes of the calculated standard deviations (second and third washout curves) may be irrelevant, they validate the significance of the displaced La quantities (see reference 25 for a discussion of this matter).

Calculation of the quantity of La which would have been displaced by an elevation of the $[Ca]_o$ at zero time required, in each case, knowledge of the rate of exchange of that fraction of tissue La displaced by Ca. Since this rate of exchange was not determined with certainty, each uncorrected calculation will underestimate the actual or zero time quantity of La by the factor $e^{-\lambda t}$. The value of λ is equal to the unknown exchange constant and t is (approximately) the time after washout at which the $[Ca]_o$ was elevated.

For this experiment (Fig. 3) the values were 15 nmoles La/liter tissue H_2O , following the switch from 2.5 to 5.0 mM Ca, and 42 nmoles La/liter tissue H_2O , following the 2.5–7.5 mM Ca event. For the other identical experiment the values were 20 nmoles La/liter tissue H_2O and 85 nmoles La/liter tissue H_2O , respectively. Thus the data disclosed that in cardiac tissue, which had exhibited a significant attenuation of its contractile strength following exposure to 10 μM La, elevation of $[Ca]_o$ elicited two coincident events: an immediate and marked increase in systolic tension and a loss of tissue-bound La. The time course of La displacement approximately paralleled the period of greatest rate of increase in tension.

Effect of La upon ^{45}Ca Washout

The quantitative effect of La on tissue-bound ^{45}Ca was investigated in a series of 14 experiments. The data from one of these experiments are displayed in Fig. 4. Prior to the washout of the ^{45}Ca isotope, the septum had been exposed to ^{45}Ca -labeled standard perfusate for 40 min. Washout began at zero time and at some arbitrary time later the perfusate was switched to standard plus 10 μM La. Active tension began to decline immediately, and as the tension approached its new value asymptotically, the return to the standard perfusate was made. Washout time for each experiment totaled 70–90 min. Each of the 14 experiments followed this general scheme and differed only in the time of perfusion with La-containing solution.

The experiment shown in Fig. 4 displays the salient responses of a ^{45}Ca -labeled septum after exposure to 10 μM La and, for comparison, 5 μM La (the solid lines indicate the best fit, by eye, to the data points). Note that during

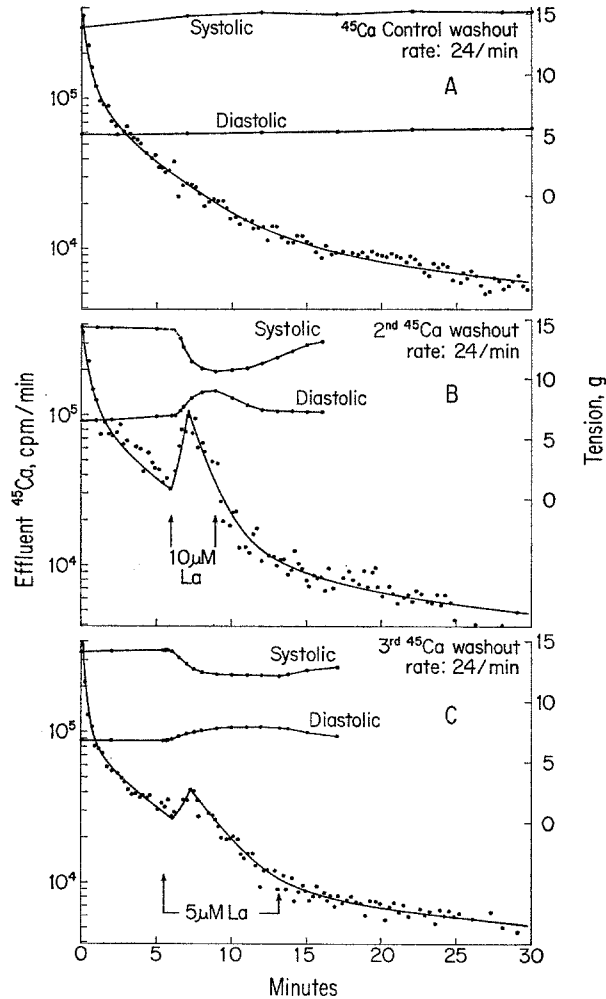


FIGURE 4. The septum (perfused with 2.5 mM Ca) from which these data were obtained was labeled for 40 min with ^{45}Ca and washed out three consecutive times (A, B, and C). Panel A displays a normal control ^{45}Ca washout and tension record. In panel B the label and washout were identical to those in A except between 6.12 and 8.92 min the perfusate contained $10\ \mu\text{M}$ La. The third label and washout also were identical to those of panel A except that between 5.50 and 13.22 min the perfusate contained $5\ \mu\text{M}$ La. Note the location of the ordinate scales; i.e., washout counts per min per min on the left scale and tension in grams on the right scale.

the first washout (panel A) the muscle was not exposed to La and therefore the data simply indicate the normal rate of ^{45}Ca washout. After 6.12 min of the second washout (panel B) the perfusate was switched to standard plus $10\ \mu\text{M}$ La. The abrupt decline of active tension began within 10 sec concurrently with a gross alteration in the shape of the ^{45}Ca washout curve. It

was noted that the washout data points, after 15 min, did not lie on an extrapolated curve (not shown but see Fig. 6 for a more obvious example) through the first 6 min of data points. This was observed in many ^{45}Ca washout curves. After a third 40 min labeling period and subsequent washout, the perfusate was changed to standard plus $5\ \mu\text{M}$ La at the 5.50 min mark (panel C). As before, active tension immediately began to fall but this time the rate of decline was much slower than that following $10\ \mu\text{M}$ La. Concurrently with the tension decline the washout curve displayed a marked alteration in its expected time course.

