Calcium Exchange in Dog Ventricular Muscle: Relation to Frequency of Contraction and Maintenance of Contractility

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A previous study\(^1\) indicated five kinetically-defined phases of calcium exchange in the arterially perfused dog papillary muscle. That fraction of myocardial calcium which is important in supporting the inotropic effect of perfusion with low sodium solutions was localized in so-called phase 2. This phase was considered to be predominantly representative of calcium in a portion of the muscle designated as the "calcium transport system" which had a time constant of exchange (\(\tau\)) of 8.62 minutes. This phase was distinguished from phase 0 (\(\tau = 0.29\) min; considered to be vascular in origin), phase 1 (\(\tau = 1.72\) min; interstitial), phase 3 (\(\tau = 47.6\) min; intracellular) and phase 4 (\(\tau = 250\) min; intracellular and/or connective tissue). It was postulated that phase 2 Ca++ is also important in the altered Ca++ exchange which had been demonstrated to occur following increments in the frequency of contraction.\(^2\) In addition, on the basis of the previous study,\(^1\) it seemed likely that the major portion of the Ca++ which contributes to the maintenance of contractility resides in that portion of the myocardium which manifests itself kinetically as phase 2.

The purpose of this study is threefold: (1) to define further the alterations in Ca++ exchange associated with changes of frequency of contraction; (2) to identify that fraction of tissue Ca++ responsible for the maintenance of contractility; and (3) to define the capacity of the "calcium transport system" and to relate this capacity to the function of the muscle.

Methods

The isolated, arterially perfused dog papillary muscle preparation was used with techniques as described previously in detail.\(^1\)\(^2\) Adult mongrel dogs of either sex weighing 10 to 15 kg were anesthetized with intravenous pentobarbital (30 mg/kg). Following administration of 75 to 100 mg sodium heparin intravenously the dogs were sacrificed by overdosage of pentobarbital and the heart rapidly excised. The artery of a papillary muscle from the right side of the interventricular septum was cannulated and the muscle mounted as described.\(^2\) When mounted it was possible to record a number of parameters continuously and simultaneously: (1) frequency of contraction with the rate controlled, when desired, by external stimulation; (2) isometric systolic and diastolic tensions with a Schilling isometric capacitance transducer;\(^3\) (3) isotopic activity of the muscle as monitored by a closely apposed G-M probe (model 222A Geiger tube, Atomic Access., Inc., Valley Stream, New York); (4) isotopic activity of the venous effluent during washout of isotope from the previously Ca++-labeled muscle. The standard perfusate had the following composition: NaCl, 130 mM; KCl, 4 mM; CaCl\(_2\), 5 mM; NaHCO\(_3\), 14 mM; NaH\(_2\)PO\(_4\), 0.435 mM; MgCl\(_2\), 6H\(_2\)O, 1.0 mM; and glucose, 5.56 mM. To introduce isotope into the tissue, the perfusate was labeled with Ca\(^{45}\) (Ca\(^{45}\)-P-3, Union Carbide, Oak Ridge, Tennessee) at a specific activity of approximately 25 \(\mu\)c/ml. The perfusate was equilibrated to 98% O\(_2\)-2% CO\(_2\) at 24°C and contained approximately 0.04 ml O\(_2\)/ml at a pH of 7.3 to 7.4. At the 150 mm Hg perfusion pressures used, perfusion rate was approximately 1 ml/g tissue/ min.

Following perfusion the wet weight of the...
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muscles was determined. They were then dried in porcelain crucibles at 90 to 100°C until weight was stable (four to six hours) and the percentage tissue water calculated. The tissue was then digested in concentrated HNO₃ and the digestant uniformly distributed in planchets for counting of total isotopic activity.

If effluent was analyzed for activity during isotopic washout, the effluent droplets were collected in individual planchets. The isotopic activity of each droplet was counted and plotted semilogarithmically. The curves were analyzed graphically according to the method described by Solomon.

