*Journal of Physiology* (1988), **406**, *pp.* 359–370
*With 5 text-figures*
*Printed in Great Britain*



# THE EFFECTS OF CHANGES IN MUSCLE LENGTH DURING DIASTOLE ON THE CALCIUM TRANSIENT IN FERRET VENTRICULAR MUSCLE

BY D. G. ALLEN, C. G. NICHOLS* AND G. L. SMITH
*From the Department of Physiology, University College London, Gower Street, London WC1E 6BT*

(*Received 19 April 1988*)

SUMMARY

1. Ferret papillary muscles were isolated and injected with aequorin to measure intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). Developed tension and $[Ca^{2+}]_i$ were measured in response to length changes.

2. A maintained reduction in muscle length produced an immediate decrease in developed tension followed by slow decline over 10–20 min. This slow decline in tension was accompanied by a slow decline in the amplitude of the systolic $[Ca^{2+}]_i$ rise (the $Ca^{2+}$ transient). The immediate decrease in tension was accompanied by a prolongation of the $Ca^{2+}$ transient and an abbreviation of the twitch.

3. Repeated reductions in muscle length timed to occur only during the period of contraction (systolic shortening) produced an immediate decrease of developed tension but the subsequent slow decline was substantially smaller. The slow decline in the amplitude of the $Ca^{2+}$ transients was also smaller. The prolongation of the $Ca^{2+}$ transient and abbreviation of the twitch were similar to those observed with a maintained reduction of length.

4. Repeated reductions in muscle length during the period between contractions (diastolic shortening) did not produce the immediate decrease of tension but the slow decline of tension was present. The slow decline in the amplitude of the $Ca^{2+}$ transients was also present. However no change in the duration of the $Ca^{2+}$ transient or the twitch was present under these conditions.

5. These results suggest that diastolic muscle length can influence the amplitude of the $Ca^{2+}$ transients achieved during systole. This conclusion was confirmed by experiments in which the recovery of tension and $Ca^{2+}$ transients was observed after periods of rest. Both developed tension and $Ca^{2+}$ transients on recovery from a rest were reduced when the rest occurred at a short length in comparison with a long length.

6. We suggest that muscle length influences resting $[Ca^{2+}]_i$ and this in turn affects the $Ca^{2+}$ transients and developed tension.

* Current address: Department of Physiology, University of Maryland, 660 West Redwood Street, Baltimore, MD 21201, U.S.A.

## INTRODUCTION

It has long been recognized that diastolic filling of the heart influences the pressure developed in the ventricle during systole (Frank, 1895) and the cardiac output (Patterson & Starling, 1914) (for review see Jewell, 1977; Allen & Kentish, 1985). A study by Parmley & Chuck (1973) on isolated ventricular muscle established that the increase in developed tension following stretch occurred in two distinct phases: an immediate increase, followed by a slower increase over 10–20 min. Parmley and Chuck assumed that the immediate increase in tension was due, at least in part, to the changes in myofilament overlap. In the absence of any other obvious cause, they suggested that the slow phase of tension increase might be due to an increase in the degree of activation of the contractile machinery. This idea was supported when the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) during systole (the $Ca^{2+}$ transient) was found to increase with a similar time course (Allen & Kurihara, 1982).

Thus it appears that muscle length can influence $Ca^{2+}$ movements in cardiac muscle. This could come about if length affected processes occurring during contraction, e.g. opening of $Ca^{2+}$ channels (Lakatta & Jewell, 1977) or release of $Ca^{2+}$ from the sarcoplasmic reticulum (Allen & Kurihara, 1982). However Nichols (1985*b*) showed that an apparently similar slow change in developed tension could be produced when length was changed only during the diastolic period between contractions or in a resting preparation. His result suggests that it is the changes in diastolic, rather than systolic, processes which are the primary determinant of the slow changes in activation. The aim of the present study was to determine whether such length changes during the diastolic period or in resting muscles affect the $[Ca^{2+}]_i$. Preliminary accounts of this work have been published (Allen, Nichols & Smith, 1985; Allen, Smith & Nichols, 1988).

## METHODS

The experiments were performed on isolated papillary muscles taken from the right ventricle of ferrets. The ferrets were killed by an overdose of pentobarbitone. $[Ca^{2+}]_i$ was measured using aequorin which was injected into twenty to fifty cells on the surface of the preparation. The methods were similar to those used in previous mechanical studies from this laboratory (Allen & Kurihara, 1982). The light emission from the injected aequorin was collected with a light guide and measured with a photomultiplier tube. Because the diameter of the light guide and photocathode (10 mm) were large compared to the length of muscles (3–4 mm), light collection was insensitive to movement of the muscle.