Several points should be noted with reference to the events which followed tissue exposure to La. First, the peak of the rapid phase of the premature ^{45}Ca loss corresponded, approximately, to the period of maximum rate of tension decline. Second, whether or not perfusion with La was terminated at the time that the active tension reached a lesser asymptotic value or was allowed to continue well after the attainment of this value, the fall of the data loci from the peak of the transient was smooth and showed no discontinuities (compare the duration of La exposure, tension decline, and the shape of the transient in Fig. 4, panels B and C). Third, after the transient loss of ^{45}Ca , the loci of data points (panel B) defined a curve lying significantly below an extrapolation through the data points prior to the La-induced ^{45}Ca loss. A superposition of the control washout (panel A) on the second washout (panel B) will verify this statement (see also Fig. 6 for a more obvious example). (This finding, however, was not observed every time a septum was exposed to La; see panel A, Fig. 7.)

The semilogarithmic plot (Fig. 5) combines the data from the 14 experiments. Times for the initiation of perfusion with $10\ \mu\text{M}$ La ranged from 5.0 to 47.5 min. Calculation of the ordinate value of each datum point was based upon the following reasoning. Since the dependent variable of each washout curve is in units of counts per minute per minute, bounded areas have the units of counts per minute, a quantity of material, ^{45}Ca in this case. If La-susceptible Ca comprises, kinetically, a homogeneous body of Ca, and if its exchange with the vascular perfusate is not masked by one or more rate-limiting barriers, a premature and rapidly displaced quantity of ^{45}Ca will be seen as a transient extending above the control washout curve. If one septum could have been repeatedly exposed to $10\ \mu\text{M}$ La and restored to its control physicochemical state after each exposure, then the quantity of tissue-bound ^{45}Ca available for displacement (from a kinetically homogeneous Ca pool) at time t would be given by $B_0 e^{-\lambda t}$. B_0 is the quantity of ^{45}Ca in the pool (measured in counts per minute) at the instant isotope labeling is terminated and washout is begun and λ is the rate or exchange constant which describes the rate of isotope loss from this homogeneous pool. Further, if the Ca pool is 100% labeled, its specific activity is equal to that of the perfusate. Thus

the quotient of (quantity of ^{45}Ca (measured in counts per minute per micromole) displaced by $10\ \mu\text{M}$ La) \div (perfusate specific activity (measured in counts per minute per micromole Ca)) \times (tissue water content of the septum (measured in liters) yields a fictitious quantity of Ca with the units micromoles Ca per liter tissue H_2O . Since $10\ \mu\text{M}$ La does not displace all the ^{45}Ca (defined above by B_o) in this pool, a plot of each fictitious datum point would describe a curve given by $C_o e^{-\lambda t}$ (assuming $10\ \mu\text{M}$ La displaced the same amount of Ca each time). C_o is seen to be the actual quantity of Ca displaced by each expo-

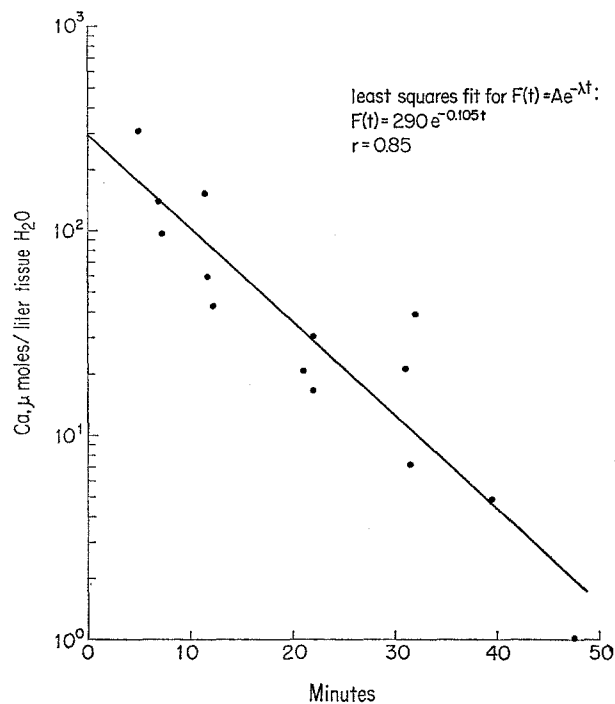


FIGURE 5. Shown here are a plot and a least squares fit of the ^{45}Ca transient displacements quantified for 14 septa exposed to $10\ \mu\text{M}$ La at times ranging from 5.0 to 47.5 min. For example the second datum point to the right of the left border (coordinates: 7 min, 139 $\mu\text{moles/liter}$ tissue H_2O) is the quantification for the transient from Fig. 4 (panel B).

sure to La (note that the zero time value on the ordinate of Fig. 5 is equivalent to C_o).

It was stated earlier that not all septa completely recover after exposure to La. Therefore only the data from the first exposure to La (some septa were given more than one exposure to La) were used to calculate the data points for the plot of Fig. 5. Langer (unpublished results) has determined that after a 40 min labeling period, phase 2 (his contractile dependent Ca phase) can be considered 100% labeled. Also, reference to the phase 2 constants listed

in Table I shows that if phase 2 Ca is labeled at the same rate at which it exchanges during washout, 40 min is an adequate time for phase 2 to achieve essential asymptotic specific activity. Thus the data from each of the 14 experiments can be treated as in the example except that the semilogarithmic plot would be expected to reflect the influence of biological variations; i.e., the B_o , C_o , and λ values for all septa would not be identical.