The following groups of experiments were done:
1. Determination of the increment in tissue Ca⁺⁺ associated with increased frequency of contraction. The value obtained from this series of experiments represents a maximum increment, when computed on a “per-beat basis,” in that the analysis was done within the first 11 minutes following a rate increase. It is during this time that the maximum increment per beat is to be expected.
2. Kinetic definition of the movements of Ca⁺⁺ associated with increased frequency of contraction.
3. Calcium exchange as a function of the absolute frequency of contraction.
4. Comparison of the exchange characteristics of tissue Ca⁺⁺ with the maintenance of contractility under the conditions of Ca⁴⁵-washout with perfusate in which all Ca⁺⁺ was replaced with an isosmolar quantity of sucrose.

Results

1. INCREMENT IN Ca⁺⁺ UPTAKE ASSOCIATED WITH INCREASED FREQUENCY OF CONTRACTION

The typical alteration in Ca⁴⁵ uptake, following a brief increase in frequency of contraction, is illustrated in figure 1. A similar pattern was defined in 10 papillary muscles that had been labeled with isotope for at least 40 minutes. Increments in frequency of contraction were varied between 12 and 50 beats/min with a mean increment in rate of 28/min. The mean period of increased stimulation rate was 11.0 min. The rate of Ca⁴⁵ uptake remained quite linear during this period but then began to decrease with maintenance of the stimulation rates noted above.

An estimate of Ca⁺⁺ uptake/beat was made for the period (mean = 11.0 min) during which the rate of increase in Ca³³ activity remained unchanged following the stimulus of increased frequency of contraction. The mean uptake for 10 muscles was 2.5 ± 0.45* μmoles/liter tissue water/beat. It should be emphasized that this represents a maximum value, as the slope representing rate of uptake usually began to decline after 10 to 12 minutes of stimulation at increased rate. The pattern of Ca⁺⁺ exchange after the initial 10- to 12-minute period of stimulation depended greatly upon the rate (vide infra).

*1 SE of mean.
2. KINETIC DEFINITION OF THE MOVEMENTS OF Ca++ ASSOCIATED WITH INCREASED FREQUENCY OF CONTRACTION

A papillary muscle was perfused with isotope for 35 min and washed out for 60 min during which time it was contracting at a rate of 7 to 8 beats/min. A portion of this washout curve with its exponential resolution is illustrated in figure 2. At end-washout the muscle was again loaded isotopically for 35 min, but was stimulated at a rate of 75 beats/min for the last 8 minutes of loading. The isotope was then, once again, washed out for 60 minutes during which time it was contracting at control rate (7 to 8 beats/min). A portion of this washout curve with its exponential resolution is also illustrated in figure 2.

It should be noted that the basic pattern of washout followed that which has been described previously in detail with phases 0 to 3 clearly represented upon graphic resolution of the effluent curves. The initial portions of the curves (first 2 min of washout) were superimposable with phase 0 (vascular phase) identical for both washouts. The slopes for phase 1 were identical, but the zero time intercept for washout no. 2 (following the period of rapid stimulation) was lower than that for washout no. 1. This indicates a lower Ca++ content for that portion of the tissue represented by phase 1 which is probably the interstitial space, chiefly. The reduction of Ca++ in this phase was approximately 15% and correlated with the appearance of slight contracture associated with the high rate of stimulation.

The major difference between the two curves appeared during the second to sixteenth minute of washout. Throughout most of this period the curves differed by more than 20% It is to be noted that upon exponential resolution this difference affected phase 2 for the two washouts. In this experiment the additional Ca++ represented by the difference in phase 2 components was 1.2 mmoles/liter tissue water. Since the muscle contracted 540 more times in the 8 minutes prior to washout no. 2 as compared to washout no. 1, the additional Ca++ in phase 2 of the second washout represented 2.2 μmoles Ca++/liter tissue water/beat.