The Tyrode solution used routinely had the following composition (mM): $Na^+$, 135; $K^+$, 5; $Mg^{2+}$, 1; $Ca^{2+}$, 2; $Cl^-$, 134; $HCO_3$, 20; $HPO_4$, 1; acetate, 20; glucose, 10; insulin, $4 \times 10^{-5}$ (i.e. 40 nM); equilibrated with 5% $CO_2$; pH, 7·4.

All the experiments were performed at 30 °C and the muscles were stimulated at 0·25 or 0·33 Hz. At the start of each experiment muscles were set to the length at which developed tension was maximal (100% $L_{max}$). Muscle length was changed with a servo-controlled lever (except in Fig. 5).

The magnitude of the slow changes in tension and aequorin light were calculated as

$$\frac{x_{20}-x_1}{x_1} \times 100\%,$$

where $x_n$ is the developed tension or peak light averaged over the $n$th minute after a length change.

## RESULTS

In the first series of experiments we compared the effects of three different shortening protocols on the slow changes in tension and aequorin light. The aim of the different protocols was to determine whether muscle length during systole or diastole was important in producing the slow changes in developed tension and

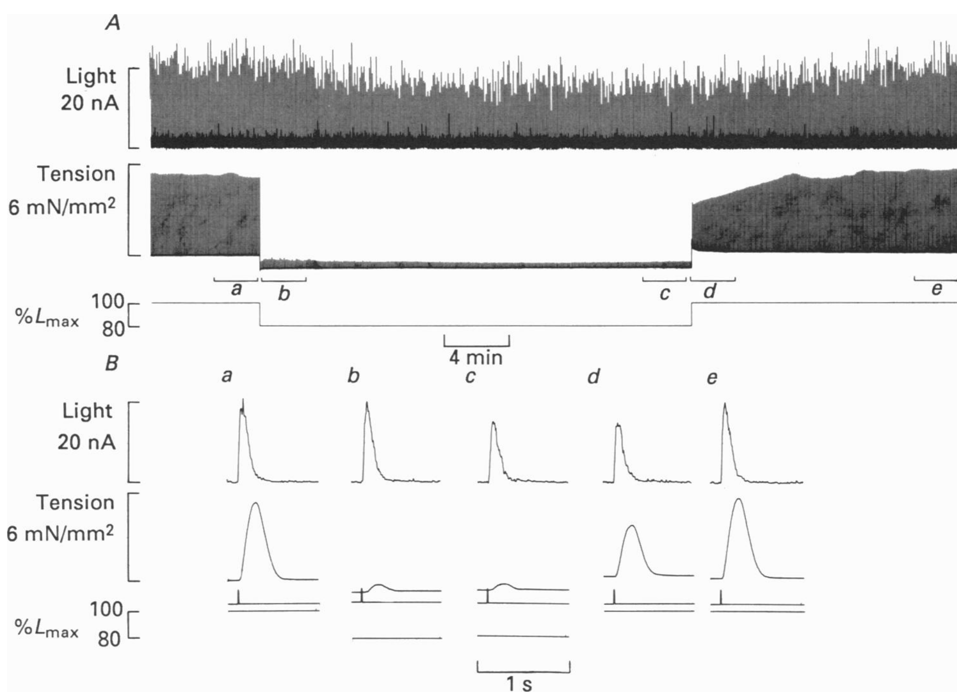


Fig. 1. Aequorin light and tension when muscle length was changed from $L_{max}$ to 80% $L_{max}$ and back. Ferret papillary muscle, 30 °C, 3 mM-extracellular $Ca^{2+}$, 0·25 Hz stimulation rate. *A*, continuous record of light, tension and length. *B*, averaged records ($n = 32$) of light, tension, stimulus marker and length taken from the periods indicated in *A*.

aequorin light. Four muscles were examined in this way and all showed the same general pattern of results. The order of the protocols was varied in the different preparations. Mean results from the four experiments are collected in Table 1. Figures 1, 2 and 3 are from the same preparation so that the different protocols can be directly compared.

### *The effects of maintained reduction in muscle length*

In one part of each experiment the muscle was shortened (over 25 ms) from 100% $L_{max}$ to 85–80% $L_{max}$ during diastole and maintained at this length for 20–30 min. Figure 1 demonstrates that developed tension alters with a complex time course. There is an immediate large decline of tension observable in the first contraction at the new length. This is followed by a very small recovery of tension which occurs over

one to five contractions. Thereafter tension declines slowly reaching a new steady state after 20 min. (Sometimes, as is just detectable in Fig. 1, an even smaller and slower recovery of tension then starts; see Hanck & Jewell, 1985.) The slow change of tension (see definition in Methods) in this case was −40%. The opposite sequence of changes was seen following stretch; in this case the slow change in tension was +75%.