A rigorous calculation of the quantity of ^{45}Ca displaced during each exposure to $10\ \mu\text{M}$ of La would have required separation of the processes occurring at the presumed site of La-Ca interaction, one describing Ca displacement by La and the other describing the exchange of ^{45}Ca with standard perfusate which did not contain La. Since little specific information regarding the former relationship was to be found, this factor was ignored. Consideration of the second process enabled a quantification to be made by integration of the area bounded by the ^{45}Ca transient above and by the curve extrapolated through the data points prior to the exposure to La below. The time corresponding to each normalized ^{45}Ca quantity was taken as the peak of the transient. These assumptions yielded a quantity not greater than the actual quantity of ^{45}Ca displaced. A least squares semilogarithmic fit of the data points generated the function $F(t) = 290 e^{-0.105t}$ $\mu\text{moles Ca/liter tissue H}_2\text{O}$; the correlation coefficient was 0.85. Thus the zero time intercept represents that mean quantity of Ca displaced from rabbit septal tissue (equilibrated with 2.5 mM Ca perfusate), following exposure to $10\ \mu\text{M}$ La. Since in 28 septa the per cent H_2O equaled 83.3 ± 0.3 (SE), this quantity was equal to 242 $\mu\text{moles Ca/kg wet tissue}$. (Note in particular that this displaced Ca is independent of the duration of exposure to La and correlates only with the period of attenuation of active tension.)

The observed consistency of the septal response to $10\ \mu\text{M}$ La suggested that more Ca would be displaced by raising $[\text{La}]_o$. The washout curve and tension record of one such experiment (exposure to $20\ \mu\text{M}$ La) are displayed in Fig. 6. Except for the unexplained data point scatter between 1 and 3 min the locus of points describes a well-defined curve. One unmistakable observation is that the curve from 18 min and beyond lies well below a smooth extrapolation through the points up to 10 min. The separation is 21 standard deviations. The disjunction in this washout curve (exposure to $20\ \mu\text{M}$ La) appears to be identical to but more exaggerated than the discrepancy described earlier for the curve of Fig. 4, panel B (exposure to $10\ \mu\text{M}$ La). Based on observations of over 200 ^{45}Ca washout curves, the predicted course of the curve between 10 and 50 min (Fig. 6) has been indicated by a dashed line. A tentative explanation for the results of this particular experiment was that La had completely displaced all the "susceptible ^{45}Ca " within the tissue.

If at 10 min $20\ \mu\text{M}$ La could displace all contractile dependent Ca, exposure of the tissue to higher $[\text{La}]_o$ prior to washout should have resulted in a wash-

out curve lacking a phase 2 component. In order to test this prediction four septa were exposed to 30–40 μM La 1–4 min before the end of the 40 min labeling period. Each successive perfusion was undertaken after the preceding one had been plotted and analyzed. Thus the procedure could be altered to reflect decisions based on the prior analyses. Although the active tension of each septum, following exposure to La, declined 81% or more, a visual inspection of the washout curves revealed that La had not displaced all phase 2 Ca. The computer resolutions verified this observation. The exact quantities of the remaining and displaced phase 2 Ca are not pertinent. The important

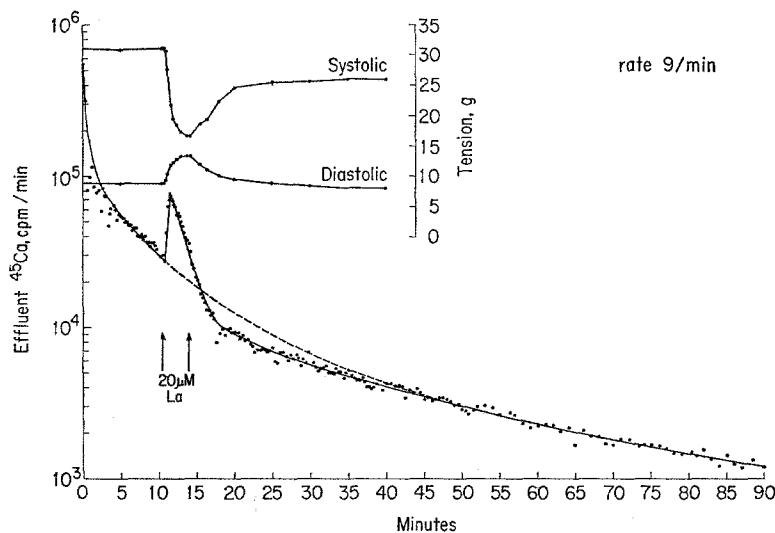


FIGURE 6. The data plotted here are from a septum (perfused with 2.5 mM Ca), labeled for 40 min with ^{45}Ca , and washed out for 90 min. Between 10.43 and 13.87 min the perfusate contained 20 μM La. An analysis of the washout curve after the 18th min indicated that no phase 2 Ca remained in the tissue beyond this time. The dashed line extending from the 10th to the 50th min indicates the expected course of the washout (had the tissue not been exposed to 20 μM La). The ordinate scale for the washout curve is on the left side and that for contractile tension is on the right side of the figure.

point is that the introduction of La just prior to washout did not eliminate the phase 2 component; quantification of each experiment revealed that although a substantial reduction of phase 2 had occurred, the amount of Ca remaining in phase 2 was significant.