The slopes representing phase 3 were iden-
tical, but again, as with phase 1, the zero time intercepts were significantly different. This difference indicated a 19% increase in the isotopic content of phase 3 for washout no. 2. This, however, does not necessarily indicate a net gain in Ca\textsuperscript{++} in the portion of the muscle represented by this phase since additional isotope is expected to derive from this phase during washout no. 2. This is due to the fact that phase 3 had a rate constant (\(\lambda\)) of 0.0198/min and, therefore, was not nearly approaching its asymptotic value following 35 minutes of isotopic loading, but was only 50% labeled prior to washout no. 1. Following washout no. 1 for 60 min and reloading for 35 min, phase 3 would be 58% labeled and therefore contribute 16% more counts during washout no. 2. This value agrees well with the experimental results.

The same basic pattern as described was defined in a total of five muscles in which the period of increased stimulation was 8 to 10 minutes. The results indicate that the additional Ca\textsuperscript{++} which is reversibly bound following a rate increase is accounted for in that portion of the myocardium which is represented kinetically by phase 2.

3. CALCIUM EXCHANGE AT VARIOUS RATES OF CONTRACTION
(a) Small Increments in Frequency

The experiments reported in the previous section indicated that additional Ca\textsuperscript{++} taken up by the muscle during an 8- to 10-minute period of stimulation was released upon cessation of increased rate of contraction according to the exchange characteristics of phase 2. Figure 3 illustrates the exchange characteris-

\[\text{FIGURE 3}\]

Ca\textsuperscript{++} activity (counts/min/min) of effluent, plotted semilogarithmically, for the initial 20 min of two 65-min washouts from a single papillary muscle. Washout 1 (filled circles) was preceded by a 35 min isotopic labeling period during which the muscle was beating at a rate of 1/min. Following washout 1 the muscle was relabeled for 35 min, the last 10 min of which the rate of contraction was increased from 1/min to 14/min and maintained throughout washout 2 (open, inverted triangles). The two effluent curves differ by >12 SD of count at the 10-min period of washout. The phase 2 portions of exponential resolution are indicated for the first (2\textsubscript{1}) and second (2\textsubscript{2}) washouts. See text for analysis.
tics of a muscle in which double-labeling and washout of isotope was done. This muscle, however, was subjected to an increment in rate of contraction (control contraction rate of 1/min increased to 14/min) for the last 10 min of the second labeling period and throughout the second washout. It is to be noted that the pattern of washout was the same as in figure 2. The second washout curve was significantly lower for the first 3 minutes but then crossed curve no. 1 at 4.5 minutes and remained above until 18 minutes of washout. Exponential resolution of these curves again demonstrated an identical phase 0 for both washouts. The slopes for phase 1 were again very nearly the same but the zero time intercept was lower for washout no. 2. This, as noted previously, indicates a lower phase 1 Ca++ content associated with stimulation. Phase 2 accounted for the expected increment of Ca++ associated with increased frequency of contraction. In this experiment the difference between the phase 2 portion of the two curves represented 1.75 μmoles Ca++/liter/beat. This pattern was confirmed in a second muscle in which the increased rate of stimulation (control rate of 1/min increased to 11/min) was maintained throughout washout.

These experiments indicate that the initial increment in myocardial Ca++, following increments in rate, is reversed not only when the increase in frequency of contraction is short in duration, but also when small increases of frequency are maintained. The time course of this Ca++ turnover depends largely, if not entirely, upon the exchange characteristics of that portion of the muscle represented by kinetic phase 2. The lower frequencies of contraction employed resulted in development of stable tension and no progressive contracture, i.e., muscle function was stable.

It is interesting that the slope of phase \( 2_2 \) is greater than slope phase \( 2_1 \) in both figures 2 and 3. This indicates that in addition to a greater Ca++ content following an increase in frequency of contraction, the rate of Ca++ exchange is also increased. Since \( \lambda_2 \) defined during washout is proportional to \( \frac{[\text{efflux}]}{[\text{Ca}^{++}\text{conc of phase 2}]} \), this implies that efflux is increased to a relatively greater extent than is concentration. However, \( \frac{[\text{slope phase } 2_2]}{[\text{slope phase } 2_1]} \) is greater in figure 2 than in figure 3. The frequency of contraction during washout was less and phase 2 Ca++ concentration greater in the experiment illustrated in figure 2 than in the experiment illustrated in figure 3. This indicates that frequency of contraction and phase 2 Ca++ concentration are not the only factors influencing rate of exchange. If they were it would be expected that the slope ratio above would be less in figure 2 than in figure 3.