The aequorin light transients recorded over the first 2 min period after the length change show no change in amplitude (*B a* and *b* in Fig. 1). However, studies in which the length change was repeated many times and the resulting changes averaged show that, following shortening, the first light transient at the shorter length has a greater amplitude (Allen & Smith, 1985) and the increase in tension over one to five contractions is also accompanied by a further small increase in amplitude (Allen *et al.* 1988). However, the most obvious effect in the present study was a slow decline in amplitude which appears to be complete after 20 min. Using the definition given above, the slow change in amplitude of the light transient was −30% in this experiment. The opposite sequence can be seen following stretch and the slow change in light transient here was +50%.

The time course of the twitch is clearly abbreviated at the short length and in addition, although not apparent without careful measurement, the light transient is prolonged. This prolongation is clearer in Fig. 4*A* where light transients from another experiment have been displayed on a faster time base. Figure 4*A* shows the superimposed light transients from a muscle at $L_{max}$ (C) and at a short length (SL) and there is a clear prolongation of the light transient at the short length. When duration of the light transient was measured (at 25% of maximum amplitude) in a number of experiments, the duration at short lengths was increased by $9{\cdot}7 \pm 1{\cdot}8$% (mean ± S.E.M., $n = 18$) compared to control ($L_{max}$).

The slow changes in tension and light transients and the changes in duration are similar to those reported by Allen & Kurihara (1982) in cat and rat ventricular preparations.

*The effects of repeated systolic shortening*

In the second protocol the same muscle was subjected to a series of shortenings to 80% $L_{max}$ but only during the systolic period (Fig. 2). Shortening started 50 ms before the stimulus and was complete in 25 ms. The short length was maintained for 450 ms, which was sufficient for contraction to be complete, and the muscle was then restretched over 25 ms. Since the stimulus interval was 4 s, the muscle spent 12% of its cycle at the short length. This sequence of shortening was continued for a time greater than that required to achieve a steady state in the first protocol, in this case about 30 min.

Figure 2*A* shows a continuous record of aequorin light, tension and muscle length during the experiment. Note that in this continuous record only the changes in resting tension can be resolved in the tension record during the period of shortening. However comparison of *b* and *c* in Fig. 2*B* shows that the developed tension changes little over this period and the slow change in tension as defined above was only −12%. The amplitude of the light transients also showed little change over this period and the slow change in light was −7%.

After the period of systolic shortening the muscle was returned to 100% $L_{max}$. There was a small slow change in tension amounting to +10% and the amplitude of the light transient also showed a small slow change of +7%. The fact that small slow changes in tension and light are present (see also Table 1) is probably because the

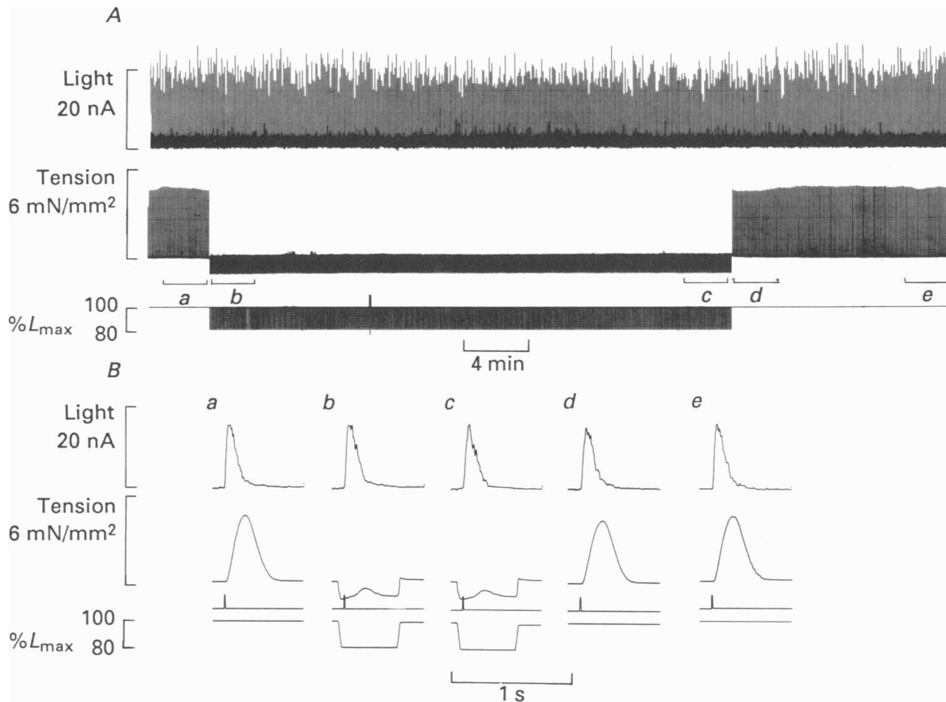


Fig. 2. Aequorin light and tension from before, during and after a period of systolic shortening. Same muscle and conditions as Fig. 1. *A*, continuous record of light, tension and length. Note that during the period of systolic shortening only the passive tension changes are visible on this slow time-base record. *B*, averaged record ($n = 32$) of light, tension, stimulus marker and length from periods indicated in *A*. The time course of systolic shortening protocol can be seen in *b* and *c*.

muscle was at a short length for 12% of its cycle in this experiment (see Discussion in Nichols, 1985*b*). In the other experiments averaged in Table 1 the fraction of the cycle at the short length varied between 12 and 18% depending on stimulus frequency and the duration of shortening.