If almost zero active tension were indicative of depleted stores of contractile dependent Ca, exposure to La after an appropriate period of Ca-free perfusion should have displaced little Ca. Again four septa were sequentially labeled with ^{45}Ca , washed out, and exposed to 20 μM La. Two muscles served as controls and were not exposed to zero $[\text{Ca}]_o$. The results of both sets of experiments were quantitatively identical and the data from one set are presented

in Fig. 7. Both septa were labeled for 40 min with ^{45}Ca standard perfusate and washed out beginning at zero time. The control muscle (panel A) was perfused with 2.5 mM Ca for the entire 90 min except for the addition of 20 μM La between 10.15 and 12.82 min. The second muscle (panel B) was perfused with Ca-free perfusate beginning at 3.25 min and lasting through the washout except for the addition of 20 μM La during the interval from 10.20 to 13.17 min.

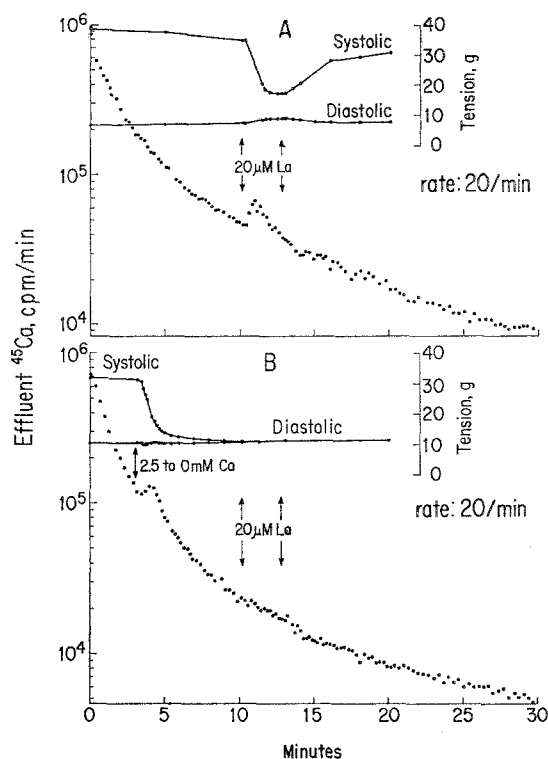


FIGURE 7. Panels A and B display the data from two septa perfused with 2.5 mM Ca labeled for 40 min with ^{45}Ca , and washed out for 90 min (only 30 min of the records are reproduced). Between 10.15 and 12.82 min (panel A) the perfusate contained 20 μM La. Beginning at 3.25 min (panel B) the $[\text{Ca}]_o$ in the perfusate was decreased to 0 mM and maintained throughout the rest of the washout. Between 10.20 and 13.10 min the perfusate contained 20 μM La. The ordinate scales for the washout curves are on the left side and those for contractile tension are on the right side of the figure.

Quantification of the ^{45}Ca transient from the first washout (panel A) yields a normalized fictitious value of 47 $\mu\text{moles Ca/liter tissue H}_2\text{O}$. An analysis of the zero Ca curve (panel B) indicates that the quantity of Ca displaced following exposure to 20 μM La is of questionable significance. If a smooth curve is first drawn (by eye) through the data points prior to 10 min

and after 14 min (i.e., ignoring the data points between 10 and 14 min and extrapolating the curve through this interval) and then compared to a smooth curve drawn through the data points included in the 10–14 min interval, the maximum vertical separation will be found to equal 4 standard deviations. On the other hand one continuous smooth curve can be drawn through the entire data loci and the data points between 10 and 14 min will lie no more than 3 standard deviations above the curve. If the separation of 4 standard deviations is significant, the normalized fictitious quantity of Ca equals 10 μ moles Ca/liter tissue H₂O. The selection of a single smooth curve to describe the data loci means that the quantity of displaced Ca equals zero. Thus whether or not 20 μ M La caused the release of a measurable quantity of Ca from a septum first perfused with zero [Ca]_o solution depended upon the criteria for drawing the best fit curve. For the case of the normally perfused septum (2.5 mM Ca) shown in panel A, the peak of the transient is 14 standard deviations above the extrapolated curve and the effect of La on tissue-bound Ca is clearly evident. The data from the other experimental pair of septa (zero [Ca]_o washout begun at zero time and perfusion with 20 μ M La initiated at approximately 4.0 min) were identical. Therefore once a septum has been perfused first with zero [Ca]_o solution for a period sufficient to cause an approximately 98% reduction in contractile tension and then exposed to 20 μ M La, the quantity of Ca displaced is no more than $\frac{1}{5}$ (10 μ moles Ca/liter tissue H₂O \div 47 μ moles Ca/liter tissue H₂O) the amount which is displaced from a normally perfused (2.5 mM Ca) septum, identically exposed to La. Dependent upon the significance given to the quantity of Ca displaced by La following perfusion with zero [Ca]_o, the fraction may be considerably less.

DISCUSSION

The observations, data, and analyses pertinent to the effects of La on mammalian cardiac tissue support the hypothesis that La effects a release of contractile dependent Ca. The physical phenomena involved in this La-Ca interaction are not well-understood at this time. If it could have been shown that intercellular conduction was systematically blocked or that regenerative depolarization of the sarcolemmal membrane was adversely affected during perfusion with 5–40 μ M La, the hypothetical foundations of this investigation would have been groundless.