(b) Greater Increments in Frequency

Figure 4 illustrates an uptake curve for Ca\(^{45} \) in a papillary muscle in which a 49 beats/min increment in rate was introduced and maintained for 31 minutes (absolute frequency of contraction was increased to 70/min). Muscle function deteriorated over this time as indicated by a reduction of actively developed isometric tension from 5.5 g to 0.7 g and by a 2 g increase of resting tension.

![Figure 4](https://example.com/figure4.png)

**FIGURE 4**

Ca\(^{45} \) uptake (counts/min) correlated with frequency of contraction during a 60-min period of Ca\(^{45} \)-labeling in a papillary muscle. The rate was increased from 21 to 70/min at 27 min, and maintained through the 60-min period. Systolic and relative diastolic tensions at 27 and 80 min are indicated. Dashed line indicates extrapolation of control loading curve. Dotted line indicates linear extrapolation of the initial portion of the uptake curve during increased frequency of contraction.

_Circulation Research, Vol. XVII, July 1965_
It is to be noted that the rate of uptake of Ca\textsuperscript{45} increased but that the rate began to decline at about 13 minutes following onset of increased rate of contraction. The increment in Ca\textsuperscript{45} over the 31-minute period represented 0.34 μmoles Ca\textsuperscript{2+}/liter/beat, significantly below the average 2.5 μmolar increment found for shorter periods of stimulation.

Further definition of the Ca\textsuperscript{2+} exchange associated with rates of contraction which produce deterioration in function is found in the experiment illustrated in figure 5. This shows three successive washout curves from the same muscle. This muscle had been labeled with Ca\textsuperscript{45} for 40 minutes preceding each washout. The periods of time during which the kinetic phases 1 and 2 contributed predominantly to the effluent curve, based on previous experiments, are indicated. The second and third washout curves have been corrected for the additional counts deriving from phase 3 as explained previously.

The control isotopic labeling and washout were done while the muscle was contracting at a rate of 1 beat/min. During the last 10 minutes of the second isotopic labeling the frequency of contraction was increased by 12 beats/min (absolute rate of 13 beats/min) and returned to control rate (1 beat/min) during the washout. Diastolic tension increased slightly (0.5 g) during the period of increased frequency without evidence of declining tension when compared to that during the control isotopic labeling and washout.

That portion of the curve representing, predominantly, phase 1 (interstitium) was slightly lower than the control curve and presumably was related to the slight increase of diastolic tension. That portion representative of phase 2 was significantly (>7 3 SD throughout most of the period) higher than control and represented an additional 0.32 mmoles Ca\textsuperscript{2+}/liter or 2.7 μmoles Ca\textsuperscript{2+}/liter/beat. As the curves passed into phase 3 their differences became much less significant. This was similar to the pattern illustrated in figures 2 and 3. Following the second washout the muscle was again isotopically labeled for 40 minutes but the rate was increased by 29 beats/min (ab-
solute rate = 30 beats/min) during the last 10 minutes of labeling and throughout washout. The uptake curve for the third labeling period indicated a 0.92 mmole/liter increase in tissue Ca++ for the 10-minute period immediately prior to washout which was equivalent to 3.2 μmoles/liter/beat during this period. During the period of increased stimulation, systolic tension declined from 13.0 g to 4.9 g and diastolic tension increased by 8.0 g so that the muscle was in marked contracture at end-washout.*

The increment in Ca15 activity associated with the 10-minute period of increased stimulation during the third loading was about 2.9 times that which was taken up during the last 10 minutes of load no. 2. This correlates, approximately, with the 2.4-fold increment in rate of contraction (12 vs. 29/min). Despite this additional increment in Ca15 activity, washout curves nos. 2 and 3 were virtually superimposable except for a significant depression in that portion of washout no. 3 associated, predominantly, with phase 1. As previously noted, this may be related to the marked contracture which developed. Therefore only 35% of the increment in Ca15 taken up during the loading was released during the subsequent washout.