It is clear from Fig. 2 (compare twitches in *B a* and *b* and in *c* and *d*) that the twitch is greatly abbreviated by this protocol. The light transient is slightly prolonged and this is clearer in the superimposed records in Fig. 4*B*. Measurement of the duration of the light transient (at 25% of maximum amplitude) in a number of experiments showed that on average the duration was increased by $9{\cdot}5 \pm 3{\cdot}1$% (mean ± S.E.M., $n = 10$) compared to controls ($L_{max}$).

*The effects of repeated diastolic shortening*

In the third protocol the muscle was subjected to a series of shortenings to 80% $L_{max}$ but this time confined to the diastolic period (Fig. 3). The timing of the length changes was identical to the previous section except that shortening and stretching

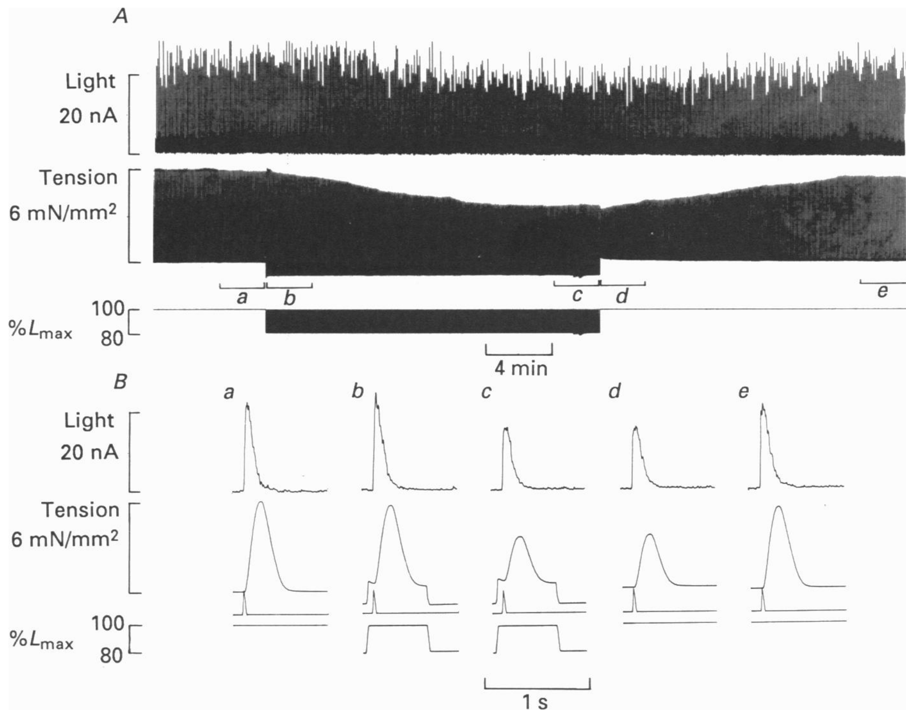


Fig. 3. Aequorin light and tension before, during and after a period of diastolic shortening. Same muscle and conditions as Fig. 1. *A*, continuous record of light, tension and length. *B*, averaged records ($n = 32$) of light, tension, stimulus marker and length from the periods indicated in *A*. The time course of diastolic shortening can be seen in *b* and *c*.

were reversed. In this sequence the muscle spent 88% of the cycle at the short length and this period of diastolic shortening continued for 20 min. It is clear from Fig. 3 that although contraction occurred at 100% $L_{max}$ throughout the whole period there were slow changes in developed tension and light transients with a time course which is similar to that in Fig. 1. The slow change in developed tension amounted to $-46$% and over the same period the slow change in amplitude of the light transients amounted to $-35$%. When the muscle was returned to $L_{max}$, there was a slow recovery of tension of $+52$% and a slow recovery of light of $+42$%.

In this experiment the slow changes in tension and light were not greatly different in the present protocol when compared to the maintained length reduction. Table 1 shows that when all the experiments are considered, the slow phases were generally smaller (three out of four) but this difference was not generally significant (three out of four). In the present protocol the fraction of the cycle at the short length was

0·82–0·88 of the maximum possible so one might predict that on average the slow phases would be smaller than those during maintained reduction by 0·82–0·88. Our results are too variable to state whether this prediction is correct or not (but see Nichols, 1985*b*).