The results of multiple cell impalements with a microelectrode obtained during bath perfusion of two papillary muscles, one exposed to 10 μ M La and the other to 40 μ M La, showed that, aside from a systematic lengthening of the plateau coincident with the duration of exposure to La, the action potentials were essentially normal and that all impaled cells were undergoing rhythmic depolarization. The data of Fig. 2 are in agreement, both qualitatively and quantitatively, with the work of Nayler and Anderson (10) and

Ciofalo and Thomas (7). Neither group could demonstrate that the graded negative contractile responses following perfusion of cardiac tissue with Zn were attributable to impaired cellular electrical activity. Although Nayler and Anderson did not record dP/dt , their tension records indicate that dP/dt and not time to peak tension was the primary factor related to diminishing tension. During the present study dP/dt was recorded and the results suggest that La affected the intensity of the active state rather than the duration of the active state. These findings are commensurate with the hypothesis that La diminished the quantity of Ca released during each depolarization.

The possibility that the effects of lanthanum are other than the direct displacement of contractile dependent Ca must be given consideration. Since the data and subsequent analyses do not tend to support this contention, the body of the discussion will be devoted to the development of the "lanthanum-contractile dependent Ca" aspect.

In addition to the observation that in septa perfused with zero $[Ca]_o$, La displaces little significant Ca after active tension declines to almost zero, the data support the hypothesis that phase 2, the kinetic manifestation of contractile dependent Ca, is composed of two (? or more) fractions. Ca from both fractions apparently exchanges with the same rate constant. This suggestion will be expanded toward the end of the discussion which is concluded with a speculation on the physical basis for the observed action of La on contractile dependent Ca.

The correlation coefficient of 0.85 for the semilogarithmic curve fitted to the data plot of Fig. 5 supports the conclusion that La-susceptible Ca comprises a kinetically homogeneous pool. The temporal concurrence of the Ca loss from this pool, and the decline of systolic tension, taken together with the demonstrable Ca-La interaction, lead to the conclusion that La specifically displaces contractile dependent Ca.

Several investigators agree that contractile dependent Ca is kinetically distinguishable from other tissue Ca fractions (e.g., extracellular, mitochondrial, and connective tissue). Langer (16, 26) discovered that in every instance in which increased contractile tension (positive inotropism) occurred concurrently with increased tissue Ca uptake, the extra Ca was confined to a specific kinetic phase (phase 2) whose rate constant was not significantly different from the value determined from a prior control washout. This phase represented Ca which exchanged less rapidly than that from the interstitial space but more rapidly than that percentage (40–50%) of total tissue Ca which exchanged with a time constant greater than 8 min. Identical findings were reported for vascularly perfused rabbit interventricular septal tissue (17). Niedergerke (27) termed a similar fraction in the frog heart "superficial Ca" and later (13) provided additional evidence that this Ca was localized to one compartment or was at least homogeneously distributed.

In their studies on guinea pig atria with ^{45}Ca Winegrad and Shanes (12) also found a similar contractile dependent Ca pool and identified it as an "intermediary locus."

If the homogeneous kinetic fraction of tissue Ca that La affects is contractile dependent Ca, and if contractile dependent Ca is manifest in the ^{45}Ca washout curves as phase 2, then the kinetic Ca pool represented by Fig. 5 and the phase 2 kinetic Ca pool identified in Table I must be equivalent. Several lines of evidence validate this equivalence. Comparisons of the washout curves obtained from septa perfused with ^{45}Ca -labeled standard solution and washed out with standard solution and then relabeled and washed out with zero $[\text{Ca}]_o$ solution show that after approximately 50 min of washout the two curves are superimposable. This means that phases 3 and 4 (computer resolution) or phase 3 (hand resolution) are independent of the $[\text{Ca}]_o$ in the washout solution. The data of Fig. 5 show that $10\ \mu\text{M}$ La displaces negligible quantities of ^{45}Ca after 50 min of washout, and the data of Fig. 7 (panel B) show that after approximately 7 min of perfusion with zero $[\text{Ca}]_o$ $20\ \mu\text{M}$ La displaces a questionably significant quantity of Ca. Since the data from these septa, perfused with zero Ca and exposed to La indicate a normal phase 3, phase 3 was not disturbed by perfusion with La. After 50 min of washout the Ca fraction shown in Fig. 5 would be 99.5% exhausted whereas phase 3 would be 59.3% ($\lambda = 0.018\ \text{min}^{-1}$, hand resolution) or 90.5% ($\lambda = 0.047\ \text{min}^{-1}$, computer resolution) exhausted. Therefore, both the washout and rate constant data argue against the choice of phase 3 as the equivalent of the Ca pool determined by the data of Fig. 5. The rate constant for phase 1 is $0.52\ \text{min}^{-1}$ (Table I, rabbit septa). Phase 1 is not the source of La-susceptible Ca because only 0.1% of its original ^{45}Ca would remain after 13.3 min of washout whereas the pool determined by Fig. 5 would still be 50% of its original size. The conclusion is that no other phase but phase 2 can be related to the Ca fraction determined by the data of Fig. 5. Data from the present experiments (exposure to La and perfusion with zero Ca) support Langer's conclusion (3) that ^{45}Ca in dog papillary muscle can be considered to exchange from compartments arranged in parallel. A rigorous treatment of the ^{45}Ca kinetics, however, would require slight corrections for series exchanging compartments, and since this was not done the rate constant values of the washout curve (hand or computer resolution) are insufficiently accurate (although they are reproducible) to be statistically compared to the rate constant for the data of Fig. 5. For this reason the arguments supporting the equivalence of phase 2 and the Ca pool identified by the data of Fig. 5 were based on the more qualitative rather than the strict quantitative significance of the experimental data.