The uptake and washout experiments indicate that an increase in rate of contraction, which results in a progressive fall in tension and progressive contracture, is associated with irreversible binding of Ca++ by some portion of the tissue. Therefore, the initial increment in myocardial Ca++ following a rate increase is not completely reversed in the face of deteriorating tension development and of increasing contracture.

The ability of a papillary muscle, which had been perfused with 5 mM Ca++ for 4 hours, to take up additional Ca++ prior to the onset of contracture was tested. The muscle was labeled isotopically and then washed out over the second to fourth hour of perfusion (40 min of isotopic loading and 80 min of washout) to establish a control washout curve. The muscle demonstrated stable contractile tension and no increase of diastolic tension over this period during which it was contracting spontaneously at an average frequency of 13 beats/min. The muscle was then relabeled for 40 min but was stimulated at a rate of 50/min during the last 10 min of isotopic labeling. This rate was associated with the onset of contracture during the last minute of labeling. Control rate of stimulation was then re-established and the muscle was once again washed out for 80 min. As indicated in the footnote on page 81, no significant difference would be expected in the two washout curves in the absence of the 10-min period of increased stimulation. The second washout did, however, demonstrate an increment in Ca15 activity which represented 1.5 mmoles Ca++/liter tissue water, all localized to kinetic phase 2. This experiment indicates that the region of muscle represented by phase 2 is capable of taking up, and subsequently releasing, 1.5 mmoles/liter of additional Ca++ prior to the onset of contracture.

4. ISOTOPIC WASHOUT WITH Ca++-FREE PERFUSATE

A series of five muscles was loaded isotopically for periods which varied between 35 and 48 minutes. Washout was then commenced with perfusate in which all CaCl2 had been replaced with an isosmolar quantity of sucrose. The muscle was stimulated at basal rates throughout the washout and development of isometric tension was recorded continuously. Figure 6 illustrates one experiment typical of the series.

The effluent curve, with its exponential resolution into four phases, is plotted on the
Ca\textsuperscript{45} activity (counts/min/min) of effluent (filled circles) plotted semilogarithmically, for the initial 35 min of an 85-min washout from a papillary muscle. The isotopic washout was done with perfusate containing zero Ca\textsuperscript{2+} and is correlated with peak systolic tension (open circles) in grams. The exponential resolution of the effluent curve is indicated (filled squares). The muscle was perfused for 35 min prior to washout with standard (5mM Ca\textsuperscript{2+}), isotopically labeled perfusate. See text for analysis.

same time scale as the peak systolic tension. This muscle was stimulated at a rate of 6/min. A number of points should be noted: (1) A very rapid phase 1 appeared (\(\lambda = 2.3\)/min). (2) Essentially no change in development of tension was present for the first 2 to 3 minutes of washout after which time the fall in tension followed an exponential course until the twentieth minute of washout. (3) The rate constant (\(\lambda = 0.115/\)min) for this exponential portion of the tension plot was very nearly equal to the rate constant (\(\lambda = 0.108/\)min) for phase 2. (4) After 20 minutes of Ca\textsuperscript{2+}-free washout, tension had fallen to 14% of its control value at which time phase 2 had washed out all but 12% of its isotopic activity (and, presumably, all but 12% of its Ca\textsuperscript{2+}). It should be noted that the smooth descent of the effluent curve is interrupted at the fifth minute of washout. This represents a brief transitional phase between phases 1 and 2 and is evident only in muscles washed out with Ca\textsuperscript{2+}-free perfusate.