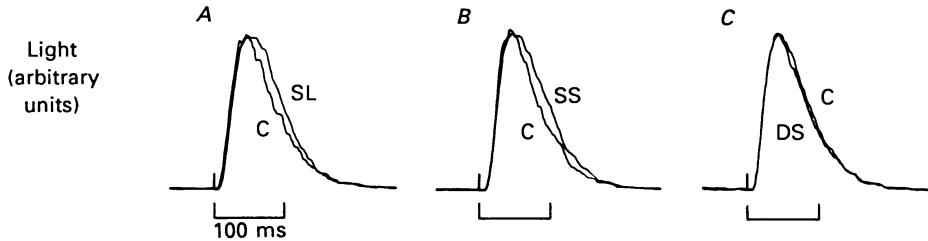


Fig. 4. The duration of the $Ca^{2+}$ transient during various shortening protocols. Each panel shows averaged ($n = 128$) and normalized aequorin light transients from a control period of isometric contraction at $L_{max}$ (C) and from a subsequent period of shortening to 82% $L_{max}$ with one of the shortening protocols. *A*, maintained shortening (SL). *B*, repeated systolic shortening (SS). *C*, repeated diastolic shortening (DS).

There was no change in the duration of the twitch or the light transient either immediately after starting the diastolic length changes or during the development of the slow changes. Figure 4*C* shows more clearly that the duration of light transients was not significantly different during this protocol. Measurements of the duration of the light transient (at 25% of maximum amplitude) showed no significant change ($-1·4 \pm 1·4$%, mean $\pm$ S.E.M., $n = 16$) when compared to controls at $L_{max}$.

*Length changes in resting muscles*

The above results demonstrate that the amplitude of the light transients is affected by muscle length irrespective of whether the muscle is in systole or diastole. This suggests that muscle length has some effect on $Ca^{2+}$ handling in the cell which is not dependent on systolic activation. A further experiment to confirm this possibility is shown in Fig. 5. In *A* the muscle was rested for 7 min and then regular stimulation restarted. The amplitude of the first contraction after a rest was little affected in this particular muscle but subsequently tension declined for five contractions and then slowly recovered. The pattern of recovery of the light transients is obscured by noise but the first light transient is large and subsequent transients are somewhat smaller than control and return to control over 5–10 min. In *B* the same duration of rest was applied but muscle length was reduced to 80% $L_{max}$ 10 s before the rest started and was returned to 100% $L_{max}$ 10 s before stimulation was restarted. The tension developed in the first beat after the rest in *B* was reduced to less than 50% of the equivalent contraction in *A* and this difference gradually declined over 5–10 min. Likewise the light transients were substantially depressed to less than 50 % when *B* is compared to *A* and this difference also declined over 5–10 min. Similar results were seen in all preparations examined in this way ($n = 4$).

One possible explanation for the changes in light transients following changes in diastolic or resting length is that the resting myoplasmic [$Ca^{2+}$] changes as a function