Several lines of evidence suggested that more Ca could be displaced if the $[\text{La}]_o$ were elevated above $10\ \mu\text{M}$: La is apparently specific for contractile

dependent Ca; the diminution of active tension is related to the $[La]_o$; 10 μM La does not diminish active tension to zero; and the ratio the quantity of Ca which is displaced by 10 μM La (290 $\mu\text{moles Ca/liter tissue H}_2\text{O}$) divided by the quantity of Ca which is localized to phase 2 (812 ± 63.9^2 $\mu\text{moles Ca/liter tissue H}_2\text{O}$) is less than unity. The data of Fig. 6 are the most convincing demonstration of this prediction. Although active tension was not abolished (15% remained at the termination of exposure to La), comparative calculations indicate that 20 μM La did effect a displacement of nearly all the phase 2 Ca. The variability of Ca displacement at constant $[La]_o$ (and constant infusion time) can be seen by comparing Fig. 6 and Fig. 7 (panel A). These responses are, respectively, typical of summer and winter septa. The data of Fig. 6 and their detailed interpretation are important for two reasons: (a) the location of contractile dependent Ca (or at least the major source of it at 2.5 mm Ca perfusion) is clearly established, and (b) the specific and rapid displacement of Ca defines a washout curve significantly different from those showing lesser Ca losses. The quantitative calculations are based on the following computer-derived function $F(t)$ for the actual (from 0 to 10 and from 50 to 90 min) and the predicted (from 10 to 50 min) washout curve of Fig. 6:

$$F(t) = 10^8 \times [91.2 e^{-0.642t} + 71.3 e^{-0.142t} + 17.1 e^{-0.0455t} + 1.55 e^{-0.006t}] \text{cpm/min}$$

Even though phase zero (primarily vascular ^{45}Ca) has not been included, this function fits the handdrawn curve from 2 to 90 min. Four independent quantifications substantiate the contention that 20 μM La displaced nearly all the phase 2 Ca in this muscle:

1. Upon visual inspection and after comparison with previously analyzed washout curves it was concluded that the loci of data points from 20 to 90 min defined a curve which was flatter than expected. A comparison of the sum of the phase 3 and 4 values over this interval with the values taken from the best fit curve (solid line) showed that all calculated points fit better than ± 1 SD. No control curve can be fit between the 20th and 90th min by a two-term function ($A_3 e^{-\lambda_3 t} + A_4 e^{-\lambda_4 t}$).

2. The quantity of ^{45}Ca contained in phase 2 from 10.8 min to infinity is simply the integral of the second term in $F(t)$ between these limits. The calculation yields a value of 108×10^8 cpm. The quantity of ^{45}Ca under the transient is 100×10^8 cpm.

3. The slope of the curve from the peak of the transient to 17 min, after subtracting the contributions of phases 3 and 4, is 0.62 min^{-1} . This value is just slightly less than the phase 1 rate constant, 0.64 min^{-1} . It appears that the steep rise of the ^{45}Ca loss induced by La represents a nearly instantaneous and total displacement of ^{45}Ca directly into the inter-

² SE.

stitial space. The size and shape of this ^{45}Ca displacement (exposure to 20 μM La) represent an extreme condition with respect to that normally encountered following exposure to 10 μM La. In general, the falling phase of a ^{45}Ca transient is less steep than that observed in Fig. 6 and reflects the contribution of that fraction of phase 2 Ca (remaining after the peak) which is being more slowly displaced by La or which is, for whatever reason, insensitive to La and hence still exchanging with the phase 2 rate constant.

4. If the total displacement of phase 2 ^{45}Ca by La were from a superficial or external site in equilibrium with the interstitial space and this displacement took place instantaneously, the event would be marked by a vertical step in the washout curve to a higher ordinate value followed by a decline which would be described by the sum of three rate constants: that for the interstitial space (phase 1) and phases 3 and 4. Although the resolution time for this washout technique most certainly is too slow to follow a step displacement of ^{45}Ca , the comparison between the expected peak value of the transient (were total displacement to occur at 10.8 min), 81.4×10^3 cpm, and the value taken from the peak of the transient, 74.1×10^3 cpm, supports the general hypothesis.³

If, in general, exposure to 20 μM La was sufficient to induce the total displacement of phase 2 Ca, two additional experiments should have verified this. The first, exposure to 30–40 μM La just prior to initiation of washout, should have yielded a washout curve lacking a phase 2 component. The second experiment, exposure to 20 μM La following Ca-free perfusion (Fig. 7, panel B), should have yielded a washout curve lacking a La-induced Ca transient.