The mean rate constants (\(\lambda\)) for phases 1 to 3 in this series were: phase 1, 1.2/min; phase 2, 0.113/min; phase 3, 0.019/min. As noted above, the fall in actively developed tension coincided, predominantly, with phase 2 washout. The fall in tension had an average rate constant of 0.130/min in this series. All muscles maintained some ability to contract under zero Ca\textsuperscript{2+} perfusion for the duration of the washout (80 to 90 min).

Discussion

RELATION OF Ca\textsuperscript{2+} TO FREQUENCY OF CONTRACTION

A previous study\textsuperscript{2} of calcium exchange in the dog papillary muscle indicated that the initial gain in myocardial Ca\textsuperscript{2+} which occurred following an increase in frequency of contraction was later reversed. This reversal occurred in the face of continued increment in rate, if the rate increase did not result in progressive fall of contractile tension and the onset of contracture. It was also noted that the maximum increment in myocardial Ca\textsuperscript{2+} was approximately proportional to the increment in rate.

The present study confirms these findings and further defines the quantity and kinetics of the calcium exchange which occur secondary to increases in frequency of contraction. During the first 10 to 12 minutes the increment of myocardial calcium was 2.5 \(\mu\)moles \(\pm 0.45 \mu\)moles/liter/beat. This increment was fully reversed over the next 15 to 20 minutes if control rates of stimulation were re-established, or if the increased rate did not produce deterioration of muscle function. The calcium was exchanged according to the charac-
teristics of the previously well-defined kinetic phase 2. This supports the suggestion that the "Ca++ transport system" involved in the inotropic response to low sodium perfusion is also involved in the augmented Ca++ uptake (and later release) that occurs secondary to rate increase.

Co++ CAPACITY OF THE PHASE 2 SYSTEM

The Ca++ taken up by that portion of myocardium which is represented kinetically by phase 2 is labile and remains reversibly bound. Additional increments, however, appear to be bound firmly by some portion of the tissue and seem to correlate with a rising diastolic tension. The additional capacity of the phase 2 system was estimated by the experiment in which increased stimulation was carried to the point at which contracture first became evident. This was found to be 1.5 mmoles/liter tissue H₂O. A previous study estimated the resting or basal capacity of this system at 1.7 mmoles/liter. Therefore, the total capacity would appear to be about 3.2 mmoles/liter in muscles perfused with 5 mM Ca++ solution. This compares well with the previous estimate of 2.7 mmoles/liter in which the Ca++ increment was produced by low Na+ perfusion. Both in the present study (Figs. 4 and 5), and in those in which low Na+ perfusion was used, additional increments of tissue Ca++ were associated with the onset of contracture. This Ca++ was released very slowly. This is based upon the finding that none of this fraction could be identified in subsequent washouts.

POSSIBLE RELATION OF PHASE 2 TO THE SARCOTUBULAR SYSTEM

Much evidence indicates that elements of the sarcoplasmic reticulum may be responsible for the specialized transport of Ca++ which relates to the control of the contractile response. This is based upon the demonstration of the marked ability of this system to pump Ca++. It has been postulated that phase 2 is the kinetic representation of the sarcotubular system in dog papillary muscle. The volume of transverse elements of the sarcotubular system has been estimated at about 30% of the sucrose-measured extracellular space in rabbit heart. If phase 2 were distributed in this volume its concentration would range from 12 to 25 mM under the conditions of 5 mM Ca++ perfusion. Since the transverse system has been demonstrated to represent invaginations of the interstitial space, it is perhaps more likely that the longitudinal elements of the sarcotubular system, in juxtaposition to the myofilaments, hold the Ca++ represented by phase 2. In this case Ca++ concentration might be somewhat greater because of the possibly smaller volume of the longitudinal, as compared to the transverse, system.