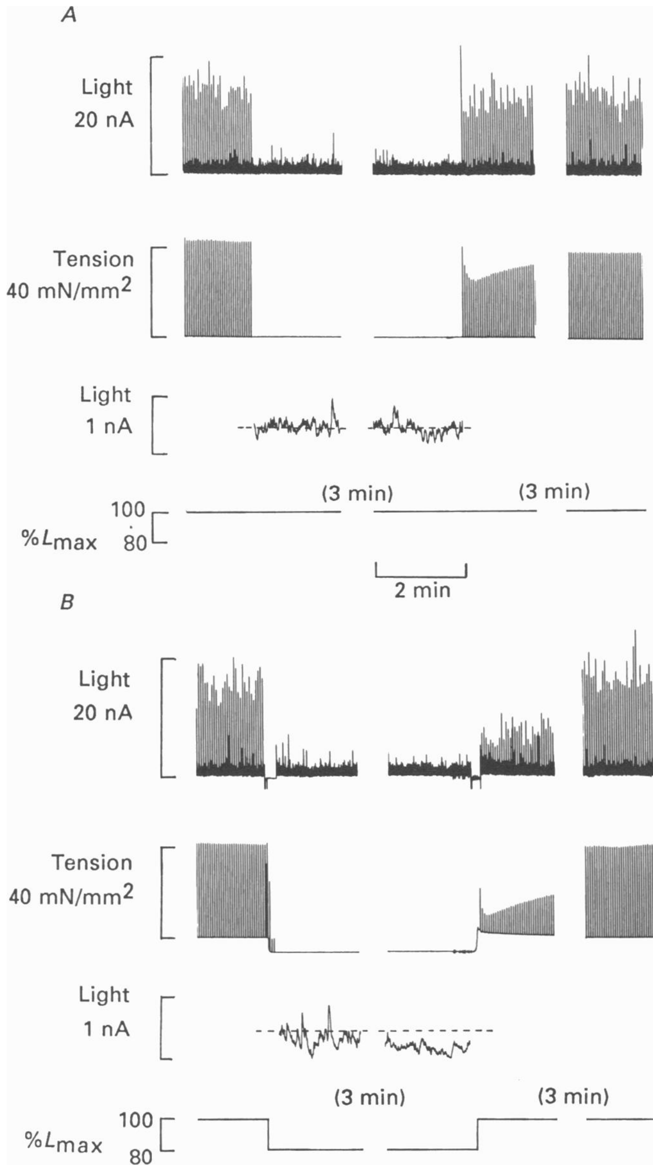


Fig. 5. The effect of muscle length during a rest on subsequent recovery of aequorin light and tension. Note two breaks of 3 min in the record. *A*, muscle length at $L_{max}$ throughout. Rest duration was 7 min. High-gain light record is shown during the rest period and was low-pass filtered (bandwidth 0–0·1 Hz). *B*, muscle length reduced to 80 % $L_{max}$ from 10 s before the start of the rest to 10 s before stimulation restarted. The muscle length was changed manually in this experiment – to protect the photomultiplier it was necessary to close its shutter during these periods. Dashed line through both high-gain light records indicates the mean light signal during the rest period in *A*.

TABLE 1. Tension and light during the three shortening protocols

Maintained reduction in length

| | Control | 1st min after shortening | 20th min after shortening | 1st min after restretch | 20th min after restretch |
|---|---|---|---|---|---|
| Developed tension (%) | 100 | 12 ± 3 | 4·5 ± 1·3 | 56 ± 3 | 107 ± 4 |
| Amplitude of light transient (%) | 100 | 106 ± 11 | 79 ± 5 | 64 ± 1 | 99 ± 7 |
| Slow phase (%) | Tension | −59 ± 11 | | +92 ± 9 | |
| | Light | −25 ± 3 | | +55 ± 7 | |

Repeated systolic shortening

| | Control | 1st min of shortening | 20th min of shortening | 1st min at control length | 20th min at control length |
|---|---|---|---|---|---|
| Developed tension (%) | 100 | 10 ± 1 | 7·0 ± 1·5 | 76 ± 6 | 92 ± 4 |
| Amplitude of light transient (%) | 100 | 113 ± 15 | 97 ± 7 | 76 ± 5 | 90 ± 5 |
| Slow phase (%) | Tension | −31 ± 8* | | +21 ± 8** | |
| | Light | −13 ± 5* | | +18 ± 5** | |

Repeated diastolic shortening

| | Control | 1st min of shortening | 20th min of shortening | 1st min at control length | 20th min at control length |
|---|---|---|---|---|---|
| Developed tension (%) | 100 | 95 ± 2 | 58 ± 4 | 63 ± 4 | 95 ± 4 |
| Amplitude of light transient (%) | 100 | 99 ± 3 | 71 ± 4 | 74 ± 5 | 98 ± 6 |
| Slow phase (%) | Tension | −43 ± 2 | | +51 ± 7* | |
| | Light | −28 ± 4 | | +31 ± 4 | |

Peak developed tension and amplitude of aequorin light transient from the three shortening protocols described in Results. The table shows averaged results from four complete experiments; each value in the table is the mean ± S.E.M. In each panel the values of tension and light at the start were defined as 100%. The slow phase was calculated as defined in the Methods and the S.E.M. calculated on these values. In all cases the control length was 100% $L_{max}$ while the shorter length varied between 80 and 85% $L_{max}$. Top panel, maintained reduction in length. Middle panel, repeated systolic shortening. The fraction of the cycle at the short length varied from 12 to 18% in the different experiments. Bottom panel, repeated diastolic shortening. The fraction of the cycle at the short length varied from 82 to 88% in the different experiments. In the middle and bottom panels asterisks against the slow phases of tension and light indicate the result of a paired $t$ test (two tails) in which each slow phase was compared to the equivalent slow phase associated with maintained length reduction (top panel). No asterisk, $P > 0·05$; *$P$ between 0·05 and 0·01; **$P < 0·01$.

of muscle length. Accordingly in five experiments we recorded resting light with high gain and increased filtering (bandwidth 0–0·1 Hz) during rest periods at either $L_{max}$ or at 80% $L_{max}$. In four experiments we observed no significant change in resting light. In one experiment, which is that shown in Fig. 5, resting light remained constant when the rest was at the same length ($L_{max}$) as the preceding period of stimulation. However when the muscle was shortened in the rest period, there was significant decline in resting light over 5–6 min. In this preparation the decline of resting light when muscle length was reduced was observed on two occasions.

## DISCUSSION

This study confirms earlier observations (Allen & Kurihara, 1982) that muscle length influences the amplitude of $Ca^{2+}$ transients. It also confirms the finding (Nichols, 1985*b*) that the slow changes in tension caused by stretch or shortening develop whether or not contractions occur. The main new finding is that changes in $Ca^{2+}$ transients appear to underlie the influence of diastolic or resting muscle length on tension. The evidence that changes in $Ca^{2+}$ transient cause the changes in tension is (i) the changes in $Ca^{2+}$ transient are of approximately the correct amplitude to explain the changes in tension (Allen & Kurihara, 1980; Wier & Yue, 1986) and (ii) the time course of the changes in $Ca^{2+}$ transient is similar to the time course of the changes in tension.