The data analysis of the four septa exposed to 30–40 μM La prior to washout showed that even though tension had been reduced by 81–96%, undisplaced phase 2 Ca ranged from one-third to two-thirds of the amount determined from normal control washout curves. The data analysis of the two experimental sets in which exposure to La followed Ca-free perfusion (see Fig. 7, panel B) showed that little, if any, Ca was displaced by 20 μM La. Although contractile tension registered zero after approximately 3 min of perfusion with La, phase 2 was not depleted of its Ca. The amount remaining was approximately one-half that determined from control washout curves

³ It is pertinent to note the implications of the zero Ca washout data and the data of Fig. 6. In septa twice labeled with ^{45}Ca and washed out first with standard perfusate and then with zero $[\text{Ca}]_o$ perfusate, the two washout curves are superimposable beginning at approximately 50 min of washout. Since the computer resolution begins with the 50–70 min interval, the data prior to 50 min do not influence the solution of the terms $A_3e^{-\lambda_3 t}$ and $A_4e^{-\lambda_4 t}$. The data of Fig. 6 show the shape of a washout curve which has had an intermediate phase (phase 2) removed. Because the data loci from 20 to 50 min are accurately defined by the sum of the terms $A_3e^{-\lambda_3 t}$ and $A_4e^{-\lambda_4 t}$ (and are not influenced by the loss of phase 2), it can be concluded that, kinetically, phase 2 and phases 3 and 4 exchange in parallel (3).

obtained at the same time (e.g., the comparison of phase 2 from the curves of panels A and B in Fig. 7).

The data of Fig. 6, the data from exposure to La 1–4 min prior to washout and the data from exposure to La following Ca-free perfusion appear to be in conflict: La is specific for contractile dependent Ca; phase 2 and contractile dependent Ca are equivalent; but although La and/or Ca-free perfusion can nearly abolish active tension, neither can consistently deplete the store of phase 2 Ca. This conflict can be reconciled by assuming that phase 2 is composed of two or more morphologically distinct but kinetically unresolvable fractions.

At the onset of the present study the inhomogeneity of phase 2 was not recognized and hence no experiments were specifically designed to investigate this question. Several additional lines of evidence, however, do support this hypothesis.

Langer (26) equilibrated dog papillary muscles with 5.0 mM Ca solution and investigated the effects of Ca-free perfusion on the ^{45}Ca washout curve and on contractile tension. The implications from Langer's study were that twitch tension reflected the amount of Ca remaining in phase 2, at least until 86% of it washed out. Twitch tension thereafter declined more slowly roughly paralleling the washout of Ca from the tissue. The present study shows that active tension fell to 10% of its steady-state value with a rate constant greater than that calculated for phase 1. Thereafter the rate of decline lessened as active tension fell further to 2.3% of its 2.5 mM Ca-stable value. This finding implies that Ca exchanging with the phase 2 rate constant did not measurably support tension, at least during the time that tension fell by 90%. In fact a more liberal interpretation of the data (24) suggests that at the instant after perfusion with zero $[\text{Ca}]_o$ was begun the superficial or external store of contractile dependent Ca became indistinguishable from phase 1 Ca. With respect to Langer's data the discrepancies may be attributable to a species difference of the myocardium and/or the $[\text{Ca}]_o$ of the perfusate. Langer's dog papillary muscle perfusate contained 5 mM Ca and the rabbit septal perfusate prepared for the present study contained 2.5 mM Ca. This factor of 2 appeared in the respective phase 2 quantifications: the quantity of Ca localized to phase 2 for dog papillary muscle was approximately twice that for the rabbit septum.

Langer and Serena⁴ found that after exposure to high doses of cardiac glycoside, which produced a large net uptake of Ca, the rabbit septum (perfused with 2.5 mM Ca) was able to maintain nearly constant active tension for several minutes when the $[\text{Ca}]_o$ was decreased to zero. Comparison of a control

⁴Langer, G. A., and S. D. Serena. 1970. The effects of strophanthidin upon contraction and ionic exchange in rabbit ventricular myocardium; relation to control of active state. Manuscript submitted for publication. *J. Mol. Cell. Cardiol.*

zero Ca washout with a zero Ca washout after inducing a net Ca uptake with glycoside showed that the two ^{45}Ca washout curves were superimposable. They concluded that this could have occurred only if a large fraction of coupling Ca was located at a "deeper" or internal site not directly accessible to the extracellular space. Thus the available evidence suggests that phase 2 Ca, although kinetically homogeneous, is morphologically inhomogeneous and is comprised of two (or more) sources of exchangeable Ca: the superficial depot (most probably the sarcolemmal membrane, ? T system as well) and the "internal" depot (most probably the sarcoplasmic reticulum).

In summary, the data from the present study together with additional selected data from Langer's laboratory support six conclusions. Three are substantially supported: (a) La effects a displacement of tissue-bound Ca. (b) This La-susceptible Ca is kinetically homogeneous and is identifiable as contractile dependent Ca. (c) The site of this La-Ca interaction (perfusion with 2.5 mM Ca) is superficial (i.e., in direct equilibrium with the extracellular space) rather than internal. Three are circumstantially supported: (d) La, in general and in concentrations up to 40 μM , does not effect the total displacement of phase 2 Ca (that kinetic phase which includes contractile dependent Ca). (e) A significant portion of phase 2 Ca may be internally located. (f) Contractility can be sustained or at least partially sustained by an "internal" Ca source which may be the "internal" portion of phase 2 Ca. (Under physiological conditions, however, the contribution of this "internal" Ca to contractile activation is relatively insignificant.)⁵

In order to satisfy all the data relevant to this study a model incorporating more than one source of contractile dependent Ca is necessary. The movement of the major portion of contractile Ca (during perfusion with 2.5 mM Ca) would be from the superficial site (most probably the sarcolemmal membrane, ? including T tubules) to the myofilaments, to the internal site (most probably the sarcoplasmic reticulum), and back out to the interstitial space. Thus both Ca-free perfusion and La could interrupt contraction by depleting the stores of sarcolemmal Ca. The remaining quantity of Ca, bound to the sarcoplasmic reticulum, would be insensitive to these interventions and sufficient to support only a small fraction of control tension.