REGULATION OF CONTRACTILE TENSION

The portion of myocardium represented kinetically by phase 2 seems to be of major importance in the regulation of Ca++ exchange associated with changes in frequency of contraction, and with the inotropism produced by low Na+ perfusion. The series of experiments represented by figure 6 indicates that phase 2 Ca++ plays a significant role in the ability of the muscle to develop tension. As illustrated, contractile tension remained unaffected while Ca++ was washing out of phases 0 and 1. Because these phases seem to represent chiefly the vascular and interstitial spaces, it is evident that the ability to develop contractile tension is not primarily dependent upon Ca++ in these regions. It is noteworthy that the mean rate constant for phase 1 doubled (1.2/min) with Ca++-free perfusion when compared to the mean value derived previously (0.59/min) during washout with perfusate containing 5 mM Ca++. The Ca++-free washout also introduced a brief transitional phase between phases 1 and 2. These observations point to a definite concentration dependence for Ca++ exchange of phase 1, probably representing Ca++ in the interstitial space. This is in marked contrast to phases 2 and 3 whose mean rate constants (0.113/min and 0.019/min, respectively) remained unchanged, in the face of Ca++-free perfusion, with respect to those previously derived (0.116/min and 0.021/min). This indicates, for the phases, a process of exchange.
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which is independent of concentration gradient.

Systolic tension began to fall as phase 2 commenced to dominate the washout of Ca++. The rate constant for fall of tension was very nearly equal to the rate constant for phase 2 (0.130/min and 0.113/min, respectively). This suggests that tension amplitude was directly proportional to the Ca++ content of that region of the muscle represented by phase 2, at least until 90% of its Ca++ had been cleared. At this time the rate of decline of tension decreased markedly with some active development of tension remaining for 80 to 90 minutes, though at greatly reduced amplitude. This suggests an exchange between the phase 2 region and more slowly exchanging fractions of the muscle, most probably wholly intracellular. (The region represented by phase 3 (λ = 0.019/min) would not be 90% depleted of Ca++ until the muscle had been perfused for 120 min with Ca++-free perfusate.)

HYPOTHESIS FOR THE CONTROL OF PHASE 2 Ca++ EXCHANGE

Much evidence indicates that there is a region in heart muscle where Na+ and Ca++ ions compete for sites. Previous study indicated that this region was represented kinetically by phase 2 in dog papillary muscle. The present study indicates that this same region is the locus for those alterations which occur in Ca++ exchange following increase in frequency of contraction. This raises the possibility that change in Na+ concentration in the region represented by phase 2 may be one of the controlling factors in the altered Ca++ exchange associated with increased frequency of contraction.

Na+ depletion in some portion of the cell membrane, occurring secondary to increased frequency of contraction, is a possibility if the hypothesis of Woodbury is correct. He has postulated a time lag in the ability of the Na+ pump of the muscle to compensate for the increased influx of Na+ that occurs when frequency of contraction is increased. Woodbury has calculated, on the basis of data from Hodgkin and Horowicz, that stimulation of frog skeletal muscle fiber at a rate of 1/sec necessitates a fivefold increase in Na+ efflux over that of the resting state. This augmentation of Na+ pump activity requires time during which, it is presently suggested, a depletion of Na+ ion might occur at the membrane. On the basis of this theory it is proposed that Ca++, derived from the region of the muscle represented by phase 2, immediately occupies the membrane sites vacated by Na+. The phase 2 system would then be replenished with Ca++ from the vascular space at a rate defined by λ. If the Na+ pump is able, eventually, to compensate for the increased frequency, Na+ would return to the membrane and displace the added Ca++ to the phase 2 system. The additional Ca++ would subsequently be lost to the vascular space at a rate defined by λ. This hypothesis is consistent with the sequence of Ca++ exchange that occurs when increased frequency of contraction does not result in decreasing systolic tension and progressive contracture (figs. 2 and 3).

It was proposed above that phase 2 might represent the sarcotubular system. Relevant to the hypothesis presented, it has been demonstrated that the Ca++ binding activity of sarcotubular elements is sensitive to Na+ concentration. This suggests, at least, that the competition between Na+ and Ca++ for sites may take place on a segment of sarcoplasmic membrane.