This study shows that abbreviation of the twitch and prolongation of the $Ca^{2+}$ transient are only observed when the muscle is shortened during systole. One of the possible mechanisms considered by Allen & Kurihara (1982) for the slow changes in the amplitude of the $Ca^{2+}$ transients was that they were caused by the changes in the duration of the $Ca^{2+}$ transient. This possibility is excluded by the present results because, during repeated diastolic shortening, the slow changes in $Ca^{2+}$ transient amplitude occur in the absence of changes in the duration of the $Ca^{2+}$ transient. The present result is however consistent with the hypothesis that abbreviation of the twitch and prolongation of the $Ca^{2+}$ transient are caused by the reduced developed tension which reduces the binding constant of troponin for $Ca^{2+}$ (Allen & Kurihara, 1982; Housmans, Lee & Blinks, 1983; Allen & Kentish, 1988).

An unresolved question is how the diastolic or resting length influences the subsequent $Ca^{2+}$ transients. Our working hypothesis is that when muscle length is decreased, the resting myoplasmic $[Ca^{2+}]$ declines with a time course of 10–20 min. This would lead to decreased loading of the sarcoplasmic reticulum and the $Ca^{2+}$ transients would be reduced when stimulation restarted. Such changes in resting myoplasmic $[Ca^{2+}]$ have been observed in skeletal muscle (Snowdowne & Lee, 1980; Lopez, Alamo & Caputo, 1985) which, of course, can be stretched over a much greater range of lengths. Unfortunately, our own experiments on this point were suggestive but inconclusive. In one experiment we observed a decline in resting $[Ca^{2+}]$ after shortening to 80% $L_{max}$ with a time course similar to the decline in $Ca^{2+}$ transients which would have occurred if stimulation had continued. However in four other experiments no such effect could be detected. The problem may be that resting $[Ca^{2+}]$ is at the limits of detectability using aequorin in heart muscle. It is relatively easy to detect rises in resting $[Ca^{2+}]$ (e.g. Allen, Eisner & Orchard, 1984) but it is very difficult to detect decreases in resting $[Ca^{2+}]$ such as may occur when resting length is reduced to below $L_{max}$. A definitive answer to this question will probably require methods which are much more sensitive to resting $[Ca^{2+}]$.

There are a number of mechanisms by which changes in muscle length might affect resting $[Ca^{2+}]$. An attractive possibility is that stretch-sensitive channels, such as those described by Guharay & Sachs (1984) in skeletal muscle or by Lansman, Hallam & Rink (1987) in vascular endothelial cells, might be involved. These channels are relatively non-specific allowing passage of $Na^+$, $Ca^{2+}$ and $K^+$. Consequently activation of such channels by stretch in a resting muscle would be

expected to lead to an influx of $Na^+$ and $Ca^{2+}$ (since these have large inward gradients). Influx of $Ca^{2+}$ would directly raise the $[Ca^{2+}]_i$ whereas influx of $Na^+$ would raise $[Ca^{2+}]_i$ by operation of the $Na^+$–$Ca^{2+}$ exchanger.

*Physiological significance of slow changes in tension*

The slow changes in tension following a length change may be of considerable importance in the response of the heart to increases in venous filling pressure or in arterial pressure. Either of these interventions will lead to increases in heart size (and therefore muscle length) which would be expected to lead to an immediate increase in developed tension followed by a slower phase. However, Blinks & Endoh (1986) regard the slow changes in tension in response to length changes as 'an epiphenomenon, rather than an essential aspect of the length–tension relationship'. The basis for their view is that the slow changes in tension are considerably smaller at 37 °C than at 30 °C (Parmely & Chuck, 1973) and are not invariably present in isolated preparations. In our experience, slow changes in tension following length changes are variable in magnitude but nearly always present in mammalian ventricular preparations. One exception are preparations which are close to maximum activation, e.g. rat papillary muscles at 30 °C and 2 mM-extracellular $Ca^{2+}$: in such cases the slow changes can be revealed by lowering extracellular $Ca^{2+}$ (Allen & Kurihara, 1982). Slow changes in tension following a length change have been reported in isolated ventricular preparations of cat, rat, ferret, rabbit, chicken and dog (Parmley & Chuck, 1973; Allen & Kurihara, 1982; Nichols, 1985*a*; present study). More important, an apparently similar phenomenon has now been observed in intact, blood-perfused dog hearts (Tucci, Bregagnollo, Spadaro, Cicogna & Ribeiro, 1984; Nichols, 1985*a*). In the experiments of Tucci *et al.* the increment of left ventricular pressure associated with the slow phase (defined as in Methods) for a moderate volume expansion was 38%. Thus the phenomenon appears to be of considerable quantitative importance in intact, blood-perfused heart at 37 °C as well as in isolated ventricular preparations.