Although the data justify assigning causality to the sequence,

La \rightarrow displacement of contractile Ca \rightarrow diminution of contractile tension

the following possibilities must also be considered:

⁵ Shine (manuscript in preparation) has shown that the rate of fall of maximum dP/dt in many, but not all septa during blood perfusion with zero Ca solution (EDTA-washed glassware used throughout) can be described by a single rate constant. In these cases these dP/dt rate constants are identical to the phase 1 rate constants which have been derived from the washout curve resolution. This implies that little if any contractile activation is being supported by an "internal" Ca store.

1. La \rightarrow displacement of noncontractile Ca \rightarrow inactivation
of some contractile dependent mechanism

or

2. La $\left\{ \begin{array}{l} \xrightarrow{\text{(Primary)}} \text{inactivation of some contractile dependent mechanism} \\ \xrightarrow{\text{(Secondary)}} \text{displacement of contractile Ca} \end{array} \right.$

The data shown in Figs. 5–7 together with those from Langer's (16) experiments with low Na indicate that only one kinetic phase represents contractile dependent Ca. La affects only this fraction. The first alternate hypothesis is, therefore, unsupported since the displacement of noncontractile Ca is not observed. The second alternate scheme is not easily refuted since the biochemical effects of La are not yet known. The data do show that repeated or long-term exposure may cause irreversible impairment of the muscle's contractile capability. However, if the exposure period lasts only long enough to diminish tension, as shown in Figs. 1 and 3, a viable muscle, beating at less than 30 per min, will recover at least 90% of its prelanthanum tension within approximately 20 min. Even then considerable La remains in the tissue. For example, the quantity of La which was taken up by the muscle (Fig. 3) during each labeling period was 10 μ moles La/kg wet tissue, and after termination of washout, 9 of the 10 μ moles La/kg wet tissue still remained bound within the tissue. Thus the removal of approximately 1 μ mole La/kg wet tissue per washout was associated with the return of near-control tension. The sequence of events following exposure to La suggests that the first physical interaction of La is with contractile dependent Ca.

Until now, the mechanism of lanthanum's action on tissue-bound Ca has been unspecified. Little of the data from the present study can be used to construct a relevant model. However, this study together with the recent review by Diamond and Wright (30), in which the authors discuss the physical basis of a membrane's electrolyte and nonelectrolyte permselective properties, and the 1910 paper by Mines (20), in which he discusses the effects of Y, La, and Ce on frog ventricle and artificial membrane, do provide a rationale for further speculation. Diamond and Wright (31) found, from their experiments on gallbladder epithelium, that at pH 7 the epithelium was more permeable to cations than to anions, whereas at pH 2.4 the epithelium was more permeable to anions than to cations. They concluded that their data supported a membrane model in which ionizable positive and negative groups were the determinants of the observed permselectivity patterns. Mines' data showed that the contractile response of frog ventricle perfused with 10 μ M Y, La, or, Ce was exactly the same as that determined during the

present study for rabbit ventricle perfused with $10 \mu\text{M}$ La. His data demonstrated that whereas pH 4.5 Ringer ($[\text{H}] = 0.00003 \text{ N}$) did not affect contractility during 4 min of perfusion, pH 4.0 Ringer ($[\text{H}] = 0.0001 \text{ N}$) abolished active tension within 2 min. Mines concluded: "... it is clear that in relation to the frog's heart they [Y, La. and Ce] show a close resemblance to the hydrogen ion, though producing the same effects in much smaller concentration." To investigate this phenomenon further Mines developed a galvanic cell from which he could determine the polarity of a membrane after it had been exposed to selected cations. His findings are quoted: "A neutral solution containing [10 mM] La, Y or Ce suffices to alter the membrane in one or two minutes so as to convert the deflection from $-$ to $+$."

"Solutions of divalent positive ions, e.g., Ca, Sr, Mg produce a much smaller effect even in [125 mM] concentration and do not, in the same time, succeed in reversing the direction of the deflection." Mines found that solutions of low pH produced the same effect.

It appears reasonable to speculate that the regulation of excitation-contraction coupling Ca is dependent upon the electronic configuration of a membrane which contains fixed ionizable groups. If the La and H effects are identical this should be demonstrable by the same techniques employed in this study.

Note Added in Proof Studies in progress show that La is capable of E-C uncoupling without producing a quantifiable displacement of tissue-bound Ca. This was observed in septa that were perfused with solutions equilibrated to a higher pO_2 (approximately 1140 mm Hg compared to approximately 885 mm Hg for the present study). The ^{45}Ca washout curves that were obtained from these "hyperbarically" perfused septa are identical to the curves described for the present study. Although the contractile response of the tissue to La during both types of perfusion is identical there is no quantifiable displacement of tissue-bound Ca by La during "hyperbaric" perfusion. We interpret these data to mean that contractile dependent Ca is bound to two loci (both external). For reasons not yet understood, La, during "normobaric" perfusion is capable of displacing the bulk of contractile dependent Ca from phase 2. On the other hand, during hyperbaric perfusion La blocks or displaces the significantly smaller store of contractile dependent Ca which is not definable by the kinetic analysis of the washout curve. It is, however, to be emphasized that, for the present study, septa were perfused only with normobaric aqueous solutions.

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