Prolonged depletion of membrane Na+ would be expected to occur during maintenance of low Na+ perfusion and with increments in frequency of contraction for which the Na+ pump was unable to compensate. Based on present theory, this would allow the phase 2 system to fill with Ca++ to its capacity. Further Ca++ uptake might then be associated with accumulation in the region of the myofilaments and onset of contracture. The decrease of systolic tension in the muscles illustrated in figures 4 and 5 indicates a compromise in oxygen-dependent contractile mechanisms. This is predicted in the perfusion system used (O2 supply = 0.04 ml/g tissue/min). It would be expected that the oxygen-dependent Na+
pump would also be compromised. The accumulation and retention of Ca++ as well as the onset of contracture demonstrated in these experiments is compatible with the proposed failure of the Na++ pump to compensate for the higher frequencies of contraction. If 1.5 mmoles Ca++/liter is the additional capacity of the phase 2 system (?sarcotubules) a total of 600 beats (at 2.5 μmoles Ca++/beat) could be accommodated in the period during which the Na+ pump is supposed to be lagging and before contracture would be expected to appear.

Consistent with the proposal above, it would follow that changes in Ca++ content of the tissue that accompany increased frequency of contraction would be an indication of the competence of the Na+ pump. It is also possible that other factors, e.g., changes of intracellular pH secondary to rate change, may significantly influence Ca++ binding and thereby alter its exchange characteristics. These possibilities are subjects of current investigation.

The proposed system has much in common with a more general model for Ca++ exchange suggested by Niedergerke for the frog heart. It should be emphasized that the exchange constant (λ_2) which has been defined for phase 2 is proposed to represent the exchange between a Ca++ storage area (?sarcotubules) and the vascular space. As postulated by Niedergerke, it is likely that there is another much more rapid exchange (time constant ~ 500 msec) that occurs on a beat-to-beat basis. In the system presently suggested this would represent exchange between the Ca++ storage area (?sarcotubules) and the myofilament region.

**Summary**

Calcium exchange was studied in the arterially-perfused papillary muscle of the dog under the conditions of varying frequency of contraction as well as calcium-free perfusion. The following conclusions are drawn from these studies: (1) The maximum increment in rate of Ca++ uptake associated with increased rate of contraction occurs in the first 10 to 12 minutes following rate increase. The mean increment over this period of time is 2.5 μmoles/liter tissue water/beat. (2) The increased Ca++ exchange which occurs in association with the increases in rate of contraction occurs within that portion of the myocardium represented kinetically by phase 2. (3) The Ca++ associated with phase 2 remains reversibly bound. The maximum capacity of the "phase 2 system" under the present experimental conditions, is approximately 3.2 mmoles Ca++/liter tissue water. (4) Exceeding the capacity of the "phase 2 system" for Ca++ is associated with the onset of contracture in the muscle. This occurs at rates of contraction which produce progressive fall in contractile tension and is associated, presumably, with myocardial hypoxia. That fraction of Ca++ taken up in excess of the capacity of the "phase 2 system" is released very slowly. (5) The initial increment of Ca++ uptake, following an increase in rate, is reversed if the rate increment is not associated with progressive fall in systolic tension and contracture. (6) The maintenance of the ability to develop contractile tension depends significantly upon the presence of Ca++ in the "phase 2 system." The rate of loss of contractile tension is very nearly the same as the rate of loss of Ca++ from this system. (7) Ca++ exchange between vascular and interstitial regions depends upon concentration gradient. In contrast, Ca++ exchange of phases 2 and 3 (?sarcotubular system and intracellular region respectively) is unaffected by change of concentration gradient.

**Acknowledgment**

The author expresses his thanks to Dr. Edward Leonard, National Heart Institute, Bethesda, Maryland, for the interesting discussion which was the origin of the Ca++-free experiments.

**References**


Circulation Research, Vol. XVII, July 1965
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Circ Res. 1965;17:78-89
doi: 10.1161/01.RES.17.1.78

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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