Supported by grants from the Medical Research Council and the British Heart Foundation. We thank Drs J. C. Kentish and J. A. Lee for comments on the manuscript. The aequorin used in this study was prepared in the laboratory of Dr J. R. Blinks with support from NIH Grant HL 12186.

REFERENCES

ALLEN, D. G., EISNER, D. A. & ORCHARD, C. H. (1984). Factors affecting free intracellular calcium concentration in quiescent ferret ventricular muscle. *Journal of Physiology* **350**, 615–630.

ALLEN, D. G. & KENTISH, J. C. (1985). The cellular basis of the length–tension relation in cardiac muscle. *Journal of Molecular and Cellular Cardiology* **17**, 821–840.

ALLEN, D. G. & KENTISH, J. C. (1988). Calcium concentration in the myoplasm of skinned ferret ventricular muscle following changes in muscle length. *Journal of Physiology* (in the Press).

ALLEN, D. G. & KURIHARA, S. (1980). Calcium transients in mammalian ventricular muscle. *European Heart Journal* 1, suppl. A, 5–15.

ALLEN, D. G. & KURIHARA, S. (1982). The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *Journal of Physiology* **327**, 79–94.

ALLEN, D. G., NICHOLS, C. G. & SMITH, G. L. (1985). The effect of diastolic length on calcium transients in isolated ferret ventricular muscle. *Journal of Physiology* **365**, 57*P*.

ALLEN, D. G. & SMITH, G. L. (1985). The first calcium transient following shortening in isolated ferret ventricular muscle. *Journal of Physiology* **366**, 83*P*.

ALLEN, D. G., SMITH, G. L. & NICHOLS, C. G. (1988). Intracellular calcium concentration following length changes in mammalian cardiac muscle. In *Starling's Law of the Heart Revisited*, ed. TER KEURS, H. E. D. J. & TYBERG, J. V. Dordrecht, Netherlands: Martinus Nijhoff.

BLINKS, J. R. & ENDOH, M. (1986). Modification of myofibrillar responsiveness to $Ca^{2+}$ as an inotropic mechanism. *Circulation* **73**, suppl. III, 85–98.

FRANK, O. (1895). Zur Dynamik des Herzmuskels. *Zeitschrift für Biologie* **32**, 370–447.

GUHARAY, F. & SACHS, F. (1984). Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *Journal of Physiology* **352**, 685–701.

HANCK, D. A. & JEWELL, B. R. (1985). Effects of physiological beating on the contractility of cat ventricular muscle. *American Journal of Physiology* **248**, H894–900.

HOUSMANS, P. K., LEE, N. K. & BLINKS, J. R. (1983). Active shortening retards the decline of the intracellular calcium transient in mammalian heart muscle. *Science* **221**, 159–161.

JEWELL, B. R. (1977). A re-examination of the influence of muscle length on myocardial performance. *Circulation Research* **40**, 221–230.

LAKATTA, E. G. & JEWELL, B. R. (1977). Length-dependent activation; its effects on the length–tension relation in cat ventricular muscle. *Circulation Research* **40**, 251–257.

LANSMAN, J. B., HALLAM, T. J. & RINK, T. J. (1987). Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature* **325**, 811–813.

LOPEZ, J. R., ALAMO, L. & CAPUTO, C. (1985). The increase in metabolic rate associated with stretching in skeletal muscle might be related to an increment in free [$Ca^{2+}$]. *Biophysical Journal* **47**, 378a.

NICHOLS, C. G. (1985*a*). The effects of changes of length and load on contractility in mammalian myocardium. Ph.D. Thesis, University of Leeds.

Nichols, C. G. (1985*b*). The influence of 'diastolic' length on the contractility of isolated cat papillary muscle. *Journal of Physiology* **361**, 269–279.

PARMLEY, W. W. & CHUCK, L. (1973). Length-dependent changes in myocardial contractile state. *American Journal of Physiology* **224**, 1195–1199.

PATTERSON, S. & STARLING, E. H. (1914). On the mechanical factors which determine the output of the ventricles. *Journal of Physiology* **48**, 357–379.

SNOWDOWNE, K. W. & LEE, N. K. M. (1980). Subcontracture concentrations of potassium and stretch cause an increase in the activity of intracellular calcium in frog skeletal muscle. *Federation Proceedings* **39**, 1733.

TUCCI, P. F. J., BREGAGNOLLO, E. A., SPADARO, J., CICOGNA, A. C. & RIBEIRO, M. C. L. (1984). Length-dependence of activation studied in the iso-volumic blood-perfused heart. *Circulation Research* **55**, 59–66.

WIER, W. G. & YUE, D. T. (1986). Intracellular calcium transients underlying the short-term force–interval relationship in ferret ventricular myocardium. *Journal of Physiology* **376**, 507–530.